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Attorneys for Plaintiffs

UNITED STATES DISTRICT COURT

CENTRAL DISTRICT OF CALIFORNIA, WESTERN DIVISION

BIOGEN INTERNATIONAL GMBH and BIOGEN MA INC.,

Plaintiffs,

v.

STASON PHARMACEUTICALS, INC. and SAWAI PHARMACEUTICAL CO., LTD.,

Defendants.

Case No.

COMPLAINT FOR PATENT INFRINGEMENT

Plaintiffs Biogen International GmbH and Biogen MA Inc. (collectively, "Biogen" or "Plaintiffs"), by way of Complaint against Defendants Stason Pharmaceuticals, Inc. ("Stason") and Sawai Pharmaceutical Co., Ltd. ("Sawai Pharmaceutical") (collectively, "Sawai" or "Defendants"), allege as follows:

BASIS FOR JURISDICTION STATEMENT

1. Biogen received a letter dated May 31, 2017 ("the Notice Letter"), purporting to include a Notice of Certification for ANDA No. 210285 under 21 U.S.C. § 355(j)(2)(B)(ii) and 21 C.F.R. § 314.95(c). The Notice Letter states:

> Stason Pharmaceuticals, Inc., acting as the U.S. agent for Sawai USA, Inc. (collectively hereinafter "Sawai) is providing …notice that Sawai has submitted … an Abbreviated New Drug Application.

2. Biogen believes that this suit is properly placed in Delaware because Sawai USA, Inc. is incorporated there. Biogen is therefore filing concurrently herewith a case in Delaware. Biogen is filing the present suit in this district merely out of an abundance of caution given the confusing language in the Notice Letter suggesting that Stason participated in the filing of the noted ANDA.

THE PARTIES

3. Plaintiff Biogen International GmbH is a Swiss corporation with its principal place of business in Zug, Switzerland at Landis + Gyr-Strasse 3, 6300 Zug, Switzerland.

4. Plaintiff Biogen MA Inc. is a corporation organized and existing under the laws of the Commonwealth of Massachusetts with its principal place of business at 225 Binney Street, Cambridge, Massachusetts 02142.

5. Biogen is in the business of developing, manufacturing and marketing innovative therapies for patients living with serious neurological, autoimmune, and rare diseases, including therapies for multiple sclerosis. Biogen's asserted patents

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cover Tecfidera®, which is marketed and sold in this judicial district and throughout the united states for the treatment of relapsing forms of multiple sclerosis.

6. Upon information and belief, Stason is a corporation organized under the laws of California, having a principal place of business at 11 Morgan, Irvine, CA 92618.

7. Upon information and belief, Stason is a generic pharmaceutical company that develops, manufactures, markets, and distributes generic pharmaceutical products for sale in the State of California and throughout the United States.

8. Upon information and belief, Sawai Pharmaceutical is a corporation organized under the laws of Japan, having a principal place of business in Osaka, Japan.

9. Upon information and belief, Sawai Pharmaceutical is a generic pharmaceutical company that develops, manufactures, markets, and distributes generic pharmaceutical products for sale in the State of California and throughout the United States.

10. A press release by Sawai Pharmaceutical states that "Sawai Pharmaceutical Co., Ltd. … through its subsidiary Sawai USA, Inc. (Headquarters: Delaware, USA), …submitted Abbreviated New Drug Application (ANDA) with Paragraph IV certificate attached for the therapeutic agent 'Dimethyl Fumarate Delayed-Release Capsules, 120 mg and 240 mg." (emphasis added).

11. Upon information and belief, Stason provided the Notice Letter to Biogen, but Sawai Pharmaceutical submitted its ANDA through its Delaware subsidiary Sawai USA, Inc.

12. Upon information and belief, the acts of Stason complained of herein were done with the cooperation, participation, and assistance of, and at the direction of, Sawai Pharmaceutical and Sawai USA, Inc.

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NATURE OF THE ACTION

13. This is an action for patent infringement of U.S. Patent Nos. 6,509,376 ("the '376 patent"), 7,320,999 ("the '999 patent"), 7,619,001 ("the '001 patent"), 7,803,840 ("the '840 patent"), 8,759,393 ("the '393 patent") and 8,399,514 ("the '514 patent") arising under the patent laws of the United States, Title 35, United States Code, §§ 100 et seq., including 35 U.S.C. § 271. This action relates to Sawai's filing of Abbreviated New Drug Application ("ANDA") No. 210285 under Section 505(j) of the Federal Food, Drug and Cosmetic Act ("the Act"), 21 U.S.C. § 355(j), seeking U.S. Food and Drug Administration ("FDA") approval to manufacture, use, sell, offer to sell, and import dimethyl fumarate delayed-release capsules prior to the expiration of the asserted patents.

JURISDICTION AND VENUE

14. This Court has subject matter jurisdiction under 28 U.S.C. §§ 1331 and 1338(a).

15. Venue is proper in this Court under 28 U.S.C. § 1391(b) and (c), and § 1400(b) because Stason is incorporated in California and Sawai Pharmaceutical is incorporated in Japan and may be sued in any judicial district in the United States in which the defendant is subject to the court's personal jurisdiction.

16. This Court has personal jurisdiction over Stason because Stason is incorporated in California.

17. This Court has personal jurisdiction over Sawai Pharmaceutical under Federal Rule of Civil Procedure 4(k)(2), because, upon information and belief, Sawai Pharmaceutical is organized under the laws of Japan.

18. Upon information and belief, Stason and Sawai Pharmaceuticals have been, and continue to be, joint and prime actors, together with Sawai USA, Inc., in the drafting, submission, approval and maintenance of ANDA No. 210285.

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19. For these reasons and for other reasons that will be presented to the Court if jurisdiction is challenged, the Court has personal jurisdiction over Defendants.

FIRST CLAIM FOR RELIEF

(Patent Infringement ('376 Patent))

20. Biogen realleges, and incorporates in full herein, each preceding paragraph.

21. The U.S. Patent and Trademark Office ("PTO") issued the '376 patent on January 21, 2003, entitled "Utilization of Dialkyfumarates." The '376 patent identifies Rajendra Kumar Joshi and Hans-Peter Strebel as inventors of the claimed subject matter. A copy of the '376 patent is attached hereto as Exhibit A.

22. Biogen International GmbH is the owner of the '376 patent by virtue of assignment.

23. The '376 patent expires on October 29, 2019, excluding any pediatric exclusivity or patent term extension.

24. The '376 patent is directed to and claims, inter alia, pharmaceutical preparations and compositions.

25. The '376 patent is listed in Approved Drug Products with Therapeutic Equivalence Evaluations ("the Orange Book") for New Drug Application ("NDA") No. 204063 for dimethyl fumarate delayed-release capsules.

26. The FDA approved NDA No. 204063 on March 27, 2013, for the treatment of relapsing forms of multiple sclerosis.

27. Dimethyl fumarate delayed-release capsules are marketed in the United States under the trademark Tecfidera®.

28. Upon information and belief, Sawai submitted ANDA No. 210285 to the FDA, under Section 505(j) of the Act, 21 U.S.C. § 355(j), seeking approval to manufacture, use, import, offer to sell and sell dimethyl fumarate delayed-release

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capsules containing 120 mg and 240 mg of dimethyl fumarate ("Sawai's generic products") in the United States.

29. The Notice Letter dated May 31, 2017 purported to include a Notice of Certification for ANDA No. 210285 under 21 U.S.C. § 355(j)(2)(B)(ii) and 21 C.F.R. § 314.95(c) as to the '376 patent. The Notice Letter did not allege non-infringement as to at least one claim of the '376 patent.

30. Sawai thus has actual knowledge of the '376 patent.

31. Upon information and belief, Sawai's generic products, if approved and marketed, will infringe, either literally or under the doctrine of equivalents, at least one claim including at least claim 1 of the '376 patent under at least one of 35 U.S.C. § 271(a), (b), and/or (c).

32. Upon information and belief, under 35 U.S.C. § 271(e)(2)(A), Sawai has infringed at least one claim including at least claim 1 of the '376 patent by submitting, or causing to be submitted, to the FDA, ANDA No. 210285 seeking approval to manufacture, use, import, offer to sell or sell Sawai's generic products before the expiration date of the '376 patent. Upon information and belief, the products described in ANDA No. 210285 would infringe, either literally or under the doctrine of equivalents, at least one claim including at least claim 1 of the '376 patent under 35 U.S.C. § 271(e)(2)(A).

33. Upon information and belief, Sawai will manufacture, market, import, use, sell and/or offer to sell Sawai's generic products in the United States in connection with ANDA No. 210285 upon approval.

34. Upon information and belief, Sawai will directly infringe at least one claim including at least claim 1 of the '376 patent when it proceeds to manufacture, market, import, use, sell and/or offer to sell Sawai's generic products in the United States in connection with ANDA No. 210285 upon approval.

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35. Upon information and belief, Sawai's actions relating to Sawai's ANDA No. 210285 complained of herein were done with the cooperation, participation, assistance, and for the benefit of Sawai.

36. If Sawai's marketing and sale of generic dimethyl fumarate delayed-release capsules prior to expiration of the '376 patent and all other relevant activities are not enjoined, Biogen will suffer substantial and irreparable harm for which there is no adequate remedy at law.

SECOND CLAIM FOR RELIEF

(Patent Infringement ('999 Patent))

37. Biogen realleges, and incorporates in full herein, each preceding paragraph.

38. The PTO issued the '999 patent on January 22, 2008, entitled "Dimethyl Fumarate for the Treatment of Multiple Sclerosis." The '999 patent identifies Rajendra Kumar Joshi and Hans-Peter Strebel as inventors of the claimed subject matter. A copy of the '999 patent is attached hereto as Exhibit B.

39. Biogen International GmbH is the owner of the '999 patent by virtue of assignment.

40. The '999 patent expires on May 18, 2020, which includes 202 days of Patent Term Adjustment under 35 U.S.C. § 154(b), excluding any pediatric exclusivity or patent term extension.

41. The '999 patent is directed to and claims, inter alia, methods of treating multiple sclerosis.

42. The '999 patent is listed in the Orange Book for NDA No. 204063 for dimethyl fumarate delayed-release capsules.

43. The Notice Letter dated May 31, 2017, purported to include a Notice of Certification for ANDA No. 210285 under 21 U.S.C. § 355(j)(2)(B)(ii) and 21 C.F.R. § 314.95(c) as to the '999 patent. The Notice Letter did not allege non-infringement as to any claim of the '999 patent.

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44. Sawai thus has actual knowledge of the '999 patent.

45. Upon information and belief, Sawai's generic products, if approved and marketed, will infringe, either literally or under the doctrine of equivalents, at least one claim including at least claim 1 of the '999 patent under at least one of 35 U.S.C. § 271(a), (b), and/or (c).

46. Upon information and belief, under 35 U.S.C. § 271(e)(2)(A), Sawai has infringed at least one claim including at least claim 1 of the '999 patent by submitting, or causing to be submitted, to the FDA, ANDA No. 210285 seeking approval to manufacture, use, import, offer to sell or sell Sawai's generic products before the expiration date of the '999 patent. Upon information and belief, the products described in ANDA No. 210285 would infringe, either literally or under the doctrine of equivalents, at least one claim including at least claim 1 of the '999 patent under 35 U.S.C. § 271(e)(2)(A).

47. Upon information and belief, physicians and/or patients will directly infringe at least one claim including at least claim 1 of the '999 patent by the use of Sawai's generic products upon approval.

48. Upon information and belief, upon approval, Sawai will take active steps to encourage the use of Sawai's generic products by physicians and/or patients with the knowledge and intent that Sawai's generic products will be used by physicians and/or patients, in a manner that infringes at least one claim including at least claim 1 of the '999 patent, for the pecuniary benefit of Sawai. Pursuant to 21 C.F.R. § 314.94, Sawai is required to copy the FDA approved Tecfidera® labeling. Upon information and belief, Sawai will thus induce the infringement of at least one claim including at least claim 1 of the '999 patent.

49. Upon information and belief, if the FDA approves ANDA No. 210285, Sawai will sell or offer to sell its generic products specifically labeled for use in practicing at least one claim including at least claim 1 of the '999 patent, wherein Sawai's generic products are a material part of the claimed invention, wherein

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Sawai knows that physicians will prescribe and patients will use Sawai's generic products in accordance with the instructions and/or label provided by Sawai in practicing at least one claim including at least claim 1 of the '999 patent, and wherein dimethyl fumarate delayed-release capsules are not staple articles or commodities of commerce suitable for substantial non-infringing use. Upon information and belief, Sawai will thus contribute to the infringement of at least one claim including at least claim 1 of the '999 patent.

50. Upon information and belief, Sawai's actions relating to Sawai's ANDA No. 210285 complained of herein were done with the cooperation, participation, assistance, and for the benefit of Sawai.

51. If Sawai's marketing and sale of generic dimethyl fumarate delayed-release capsules prior to expiration of the '999 patent and all other relevant activities are not enjoined, Biogen will suffer substantial and irreparable harm for which there is no adequate remedy at law.

THIRD CLAIM FOR RELIEF

(Patent Infringement ('001 Patent))

52. Biogen realleges, and incorporates in full herein, each preceding paragraph.

53. The PTO issued the '001 patent on November 17, 2009, entitled "Utilization of Dialkylfumarates." The '001 patent identifies Rajendra Kumar Joshi and Hans-Peter Strebel as inventors of the claimed subject matter. A copy of the '001 patent is attached hereto as Exhibit C.

54. Biogen International GmbH is the owner of the '001 patent by virtue of assignment.

55. The '001 patent expires on April 1, 2018, excluding any pediatric exclusivity or patent term extension.

56. The '001 patent is directed to and claims, inter alia, methods of treating multiple sclerosis.

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57. The '001 patent is listed in the Orange Book for NDA No. 204063 for dimethyl fumarate delayed-release capsules.

58. The Notice Letter dated May 31, 2017, purported to include a Notice of Certification for ANDA No. 210285 under 21 U.S.C. § 355(j)(2)(B)(ii) and 21 C.F.R. § 314.95(c) as to the '001 patent. The Notice Letter did not allege non-infringement as to at least one claim of the '001 patent.

59. Sawai thus has actual knowledge of the '001 patent.

60. Upon information and belief, Sawai's generic products, if approved and marketed, will infringe, either literally or under the doctrine of equivalents, at least one claim including at least claim 1 of the '001 patent under at least one of 35 U.S.C. § 271(a), (b), and/or (c).

61. Upon information and belief, under 35 U.S.C. § 271(e)(2)(A), Sawai has infringed at least one claim including at least claim 1 of the '001 patent by submitting, or causing to be submitted, to the FDA, ANDA No. 210285 seeking approval to manufacture, use, import, offer to sell or sell Sawai's generic products before the expiration date of the '001 patent. Upon information and belief, the products described in ANDA No. 210285 would infringe, either literally or under the doctrine of equivalents, at least one claim including at least claim 1 of the '001 patent under 35 U.S.C. § 271(e)(2)(A).

62. Upon information and belief, physicians and/or patients will directly infringe at least one claim including at least claim 1 of the '001 patent by the use of Sawai's generic products upon approval.

63. Upon information and belief, upon approval, Sawai will take active steps to encourage the use of Sawai's generic products by physicians and/or patients with the knowledge and intent that Sawai's generic products will be used by physicians and/or patients, in a manner that infringes at least one claim including at least claim 1 of the '001 patent, for the pecuniary benefit of Sawai. Pursuant to 21 C.F.R. § 314.94, Sawai is required to copy the FDA approved Tecfidera® labeling.

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Upon information and belief, Sawai will thus induce the infringement of at least one claim including at least claim 1 of the '001 patent.

64. Upon information and belief, if the FDA approves ANDA No. 210285, Sawai will sell or offer to sell its generic products specifically labeled for use in practicing at least one claim including at least claim 1 of the '001 patent, wherein Sawai's generic products are a material part of the claimed invention, wherein Sawai knows that physicians will prescribe and patients will use Sawai's generic products in accordance with the instructions and/or label provided by Sawai in practicing at least one claim including at least claim 1 of the '001 patent, and wherein dimethyl fumarate delayed-release capsules are not staple articles or commodities of commerce suitable for substantial non-infringing use. Upon information and belief, Sawai will thus contribute to the infringement of at least one claim including at least claim 1 of the '001 patent.

65. Upon information and belief, Sawai's actions relating to Sawai's ANDA No. 210285 complained of herein were done with the cooperation, participation, assistance, and for the benefit of Sawai.

66. If Sawai's marketing and sale of generic dimethyl fumarate delayed-release capsules prior to expiration of the '001 patent and all other relevant activities are not enjoined, Biogen will suffer substantial and irreparable harm for which there is no adequate remedy at law.

FOURTH CLAIM FOR RELIEF

(Patent Infringement ('840 Patent))

67. Biogen realleges, and incorporates in full herein, each preceding paragraph.

68. The PTO issued the '840 patent on September 28, 2010, entitled "Utilization of Dialkylfumarates." The '840 patent identifies Rajendra Kumar Joshi and Hans-Peter Strebel as inventors of the claimed subject matter. A copy of the '840 patent is attached hereto as Exhibit D.

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69. Biogen International GmbH is the owner of the '840 patent by virtue of assignment.

70. The '840 patent expires on April 1, 2018, excluding any pediatric exclusivity or patent term extension.

71. The '840 patent is directed to and claims, inter alia, methods of treating multiple sclerosis.

72. The '840 patent is listed in the Orange Book for NDA No. 204063 for dimethyl fumarate delayed-release capsules.

73. The Notice Letter dated May 31, 2017, purported to include a Notice of Certification for ANDA No. 210285 under 21 U.S.C. § 355(j)(2)(B)(ii) and 21 C.F.R. § 314.95(c) as to the '840 patent. The Notice Letter did not allege non-infringement as to any claim of the '840 patent.

74. Sawai thus has actual knowledge of the '840 patent.

75. Upon information and belief, Sawai's generic products, if approved and marketed, will infringe, either literally or under the doctrine of equivalents, at least one claim including at least claim 1 of the '840 patent under at least one of 35 U.S.C. § 271(a), (b), and/or (c).

76. Upon information and belief, under 35 U.S.C. § 271(e)(2)(A), Sawai has infringed at least one claim including at least claim 1 of the '840 patent by submitting, or causing to be submitted, to the FDA, ANDA No. 210285 seeking approval to manufacture, use, import, offer to sell or sell Sawai's generic products before the expiration date of the '840 patent. Upon information and belief, the products described in ANDA No. 210285 would infringe, either literally or under the doctrine of equivalents, at least one claim including at least claim 1 of the '840 patent under 35 U.S.C. § 271(e)(2)(A).

77. Upon information and belief, physicians and/or patients will directly infringe at least one claim including at least claim 1 of the '840 patent by the use of Sawai's generic products upon approval.

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78. Upon information and belief, upon approval, Sawai will take active steps to encourage the use of Sawai's generic products by physicians and/or patients with the knowledge and intent that Sawai's generic products will be used by physicians and/or patients, in a manner that infringes at least one claim including at least claim 1 of the '840 patent, for the pecuniary benefit of Sawai. Pursuant to 21 C.F.R. § 314.94, Sawai is required to copy the FDA approved Tecfidera® labeling. Upon information and belief, Sawai will thus induce the infringement of at least one claim including at least claim 1 of the '840 patent.

79. Upon information and belief, if the FDA approves ANDA No. 210285, Sawai will sell or offer to sell its generic products specifically labeled for use in practicing at least one claim including at least claim 1 of the '840 patent, wherein Sawai's generic products are a material part of the claimed invention, wherein Sawai knows that physicians will prescribe and patients will use Sawai's generic products in accordance with the instructions and/or label provided by Sawai in practicing at least one claim including at least claim 1 of the '840 patent, and wherein dimethyl fumarate delayed-release capsules are not staple articles or commodities of commerce suitable for substantial non-infringing use. Upon information and belief, Sawai will thus contribute to the infringement of at least one claim including at least claim 1 of the '840 patent.

80. Upon information and belief, Sawai's actions relating to Sawai's ANDA No. 210285 complained of herein were done with the cooperation, participation, assistance, and for the benefit of Sawai.

81. If Sawai's marketing and sale of generic dimethyl fumarate delayed-release capsules prior to expiration of the '840 patent and all other relevant activities are not enjoined, Biogen will suffer substantial and irreparable harm for which there is no adequate remedy at law.

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FIFTH CLAIM FOR RELIEF

(Patent Infringement ('393 Patent))

82. Biogen realleges, and incorporates in full herein, each preceding paragraph.

83. The PTO issued the '393 patent on June 24, 2014, entitled "Utilization of Dialkylfumarates." The '393 patent identifies Rajendra Kumar Joshi and Hans-Peter Strebel as inventors of the claimed subject matter. A copy of the '393 patent is attached hereto as Exhibit E.

84. Biogen International GmbH is the owner of the '393 patent by virtue of assignment.

85. The '393 patent expires on October 29, 2019, excluding any pediatric exclusivity.

86. The '393 patent is directed to and claims, inter alia, pharmaceutical preparations.

87. The '393 patent is listed in the Orange Book for NDA No. 204063 for dimethyl fumarate delayed-release capsules.

88. The Notice Letter dated May 31, 2017, purported to include a Notice of Certification for ANDA No. 210285 under 21 U.S.C. § 355(j)(2)(B)(ii) and 21 C.F.R. § 314.95(c) as to the '393 patent. The Notice Letter did not allege non-infringement as to any claim of the '393 patent.

89. Sawai thus has actual knowledge of the '393 patent.

90. Upon information and belief, Sawai's generic products, if approved and marketed, will infringe, either literally or under the doctrine of equivalents, at least one claim including at least claim 1 of the '393 patent under at least one of 35 U.S.C. § 271(a), (b), and/or (c).

91. Upon information and belief, under 35 U.S.C. § 271(e)(2)(A), Sawai has infringed at least one claim including at least claim 1 of the '393 patent by submitting, or causing to be submitted, to the FDA, ANDA No. 210285 seeking

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approval to manufacture, use, import, offer to sell or sell Sawai's generic products before the expiration date of the '393 patent. Upon information and belief, the products described in ANDA No. 210285 would infringe, either literally or under the doctrine of equivalents, at least one claim including at least claim 1 of the '393 patent under 35 U.S.C. § 271(e)(2)(A).

92. Upon information and belief, Sawai will directly infringe at least one claim including at least claim 1 of the '393 patent when it proceeds to manufacture, market, import, use, sell and/or offer to sell Sawai's generic products in the United States in connection with ANDA No. 210285 upon approval.

93. Upon information and belief, Sawai's actions relating to Sawai's ANDA No. 210285 complained of herein were done with the cooperation, participation, assistance, and for the benefit of Sawai.

94. If Sawai's marketing and sale of generic dimethyl fumarate delayed-release capsules prior to expiration of the '393 patent and all other relevant activities are not enjoined, Biogen will suffer substantial and irreparable harm for which there is no adequate remedy at law.

SIXTH CLAIM FOR RELIEF

(Patent Infringement ('514 Patent))

95. Biogen realleges, and incorporates in full herein, each preceding paragraph.

96. The PTO issued the '514 patent on March 19, 2013, entitled "Treatment for Multiple Sclerosis." The '514 patent identifies Matvey E. Lukashev and Gilmore O'Neill as inventors of the claimed subject matter. A copy of the '514 patent is attached hereto as Exhibit F.

97. Biogen MA Inc. is the owner of the '514 patent by virtue of assignment.

98. The '514 patent expires on February 7, 2028, excluding any pediatric exclusivity.

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99. The '514 patent is directed to and claims, inter alia, methods of treating multiple sclerosis.

100. The '514 patent is listed in the Orange Book for NDA No. 204063 for dimethyl fumarate delayed-release capsules.

101. The Notice Letter dated May 31, 2017, purported to include a Notice of Certification for ANDA No. 210285 under 21 U.S.C. § 355(j)(2)(B)(ii) and 21 C.F.R. § 314.95(c) as to the '514 patent. The Notice Letter did not allege non-infringement as to at least one claim of the '514 patent.

102. Sawai thus has actual knowledge of the '514 patent.

103. Upon information and belief, Sawai's generic products, if approved and marketed, will infringe, either literally or under the doctrine of equivalents, at least one claim including at least claim 1 of the '514 patent under at least one of 35 U.S.C. § 271(a), (b), and/or (c).

104. Upon information and belief, under 35 U.S.C. § 271(e)(2)(A), Sawai has infringed at least one claim including at least claim 1 of the '514 patent by submitting, or causing to be submitted, to the FDA, ANDA No. 210285 seeking approval to manufacture, use, import, offer to sell or sell Sawai's generic products before the expiration date of the '514 patent. Upon information and belief, the products described in ANDA No. 210285 would infringe, either literally or under the doctrine of equivalents, at least one claim including at least claim 1 of the '514 patent under 35 U.S.C. § 271(e)(2)(A).

105. Upon information and belief, physicians and/or patients will directly infringe at least one claim including at least claim 1 the '514 patent by the use of Sawai's generic products upon approval.

106. Upon information and belief, upon approval, Sawai will take active steps to encourage the use of Sawai's generic products by physicians and/or patients with the knowledge and intent that Sawai's generic products will be used by physicians and/or patients, in a manner that infringes at least one claim including at

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least claim 1 of the '514 patent, for the pecuniary benefit of Sawai. Pursuant to 21 C.F.R. § 314.94, Sawai is required to copy the FDA approved Tecfidera® labeling. Upon information and belief, Sawai will thus induce the infringement of at least one claim including at least claim 1 of the '514 patent.

107. Upon information and belief, if the FDA approves ANDA No. 210285, Sawai will sell or offer to sell its generic products specifically labeled for use in practicing at least one claim including at least claim 1 of the '514 patent, wherein Sawai's generic products are a material part of the claimed invention, wherein Sawai knows that physicians will prescribe and patients will use Sawai's generic products in accordance with the instructions and/or label provided by Sawai in practicing at least one claim including at least claim 1 of the '514 patent, and wherein dimethyl fumarate delayed-release capsules are not staple articles or commodities of commerce suitable for substantial non-infringing use. Upon information and belief, Sawai will thus contribute to the infringement of at least one claim including at least claim 1 of the '514 patent.

108. Upon information and belief, Sawai's actions relating to Sawai's ANDA No. 210285 complained of herein were done with the cooperation, participation, assistance, and for the benefit of Sawai.

109. If Sawai's marketing and sale of generic dimethyl fumarate delayed-release capsules prior to expiration of the '514 patent and all other relevant activities are not enjoined, Biogen will suffer substantial and irreparable harm for which there is no adequate remedy at law.

PRAYER FOR RELIEF

WHEREFORE, Biogen respectfully prays that the Court enter judgment in its favor and against Defendants Sawai Pharmaceutical and Sawai USA on the patent infringement claims set forth above and respectfully requests that this Court:

A. enter judgment under 35 U.S.C. § 271(e)(2)(A) that Sawai has infringed at least one claim including at least claim 1 of the '376 patent through

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Sawai's submission of ANDA No. 210285 to the FDA to obtain approval to manufacture, use, import, offer to sell and sell Sawai's generic products in the United States before the expiration of the '376 patent;

B. enter judgment under 35 U.S.C. § 271(a) that Sawai's commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of Sawai's generic products prior to the expiration of the '376 patent constitutes infringement of one or more claims of said patent under 35 U.S.C. § 271(a);

C. order that the effective date of any approval by the FDA of Sawai's generic products be a date that is not earlier than the expiration date of the '376 patent, or such later date as the Court may determine;

D. enjoin Sawai, and all persons acting in concert with Sawai, from the manufacture, use, import, offer for sale and sale of Sawai's generic products until the expiration of the '376 patent, or such later date as the Court may determine;

E. enjoin Sawai, and all persons acting in concert with Sawai, from seeking, obtaining or maintaining approval of Sawai's ANDA No. 210285 until the expiration of the '376 patent, or such later date as the Court may determine;

F. enter judgment under 35 U.S.C. § 271(e)(2)(A) that Sawai has infringed at least one claim including at least claim 1 of the '999 patent through Sawai's submission of ANDA No. 210285 to the FDA to obtain approval to manufacture, use, import, offer to sell and sell Sawai's generic products in the United States before the expiration of the '999 patent;

G. enter judgment under 35 U.S.C. § 271(b) and/or (c) that Sawai's commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of Sawai's generic products

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prior to the expiration of the '999 patent constitutes infringement of one or more claims of said patent under 35 U.S.C. § 271(b) and/or (c);

H. order that the effective date of any approval by the FDA of Sawai's generic products be a date that is not earlier than the expiration date of the '999 patent, or such later date as the Court may determine;

I. enjoin Sawai, and all persons acting in concert with Sawai, from the manufacture, use, import, offer for sale and sale of Sawai's generic products until the expiration of the '999 patent, or such later date as the Court may determine;

J. enjoin Sawai, and all persons acting in concert with Sawai, from seeking, obtaining or maintaining approval of Sawai's ANDA No. 210285 until the expiration of the '999 patent, or such later date as the Court may determine;

K. enter judgment under 35 U.S.C. § 271(e)(2)(A) that Sawai has infringed at least one claim including at least claim 1 of the '001 patent through Sawai's submission of ANDA No. 210285 to the FDA to obtain approval to manufacture, use, import, offer to sell and sell Sawai's generic products in the United States before the expiration of the '001 patent;

L. enter judgment under 35 U.S.C. § 271(b) and/or (c) that Sawai's commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of Sawai's generic products prior to the expiration of the '001 patent constitutes infringement of one or more claims of said patent under 35 U.S.C. § 271(b) and/or (c);

M. order that the effective date of any approval by the FDA of Sawai's generic products be a date that is not earlier than the expiration date of the '001 patent, or such later date as the Court may determine;

N. enjoin Sawai, and all persons acting in concert with Sawai, from the manufacture, use, import, offer for sale and sale of Sawai's generic

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products until the expiration of the '001 patent, or such later date as the Court may determine;

O. enjoin Sawai, and all persons acting in concert with Sawai, from seeking, obtaining or maintaining approval of Sawai's ANDA No. 210285 until the expiration of the '001 patent, or such later date as the Court may determine;

P. enter judgment under 35 U.S.C. § 271(e)(2)(A) that Sawai has infringed at least one claim including at least claim 1 of the '840 patent through Sawai's submission of ANDA No. 210285 to the FDA to obtain approval to manufacture, use, import, offer to sell and sell Sawai's generic products in the United States before the expiration of the '840 patent;

Q. enter judgment under 35 U.S.C. § 271(b) and/or (c) that Sawai's commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of Sawai's generic products prior to the expiration of the '840 patent constitutes infringement of one or more claims of said patent under 35 U.S.C. § 271(b) and/or (c);

R. order that the effective date of any approval by the FDA of Sawai's generic products be a date that is not earlier than the expiration date of the '840 patent, or such later date as the Court may determine;

S. enjoin Sawai, and all persons acting in concert with Sawai, from the manufacture, use, import, offer for sale and sale of Sawai's generic products until the expiration of the '840 patent, or such later date as the Court may determine;

T. enjoin Sawai, and all persons acting in concert with Sawai, from seeking, obtaining or maintaining approval of Sawai's ANDA No. 210285 until the expiration of the '840 patent, or such later date as the Court may determine;

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U. enter judgment under 35 U.S.C. § 271(e)(2)(A) that Sawai has infringed at least one claim including at least claim 1 of the '393 patent through Sawai's submission of ANDA No. 210285 to the FDA to obtain approval to manufacture, use, import, offer to sell and sell Sawai's generic products in the United States before the expiration of the '393 patent;

V. enter judgment under 35 U.S.C. § 271(a) that Sawai's commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of Sawai's generic products prior to the expiration of the '393 patent constitutes infringement of one or more claims of said patent under 35 U.S.C. § 271(a);

W. order that the effective date of any approval by the FDA of Sawai's generic products be a date that is not earlier than the expiration date of the '393 patent, or such later date as the Court may determine;

X. enjoin Sawai, and all persons acting in concert with Sawai, from the manufacture, use, import, offer for sale and sale of Sawai's generic products until the expiration of the '393 patent, or such later date as the Court may determine;

Y. enjoin Sawai, and all persons acting in concert with Sawai, from seeking, obtaining or maintaining approval of Sawai's ANDA No. 210285 until the expiration of the '393 patent, or such later date as the Court may determine;

Z. enter judgment under 35 U.S.C. § 271(e)(2)(A) that Sawai has infringed at least one claim including at least claim 1 of the '514 patent through Sawai's submission of ANDA No. 210285 to the FDA to obtain approval to manufacture, use, import, offer to sell and sell Sawai's generic products in the United States before the expiration of the '514 patent;

AA. enter judgment under 35 U.S.C. § 271(b) and/or (c) that Sawai's commercial manufacture, use, offer for sale, or sale within the United

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States, or importation into the United States of Sawai's generic products prior to the expiration of the '514 patent constitutes infringement of one or more claims of said patent under 35 U.S.C. § 271(b) and/or (c);

BB. order that the effective date of any approval by the FDA of Sawai's generic products be a date that is not earlier than the expiration date of the '514 patent, or such later date as the Court may determine;

CC. enjoin Sawai, and all persons acting in concert with Sawai, from the manufacture, use, import, offer for sale and sale of Sawai's generic products until the expiration of the '514 patent, or such later date as the Court may determine;

DD. enjoin Sawai and all persons acting in concert with Sawai, from seeking, obtaining or maintaining approval of Sawai's ANDA No. 210285 until the expiration of the '514 patent, or such later date as the Court may determine;

EE. declare this to be an exceptional case under 35 U.S.C. §§ 285 and 271(e)(4) and award Biogen costs, expenses and disbursements in this action, including reasonable attorney fees; and

FF. award such further and other relief as this Court deems proper and just.

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DATED: June 30, 2017

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EXHIBIT A

US006509376B1

(12) **United States Patent**
Joshi et al.

(10) **Patent No.: US 6,509,376 B1**
(45) **Date of Patent: Jan. 21, 2003**

(54) **UTILIZATION OF DIALKYFUMARATES**

(75) Inventors: **Rajendra Kumar Joshi**, Zürich (CH); **Hans-Peter Strebel**, Muri (CH)

(73) Assignee: **Fumapharm AG**, Muri (CH)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/831,620**

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§ 371 (c)(1),
(2), (4) Date: **May 10, 2001**

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PCT Pub. Date: **Jun. 2, 2000**

(30) **Foreign Application Priority Data**

Nov. 19, 1998 (DE) 198 53 487

(51) **Int. Cl.**[7] **A61K 31/225**

(52) **U.S. Cl.** **514/547**; 514/960

(58) **Field of Search** 514/547, 960

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Primary Examiner—Kevin E. Weddington
(74) *Attorney, Agent, or Firm*—Sieberth & Patty, L.L.C.

(57) **ABSTRACT**

The present invention relates to the use of certain dialkyl fumarates for the preparation of pharmaceutical preparations for use in transplantation medicine or for the therapy of autoimmune diseases and said compositions in the form of micro-tablets or pellets. For this purpose, the dialkyl fumarates may also be used in combination with conventional preparations used in transplantation medicine and immunosuppressive agents, especially cyclosporines.

16 Claims, No Drawings

UTILIZATION OF DIALKYFUMARATES

REFERENCE TO RELATED APPLICATIONS

This application is a 371 continuation of PCT Application PCT/EP99/08215, filed Oct. 29, 1999, the text of which is not in English, which PCT Application claims priority on German Application No. 198 53 487.6 filed Nov. 19, 1998, the text of which is not in English.

DESCRIPTION

The present invention relates to the use of dialkyl fumarates for preparing pharmaceutical preparations for use in transplantation medicine or the therapy of autoimmune diseases and pharmaceutical preparations in the form of micro-tablets or micro-pellets containing dialkyl fumarates.

On the one hand, therefore, it relates especially to the use of dialkyl fumarates for preparing pharmaceutical preparations for the treatment, reduction or suppression of rejection reactions of the transplant by the recipient, i.e. host-versus graft reactions, or rejection of the recipient by the transplant, i.e. graft-versus-host reactions. On the other hand, it relates to the use of dialkyl fumarates for preparing pharmaceutical preparations for treating autoimmune diseases such as polyarthritis, multiple sclerosis, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (=lupoid) hepatitis.

Both graft rejection and autoimmune diseases are based on medically undesirable reactions or dysregulation of the immune system. Cytokins such as interleukins or tumour necrose factor α (TNF-α) are substantial mediators influencing the immune system. In general, both are treated by the administration of immunosuppressive agents such as cyclosporine.

In the overall result, autoimmune diseases may be defined as the failure of the tolerance of endogenic substances or antigens. As a rule, this tolerance can be maintained only if the antigens keep coming into contact with immunological cells. When this tolerance is lost, autoantibodies are formed, i.e. a humoral immunoresponse against endogenic tissue. The exact nature of the involvement of TNF-α is not known.

Transplantations are tissue or organ transplantations, i.e. the transfer of tissues such as cornea, skin, bones (bone chips), vessels or fasciae, of organs such as kidney, heart, liver, lung, pancreas or intestines, or of individual cells such as islet cells, α-cells and liver cells, the kidney having the greatest significance as a transplanted organ.

According to the degree of relationship between the donor and the recipient we differentiate between auto-transplantation (transfer to another part of the body of the same individual), iso-transplantation (transfer to another, genetically identical individual) and allogenic transplantation (transfer to another individual of the same species). Depending on the site of origin and transplantation, we further differentiate between homotopic transplantation (transfer to the same site) and heterotopic transplantation (transfer to a different site). The above-mentioned transplantations play an important role in modern medicine.

A major problem in transplantation medicine is graft rejection after transplantation of the tissue, organ or cell by immunological defence reactions of the recipient. Such a graft rejection is also called host-versus-graft reaction. The immunological defence reaction of the organism against the heteroprotein often results in rejection or dissolution of the grafts. In host-versus-graft reactions, different stages may be distinguished. Depending on the degree of difference between the recipient and the donor, this reaction takes place at different speeds so that we speak of an acute, subacute or chronic reaction. The acute rejection process is accompanied by the irreversible loss of the transplant (necrotisation) as a result of arteriitis or arteriolitis within 48 hours and cannot be influenced by the administration of drugs. The sub-acute rejection reaction becomes manifest as a rejection crisis from day 12 to month 4 with reversible functional disorders as a result of a transplant vasculopathy. Finally, the loss of function of the transplant as a result of vascular changes such as obliterating arteriopathy, which proceeds over weeks or years and can practically not be influenced by drugs, is termed a chronic rejection reaction.

Vice-versa, rejection reactions of the transplant against the recipient, the so-called graft-versus-host reactions, may occur when immunocompetent tissues are transplanted, i.e. primarily in bone marrow transplantation. Again, the severity of the reaction is graded, and substantially similar complications result as in host-versus-graft-reactions, namely arteriopathies and necroses.

To avoid such rejection reactions, i.e. the host-versus-graft reaction and the graft-versus-host reaction, transplantation medicine essentially makes use of immunosuppression, i.e. a weakening of the normal immunoresponse. For this purpose, anti-lymphocyte sera are often used in combination with corticosteroids and so-called antimetabolites, e.g. purine analogues such as 6-mercaptopurine and thioguanine which affect the nucleic acid and protein synthesis and thus prevent cell division and proliferation. This leads to suppression or the production of antibodies and the cellular immune response. The immunosuppressive agents used for therapy are substances which suppress or weaken the immunoreaction in the body either specifically or non-specifically. Non-specific immunosuppressive agents are cytostatic agents such as, for example, alkylating agents or antimetabolites.

In addition, active ingredients are known which cause at least partial specific immunosuppression, such as corticosteroids, antisera, antibodies FK-506, tacrolimus, mycophenolatemofetil and primarily cyclosporines such as cyclosporine A. As a result of using modern immunosuppressive agents, the most important representatives of which are the cyclosporines, especially cyclosporine A, it was possible to improve the results of transplantation considerably over the last few years. At present, the survival rate after one year is about 60% for liver transplantations, about 80% for heart transplantations and over 90% for kidney transplantations.

Autoimmune diseases where the endogenic immune system attacks endogenic organs, tissues and cells are comparable to graft-versus-host reactions. These are also medically undesirable reactions of the immune system which may be treated with immunosuppressive agents, too.

The danger in using immunosuppressive agents lies in weakening the body's defence against infectious diseases and the increased risk of malignant diseases. Therefore, it is the object of the invention to provide a pharmaceutical preparation to be employed in transplantation medicine which may be used to treat, especially to suppress, weaken and/or alleviate host-versus-graft reactions and graft-versus-host reactions, but does not have the above disadvantage.

It is another object of the invention to provide a pharmaceutical preparation which may be employed for treating autoimmune diseases, particularly polyarthritis, multiple sclerosis, juvenile-onset diabetes, Hashimoto's thyroiditis,

Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (=lupoid) hepatitis, without the disadvantages of immunosuppression.

The object of the invention is achieved by using certain dialkyl fumarates for preparing pharmaceutical preparations for use in transplantation medicine and for the therapy of autoimmune diseases and pharmaceutical preparations in the form of micro-tablets and micro-pellets containing these dialkyl fumarates. The individual subject matters of the invention are characterised in detail in the claims. The preparations according to the invention do not contain any free fumaric acids per se.

It is known that pharmaceutical preparations which, upon biological degradation after administration, enter into the citric acid cycle or are part thereof gain increasing therapeutic significance—especially when given in high dosages—since they can alleviate or heal diseases caused cryptogenetically.

Fumaric acid, for example, inhibits the growth of the Ehrlich ascites tumour in mice, reduces the toxic effects of mitomycin C and aflatoxin and displays anti-psoriatic and anti-microbial activity. When administered parenterally, transdermally and especially perorally, high dosages of fumaric acid or its derivatives known so far such as dihydroxyl fumaric acid, fumaramide and fumaronitrile have such unacceptably severe side effects and high toxicity that, in most cases, such a therapy had to be abandoned in the past.

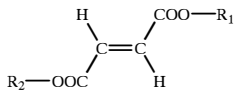
Surprisingly, investigations carried out by the applicant have shown that methyl hydrogen fumarate, a metabolite of the dimethyl fumarate, initially increases the endotoxin-stimulated TNF-α secretion in human mono-nuclear cells of periphere blood (periphere blood mono-nuclear cells= PBMC cells) and in isolated monocytes. In addition, the applicant was able to show that fumaric acid has an effect on in vitro and in vivo haemag-glutination which is comparable to that of cyclosporine.

Surprisingly, it has now been found that dialkyl fumarates are advantageous for preparing pharmaceutical compositions for use in transplantation medicine and for the therapy of autoimmune diseases. This is because compositions containing such dialkyl fumarates surprisingly permit a positive modulation of the immune system in host-versus-graft reactions, graft-versus-host reactions and other autoimmune diseases.

European Patent Application 0 188 749 already describes fumaric acid derivatives and pharmaceutical compositions containing the same for the treatment of psoriasis. Pharmaceutical compositions for the treatment of psoriasis containing a mixture of fumaric acid and other fumaric acid derivatives are known from DE-A-25 30 372. The content of free fumaric acid is obligatory for these medicaments.

DE-A-26 21 214 describes medicaments containing the fumaric acid monoethyl ester and its mineral salts as active ingredient for the treatment of psoriasis. The publication "Hautarzt (Dermatologist) (1987) 279–285" discusses the use of fumaric acid monoethyl ester salts. Pharmaceutical preparations containing a mixture of fumaric acid monoalkyl ester salts and a fumaric acid diester for the treatment of psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn are known from EP 0 312 697 B1.

Specifically, the object of the invention is achieved by the use of one or more dialkyl fumarates of the formula



wherein R_1 and R_2, which may be the same or different, independently represent a linear, branched or cyclic, saturated or unsaturated C_{1-20} alkyl radical which may be optionally substituted with halogen (Cl, F, I, Br), hydroxy, C_{1-4} alkoxy, nitro or cyano for preparing a pharmaceutical preparation for use in transplantation medicine or for the therapy of autoimmune diseases.

The C_{1-20} alkyl radicals, preferably C_{1-8} alkyl radicals, most preferably C_{1-5} alkyl radicals are, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, t-butyl, pentyl, cyclopentyl, 2-ethyl hexyl, hexyl, cyclohexyl, heptyl, cycloheptyl, octyl, vinyl, allyl, 2-hydroxy ethyl, 2 or 3-hydroxy propyl, 2-methoxy ethyl, methoxy methyl or 2- or 3-methoxy propyl. Preferably at least one of the radicals R_1 or R_2 is C_{1-5} alkyl, especially methyl or ethyl. More preferably, R_1 and R_2 are the same or different C_{1-5} alkyl radicals such as methyl, ethyl, n-propyl or t-butyl, methyl and ethyl being especially preferred. Most preferably, R_1 and R_2 are identical and are methyl or ethyl. Especially preferred are the dimethyl fumarate, methyl ethyl fumarate and diethyl fumarate.

The dialkyl fumarates to be used according to the invention are prepared by processes known in the art (see, for example, EP 0 312 697).

Preferably, the active ingredients are used for preparing oral preparations in the form of tablets, micro-tablets, pellets or granulates, optionally in capsules or sachets. Preparations in the form of micro-tablets or pellets, optionally filled in capsules or sachets are preferred and are also a subject matter of the invention. The oral preparations may be provided with an enteric coating. Capsules may be soft or hard gelatine capsules.

The dialkyl fumarates used according to the invention may be used alone or as a mixture of several compounds, optionally in combination with the customary carriers and excipients. The amounts to be used are selected in such a manner that the preparations obtained contain the active ingredient in an amount corresponding to 10 to 300 mg of fumaric acid.

Preferred preparations according to the invention contain a total amount of 10 to 300 mg of dimethyl fumarate and/or diethyl fumarate.

According to a preferred embodiment, the size or the mean diameter, respectively, of the pellets or micro-tablets is in the range from 300 to 2,000 μm, especially in the range of 500 or 1,000 μm.

In addition to graft-versus-host reactions (see above), the following autoimmune diseases to be treated may be named: polyarthritis, multiple sclerosis, graft-versus-host reactions, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (lupoid) hepatitis. Autoimmune diseases in a wider meaning also comprise psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn.

In addition to the preparations for peroral administration in the form of micro-pellets, micro-tablets, capsules (such as soft and hard gelatine capsules), granulates and tablets cited above, suitable pharmaceutical preparations are preparations for cutaneous and transdermal administration in the form of ointments, plasters, lotions or shower preparations and for

parenteral administration in the form of aqueous micro-dispersions, oil-in-water emulsions or oily solutions for rectal administration of suppositories or micro-enemas. Pharmaceutical preparations in the form of micro-tablets or micro-pellets are preferred for the therapy of all autoimmune diseases mentioned above, including psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn and are also a subject matter of the invention.

According to the invention, a therapy with dialkyl fumarates may also be carried out in combination with one or more preparations of the triple drug therapy customarily used in organ transplantations or with cyclosporine A alone. For this purpose, the preparations administered may contain a combination of the active ingredients in the known dosages or amounts, respectively. Likewise, the combination therapy may consist of parallel administration of separate preparation same or different routes. Optionally, the dosage of the active ingredient contained in addition to the dose of the fumaric acid derivative administered in accordance with the invention may be reduced advantageously.

Another embodiment of the use according to the invention is to alternate the drug therapy with immunosuporessive agents such as cyclosporine in sequence with an application of the above-mentioned dialkyl fumarate. This means that an application of fumaric acid derivatives as defined above over one or more weeks may follow a cyclosporine therapy of one or more weeks. This permits reduction of the Cyclosporine A dosage resulting in a considerable decrease of the rate of side effects in long-term therapy.

By administration of the dialkyl fumarates in the form of micro-tablets, which is preferred, gastrointestinal irritations and side effects, which are reduced already when conventional tablets are administered but is still observed, may be further reduced vis-à-vis fumaric acid derivatives and salts.

It is presumed that, upon administration of conventional tablets, the ingredients of the tablet are released in the intestine in a concentration which is too high, causing local irritation of the intestinal mucous membrane. This local irritation results in a short-term release of very high TNF-α concentrations which may be responsible for the gastrointestinal side effects. In case of application of enteric-coated micro-tablets in capsules, on the other hand, very low local concentrations of the active ingredients in the intestinal epithelial cells are achieved. The micro-tablets are incrementally released by the stomach and passed into the small intestine by peristaltic movements so that distribution of the active ingredients is improved.

This means that enteric-coated micro-tablets in the same dosage are distributed already in the stomach and passed to the intestine in portions, where the active ingredients are released in smaller dosages. This avoids local irritation of the intestinal epithelial cells and the release of TNF-α. It is assumed that this results in the improved tolerance of micro-tablets in the gastrointestinal tract vis-à-vis conventional tablets.

In addition, resorption is improved, because the dialkyl fumarates to be used according to the invention are not the active ingredient per se, but a so-called pro-drug, which must be converted into the active ingredient in the body.

In order to illustrate the use according to the invention, different examples for preparing preferred drugs are given below.

PRODUCTION EXAMPLES

In principle, the oral preparations according to the invention in the form of tablets or micro-tablets may be prepared by classical tabletting processes. Instead of such classical tabletting processes, other methods for the preparation of tablets may be used, such as direct tabletting and processes for preparing solid dispersions in according with the melt method and the spray drying method.

The tablets may be provided with an enteric coating. The enteric coating may be applied in a classical coating pan or sprayed on or applied in a fluidised bed apparatus. The tablet may also be provided with a film coat.

EXAMPLE 1

Preparation of Enteric-coated Micro-tablets in Capsules Containing 120.0 mg of Dimethyl Fumarate, which Corresponds to 96 mg of Fumaric Acid

Taking the necessary precautions (breathing mask, gloves, protective clothing, etc.), 12.000 kg of dimethyl fumarate are crushed, mixed and homogenised by means of a sieve 800. Then an excipient mixture with the following composition is prepared: 17.50 kg of starch derivative (STA-RX® 1500), 0.30 kg of micro-crystalline cellulose (Avicel® PH 101), 0.75 kg of PVP (Kollidon® 120), 4.00 kg of Primogel®, 0.25 kg of colloidal silicic acid (Aerosil®). The active ingredient is added to the entire powder mixture, mixed, homogenised by means of a sieve 200, processed in the usual manner with a 2% aqueous solution of polyvidon pyrrolidone (Kollidon® K25) to obtain a binder granulate and then mixed in the dry state with the outer phase. Said outer phase consists of 0.50 kg of Mg stearate and 1.50 kg of talcum.

Then the powder mixture is compressed in the usual manner to obtain convex tablets having a gross weight of 10.0 mg and a diameter of 2.0 mm.

One example to achieve resistance to gastric acid is to dissolve a solution of 2.250 kg of hydroxy propyl methyl cellulose phthalate (HPMCP, Pharmacoat® HP 50) in portions in a mixture of the following solvents: 13.00 l of acetone, 13.50 l of ethanol (94 wt.-%, denatured with 2% of ketone) and 1.50 l of demineralised water. As a plasticiser, castor oil (0.240 kg) is added to the finished solution and applied in portions onto the tablet cores in the customary manner.

After drying is completed, a suspension of the following composition is applied as a film coat in the same apparatus: 0.340 kg of talcum, 0.400 kg of titanium(VI) oxide Cronus RN 56, 0.324 kg of coloured lacquer L-Rotlack 86837, 4.800 kg of Eudragit E 12.5% and 0.120 kg of polyethylene glycol 6000, pH 11 XI in a solvent mixture of the following composition: 8.170 kg of 2-propanol, 0.200 kg of demineralised water and 0.600 kg of glycerine triacetate (Triacetin).

After that the enteric-coated micro-tablets are filled into hard gelatine capsules having a net weight of 400 mg and sealed.

EXAMPLE 2

Preparation of Enteric-coated Micro-tablets in Capsules Containing 120.0 mg of Dimethyl Fumarate, which Corresponds to 96 mg of Fumaric Acid

12.000 kg of dimethyl fumarate are crushed and homogenised as above. Then an excipient mixture composed as follows is prepared: 23.20 kg of microcrystalline cellulose (Avicelo® PH 200), 3.00 kg of Croscarmellose sodium (AC-Di-SOL-SD-711), 2.50 kg of talcum, 0.10 kg of anhydrous silica (Aerosil® 200) and 1.00 kg of Mg stearate. The active ingredient is then added to the entire powder mixture and mixed homogenously. By means of direct tabletting, the powder mixture is then pressed into convex tablets having a gross weight of 10.0 mg and a diameter of 2.00 mm.

After that, a solution of 0.94 Eudragit® L in isopropanol is prepared which also contains 0.07 kg of dibutyl phthalate. This solution is sprayed onto the tablet cores. After that, a dispersion of 17.32 kg of Eudragit® L D-55 and a mixture of 2.80 kg of micro-talcum, 2.00 kg of Macrogol 6000 and 0.07 kg of dimeticon in water is prepared and sprayed onto the cores.

Next, the enteric-coated micro-tablets are filled into hard gelatine capsules having a net weight of 650 mg and sealed.

EXAMPLE 3

Preparation of Micro-pellets in Capsules Containing 50.0 mg of Dimethyl Fumarate, which Corresponds to 40 mg of Fumaric Acid

5.000 kg of dimethyl fumarate are crushed and homogenised as above. In addition, 2 l of a 20% (m/v) polyvinyl pyrrolidone solution (Kollidon K-30) in ethanol are prepared. 7.250 kg of nonpareilles pellets in a coating pan are sprayed with part of the Kollidon K-30 solution until slightly humid. Then the active ingredient is added in portions until the pellets are dry. This procedure of humidification/drying is continued until all of the active ingredient mixture has been added. Then the pellets are moved around until completely dry.

After that, the pellets are filled into hard gelatine capsules (126.5 mg pellets/capsule).

EXAMPLE 4

Preparation of Enteric-coated Capsules Containing 110.0 mg of Dimethyl Fumarate, which Corresponds to 88 mg of Fumaric Acid

11.000 kg of dimethyl fumarate are intensely mixed in a mixture consisting of 14.00 kg of starch, 5.65 kg of lactose, 2.00 kg of microcrystalline cellulose (Avicel®), 1.00 kg of polyvinyl pyrrolidone (Kollidon® 25) and 2.443 kg of Primogel® and, taking the necessary precautions (breathing mask, gloves, protective clothing), homogenised by means of a sieve 800.

Using a 2% aqueous solution of polyvinyl pyrrolidone (Kollidon® K25), the entire powder mixture is processed into a binder granulate in the customary manner and mixed with the outer phase when dry. Said outer phase consists of 0.350 kg of colloidal silicic acid (Aerosil®), 0.500 kg of Mg stearate and 1.500 kg of talcum. The homogenous mixture is filled into suitable capsules in portions of 400 mg which are then provided with an enteric coating consisting of hydroxy propyl methyl cellulose stearate and castor oil as plasticiser in the customary manner. Instead of using hard gelatine capsules, the product may also be filled into suitable enteric-coated capsules consisting of a mixture of cellulose acetate phthalate (CAP) and hydroxy propyl methyl cellulose phthalate (HPMCP).

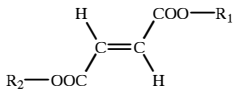
In comparison with substances of the prior art such as cyclosporine, which may cause massive kidney disorders or diseases of the lymphoproliferative system, a therapy with fumaric acid derivatives according to the invention for the indications listed above rarely results in serious side effects.

Among other things, the immunosuppressive effect of cyclosporine is caused by the inhibition of Th-1 cell formation. As in vitro experiments of the applicant have shown, fumarates cause a shift of the cytokine pattern of the Th1 type to the cytokine pattern of the Th2 type.

Especially in view of the long-term therapy and prevention which is always necessary in graft-versus-host reactions and host-versus-graft reactions or other immune diseases such as multiple sclerosis, the unexpected effect of the use according to the invention is of the greatest interest. In a combination therapy of cyclosporine with the fumaric acid derivatives, the toxic side effects of the former compounds may be unexpectedly reduces to a substantial degree. In addition, the use according to the invention is also significant in the substitution of the corticosteroid therapy of autoimmune diseases which is known to be accompanied by severe side effects.

What is claimed is:

1. Pharmaceutical preparation in the form of microtablets or micropellets comprising one or more dialkyl fumarates of the formula



wherein R_1 and R_2, which may be the same or different, independently represent a linear, branched or cyclic, saturated or unsaturated C_{1-20} alkyl radical which may be optionally substituted with halogen (Cl, F, I, Br), hydroxy, C_{1-4} alkoxy, nitro or cyano, and optionally suitable carriers and excipients for use in transplantation medicine or for the therapy of autoimmune diseases such as polyarthritis, multiple sclerosis, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia, chronic active (lupoid) hepatitis, psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn.

2. A preparation according to claim **1** comprising dimethyl fumarate, diethyl fumarate or methylethyl fumarate.

3. A preparation according to claim **1** or **2** comprising an amount of the active ingredient corresponding to 10 to 300 mg of fumaric acid.

4. A preparation according to claim **1** wherein the one or more dialkylfumarates is diethylfumarate.

5. A preparation according to claim **1** wherein the one or more dialkylfumarates is dimethylfumarate.

6. A preparation according to claims **4** or **5** wherein the amount of dialkylfumarate in said preparation corresponds to 10 to 300 mg of fumaric acid.

7. A preparation according to any of claims **1**, **2**, **4** or **5** wherein the preparation is formulated into an oral preparation in which the microtablets or micropellets are in capsules or sachets.

8. A preparation according to any of claims **1**, **2**, **4** or **5** wherein the preparation is formulated into an oral preparation in which the microtablets or micropellets are in soft or hard gelatine capsules.

9. A preparation according to any of claims **1**, **2**, **4** or **5** wherein the microtablets or micropellets are provided with an enteric coating.

10. A preparation according to claim **1** wherein the preparation is formulated into an oral preparation in which the microtablets or micropellets are in capsules or sachets and wherein the amount of dialkylfumarate in said preparation corresponds to 10 to 300 mg of fumaric acid.

11. A preparation according to claim **1** wherein the preparation is formulated into an oral preparation in which the microtablets or micropellets are in soft or hard gelatine capsules and wherein the amount of dialkylfumarate in said preparation corresponds to 10 to 300 mg of fumaric acid.

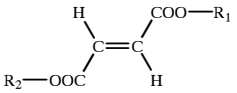
12. A preparation according to claim **1** wherein the microtablets or micropellets are provided with an enteric coating and wherein the amount of dialkylfumarate in said preparation corresponds to 10 to 300 mg of fumaric acid.

13. A pharmaceutical composition in which the active ingredient consists of one or more dialkyl fumarates, said composition being in the form of microtablets or micropellets wherein the size or mean diameter, respectively of said

microtablets or micropellets is 5,000 microns or less exclusive of any optional coating applied to said microtablets or micropellets.

14. The composition of claim **13** wherein said size or mean diameter is 2,000 microns or less.

15. The composition of claim **13** wherein said one or more dialkyl fumarates are of the formula



wherein R_1 and R_2, which may be the same or different, independently represent a linear, branched or cyclic, saturated or unsaturated C_{1-20} alkyl radical which may be optionally substituted with halogen (Cl, F, I, Br), hydroxy, C_{1-4} alkoxy, nitro or cyano.

16. The composition of claim **13** wherein said one or more dialkyl fumarates is dimethyl fumarate, or diethyl fumarate, or methylethyl fumarate, and wherein the amount thereof corresponds to 10 to 300 mg fumaric acid.

* * * * *

EXHIBIT B


US007320999B2

(12) **United States Patent**
Joshi et al.

(10) **Patent No.: US 7,320,999 B2**
(45) **Date of Patent: Jan. 22, 2008**

(54) **DIMETHYL FUMARATE FOR THE TREATMENT OF MULTIPLE SCLEROSIS**

(75) Inventors: **Rajendra Kumar Joshi**, Zürich (CH); **Hans-Peter Strebel**, Muri (CH)

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(21) Appl. No.: **10/197,077**

(22) Filed: **Jul. 17, 2002**

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Related U.S. Application Data

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(30) **Foreign Application Priority Data**

Nov. 19, 1998 (DE) 198 53 487

(51) **Int. Cl.**
A61K 31/22 (2006.01)

(52) **U.S. Cl.** **514/549**

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to the use of certain dialkyl fumarates for the preparation of pharmaceutical preparations for use in transplantation medicine or for the therapy of autoimmune diseases and said compositions in the form of micro-tablets or pellets. For this purpose, the dialkyl fumarates may also be used in combination with conventional preparations used in transplantation medicine and immunosuppressive agents, especially cyclosporines.

18 Claims, No Drawings

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DIMETHYL FUMARATE FOR THE TREATMENT OF MULTIPLE SCLEROSIS

REFERENCE TO RELATED APPLICATIONS

This is a Division of commonly-owned application Ser. No. 09/831,620, filed May 10, 2001, now U.S. Pat. No. 6,509,376, which is a 371 continuation of PCT Application PCT/EP99/08215, filed Oct. 29, 1999, the text of which is not in English, which PCT Application claims priority on German Application No. 198 53 487.6, filed Nov. 19, 1998, the text of which is not in English.

DESCRIPTION

The present invention relates to the use of dialkyl fumarates for preparing pharmaceutical preparations for use in transplantation medicine or the therapy of autoimmune diseases and pharmaceutical preparations in the form of micro-tablets or micro-pellets containing dialkyl fumarates.

On the one hand, therefore, it relates especially to the use of dialkyl fumarates for preparing pharmaceutical preparations for the treatment, reduction or suppression of rejection reactions of the transplant by the recipient, i.e. host-versus graft reactions, or rejection of the recipient by the transplant, i.e. graft-versus-host reactions. On the other hand, it relates to the use of dialkyl fumarates for preparing pharmaceutical preparations for treating autoimmune diseases such as polyarthritis, multiple sclerosis, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (=lupoid) hepatitis.

Both graft rejection and autoimmune diseases are based on medically undesirable reactions or dysregulation of the immune system. Cytokins such as interleukins or tumour necrose factor a (TNF-α) are substantial mediators influencing the immune system. In general, both are treated by the administration of immunosuppressive agents such as cyclosporine.

In the overall result, autoimmune diseases may be defined as the failure of the tolerance of endogenic substances or antigens. As a rule, this tolerance can be maintained only if the antigens keep coming into contact with immunological cells. When this tolerance is lost, autoantibodies are formed, i.e. a humoral immunoresponse against endogenic tissue. The exact nature of the involvement of TNF-α is not known.

Transplantations are tissue or organ transplantations, i.e. the transfer of tissues such as cornea, skin, bones (bone chips), vessels or fasciae, of organs such as kidney, heart, liver, lung, pancreas or intestines, or of individual cells such as islet cells, α-cells and liver cells, the kidney having the greatest significance as a transplanted organ.

According to the degree of relationship between the donor and the recipient we differentiate between autotransplantation (transfer to another part of the body of the same individual), iso-transplantation (transfer to another, genetically identical individual) and allogenic transplantation (transfer to another individual of the same species). Depending on the site of origin and transplantation, we further differentiate between homotopic transplantation (transfer to the same site) and heterotopic transplantation (transfer to a different site). The above-mentioned transplantations play an important role in modern medicine.

A major problem in transplantation medicine is graft rejection after transplantation of the tissue, organ or cell by immunological defense reactions of the recipient. Such a graft rejection is also called host-versus-graft reaction. The

immunological defense reaction of the organism against the heteroprotein often results in rejection or dissolution of the grafts. In host-verses-graft reactions, different stages may be distinguished. Depending on the degree of difference between the recipient and the donor, this reaction takes place at different speeds so that we speak of an acute, sub-acute or chronic reaction. The acute rejection process is accompanied by the irreversible loss of the transplant (necrotisation) as a result of arteriitis or arteriolitis within 48 hours and cannot be influenced by the administration of drugs. The sub-acute rejection reaction becomes manifest as a rejection crisis from day 12 to month 4 with reversible functional disorders as a result of a transplant vasculopathy. Finally, the loss of function of the transplant as a result of vascular changes such as obliterating arteriopathy, which proceeds over weeks or years and can practically not be influenced by drugs, is termed a chronic rejection reaction.

Vice-versa, rejection reactions of the transplant against the recipient, the so-called graft-versus-host reactions, may occur when immunocompetent tissues are transplanted, i.e. primarily in bone marrow transplantation. Again, the severity of the reaction is graded, and substantially similar complications result as in host-versus-graft-reactions, namely arteriopathies and necroses.

To avoid such rejection reactions, i.e. the host-versus-graft reaction and the graft-versus-host reaction, transplantation medicine essentially makes use of immunosuppression, i.e. a weakening of the normal immunoresponse. For this purpose, anti-lymphocyte sera are often used in combination with corticosteroids and so-called anti-metabolites, e.g. purine analogues such as 6-mercaptopurine and thioguanine which affect the nucleic acid and protein synthesis and thus prevent cell division and proliferation. This leads to suppression of the production of antibodies and the cellular immune response. The immunosuppressive agents used for therapy are substances which suppress or weaken the immunoreaction in the body either specifically or non-specifically. Non-specific immunosuppressive agents are cytostatic agents such as, for example, alkylating agents or antimetabolites.

In addition, active ingredients are known which cause at least partial specific immunosuppression, such as corticosteroids, antisera, antibodies FK-506, tacrolimus, mycophenolatemofetil and primarily cyclosporines such as cyclosporine A. As a result of using modern immunosuppressive agents, the most important representatives of which are the cyclosporines, especially cyclosporine A, it was possible to improve the results of transplantation considerably over the last few years. At present, the survival rate after one year is about 60% for liver transplantations, about 80% for heart transplantations and over 90% for kidney transplantations.

Autoimmune diseases where the endogenic immune system attacks endogenic organs, tissues and cells are comparable to graft-versus-host reactions. These are also medically undesirable reactions of the immune system which may be treated with immunosuppressive agents, too.

The danger in using immunosuppressive agents lies in weakening the body's defense against infectious diseases and the increased risk of malignant diseases. Therefore, it is the object of the invention to provide a pharmaceutical preparation to be employed in transplantation medicine which may be used to treat, especially to suppress weaken and/or alleviate host-versus-graft reactions and graft-versus-host reactions, but does not have the above disadvantage.

It is another object of the invention to provide a pharmaceutical preparation which may be employed for treating autoimmune diseases, particularly polyarthritis, multiple

sclerosis, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (=lupoid) hepatitis, without the disadvantages of immunosuppression.

The object of the invention is achieved by using certain dialkyl fumarates for preparing pharmaceutical preparations for use in transplantation medicine and for the therapy of autoimmune diseases and pharmaceutical preparations in the form of micro-tablets and micro-pellets containing these dialkyl fumarates. The individual subject matters of the invention are characterized in detail in the claims. The preparations according to the invention do not contain any free fumaric acids per se.

It is known that pharmaceutical preparations which, upon biological degradation after administration, enter into the citric acid cycle or are part thereof gain increasing therapeutic significance—especially when given in high dosages—since they can alleviate or heal diseases caused cryptogenetically.

Fumaric acid, for example, inhibits the growth of the Ehrlich ascites tumour in mice, reduces the toxic effects of mitomycin C and aflatoxin and displays antipsoriatic and anti-microbial activity. When administered parenterally, transdermally and especially perorally, high dosages of fumaric acid or its derivatives known so far such as dihydroxyl fumaric acid, fumaramide and fumaronitrile have such unacceptably severe side effects and high toxicity that, in most cases, such a therapy had to be abandoned in the past.

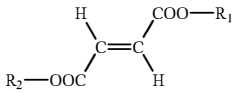
Surprisingly, investigations carried out by the applicant have shown that methyl hydrogen fumarate, a metabolite of the dimethyl fumarate, initially increases the endotoxin-stimulated TNF-α secretion in human mononuclear cells of periphere blood (periphere blood mononuclear cells=PBMC cells) and in isolated monocytes. In addition, the applicant was able to show that fumaric acid has an effect on in vitro and in vivo haemagglutination which is comparable to that of cyclosporine.

Surprisingly, it has now been found that dialkyl fumarates are advantageous for preparing pharmaceutical compositions for use in transplantation medicine and for the therapy of autoimmune diseases. This is because compositions containing such dialkyl fumarates surprisingly permit a positive modulation of the immune system in host-versus-graft reactions, graft-versus-host reactions and other autoimmune diseases.

European Patent Application 0188 749 already describes fumaric acid derivatives and pharmaceutical compositions containing the same for the treatment of psoriasis. Pharmaceutical compositions for the treatment of psoriasis containing a mixture of fumaric acid and other fumaric acid derivatives are known from DE-A-25 30 372. The content of free fumaric acid is obligatory for these medicaments.

DE-A-26 21 214 describes medicaments containing the fumaric acid monoethyl ester and its mineral salts as active ingredient for the treatment of psoriasis. The publication "Hautarzt (*Dermatologist*) (1987) 279-285" discusses the use of fumaric acid monoethyl ester salts. Pharmaceutical preparations containing a mixture of fumaric acid monoalkyl ester salts and a fumaric acid diester for the treatment of psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn are known from EP 0 312 697 B1.

Specifically, the object of the invention is achieved by the use of one or more dialkyl fumarates of the formula



wherein R_1 and R_2, which may be the same or different, independently represent a linear, branched or cyclic, saturated or unsaturated C_{1-20} alkyl radical which may be optionally substituted with halogen (Cl, F, I, Br), hydroxy, C_{1-4} alkoxy, nitro or cyano for preparing a pharmaceutical preparation for use in transplantation medicine or for the therapy of autoimmune diseases.

The C_{1-20} alkyl radicals, preferably C_{1-8} alkyl radicals, most preferably C_{1-5} alkyl radicals are, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, t-butyl, pentyl, cyclopentyl, 2-ethyl hexyl, hexyl, cyclohexyl, heptyl, cycloheptyl, octyl, vinyl, allyl, 2-hydroxyethyl, 2 or 3-hydroxy propyl, 2-methoxy ethyl, methoxy methyl or 2- or 3-methoxy propyl. Preferably at least one of the radicals R_1 or R_2 is C_{1-5} alkyl, especially methyl or ethyl. More preferably, R_1 and R_2 are the same or different C_{1-5} alkyl radicals such as methyl, ethyl, n-propyl or t-butyl, methyl and ethyl being especially preferred. Most preferably, R_1 and R_2 are identical and are methyl or ethyl. Especially preferred are the dimethyl fumarate, methyl ethyl fumarate and diethyl fumarate.

The dialkyl fumarates to be used according to the invention are prepared by processes known in the art (see, for example, EP 0 312 697).

Preferably, the active ingredients are used for preparing oral preparations in the form of tablets, micro-tablets, pellets or granulates, optionally in capsules or sachets. Preparations in the form of micro-tablets or pellets, optionally filled in capsules or sachets are preferred and are also a subject matter of the invention. The oral preparations may be provided with an enteric coating. Capsules may be soft or hard gelatine capsules.

The dialkyl fumarates used according to the invention may be used alone or as a mixture of several compounds, optionally in combination with the customary carriers and excipients. The amounts to be used are selected in such a manner that the preparations obtained contain the active ingredient in an amount corresponding to 10 to 300 mg of fumaric acid.

Preferred preparations according to the invention contain a total amount of 10 to 300 mg of dimethyl fumarate and/or diethyl fumarate.

According to a preferred embodiment, the size or the mean diameter, respectively, of the pellets or micro-tablets is in the range from 300 to 2,000 μm, especially in the range of 500 or 1,000 μm.

In addition to graft-versus-host reactions (see above), the following autoimmune diseases to be treated may be named: polyarthritis, multiple sclerosis, graft-versus-host reactions, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (lupoid) hepatitis. Autoimmune diseases in a wider meaning also comprise psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn.

In addition to the preparations for peroral administration in the form of micro-pellets, micro-tablets, capsules (such as soft and hard gelatine capsules), granulates and tablets cited above, suitable pharmaceutical preparations are preparations for cutaneous and transdermal administration in the form of ointments, plasters, lotions or shower preparations and for parenteral administration in the form of aqueous micro-

dispersions, oil-in-water emulsions or oily solutions for rectal administration of suppositories or micro-enemas. Pharmaceutical preparations in the form of micro-tablets or micro-pellets are preferred for the therapy of all autoimmune diseases mentioned above, including psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn and are also a subject matter of the invention.

According to the invention, a therapy with dialkyl fumarates may also be carried out in combination with one or more preparations of the triple drug therapy customarily used in organ transplantations or with cyclosporine A alone. For this purpose, the preparations administered may contain a combination of the active ingredients in the known dosages or amounts, respectively. Likewise, the combination therapy may consist of the parallel administration of separate preparations, by the same or different routes. Optionally, the dosage of the active ingredient contained in addition to the dose of the fumaric acid derivative administered in accordance with the invention may be reduced advantageously.

Another embodiment of the use according to the invention is to alternate the drug therapy with immunosuppressive agents such as cyclosporine in sequence with an application of the above-mentioned dialkyl fumarate. This means that an application of fumaric acid derivatives as defined above over one or more weeks may follow a cyclosporine therapy of one or more weeks. This permits reduction of the Cyclosporine A dosage resulting in a considerable decrease of the rate of side effects in long-term therapy.

By administration of the dialkyl fumarates in the form of micro-tablets, which is preferred, gastrointestinal irritations and side effects, which are reduced already when conventional tablets are administered but is still observed, may be further reduced vis-a-vis fumaric acid derivatives and salts.

It is presumed that, upon administration of conventional tablets, the ingredients of the tablet are released in the intestine in a concentration which is too high, causing local irritation of the intestinal mucous membrane. This local irritation results in a short-term release of very high TNF-α concentrations which may be responsible for the gastrointestinal side effects. In case of application of enteric-coated micro-tablets in capsules, on the other hand, very low local concentrations of the active ingredients in the intestinal epithelial cells are achieved. The micro-tablets are incrementally released by the stomach and passed into the small intestine by peristaltic movements so that distribution of the active ingredients is improved.

This means that enteric-coated micro-tablets in the same dosage are distributed already in the stomach and passed to the intestine in portions, where the active ingredients are released in smaller dosages. This avoids local irritation of the intestinal epithelial cells and the release of TNF-α. It is assumed that this results in the improved tolerance of micro-tablets in the gastrointestinal tract vis-a-vis conventional tablets.

In addition, resorption is improved, because the dialkyl fumarates to be used according to the invention are not the active ingredient per se, but a so-called prodrug, which must be converted into the active ingredient in the body.

In order to illustrate the use according to the invention, different examples for preparing preferred drugs are given below.

PRODUCTION EXAMPLES

In principle, the oral preparations according to the invention in the form of tablets or micro-tablets may be prepared by classical tabletting processes. Instead of such classical tabletting processes, other methods for the preparation of tablets may be used, such as direct tabletting and processes for preparing solid dispersions in according with the melt method and the spray drying method.

The tablets may be provided with an enteric coating. The enteric coating may be applied in a classical coating pan or sprayed on or applied in a fluidised bed apparatus. The tablet may also be provided with a film coat.

Example 1

Preparation of Enteric-coated Micro-tablets in Capsules Containing 120.0 mg of Dimethyl Fumarate, which Corresponds to 96 mg of Fumaric Acid

Taking the necessary precautions (breathing mask, gloves, protective clothing, etc.), 12.000 kg of dimethyl fumarate are crushed, mixed and homogenized by means of a sieve 800. Then an excipient mixture with the following composition is prepared: 17.50 kg of starch derivative (STA-RX® 1500), 0.30 kg of microcrystalline cellulose (Avicel® PH 101), 0.75 kg of PVP (Kollidon® 120), 4.00 kg of Primogel®, 0.25 kg of colloidal silicic acid (Aerosil®). The active ingredient is added to the entire powder mixture, mixed, homogenized by means of a sieve 200, processed in the usual manner with a 2% aqueous solution of polyvidon pyrrolidone (Kollidon® K25) to obtain a binder granulate and then mixed in the dry state with the outer phase. Said outer phase consists of 0.50 kg of Mg stearate and 1.50 kg of talcum.

Then the powder mixture is compressed in the usual manner to obtain convex tablets having a gross weight of 10.0 mg and a diameter of 2.0 mm.

One example to achieve resistance to gastric acid is to dissolve a solution of 2.250 kg of hydroxy propyl methyl cellulose phthalate (HPMCP, Pharmacoat® HP 50) in portions in a mixture of the following solvents: 13.00 1 of acetone, 13.50 1 of ethanol (94 wt.-%, denatured with 2% of ketone) and 1.50 1 of demineralised water. As a plasticiser, castor oil (0.240 kg) is added to the finished solution and applied in portions onto the tablet cores in the customary manner.

After drying is completed, a suspension of the following composition is applied as a film coat in the same apparatus: 0.340 kg of talcum, 0.400 kg of titanium(VI) oxide Cronus RN 56, 0.324 kg of coloured lacquer L-Rot-lack 86837, 4.800 kg of Eudragit E 12.5% and 0.120 kg of polyethylene glycol 6000, pH 11 XI in a solvent mixture of the following composition: 8.170 kg of 2-propanol, 0.200 kg of demineralised water and 0.600 kg of glycerine triacetate (Triacetin).

After that the enteric-coated micro-tablets are filled into hard gelatine capsules having a net weight of 400 mg and sealed.

Example 2

Preparation of Enteric-coated Micro-tablets in Capsules Containing 120.0 mg of Dimethyl Fumarate, which Corresponds to 96 mg Offumaric Acid

12.000 kg of dimethyl fumarate are crushed and homogenized as above. Then an excipient mixture composed as follows is prepared: 23.20 kg of microcrystalline cellulose (Avicel® PH 200), 3.00 kg of Croscarmellose sodium (AC-Di-SOL-SD-711), 2.50 kg of talcum, 0.10 kg of anhydrous silica (Aerosil®200) and 1.00 kg of Mg stearate. The active ingredient is then added to the entire powder mixture and mixed homogenously. By means of direct tabletting, the

powder mixture is then pressed into convex tablets having a gross weight of 10.0 mg and a diameter of 2.00 mm.

After that, a solution of 0.94 Eudragit® L in isopropanol is prepared which also contains 0.07 kg of dibutyl phthalate. This solution is sprayed onto the tablet cores. After that, a dispersion of 17.32 kg of Eudragit® L D-55 and a mixture of 2.80 kg of microtalcum, 2.00 kg of Macrogol 6000 and 0.07 kg of dimeticon in water is prepared and sprayed onto the cores.

Next, the enteric-coated micro-tablets are filled into hard gelatine capsules having a net weight of 650 mg and sealed.

Example 3

Preparation of Micro-pellets in Capsules Containing 50.0 mg of Dimethyl Fumarate, which Corresponds to 40 mg of Fumaric Acid

5.000 kg of dimethyl fumarate are crushed and homogenized as above. In addition, 2 1 of a 20% (m/v) polyvinyl pyrrolidone solution (Kollidon K-30) in ethanol are prepared. 7.250 kg of nonpareilles pellets in a coating pan are sprayed with part of the Kollidon K-30 solution until slightly humid. Then the active ingredient is added in portions until the pellets are dry. This procedure of humidification/drying is continued until all of the active ingredient mixture has been added. Then the pellets are moved around until completely dry.

After that, the pellets are filled into hard gelatine capsules (126.5 mg pellets/capsule).

Example 4

Preparation of Enteric-coated Capsules Containing 110.0 mg of Dimethylfumarate, which Corresponds to 88 mg of Fumaric Acid

11.000 kg of dimethyl fumarate are intensely mixed in a mixture consisting of 14.00 kg of starch, 5.65 kg of lactose, 2.00 kg of microcrystalline cellulose (Avicel®), 1.00 kg of polyvinyl pyrrolidone (Kollidon®25) and 2.443 kg of Primogel® and, taking the necessary precautions (breathing mask, gloves, protective clothing), homogenized by means of a sieve 800.

Using a 2% aqueous solution of polyvinyl pyrrolidone (Kollidon® K25), the entire powder mixture is processed into a binder granulate in the customary manner and mixed with the outer phase when dry. Said outer phase consists of 0.350 kg of colloidal silicic acid (Aerosil®), 0.500 kg of Mg stearate and 1.500 kg of talcum. The homogenous mixture is filled into suitable capsules in portions of 400 mg which are then provided with an enteric coating consisting of hydroxy propyl methyl cellulose stearate and castor oil as plasticiser in the customary manner. Instead of using hard gelatine capsules, the product may also be filled into suitable enteric-coated capsules consisting of a mixture of cellulose acetate phthalate (CAP) and hydroxy propyl methyl cellulose phthalate (HPMCP).

In comparison with substances of the prior art such as cyclosporine, which may cause massive kidney disorders or diseases of the lymphoproliferative system, a therapy with fumaric acid derivatives according to the invention for the indications listed above rarely results in serious side effects.

Among other things, the immunosuppressive effect of cyclosporine is caused by the inhibition of Th-1 cell formation. As in vitro experiments of the applicant have shown, fumarates cause a shift of the cytokine pattern of the Th1 type to the cytokine pattern of the Th2 type.

Especially in view of the long-term therapy and prevention which is always necessary in graft-versus-host reactions and host-versus-graft reactions or other autoimmune diseases such as multiple sclerosis, the unexpected effect of the use according to the invention is of the greatest interest. In a combination therapy of cyclosporine with the fumaric acid derivatives, the toxic side effects of the former compounds may be unexpectedly reduced to a substantial degree. In addition, the use according to the invention is also significant in the substitution of the corticosteroid therapy of autoimmune diseases which is known to be accompanied by severe side effects.

That which is claimed is:

1. A method of treating multiple sclerosis comprising treating a patient in need of treatment for multiple sclerosis with an amount of a pharmaceutical preparation effective for treating said multiple sclerosis, wherein the only active ingredient for the treatment of multiple sclerosis present in said pharmaceutical preparation is dimethyl fumarate.

2. The method of claim **1**, wherein 10 to 300 mg of dimethyl fumarate is present in said pharmaceutical preparation.

3. The method of claim **2**, wherein said pharmaceutical preparation is provided in one or more capsules.

4. The method of claim **1**, wherein at least 50 mg of dimethyl fumarate is present in said pharmaceutical preparation.

5. The method of claim **4**, wherein at least 110 mg of dimethyl fumarate is present in said pharmaceutical preparation.

6. The method of claim **5**, wherein at least 120 mg of dimethyl fumarate is present in said pharmaceutical preparation.

7. The method of claim **1**, wherein 50 mg of dimethyl fumarate is present in said pharmaceutical preparation.

8. The method of claim **1**, wherein 110 mg of dimethyl fumarate is present in said pharmaceutical preparation.

9. The method of claim **1**, wherein 120 mg of dimethyl fumarate is present in said pharmaceutical preparation.

10. The method of claim **1**, wherein the pharmaceutical preparation is formulated for oral administration.

11. The method of claim **1**, wherein the pharmaceutical preparation is formulated as a solid dosage form.

12. The method of claim **1**, wherein the pharmaceutical preparation is in the form of microtablets.

13. The method of claim **12**, wherein the microtablets are enteric-coated.

14. The method of claim **13**, wherein the microtablets have a mean diameter in the range of 0.3 mm to 2.0 mm, exclusive of any coating on the microtablets.

15. The method of claim **14**, wherein the microtablets have a mean diameter of 2.0 mm, exclusive of any coating on the microtablets.

16. The method of claim **1**, wherein the pharmaceutical preparation is in the form of a capsule containing 120 mg of dimethyl fumarate in the form of enteric-coated microtablets having a mean diameter in the range of 0.3 mm to 2.0 mm, exclusive of any coating on the microtablets.

17. The method of claim **1**, wherein the pharmaceutical preparation comprises one or more carriers.

18. The method of claim **1**, wherein the pharmaceutical preparation comprises one or more excipients.

* * * * *

EXHIBIT C


US007619001B2

(12) **United States Patent**
Joshi et al.

(10) **Patent No.: US 7,619,001 B2**
(45) **Date of Patent: *Nov. 17, 2009**

(54) **UTILIZATION OF DIALKYLFUMARATES**

(75) Inventors: **Rajendra Kumar Joshi**, Zürich (CH); **Hans-Peter Strebel**, Muri (CH)

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

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(30) **Foreign Application Priority Data**

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A61K 31/225 (2006.01)

(52) **U.S. Cl.** **514/547**; 514/903; 514/960

(58) **Field of Classification Search** 514/547, 514/903, 960
See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to the use of certain dialkyl fumarates for the preparation of pharmaceutical preparations for use in transplantation medicine or for the therapy of autoimmune diseases and said compositions in the form of micro-tablets or pellets. For this purpose, the dialkyl fumarates may also be used in combination with conventional preparations used in transplantation medicine and immunosuppressive agents, especially cyclosporines.

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UTILIZATION OF DIALKYLFUMARATES

REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 10/197,077, filed Jul. 17, 2002, which is a Division of commonly-owned application Ser. No. 09/831,620, filed May 10, 2001 now U.S. Pat. No. 6,509,376, which is a 371 continuation of PCT Application PCT/EP99/08215, filed Oct. 29, 1999, the text of which is not in English, which PCT Application claims priority on German Application No. 198 53 487.6, filed Nov. 19, 1998, the text of which is not in English, all of which are incorporated herein by reference.

DESCRIPTION

The present invention relates to the use of dialkyl fumarates for preparing pharmaceutical preparations for use in transplantation medicine or the therapy of autoimmune diseases and pharmaceutical preparations in the form of micro-tablets or micro-pellets containing dialkyl fumarates.

On the one hand, therefore, it relates especially to the use of dialkyl fumarates for preparing pharmaceutical preparations for the treatment, reduction or suppression of rejection reactions of the transplant by the recipient, i.e. host-versus graft reactions, or rejection of the recipient by the transplant, i.e. graft-versus-host reactions. On the other hand, it relates to the use of dialkyl fumarates for preparing pharmaceutical preparations for treating autoimmune diseases such as polyarthritis, multiple sclerosis, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (=lupoid) hepatitis.

Both graft rejection and autoimmune diseases are based on medically undesirable reactions or dysregulation of the immune system. Cytokins such as interleukins or tumour necrose factor α (TNF-α) are substantial mediators influencing the immune system. In general, both are treated by the administration of immunosuppressive agents such as cyclosporine.

In the overall result, autoimmune diseases may be defined as the failure of the tolerance of endogenic substances or antigens. As a rule, this tolerance can be maintained only if the antigens keep coming into contact with immunological cells. When this tolerance is lost, autoantibodies are formed, i.e. a humoral immunoresponse against endogenic tissue. The exact nature of the involvement of TNF-α is not known.

Transplantations are tissue or organ transplantations, i.e. the transfer of tissues such as cornea, skin, bones (bone chips), vessels or fasciae, of organs such as kidney, heart, liver, lung, pancreas or intestines, or of individual cells such as islet cells, α-cells and liver cells, the kidney having the greatest significance as a transplanted organ.

According to the degree of relationship between the donor and the recipient we differentiate between autotransplantation (transfer to another part of the body of the same individual), iso-transplantation (transfer to another, genetically identical individual) and allogenic transplantation (transfer to another individual of the same species). Depending on the site of origin and transplantation, we further differentiate between homotopic transplantation (transfer to the same site) and heterotopic transplantation (transfer to a different site). The above-mentioned transplantations play an important role in modern medicine.

A major problem in transplantation medicine is graft rejection after transplantation of the tissue, organ or cell by immunological defense reactions of the recipient. Such a graft rejection is also called host-versus-graft reaction. The immunological defense reaction of the organism against the heteroprotein often results in rejection or dissolution of the grafts. In host-verses-graft reactions, different stages may be distinguished. Depending on the degree of difference between the recipient and the donor, this reaction takes place at different speeds so that we speak of an acute, sub-acute or chronic reaction. The acute rejection process is accompanied by the irreversible loss of the transplant (necrotisation) as a result of arteriitis or arteriolitis within 48 hours and cannot be influenced by the administration of drugs. The sub-acute rejection reaction becomes manifest as a rejection crisis from day 12 to month 4 with reversible functional disorders as a result of a transplant vasculopathy. Finally, the loss of function of the transplant as a result of vascular changes such as obliterating arteriopathy, which proceeds over weeks or years and can practically not be influenced by drugs, is termed a chronic rejection reaction.

Vice-versa, rejection reactions of the transplant against the recipient, the so-called graft-versus-host reactions, may occur when immunocompetent tissues are transplanted, i.e. primarily in bone marrow transplantation. Again, the severity of the reaction is graded, and substantially similar complications result as in host-versus-graft-reactions, namely arteriopathies and necroses.

To avoid such rejection reactions, i.e. the host-versus-graft reaction and the graft-versus-host reaction, transplantation medicine essentially makes use of immunosuppression, i.e. a weakening of the normal immunoresponse. For this purpose, anti-lymphocyte sera are often used in combination with corticosteroids and so-called anti-metabolites, e.g. purine analogues such as 6-mercaptopurine and thioguanine which affect the nucleic acid and protein synthesis and thus prevent cell division and proliferation. This leads to suppression of the production of antibodies and the cellular immune response. The immunosuppressive agents used for therapy are substances which suppress or weaken the immunoreaction in the body either specifically or non-specifically. Non-specific immunosuppressive agents are cytostatic agents such as, for example, alkylating agents or antimetabolites.

In addition, active ingredients are known which cause at least partial specific immunosuppression, such as corticosteroids, antisera, antibodies FK-506, tacrolimus, mycophenolatemofetil and primarily cyclosporines such as cyclosporine A. As a result of using modern immunosuppressive agents, the most important representatives of which are the cyclosporines, especially cyclosporine A, it was possible to improve the results of transplantation considerably over the last few years. At present, the survival rate after one year is about 60% for liver transplantations, about 80% for heart transplantations and over 90% for kidney transplantations.

Autoimmune diseases where the endogenic immune system attacks endogenic organs, tissues and cells are comparable to graft-versus-host reactions. These are also medically undesirable reactions of the immune system which may be treated with immunosuppressive agents, too.

The danger in using immunosuppressive agents lies in weakening the body's defense against infectious diseases and the increased risk of malignant diseases. Therefore, it is the object of the invention to provide a pharmaceutical preparation to be employed in transplantation medicine which may be used to treat, especially to suppress, weaken and/or alleviate host-versus-graft reactions and graft-versus-host reactions, but does not have the above disadvantage.

It is another object of the invention to provide a pharmaceutical preparation which may be employed for treating autoimmune diseases, particularly polyarthritis, multiple

sclerosis, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (=lupoid) hepatitis, without the disadvantages of immunosuppression.

The object of the invention is achieved by using certain dialkyl fumarates for preparing pharmaceutical preparations for use in transplantation medicine and for the therapy of autoimmune diseases and pharmaceutical preparations in the form of micro-tablets and micro-pellets containing these dialkyl fumarates. The individual subject matters of the invention are characterized in detail in the claims. The preparations according to the invention do not contain any free fumaric acids per se.

It is known that pharmaceutical preparations which, upon biological degradation after administration, enter into the citric acid cycle or are part thereof gain increasing therapeutic significance—especially when given in high dosages—since they can alleviate or heal diseases caused cryptogenetically.

Fumaric acid, for example, inhibits the growth of the Ehrlich ascites tumour in mice, reduces the toxic effects of mitomycin C and aflatoxin and displays antipsoriatic and antimicrobial activity. When administered parenterally, transdermally and especially perorally, high dosages of fumaric acid or its derivatives known so far such as dihydroxyl fumaric acid, fumaramide and fumaronitrile have such unacceptably severe side effects and high toxicity that, in most cases, such a therapy had to be abandoned in the past.

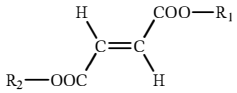
Surprisingly, investigations carried out by the applicant have shown that methyl hydrogen fumarate, a metabolite of the dimethyl fumarate, initially increases the endotoxin-stimulated TNF-α secretion in human mononuclear cells of periphere blood (periphere blood mononuclear cells=PBMC cells) and in isolated monocytes. In addition, the applicant was able to show that fumaric acid has an effect on in vitro and in vivo haemagglutination which is comparable to that of cyclosporine.

Surprisingly, it has now been found that dialkyl fumarates are advantageous for preparing pharmaceutical compositions for use in transplantation medicine and for the therapy of autoimmune diseases. This is because compositions containing such dialkyl fumarates surprisingly permit a positive modulation of the immune system in host-versus-graft reactions, graft-versus-host reactions and other autoimmune diseases.

European Patent Application 0188 749 already describes fumaric acid derivatives and pharmaceutical compositions containing the same for the treatment of psoriasis. Pharmaceutical compositions for the treatment of psoriasis containing a mixture of fumaric acid and other fumaric acid derivatives are known from DE-A-25 30 372. The content of free fumaric acid is obligatory for these medicaments.

DE-A-26 21 214 describes medicaments containing the fumaric acid monoethyl ester and its mineral salts as active ingredient for the treatment of psoriasis. The publication "Hautarzt (*Dermatologist*) (1987) 279-285" discusses the use of fumaric acid monoethyl ester salts. Pharmaceutical preparations containing a mixture of fumaric acid monoalkyl ester salts and a fumaric acid diester for the treatment of psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn are known from EP 0 312 697 B1.

Specifically, the object of the invention is achieved by the use of one or more dialkyl fumarates of the formula



wherein R_1 and R_2, which may be the same or different, independently represent a linear, branched or cyclic, saturated or unsaturated C_{1-20} alkyl radical which may be optionally substituted with halogen (Cl, F, I, Br), hydroxy, C_{1-4} alkoxy, nitro or cyano for preparing a pharmaceutical preparation for use in transplantation medicine or for the therapy of autoimmune diseases.

The C_{1-20} alkyl radicals, preferably C_{1-8} alkyl radicals, most preferably C_{1-5} alkyl radicals are, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, t-butyl, pentyl, cyclopentyl, 2-ethyl hexyl, hexyl, cyclohexyl, heptyl, cycloheptyl, octyl, vinyl, allyl, 2-hydroxyethyl, 2 or 3-hydroxy propyl, 2-methoxy ethyl, methoxy methyl or 2- or 3-methoxy propyl. Preferably at least one of the radicals R_1 or R_2 is C_{1-5} alkyl, especially methyl or ethyl. More preferably, R_1 and R_2 are the same or different C_{1-5} alkyl radicals such as methyl, ethyl, n-propyl or t-butyl, methyl and ethyl being especially preferred. Most preferably, R_1 and R_2 are identical and are methyl or ethyl. Especially preferred are the dimethyl fumarate, methyl ethyl fumarate and diethyl fumarate.

The dialkyl fumarates to be used according to the invention are prepared by processes known in the art (see, for example, EP 0 312 697).

Preferably, the active ingredients are used for preparing oral preparations in the form of tablets, micro-tablets, pellets or granulates, optionally in capsules or sachets. Preparations in the form of micro-tablets or pellets, optionally filled in capsules or sachets are preferred and are also a subject matter of the invention. The oral preparations may be provided with an enteric coating. Capsules may be soft or hard gelatine capsules.

The dialkyl fumarates used according to the invention may be used alone or as a mixture of several compounds, optionally in combination with the customary carriers and excipients. The amounts to be used are selected in such a manner that the preparations obtained contain the active ingredient in an amount corresponding to 10 to 300 mg of fumaric acid.

Preferred preparations according to the invention contain a total amount of 10 to 300 mg of dimethyl fumarate and/or diethyl fumarate.

According to a preferred embodiment, the size or the mean diameter, respectively, of the pellets or micro-tablets is in the range from 300 to 2,000 μm, especially in the range of 500 or 1,000 μm.

In addition to graft-versus-host reactions (see above), the following autoimmune diseases to be treated may be named: polyarthritis, multiple sclerosis, graft-versus-host reactions, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (lupoid) hepatitis. Autoimmune diseases in a wider meaning also comprise psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn.

In addition to the preparations for peroral administration in the form of micro-pellets, micro-tablets, capsules (such as soft and hard gelatine capsules), granulates and tablets cited above, suitable pharmaceutical preparations are preparations

for cutaneous and transdermal administration in the form of ointments, plasters, lotions or shower preparations and for parenteral administration in the form of aqueous micro-dispersions, oil-in-water emulsions or oily solutions for rectal administration of suppositories or micro-enemas. Pharmaceutical preparations in the form of micro-tablets or micro-pellets are preferred for the therapy of all autoimmune diseases mentioned above, including psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn and are also a subject matter of the invention.

According to the invention, a therapy with dialkyl fumarates may also be carried out in combination with one or more preparations of the triple drug therapy customarily used in organ transplantations or with cyclosporine A alone. For this purpose, the preparations administered may contain a combination of the active ingredients in the known dosages or amounts, respectively. Likewise, the combination therapy may consist of the parallel administration of separate preparations, by the same or different routes. Optionally, the dosage of the active ingredient contained in addition to the dose of the fumaric acid derivative administered in accordance with the invention may be reduced advantageously.

Another embodiment of the use according to the invention is to alternate the drug therapy with immunosuppressive agents such as cyclosporine in sequence with an application of the above-mentioned dialkyl fumarate. This means that an application of fumaric acid derivatives as defined above over one or more weeks may follow a cyclosporine therapy of one or more weeks. This permits reduction of the Cyclosporine A dosage resulting in a considerable decrease of the rate of side effects in long-term therapy.

By administration of the dialkyl fumarates in the form of micro-tablets, which is preferred, gastrointestinal irritations and side effects, which are reduced already when conventional tablets are administered but is still observed, may be further reduced vis-a-vis fumaric acid derivatives and salts.

It is presumed that, upon administration of conventional tablets, the ingredients of the tablet are released in the intestine in a concentration which is too high, causing local irritation of the intestinal mucous membrane. This local irritation results in a short-term release of very high TNF-α concentrations which may be responsible for the gastrointestinal side effects. In case of application of enteric-coated micro-tablets in capsules, on the other hand, very low local concentrations of the active ingredients in the intestinal epithelial cells are achieved. The micro-tablets are incrementally released by the stomach and passed into the small intestine by peristaltic movements so that distribution of the active ingredients is improved.

This means that enteric-coated micro-tablets in the same dosage are distributed already in the stomach and passed to the intestine in portions, where the active ingredients are released in smaller dosages. This avoids local irritation of the intestinal epithelial cells and the release of TNF-α. It is assumed that this results in the improved tolerance of micro-tablets in the gastrointestinal tract vis-a-vis conventional tablets.

In addition, resorption is improved, because the dialkyl fumarates to be used according to the invention are not the active ingredient per se, but a so-called prodrug, which must be converted into the active ingredient in the body.

In order to illustrate the use according to the invention, different examples for preparing preferred drugs are given below.

PRODUCTION EXAMPLES

In principle, the oral preparations according to the invention in the form of tablets or micro-tablets may be prepared by classical tabletting processes. Instead of such classical tabletting processes, other methods for the preparation of tablets may be used, such as direct tabletting and processes for preparing solid dispersions in according with the melt method and the spray drying method.

The tablets may be provided with an enteric coating. The enteric coating may be applied in a classical coating pan or sprayed on or applied in a fluidised bed apparatus. The tablet may also be provided with a film coat.

Example 1

Preparation of enteric-coated micro-tablets in capsules containing 120.0 mg of dimethyl fumarate, which corresponds to 96 mg of fumaric acid

Taking the necessary precautions (breathing mask, gloves, protective clothing, etc.), 12.000 kg of dimethyl fumarate are crushed, mixed and homogenized by means of a sieve 800. Then an excipient mixture with the following composition is prepared: 17.50 kg of starch derivative (STA-RX® 1500), 0.30 kg of microcrystalline cellulose (Avicel® PH 101), 0.75 kg of PVP (Kollidon® 120), 4.00 kg of Primogel®, 0.25 kg of colloidal silicic acid (Aerosil®). The active ingredient is added to the entire powder mixture, mixed, homogenized by means of a sieve 200, processed in the usual manner with a 2% aqueous solution of polyvidon pyrrolidone (Kollidon® K25) to obtain a binder granulate and then mixed in the dry state with the outer phase. Said outer phase consists of 0.50 kg of Mg stearate and 1.50 kg of talcum.

Then the powder mixture is compressed in the usual manner to obtain convex tablets having a gross weight of 10.0 mg and a diameter of 2.0 mm.

One example to achieve resistance to gastric acid is to dissolve a solution of 2.250 kg of hydroxy propyl methyl cellulose phthalate (HPMCP, Pharmacoat® HP 50) in portions in a mixture of the following solvents: 13.00 1 of acetone, 13.50 1 of ethanol (94 wt.-%, denatured with 2% of ketone) and 1.50 1 of demineralised water. As a plasticiser, castor oil (0.240 kg) is added to the finished solution and applied in portions onto the tablet cores in the customary manner.

After drying is completed, a suspension of the following composition is applied as a film coat in the same apparatus: 0.340 kg of talcum, 0.400 kg of titanium(VI) oxide Cronus RN 56, 0.324 kg of coloured lacquer L-Rot-lack 86837, 4.800 kg of Eudragit E 12.5% and 0.120 kg of polyethylene glycol 6000, pH 11 XI in a solvent mixture of the following composition: 8.170 kg of 2-propanol, 0.200 kg of demineralised water and 0.600 kg of glycerine triacetate (Triacetin).

After that the enteric-coated micro-tablets are filled into hard gelatine capsules having a net weight of 400 mg and sealed.

Example 2

Preparation of enteric-coated micro-tablets in capsules containing 120.0 mg of dimethyl fumarate, which corresponds to 96 mg of fumaric acid

12.000 kg of dimethyl fumarate are crushed and homogenized as above. Then an excipient mixture composed as follows is prepared: 23.20 kg of microcrystalline cellulose (Avicel® PH 200), 3.00 kg of Croscarmellose sodium (AC-Di-SOL-SD-711), 2.50 kg of talcum, 0.10 kg of anhydrous silica (Aerosil® 200) and 1.00 kg of Mg stearate. The active ingredient is then added to the entire powder mixture and mixed homogenously. By means of direct tabletting, the powder mixture is then pressed into convex tablets having a gross weight of 10.0 mg and a diameter of 2.00 mm.

After that, a solution of 0.94 Eudragit® L in isopropanol is prepared which also contains 0.07 kg of dibutyl phthalate. This solution is sprayed onto the tablet cores. After that, a dispersion of 17.32 kg of Eudragit® L D-55 and a mixture of 2.80 kg of microtalcum, 2.00 kg of Macrogol 6000 and 0.07 kg of dimeticon in water is prepared and sprayed onto the cores.

Next, the enteric-coated micro-tablets are filled into hard gelatine capsules having a net weight of 650 mg and sealed.

Example 3

Preparation of micro-pellets in capsules containing 50.0 mg of dimethyl fumarate, which corresponds to 40 mg of fumaric acid

5.000 kg of dimethyl fumarate are crushed and homogenized as above. In addition, 2l of a 20% (m/v) polyvinyl pyrrolidone solution (Kollidon K-30) in ethanol are prepared. 7.250 kg of nonpareilles pellets in a coating pan are sprayed with part of the Kollidon K-30 solution until slightly humid. Then the active ingredient is added in portions until the pellets are dry. This procedure of humidification/drying is continued until all of the active ingredient mixture has been added. Then the pellets are moved around until completely dry.

After that, the pellets are filled into hard gelatine capsules (126.5 mg pellets/capsule).

Example 4

Preparation of enteric-coated capsules containing 110.0 mg of dimethyl fumarate, which corresponds to 88 mg of fumaric acid

11.000 kg of dimethyl fumarate are intensely mixed in a mixture consisting of 14.00 kg of starch, 5.65 kg of lactose, 2.00 kg of microcrystalline cellulose (Avicel®), 1.00 kg of polyvinyl pyrrolidone (Kollidon® 25) and 2.443 kg of Primogel® and, taking the necessary precautions (breathing mask, gloves, protective clothing), homogenized by means of a sieve 800.

Using a 2% aqueous solution of polyvinyl pyrrolidone (Kollidon® K25), the entire powder mixture is processed into a binder granulate in the customary manner and mixed with the outer phase when dry. Said outer phase consists of 0.350 kg of colloidal silicic acid (Aerosil®), 0.500 kg of Mg stearate and 1.500 kg of talcum. The homogenous mixture is filled into suitable capsules in portions of 400 mg which are then provided with an enteric coating consisting of hydroxy propyl methyl cellulose stearate and castor oil as plasticiser in the customary manner. Instead of using hard gelatine capsules, the product may also be filled into suitable enteric-coated capsules consisting of a mixture of cellulose acetate phthalate (CAP) and hydroxy propyl methyl cellulose phthalate (HP-MCP).

In comparison with substances of the prior art such as cyclosporine, which may cause massive kidney disorders or diseases of the lymphoproliferative system, a therapy with fumaric acid derivatives according to the invention for the indications listed above rarely results in serious side effects.

Among other things, the immunosuppressive effect of cyclosporine is caused by the inhibition of Th-1 cell formation. As in vitro experiments of the applicant have shown, fumarates cause a shift of the cytokine pattern of the Th1 type to the cytokine pattern of the Th2 type.

Especially in view of the long-term therapy and prevention which is always necessary in graft-versus-host reactions and host-versus-graft reactions or other autoimmune diseases such as multiple sclerosis, the unexpected effect of the use according to the invention is of the greatest interest. In a combination therapy of cyclosporine with the fumaric acid derivatives, the toxic side effects of the former compounds may be unexpectedly reduced to a substantial degree. In addition, the use according to the invention is also significant in the substitution of the corticosteroid therapy of autoimmune diseases which is known to be accompanied by severe side effects.

That which is claimed is:

1. A method of treating multiple sclerosis comprising administering, to a patient in need of treatment for multiple sclerosis, an amount of a pharmaceutical preparation effective for treating multiple sclerosis, the pharmaceutical preparation comprising

at least one excipient or at least one carrier or at least one combination thereof; and

dimethyl fumarate, methyl hydrogen fumarate, or a combination thereof.

2. The method of claim **1**, wherein the dimethyl fumarate, methyl hydrogen fumarate, or a combination thereof is present in an amount of from 10 to 300 mg in the pharmaceutical preparation.

3. The method of claim **1**, wherein the dimethyl fumarate, methyl hydrogen fumarate, or a combination thereof is provided in one or more capsules.

4. The method of claim **1**, the pharmaceutical preparation comprising a combination of dimethyl fumarate and methyl hydrogen fumarate.

5. The method of claim **1**, the pharmaceutical preparation comprising methyl hydrogen fumarate.

6. The method of claim **1**, the pharmaceutical preparation comprising dimethyl fumarate.

7. The method of claim **6**, wherein at least 50 mg of dimethyl fumarate is present in the pharmaceutical preparation.

8. The method of claim **6**, wherein at least 110 mg of dimethyl fumarate is present in the pharmaceutical preparation.

9. The method of claim **6**, wherein at least 120 mg of dimethyl fumarate is present in the pharmaceutical preparation.

10. The method of claim **6**, wherein 50 mg of dimethyl fumarate is present in the pharmaceutical preparation.

11. The method of claim **6**, wherein 110 mg of dimethyl fumarate is present in the pharmaceutical preparation.

12. The method of claim **6**, wherein 120 mg of dimethyl fumarate is present in the pharmaceutical preparation.

13. The method of claim **1**, wherein the pharmaceutical preparation is formulated for oral administration.

14. The method of claim **1**, wherein the pharmaceutical preparation is formulated as a solid dosage form.

15. The method of claim **1**, wherein the pharmaceutical preparation is formulated as microtablets.

16. The method of claim **15**, wherein the microtablets are provided in one or more capsules, wherein at least 50 mg of dimethyl fumarate is present in each capsule.

17. The method of claim **16**, wherein at least 110 mg of dimethyl fumarate is present in each capsule.

18. The method of claim **17**, wherein at least 120 mg of dimethyl fumarate is present in each capsule.

19. The method of claim **15**, wherein the microtablets are enteric-coated.

20. The method of claim **19**, wherein the microtablets have a mean diameter in the range of 0.3 mm to 2.0 mm, exclusive of any coating on the microtablets.

21. The method of claim **20**, wherein the microtablets have a mean diameter of 2.0 mm, exclusive of any coating on the microtablets.

22. The method of claim **1**, the pharmaceutical preparation comprising at least one carrier.

23. The method of claim **1**, the pharmaceutical preparation comprising at least one excipient.

24. The method of claim **1**, the pharmaceutical preparation comprising at least one excipient and at least one carrier.

* * * * *

EXHIBIT D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(12) **United States Patent**
Joshi et al.

(10) **Patent No.: US 7,803,840 B2**
(45) **Date of Patent: *Sep. 28, 2010**

(54) **UTILIZATION OF DIALKYLFUMARATES**

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(57) **ABSTRACT**

The present invention relates to the use of certain dialkyl fumarates for the preparation of pharmaceutical preparations for use in transplantation medicine or for the therapy of autoimmune diseases and said compositions in the form of micro-tablets or pellets. For this purpose, the dialkyl fumarates may also be used in combination with conventional preparations used in transplantation medicine and immunosuppressive agents, especially cyclosporines.

21 Claims, No Drawings

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UTILIZATION OF DIALKYLFUMARATES

REFERENCE TO RELATED APPLICATIONS

This is a continuation of application Ser. No. 11/765,578, filed Jun. 20, 2007, now U.S. Pat. No. 7,619,001 which is a continuation of application Ser. No. 10/197,077, filed Jul. 17, 2002 (now U.S. Pat. No. 7,320,999), which is a division of application Ser. No. 09/831,620, filed May 10, 2001 (now U.S. Pat. No. 6,509,376), which is a national stage (section 371) application of PCT application Ser. No. PCT/EP99/08215, filed Oct. 29, 1999, which claims priority to German Application No. 198 53 487.6, filed Nov. 19, 1998, all of which are hereby incorporated herein by reference.

The present invention relates to the use of dialkyl fumarates for preparing pharmaceutical preparations for use in transplantation medicine or the therapy of autoimmune diseases and pharmaceutical preparations in the form of micro-tablets or micro-pellets containing dialkyl fumarates.

On the one hand, therefore, it relates especially to the use of dialkyl fumarates for preparing pharmaceutical preparations for the treatment, reduction or suppression of rejection reactions of the transplant by the recipient, i.e. host-versus graft reactions, or rejection of the recipient by the transplant, i.e. graft-versus-host reactions. On the other hand, it relates to the use of dialkyl fumarates for preparing pharmaceutical preparations for treating autoimmune diseases such as polyarthritis, multiple sclerosis, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (=lupoid) hepatitis.

Both graft rejection and autoimmune diseases are based on medically undesirable reactions or dysregulation of the immune system. Cytokins such as interleukins or tumour necrose factor α (TNF-α) are substantial mediators influencing the immune system. In general, both are treated by the administration of immunosuppressive agents such as cyclosporine.

In the overall result, autoimmune diseases may be defined as the failure of the tolerance of endogenic substances or antigens. As a rule, this tolerance can be maintained only if the antigens keep coming into contact with immunological cells. When this tolerance is lost, autoantibodies are formed, i.e. a humoral immunoresponse against endogenic tissue. The exact nature of the involvement of TNF-α is not known.

Transplantations are tissue or organ transplantations, i.e. the transfer of tissues such as cornea, skin, bones (bone chips), vessels or fasciae, of organs such as kidney, heart, liver, lung, pancreas or intestines, or of individual cells such as islet cells, α-cells and liver cells, the kidney having the greatest significance as a transplanted organ.

According to the degree of relationship between the donor and the recipient we differentiate between autotransplantation (transfer to another part of the body of the same individual), iso-transplantation (transfer to another, genetically identical individual) and allogenic transplantation (transfer to another individual of the same species). Depending on the site of origin and transplantation, we further differentiate between homotopic transplantation (transfer to the same site) and heterotopic transplantation (transfer to a different site). The above-mentioned transplantations play an important role in modern medicine.

A major problem in transplantation medicine is graft rejection after transplantation of the tissue, organ or cell by immunological defense reactions of the recipient. Such a graft rejection is also called host-versus-graft reaction. The immunological defense reaction of the organism against the heteroprotein often results in rejection or dissolution of the grafts. In host-verses-graft reactions, different stages may be distinguished. Depending on the degree of difference between the recipient and the donor, this reaction takes place at different speeds so that we speak of an acute, sub-acute or chronic reaction. The acute rejection process is accompanied by the irreversible loss of the transplant (necrotisation) as a result of arteriitis or arteriolitis within 48 hours and cannot be influenced by the administration of drugs. The sub-acute rejection reaction becomes manifest as a rejection crisis from day 12 to month 4 with reversible functional disorders as a result of a transplant vasculopathy. Finally, the loss of function of the transplant as a result of vascular changes such as obliterating arteriopathy, which proceeds over weeks or years and can practically not be influenced by drugs, is termed a chronic rejection reaction.

Vice-versa, rejection reactions of the transplant against the recipient, the so-called graft-versus-host reactions, may occur when immunocompetent tissues are transplanted, i.e. primarily in bone marrow transplantation. Again, the severity of the reaction is graded, and substantially similar complications result as in host-versus-graft-reactions, namely arteriopathies and necroses.

To avoid such rejection reactions, i.e. the host-versus-graft reaction and the graft-versus-host reaction, transplantation medicine essentially makes use of immunosuppression, i.e. a weakening of the normal immunoresponse. For this purpose, anti-lymphocyte sera are often used in combination with corticosteroids and so-called anti-metabolites, e.g. purine analogues such as 6-mercaptopurine and thioguanine which affect the nucleic acid and protein synthesis and thus prevent cell division and proliferation. This leads to suppression of the production of antibodies and the cellular immune response. The immunosuppressive agents used for therapy are substances which suppress or weaken the immunoreaction in the body either specifically or non-specifically. Non-specific immunosuppressive agents are cytostatic agents such as, for example, alkylating agents or antimetabolites.

In addition, active ingredients are known which cause at least partial specific immunosuppression, such as corticosteroids, antisera, antibodies FK-506, tacrolimus, mycophenolatemofetil and primarily cyclosporines such as cyclosporine A. As a result of using modern immunosuppressive agents, the most important representatives of which are the cyclosporines, especially cyclosporine A, it was possible to improve the results of transplantation considerably over the last few years. At present, the survival rate after one year is about 60% for liver transplantations, about 80% for heart transplantations and over 90% for kidney transplantations.

Autoimmune diseases where the endogenic immune system attacks endogenic organs, tissues and cells are comparable to graft-versus-host reactions. These are also medically undesirable reactions of the immune system which may be treated with immunosuppressive agents, too.

The danger in using immunosuppressive agents lies in weakening the body's defense against infectious diseases and the increased risk of malignant diseases. Therefore, it is the object of the invention to provide a pharmaceutical preparation to be employed in transplantation medicine which may be used to treat, especially to suppress weaken and/or alleviate host-versus-graft reactions and graft-versus-host reactions, but does not have the above disadvantage.

It is another object of the invention to provide a pharmaceutical preparation which may be employed for treating autoimmune diseases, particularly polyarthritis, multiple sclerosis, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (=lupoid) hepatitis, without the disadvantages of immunosuppression.

The object of the invention is achieved by using certain dialkyl fumarates for preparing pharmaceutical preparations for use in transplantation medicine and for the therapy of autoimmune diseases and pharmaceutical preparations in the form of micro-tablets and micro-pellets containing these dialkyl fumarates. The individual subject matters of the invention are characterized in detail in the claims. The preparations according to the invention do not contain any free fumaric acids per se.

It is known that pharmaceutical preparations which, upon biological degradation after administration, enter into the citric acid cycle or are part thereof gain increasing therapeutic significance—especially when given in high dosages—since they can alleviate or heal diseases caused cryptogenetically.

Fumaric acid, for example, inhibits the growth of the Ehrlich ascites tumour in mice, reduces the toxic effects of mitomycin C and aflatoxin and displays antipsoriatic and antimicrobial activity. When administered parenterally, transdermally and especially perorally, high dosages of fumaric acid or its derivatives known so far such as dihydroxyl fumaric acid, fumaramide and fumaronitrile have such unacceptably severe side effects and high toxicity that, in most cases, such a therapy had to be abandoned in the past.

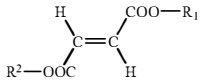
Surprisingly, investigations carried out by the applicant have shown that methyl hydrogen fumarate, a metabolite of the dimethyl fumarate, initially increases the endotoxin-stimulated TNF-α secretion in human mononuclear cells of periphere blood (periphere blood mononuclear cells=PBMC cells) and in isolated monocytes. In addition, the applicant was able to show that fumaric acid has an effect on in vitro and in vivo haemagglutination which is comparable to that of cyclosporine.

Surprisingly, it has now been found that dialkyl fumarates are advantageous for preparing pharmaceutical compositions for use in transplantation medicine and for the therapy of autoimmune diseases. This is because compositions containing such dialkyl fumarates surprisingly permit a positive modulation of the immune system in host-versus-graft reactions, graft-versus-host reactions and other autoimmune diseases.

European Patent Application 0188 749 already describes fumaric acid derivatives and pharmaceutical compositions containing the same for the treatment of psoriasis. Pharmaceutical compositions for the treatment of psoriasis containing a mixture of fumaric acid and other fumaric acid derivatives are known from DE-A-25 30 372. The content of free fumaric acid is obligatory for these medicaments.

DE-A-26 21 214 describes medicaments containing the fumaric acid monoethyl ester and its mineral salts as active ingredient for the treatment of psoriasis. The publication "Hautarzt (*Dermatologist*) (1987) 279-285" discusses the use of fumaric acid monoethyl ester salts. Pharmaceutical preparations containing a mixture of fumaric acid monoalkyl ester salts and a fumaric acid diester for the treatment of psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn are known from EP 0 312 697 B1.

Specifically, the object of the invention is achieved by the use of one or more dialkyl fumarates of the formula



wherein R_1 and R_2, which may be the same or different, independently represent a linear, branched or cyclic, saturated or unsaturated C_{1-20} alkyl radical which may be optionally substituted with halogen (Cl, F, I, Br), hydroxy, C_{1-4} alkoxy, nitro or cyano for preparing a pharmaceutical preparation for use in transplantation medicine or for the therapy of autoimmune diseases.

The C_{1-20} alkyl radicals, preferably C_{1-8} alkyl radicals, most preferably C_{1-5} alkyl radicals are, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, t-butyl, pentyl, cyclopentyl, 2-ethyl hexyl, hexyl, cyclohexyl, heptyl, cycloheptyl, octyl, vinyl, allyl, 2-hydroxyethyl, 2 or 3-hydroxy propyl, 2-methoxy ethyl, methoxy methyl or 2- or 3-methoxy propyl. Preferably at least one of the radicals R_1 or R_2 is C_{1-5} alkyl, especially methyl or ethyl. More preferably, R_1 and R_2 are the same or different C_{1-5} alkyl radicals such as methyl, ethyl, n-propyl or t-butyl, methyl and ethyl being especially preferred. Most preferably, R_1 and R_2 are identical and are methyl or ethyl. Especially preferred are the dimethyl fumarate, methyl ethyl fumarate and diethyl fumarate.

The dialkyl fumarates to be used according to the invention are prepared by processes known in the art (see, for example, EP 0 312 697).

Preferably, the active ingredients are used for preparing oral preparations in the form of tablets, micro-tablets, pellets or granulates, optionally in capsules or sachets. Preparations in the form of micro-tablets or pellets, optionally filled in capsules or sachets are preferred and are also a subject matter of the invention. The oral preparations may be provided with an enteric coating. Capsules may be soft or hard gelatine capsules.

The dialkyl fumarates used according to the invention may be used alone or as a mixture of several compounds, optionally in combination with the customary carriers and excipients. The amounts to be used are selected in such a manner that the preparations obtained contain the active ingredient in an amount corresponding to 10 to 300 mg of fumaric acid.

Preferred preparations according to the invention contain a total amount of 10 to 300 mg of dimethyl fumarate and/or diethyl fumarate.

According to a preferred embodiment, the size or the mean diameter, respectively, of the pellets or micro-tablets is in the range from 300 to 2,000 μm, especially in the range of 500 or 1,000 μm.

In addition to graft-versus-host reactions (see above), the following autoimmune diseases to be treated may be named: polyarthritis, multiple sclerosis, graft-versus-host reactions, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (lupoid) hepatitis. Autoimmune diseases in a wider meaning also comprise psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn.

In addition to the preparations for peroral administration in the form of micro-pellets, micro-tablets, capsules (such as soft and hard gelatine capsules), granulates and tablets cited above, suitable pharmaceutical preparations are preparations

for cutaneous and transdermal administration in the form of ointments, plasters, lotions or shower preparations and for parenteral administration in the form of aqueous micro-dispersions, oil-in-water emulsions or oily solutions for rectal administration of suppositories or micro-enemas. Pharmaceutical preparations in the form of micro-tablets or micro-pellets are preferred for the therapy of all autoimmune diseases mentioned above, including psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn and are also a subject matter of the invention.

According to the invention, a therapy with dialkyl fumarates may also be carried out in combination with one or more preparations of the triple drug therapy customarily used in organ transplantations or with cyclosporine A alone. For this purpose, the preparations administered may contain a combination of the active ingredients in the known dosages or amounts, respectively. Likewise, the combination therapy may consist of the parallel administration of separate preparations, by the same or different routes. Optionally, the dosage of the active ingredient contained in addition to the dose of the fumaric acid derivative administered in accordance with the invention may be reduced advantageously.

Another embodiment of the use according to the invention is to alternate the drug therapy with immunosuppressive agents such as cyclosporine in sequence with an application of the above-mentioned dialkyl fumarate. This means that an application of fumaric acid derivatives as defined above over one or more weeks may follow a cyclosporine therapy of one or more weeks. This permits reduction of the Cyclosporine A dosage resulting in a considerable decrease of the rate of side effects in long-term therapy.

By administration of the dialkyl fumarates in the form of micro-tablets, which is preferred, gastrointestinal irritations and side effects, which are reduced already when conventional tablets are administered but is still observed, may be further reduced vis-a-vis fumaric acid derivatives and salts.

It is presumed that, upon administration of conventional tablets, the ingredients of the tablet are released in the intestine in a concentration which is too high, causing local irritation of the intestinal mucous membrane. This local irritation results in a short-term release of very high TNF-α concentrations which may be responsible for the gastrointestinal side effects. In case of application of enteric-coated micro-tablets in capsules, on the other hand, very low local concentrations of the active ingredients in the intestinal epithelial cells are achieved. The micro-tablets are incrementally released by the stomach and passed into the small intestine by peristaltic movements so that distribution of the active ingredients is improved.

This means that enteric-coated micro-tablets in the same dosage are distributed already in the stomach and passed to the intestine in portions, where the active ingredients are released in smaller dosages. This avoids local irritation of the intestinal epithelial cells and the release of TNF-α. It is assumed that this results in the improved tolerance of micro-tablets in the gastrointestinal tract vis-a-vis conventional tablets.

In addition, resorption is improved, because the dialkyl fumarates to be used according to the invention are not the active ingredient per se, but a so-called prodrug, which must be converted into the active ingredient in the body.

In order to illustrate the use according to the invention, different examples for preparing preferred drugs are given below.

PRODUCTION EXAMPLES

In principle, the oral preparations according to the invention in the form of tablets or micro-tablets may be prepared by classical tabletting processes. Instead of such classical tabletting processes, other methods for the preparation of tablets may be used, such as direct tabletting and processes for preparing solid dispersions in according with the melt method and the spray drying method.

The tablets may be provided with an enteric coating. The enteric coating may be applied in a classical coating pan or sprayed on or applied in a fluidised bed apparatus. The tablet may also be provided with a film coat.

Example 1

Preparation of Enteric-coated Micro-tablets in Capsules Containing 120.0 mg of Dimethyl Fumarate, which Corresponds to 96 mg of Fumaric Acid

Taking the necessary precautions (breathing mask, gloves, protective clothing, etc.), 12.000 kg of dimethyl fumarate are crushed, mixed and homogenized by means of a sieve 800. Then an excipient mixture with the following composition is prepared: 17.50 kg of starch derivative (STA-RX® 1500), 0.30 kg of microcrystalline cellulose (Avicel® PH 101), 0.75 kg of PVP (Kollidon® 120), 4.00 kg of Primogel®, 0.25 kg of colloidal silicic acid (Aerosil®). The active ingredient is added to the entire powder mixture, mixed, homogenized by means of a sieve 200, processed in the usual manner with a 2% aqueous solution of polyvidon pyrrolidone (Kollidon® K25) to obtain a binder granulate and then mixed in the dry state with the outer phase. Said outer phase consists of 0.50 kg of Mg stearate and 1.50 kg of talcum.

Then the powder mixture is compressed in the usual manner to obtain convex tablets having a gross weight of 10.0 mg and a diameter of 2.0 mm.

One example to achieve resistance to gastric acid is to dissolve a solution of 2.250 kg of hydroxy propyl methyl cellulose phthalate (HPMCP, Pharmacoat® HP 50) in portions in a mixture of the following solvents: 13.00 l of acetone, 13.50 l of ethanol (94 wt.-%, denatured with 2% of ketone) and 1.50 l of demineralised water. As a plasticiser, castor oil (0.240 kg) is added to the finished solution and applied in portions onto the tablet cores in the customary manner.

After drying is completed, a suspension of the following composition is applied as a film coat in the same apparatus: 0.340 kg of talcum, 0.400 kg of titanium(VI) oxide Cronus RN 56, 0.324 kg of coloured lacquer L-Rot-lack 86837, 4.800 kg of Eudragit E 12.5% and 0.120 kg of polyethylene glycol 6000, pH 11 XI in a solvent mixture of the following composition: 8.170 kg of 2-propanol, 0.200 kg of demineralised water and 0.600 kg of glycerine triacetate (Triacetin).

After that the enteric-coated micro-tablets are filled into hard gelatine capsules having a net weight of 400 mg and sealed.

Example 2

Preparation of Enteric-coated Micro-tablets in Capsules Containing 120.0 mg of Dimethyl Fumarate, which Corresponds to 96 mg of Fumaric Acid

12.000 kg of dimethyl fumarate are crushed and homogenized as above. Then an excipient mixture composed as follows is prepared: 23.20 kg of microcrystalline cellulose (Avicel® PH 200), 3.00 kg of Croscarmellose sodium (AC-Di-SOL-SD-711), 2.50 kg of talcum, 0.10 kg of anhydrous silica (Aerosil® 200) and 1.00 kg of Mg stearate. The active ingredient is then added to the entire powder mixture and mixed homogenously. By means of direct tabletting, the powder mixture is then pressed into convex tablets having a gross weight of 10.0 mg and a diameter of 2.00 mm.

After that, a solution of 0.94 Eudragit® L in isopropanol is prepared which also contains 0.07 kg of dibutyl phthalate. This solution is sprayed onto the tablet cores. After that, a dispersion of 17.32 kg of Eudragit® L D-55 and a mixture of 2.80 kg of microtalcum, 2.00 kg of Macrogol 6000 and 0.07 kg of dimeticon in water is prepared and sprayed onto the cores.

Next, the enteric-coated micro-tablets are filled into hard gelatine capsules having a net weight of 650 mg and sealed.

Example 3

Preparation of Micro-pellets in Capsules Containing 50.0 mg of Dimethyl Fumarate, which Corresponds to 40 mg of Fumaric Acid

5.000 kg of dimethyl fumarate are crushed and homogenized as above. In addition, 2 of a 20% (m/v) polyvinyl pyrrolidone solution (Kollidon K-30) in ethanol are prepared. 7.250 kg of nonpareilles pellets in a coating pan are sprayed with part of the Kollidon K-30 solution until slightly humid. Then the active ingredient is added in portions until the pellets are dry. This procedure of humidification/drying is continued until all of the active ingredient mixture has been added. Then the pellets are moved around until completely dry.

After that, the pellets are filled into hard gelatine capsules (126.5 mg pellets/capsule).

Example 4

Preparation of Enteric-coated Capsules Containing 110.0 Mg of Dimethyl Fumarate, which Corresponds to 88 mg of Fumaric Acid

11.000 kg of dimethyl fumarate are intensely mixed in a mixture consisting of 14.00 kg of starch, 5.65 kg of lactose, 2.00 kg of microcrystalline cellulose (Avicel®), 1.00 kg of polyvinyl pyrrolidone (Kollidon® 25) and 2.443 kg of Primogel® and, taking the necessary precautions (breathing mask, gloves, protective clothing), homogenized by means of a sieve 800.

Using a 2% aqueous solution of polyvinyl pyrrolidone (Kollidon® K25), the entire powder mixture is processed into a binder granulate in the customary manner and mixed with the outer phase when dry. Said outer phase consists of 0.350 kg of colloidal silicic acid (Aerosil®), 0.500 kg of Mg stearate and 1.500 kg of talcum. The homogenous mixture is filled into suitable capsules in portions of 400 mg which are then provided with an enteric coating consisting of hydroxy propyl methyl cellulose stearate and castor oil as plasticiser in the customary manner. Instead of using hard gelatine capsules, the product may also be filled into suitable enteric-coated capsules consisting of a mixture of cellulose acetate phthalate (CAP) and hydroxy propyl methyl cellulose phthalate (HP-MCP).

In comparison with substances of the prior art such as cyclosporine, which may cause massive kidney disorders or diseases of the lymphoproliferative system, a therapy with fumaric acid derivatives according to the invention for the indications listed above rarely results in serious side effects.

Among other things, the immunosuppressive effect of cyclosporine is caused by the inhibition of Th-1 cell formation. As in vitro experiments of the applicant have shown, fumarates cause a shift of the cytokine pattern of the Th1 type to the cytokine pattern of the Th2 type.

Especially in view of the long-term therapy and prevention which is always necessary in graft-versus-host reactions and host-versus-graft reactions or other autoimmune diseases such as multiple sclerosis, the unexpected effect of the use according to the invention is of the greatest interest. In a combination therapy of cyclosporine with the fumaric acid derivatives, the toxic side effects of the former compounds may be unexpectedly reduced to a substantial degree. In addition, the use according to the invention is also significant in the substitution of the corticosteroid therapy of autoimmune diseases which is known to be accompanied by severe side effects.

That which is claimed is:

1. A method of treating at least one autoimmune disease selected from autoimmune polyarthritis and multiple sclerosis, but not treating psoriatic arthritis, comprising administering, to a patient in need of treatment for said at least one autoimmune disease but not for said psoriatic arthritis, an amount of a pharmaceutical preparation effective for treating said at least one autoimmune disease, the pharmaceutical preparation comprising

at least one excipient or at least one carrier or at least one combination thereof; and

dimethyl fumarate,

wherein the only active ingredient for the treatment of the at least one autoimmune disease present in said pharmaceutical preparation is the dimethyl fumarate.

2. The method of claim **1**, wherein the dimethyl fumarate is present in an amount of from 10 to 300 mg in the pharmaceutical preparation.

3. The method of claim **1**, wherein the dimethyl fumarate is provided in one or more capsules.

4. The method of claim **2**, wherein at least 50 mg of dimethyl fumarate is present in the pharmaceutical preparation.

5. The method of claim **2**, wherein at least 110 mg of dimethyl fumarate is present in the pharmaceutical preparation.

6. The method of claim **2**, wherein at least 120 mg of dimethyl fumarate is present in the pharmaceutical preparation.

7. The method of claim **2**, wherein 50 mg of dimethyl fumarate is present in the pharmaceutical preparation.

8. The method of claim **2**, wherein 110 mg of dimethyl fumarate is present in the pharmaceutical preparation.

9. The method of claim **2**, wherein 120 mg of dimethyl fumarate is present in the pharmaceutical preparation.

10. The method of claim **1**, wherein the pharmaceutical preparation is formulated for oral administration.

11. The method of claim **1**, wherein the pharmaceutical preparation is formulated as a solid dosage form.

12. The method of claim **1**, wherein the pharmaceutical preparation is formulated as microtablets.

13. The method of claim 12, wherein the microtablets are provided in one or more capsules, wherein at least 50 mg of dimethyl fumarate is present in each capsule.

14. The method of claim 13, wherein at least 110 mg of dimethyl fumarate is present in each capsule.

15. The method of claim 14, wherein at least 120 mg of dimethyl fumarate is present in each capsule.

16. The method of claim 12, wherein the microtablets are enteric-coated.

17. The method of claim 16, wherein the microtablets have a mean diameter in the range of 0.3 mm to 2.0 mm, exclusive of any coating on the microtablets.

18. The method of claim 17, wherein the microtablets have a mean diameter of 2.0 mm, exclusive of any coating on the microtablets.

19. The method of claim 1, the pharmaceutical preparation comprising at least one carrier.

20. The method of claim 1, the pharmaceutical preparation comprising at least one excipient.

21. The method of claim 1, the pharmaceutical preparation comprising at least one excipient and at least one carrier.

* * * * *

EXHIBIT E


US008759393B2

(12) **United States Patent**
Joshi et al.

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(45) **Date of Patent: *Jun. 24, 2014**

(54) **UTILIZATION OF DIALKYLFUMARATES**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 94 days.

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(57) **ABSTRACT**

The present invention relates to the use of certain dialkyl fumarates for the preparation of pharmaceutical preparations for use in transplantation medicine or for the therapy of autoimmune diseases and said compositions in the form of micro-tablets or pellets. For this purpose, the dialkyl fumarates may also be used in combination with conventional preparations used in transplantation medicine and immunosuppressive agents, especially cyclosporines.

13 Claims, No Drawings

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UTILIZATION OF DIALKYLFUMARATES

REFERENCE TO RELATED APPLICATIONS

This is a Division of commonly-owned copending application Ser. No. 09/831,620, filed May 10, 2001, which is a 371 continuation of PCT Application PCT/EP99/08215, filed Oct. 29, 1999, the text of which is not in English, which PCT Application claims priority on German Application No. 198 53 487.6, filed Nov. 19, 1998, the text of which is not in English.

DESCRIPTION

The present invention relates to the use of dialkyl fumarates for preparing pharmaceutical preparations for use in transplantation medicine or the therapy of autoimmune diseases and pharmaceutical preparations in the form of micro-tablets or micro-pellets containing dialkyl fumarates.

On the one hand, therefore, it relates especially to the use of dialkyl fumarates for preparing pharmaceutical preparations for the treatment, reduction or suppression of rejection reactions of the transplant by the recipient, i.e. host-versus graft reactions, or rejection of the recipient by the transplant, i.e. graft-versus-host reactions. On the other hand, it relates to the use of dialkyl fumarates for preparing pharmaceutical preparations for treating autoimmune diseases such as polyarthritis, multiple sclerosis, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (=lupoid) hepatitis.

Both graft rejection and autoimmune diseases are based on medically undesirable reactions or dysregulation of the immune system. Cytokins such as interleukins or tumour necrose factor α (TNF-α) are substantial mediators influencing the immune system. In general, both are treated by the administration of immunosuppressive agents such as cyclosporine.

In the overall result, autoimmune diseases may be defined as the failure of the tolerance of endogenic substances or antigens. As a rule, this tolerance can be maintained only if the antigens keep coming into contact with immunological cells. When this tolerance is lost, autoantibodies are formed, i.e. a humoral immunoresponse against endogenic tissue. The exact nature of the involvement of TNF-α is not known.

Transplantations are tissue or organ transplantations, i.e. the transfer of tissues such as cornea, skin, bones (bone chips), vessels or fasciae, of organs such as kidney, heart, liver, lung, pancreas or intestines, or of individual cells such as islet cells, α-cells and liver cells, the kidney having the greatest significance as a transplanted organ.

According to the degree of relationship between the donor and the recipient we differentiate between autotransplantation (transfer to another part of the body of the same individual), iso-transplantation (transfer to another, genetically identical individual) and allogenic transplantation (transfer to another individual of the same species). Depending on the site of origin and transplantation, we further differentiate between homotopic transplantation (transfer to the same site) and heterotopic transplantation (transfer to a different site). The above-mentioned transplantations play an important role in modern medicine.

A major problem in transplantation medicine is graft rejection after transplantation of the tissue, organ or cell by immunological defense reactions of the recipient. Such a graft rejection is also called host-versus-graft reaction. The immunological defense reaction of the organism against the heteroprotein often results in rejection or dissolution of the grafts. In host-verses-graft reactions, different stages may be distinguished. Depending on the degree of difference between the recipient and the donor, this reaction takes place at different speeds so that we speak of an acute, sub-acute or chronic reaction. The acute rejection process is accompanied by the irreversible loss of the transplant (necrotisation) as a result of arteritis or arteriolitis within 48 hours and cannot be influenced by the administration of drugs. The sub-acute rejection reaction becomes manifest as a rejection crisis from day 12 to month 4 with reversible functional disorders as a result of a transplant vasculopathy. Finally, the loss of function of the transplant as a result of vascular changes such as obliterating arteriopathy, which proceeds over weeks or years and can practically not be influenced by drugs, is termed a chronic rejection reaction.

Vice-versa, rejection reactions of the transplant against the recipient, the so-called graft-versus-host reactions, may occur when immunocompetent tissues are transplanted, i.e. primarily in bone marrow transplantation. Again, the severity of the reaction is graded, and substantially similar complications result as in host-versus-graft-reactions, namely arteriopathies and necroses.

To avoid such rejection reactions, i.e. the host-versus-graft reaction and the graft-versus-host reaction, transplantation medicine essentially makes use of immunosuppression, i.e. a weakening of the normal immunoresponse. For this purpose, anti-lymphocyte sera are often used in combination with corticosteroids and so-called anti-metabolites, e.g. purine analogues such as 6-mercaptopurine and thioguanine which affect the nucleic acid and protein synthesis and thus prevent cell division and proliferation. This leads to suppression of the production of antibodies and the cellular immune response. The immunosuppressive agents used for therapy are substances which suppress or weaken the immunoreaction in the body either specifically or non-specifically. Non-specific immunosuppressive agents are cytostatic agents such as, for example, alkylating agents or antimetabolites.

In addition, active ingredients are known which cause at least partial specific immunosuppression, such as corticosteroids, antisera, antibodies FK-506, tacrolimus, mycophenolatemofetil and primarily cyclosporines such as cyclosporine A. As a result of using modern immunosuppressive agents, the most important representatives of which are the cyclosporines, especially cyclosporine A, it was possible to improve the results of transplantation considerably over the last few years. At present, the survival rate after one year is about 60% for liver transplantations, about 80% for heart transplantations and over 90% for kidney transplantations.

Autoimmune diseases where the endogenic immune system attacks endogenic organs, tissues and cells are comparable to graft-versus-host reactions. These are also medically undesirable reactions of the immune system which may be treated with immunosuppressive agents, too.

The danger in using immunosuppressive agents lies in weakening the body's defense against infectious diseases and the increased risk of malignant diseases. Therefore, it is the object of the invention to provide a pharmaceutical preparation to be employed in transplantation medicine which may be used to treat, especially to suppress, weaken and/or alleviate host-versus-graft reactions and graft-versus-host reactions, but does not have the above disadvantage.

It is another object of the invention to provide a pharmaceutical preparation which may be employed for treating autoimmune diseases, particularly polyarthritis, multiple sclerosis, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (=lupoid) hepatitis, without the disadvantages of immunosuppression.

The object of the invention is achieved by using certain dialkyl fumarates for preparing pharmaceutical preparations for use in transplantation medicine and for the therapy of autoimmune diseases and pharmaceutical preparations in the form of micro-tablets and micro-pellets containing these dialkyl fumarates. The individual subject matters of the invention are characterized in detail in the claims. The preparations according to the invention do not contain any free fumaric acids per se.

It is known that pharmaceutical preparations which, upon biological degradation after administration, enter into the citric acid cycle or are part thereof gain increasing therapeutic significance—especially when given in high dosages—since they can alleviate or heal diseases caused cryptogenetically.

Fumaric acid, for example, inhibits the growth of the Ehrlich ascites tumour in mice, reduces the toxic effects of mitomycin C and aflatoxin and displays antipsoriatic and antimicrobial activity. When administered parenterally, transdermally and especially perorally, high dosages of fumaric acid or its derivatives known so far such as dihydroxyl fumaric acid, fumaramide and fumaronitrile have such unacceptably severe side effects and high toxicity that, in most cases, such a therapy had to be abandoned in the past.

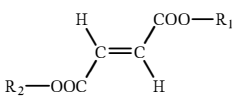
Surprisingly, investigations carried out by the applicant have shown that methyl hydrogen fumarate, a metabolite of the dimethyl fumarate, initially increases the endotoxin-stimulated TNF-α, secretion in human mononuclear cells of periphere blood (periphere blood mononuclear cells=PBMC cells) and in isolated monocytes. In addition, the applicant was able to show that fumaric acid has an effect on in vitro and in vivo haemagglutination which is comparable to that of cyclosporine.

Surprisingly, it has now been found that dialkyl fumarates are advantageous for preparing pharmaceutical compositions for use in transplantation medicine and for the therapy of autoimmune diseases. This is because compositions containing such dialkyl fumarates surprisingly permit a positive modulation of the immune system in host-versus-graft reactions, graft-versus-host reactions and other autoimmune diseases.

European Patent Application 0188 749 already describes fumaric acid derivatives and pharmaceutical compositions containing the same for the treatment of psoriasis. Pharmaceutical compositions for the treatment of psoriasis containing a mixture of fumaric acid and other fumaric acid derivatives are known from DE-A-25 30 372. The content of free fumaric acid is obligatory for these medicaments.

DE-A-26 21 214 describes medicaments containing the fumaric acid monoethyl ester and its mineral salts as active ingredient for the treatment of psoriasis. The publication "Hautarzt (*Dermatologist*) (1987) 279-285" discusses the use of fumaric acid monoethyl ester salts. Pharmaceutical preparations containing a mixture of fumaric acid monoalkyl ester salts and a fumaric acid diester for the treatment of psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn are known from EP 0 312 697 B1.

Specifically, the object of the invention is achieved by the use of one or more dialkyl fumarates of the formula



wherein R_1 and R_2, which may be the same or different, independently represent a linear, branched or cyclic, saturated or unsaturated C_{1-20} alkyl radical which may be optionally substituted with halogen (Cl, F, I, Br), hydroxy, C_{1-4} alkoxy, nitro or cyano for preparing a pharmaceutical preparation for use in transplantation medicine or for the therapy of autoimmune diseases.

The C_{1-20} alkyl radicals, preferably C_{1-8} alkyl radicals, most preferably C_{4-5} alkyl radicals are, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, t-butyl, pentyl, cyclopentyl, 2-ethyl hexyl, hexyl, cyclohexyl, heptyl, cycloheptyl, octyl, vinyl, allyl, 2-hydroxyethyl, 2 or 3-hydroxy propyl, 2-methoxy ethyl, methoxy methyl or 2- or 3-methoxy Propyl. Preferably at least one of the radicals R_1 or R_2 is C_{1-5} alkyl, especially methyl or ethyl. More preferably, R_1 and R_2 are the same or different C_{1-5} alkyl radicals such as methyl, ethyl, n-propyl or t-butyl, methyl and ethyl being especially preferred. Most preferably, R_1 and R_2 are identical and are methyl or ethyl. Especially preferred are the dimethyl fumarate, methyl ethyl fumarate and diethyl fumarate.

The dialkyl fumarates to be used according to the invention are prepared by processes known in the art (see, for example, EP 0 312 697).

Preferably, the active ingredients are used for preparing oral preparations in the form of tablets, micro-tablets, pellets or granulates, optionally in capsules or sachets. Preparations in the form of micro-tablets or pellets, optionally filled in capsules or sachets are preferred and are also a subject matter of the invention. The oral preparations may be provided with an enteric coating. Capsules may be soft or hard gelatine capsules.

The dialkyl fumarates used according to the invention may be used alone or as a mixture of several compounds, optionally in combination with the customary carriers and excipients. The amounts to be used are selected in such a manner that the preparations obtained contain the active ingredient in an amount corresponding to 10 to 300 mg of fumaric acid.

Preferred preparations according to the invention contain a total amount of 10 to 300 mg of dimethyl fumarate and/or diethyl fumarate.

According to a preferred embodiment, the size or the mean diameter, respectively, of the pellets or micro-tablets is in the range from 300 to 2,000 μm, especially in the range of 500 or 1,000 μm.

In addition to graft-versus-host reactions (see above), the following autoimmune diseases to be treated may be named: polyarthritis, multiple sclerosis, graft-versus-host reactions, juvenile-onset diabetes, Hashimoto's thyroiditis, Grave's disease, systemic Lupus erythematodes (SLE), Sjogren's syndrome, pernicious anaemia and chronic active (lupoid) hepatitis. Autoimmune diseases in a wider meaning also comprise psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn.

In addition to the preparations for peroral administration in the form of micro-pellets, micro-tablets, capsules (such as soft and hard gelatine capsules), granulates and tablets cited above, suitable pharmaceutical preparations are preparations for cutaneous and transdermal administration in the form of ointments, plasters, lotions or shower preparations and for parenteral administration in the form of aqueous micro-dispersions, oil-in-water emulsions or oily solutions for rectal administration of suppositories or micro-enemas. Pharmaceutical preparations in the form of micro-tablets or micro-pellets are preferred for the therapy of all autoimmune diseases mentioned above, including psoriasis, psoriatic arthritis, neurodermatitis and enteritis regionalis Crohn and are also a subject matter of the invention.

According to the invention, a therapy with dialkyl fumarates may also be carried out in combination with one or more preparations of the triple drug therapy customarily used in organ transplantations or with cyclosporine A alone. For this purpose, the preparations administered may contain a combination of the active ingredients in the known dosages or amounts, respectively. Likewise, the combination therapy may consist of the parallel administration of separate preparations, by the same or different routes. Optionally, the dosage of the active ingredient contained in addition to the dose of the fumaric acid derivative administered in accordance with the invention may be reduced advantageously.

Another embodiment of the use according to the invention is to alternate the drug therapy with immunosuppressive agents such as cyclosporine in sequence with an application of the above-mentioned dialkyl fumarate. This means that an application of fumaric acid derivatives as defined above over one or more weeks may follow a cyclosporine therapy of one or more weeks. This permits reduction of the Cyclosporine A dosage resulting in a considerable decrease of the rate of side effects in long-term therapy.

By administration of the dialkyl fumarates in the form of micro-tablets, which is preferred, gastrointestinal irritations and side effects, which are reduced already when conventional tablets are administered but is still observed, may be further reduced vis-a-vis fumaric acid derivatives and salts.

It is presumed that, upon administration of conventional tablets, the ingredients of the tablet are released in the intestine in a concentration which is too high, causing local irritation of the intestinal mucous membrane. This local irritation results in a short-term release of very high TNF-α concentrations which may be responsible for the gastrointestinal side effects. In case of application of enteric-coated micro-tablets in capsules, on the other hand, very low local concentrations of the active ingredients in the intestinal epithelial cells are achieved. The micro-tablets are incrementally released by the stomach and passed into the small intestine by peristaltic movements so that distribution of the active ingredients is improved.

This means that enteric-coated micro-tablets in the same dosage are distributed already in the stomach and passed to the intestine in portions, where the active ingredients are released in smaller dosages. This avoids local irritation of the intestinal epithelial cells and the release of TNF-α. It is assumed that this results in the improved tolerance of micro-tablets in the gastrointestinal tract vis-a-vis conventional tablets.

In addition, resorption is improved, because the dialkyl fumarates to be used according to the invention are not the active ingredient per se, but a so-called prodrug, which must be converted into the active ingredient in the body.

In order to illustrate the use according to the invention, different examples for preparing preferred drugs are given below.

PRODUCTION EXAMPLES

In principle, the oral preparations according to the invention in the form of tablets or micro-tablets may be prepared by classical tabletting processes. Instead of such classical tabletting processes, other methods for the preparation of tablets may be used, such as direct tabletting and processes for preparing solid dispersions in according with the melt method and the spray drying method.

The tablets may be provided with an enteric coating. The enteric coating may be applied in a classical coating pan or sprayed on or applied in a fluidised bed apparatus. The tablet may also be provided with a film coat.

Example 1

Preparation of Enteric-Coated Micro-Tablets in Capsules Containing 120.0 Mg of Dimethyl Fumarate, which Corresponds to 96 Mg of Fumaric Acid

Taking the necessary precautions (breathing mask, gloves, protective clothing, etc.), 12.000 kg of dimethyl fumarate are crushed, mixed and homogenized by means of a sieve 800. Then an excipient mixture with the following composition is prepared: 17.50 kg of starch derivative (STA-RX® 1500), 0.30 kg of microcrystalline cellulose (Avicel® PH 101), 0.75 kg of PVP (Kollidon® 120), 4.00 kg of Primogel®, 0.25 kg of colloidal silicic acid (Aerosil®). The active ingredient is added to the entire powder mixture, mixed, homogenized by means of a sieve 200, processed in the usual manner with a 2% aqueous solution of polyvidon pyrrolidone (Kollidon® K25) to obtain a binder granulate and then mixed in the dry state with the outer phase. Said outer phase consists of 0.50 kg of Mg stearate and 1.50 kg of talcum.

Then the powder mixture is compressed in the usual manner to obtain convex tablets having a gross weight of 10.0 mg and a diameter of 2.0 mm.

One example to achieve resistance to gastric acid is to dissolve a solution of 2.250 kg of hydroxy propyl methyl cellulose phthalate (HPMCP, Pharmacoat® HP 50) in portions in a mixture of the following solvents: 13.00 l of acetone, 13.50 l of ethanol (94 wt.-%, denatured with 2% of ketone) and 1.50 l of demineralised water. As a plasticiser, castor oil (0.240 kg) is added to the finished solution and applied in portions onto the tablet cores in the customary manner.

After drying is completed, a suspension of the following composition is applied as a film coat in the same apparatus: 0.340 kg of talcum, 0.400 kg of titanium(VI) oxide Cronus RN 56, 0.324 kg of coloured lacquer L-Rot-lack 86837, 4.800 kg of Eudragit E 12.5% and 0.120 kg of polyethylene glycol 6000, pH 11 XI in a solvent mixture of the following composition: 8.170 kg of 2-propanol, 0.200 kg of demineralised water and 0.600 kg of glycerine triacetate (Triacetin).

After that the enteric-coated micro-tablets are filled into hard gelatine capsules having a net weight of 400 mg and sealed.

Example 2

Preparation of Enteric-Coated Micro-Tablets in Capsules Containing 120.0 Mg of Dimethyl Fumarate, which Corresponds to 96 Mg of Fumaric Acid

12.000 kg of dimethyl fumarate are crushed and homogenized as above. Then an excipient mixture composed as follows is prepared: 23.20 kg of microcrystalline cellulose (Avicel® PH 200), 3.00 kg of Croscarmellose sodium (AC-Di-SOL-SD-711), 2.50 kg of talcum, 0.10 kg of anhydrous silica (Aerosil® 200) and 1.00 kg of Mg stearate. The active ingredient is then added to the entire powder mixture and mixed homogenously. By means of direct tabletting, the powder mixture is then pressed into convex tablets having a gross weight of 10.0 mg and a diameter of 2.00 mm.

After that, a solution of 0.94 Eudragit® L in isopropanol is prepared which also contains 0.07 kg of dibutyl phthalate.

This solution is sprayed onto the tablet cores. After that, a dispersion of 17.32 kg of Eudragit® L D-55 and a mixture of 2.80 kg of microtalcum, 2.00 kg of Macrogol 6000 and 0.07 kg of dimeticon in water is prepared and sprayed onto the cores.

Next, the enteric-coated micro-tablets are filled into hard gelatine capsules having a net weight of 650 mg and sealed.

Example 3

Preparation of Micro-Pellets in Capsules Containing 50.0 Mg of Dimethyl Fumarate, which Corresponds to 40 Mg of Fumaric Acid

5.000 kg of dimethyl fumarate are crushed and homogenized as above. In addition, 2 l of a 20% (m/v) polyvinyl pyrrolidone solution (Kollidon K-30) in ethanol are prepared. 7.250 kg of nonpareilles pellets in a coating pan are sprayed with part of the Kollidon K-30 solution until slightly humid. Then the active ingredient is added in portions until the pellets are dry. This procedure of humidification/drying is continued until all of the active ingredient mixture has been added. Then the pellets are moved around until completely dry.

After that, the pellets are filled into hard gelatine capsules (126.5 mg pellets/capsule).

Example 4

Preparation of Enteric-Coated Capsules Containing 110.0 Mg of Dimethyl Fumarate, which Corresponds to 88 Mg of Fumaric Acid

11.000 kg of dimethyl fumarate are intensely mixed in a mixture consisting of 14.00 kg of starch, 5.65 kg of lactose, 2.00 kg of microcrystalline cellulose (Avicel®), 1.00 kg of polyvinyl pyrrolidone (Kollidon® 25) and 2.443 kg of Primogel® and, taking the necessary precautions (breathing mask, gloves, protective clothing), homogenized by means of a sieve 800.

Using a 2% aqueous solution of polyvinyl pyrrolidone (Kollidon® K25), the entire powder mixture is processed into a binder granulate in the customary manner and mixed with the outer phase when dry. Said outer phase consists of 0.350 kg of colloidal silicic acid (Aerosil®), 0.500 kg of Mg stearate and 1.500 kg of talcum. The homogenous mixture is filled into suitable capsules in portions of 400 mg which are then provided with an enteric coating consisting of hydroxy propyl methyl cellulose stearate and castor oil as plasticiser in the customary manner. Instead of using hard gelatine capsules, the product may also be filled into suitable enteric-coated capsules consisting of a mixture of cellulose acetate phthalate (CAP) and hydroxy propyl methyl cellulose phthalate (HP-MCP).

In comparison with substances of the prior art such as cyclosporine, which may cause massive kidney disorders or diseases of the lymphoproliferative system, a therapy with fumaric acid derivatives according to the invention for the indications listed above rarely results in serious side effects.

Among other things, the immunosuppressive effect of cyclosporine is caused by the inhibition of Th-1 cell formation. As in vitro experiments of the applicant have shown, fumarates cause a shift of the cytokine pattern of the Th1 type to the cytokine pattern of the Th2 type.

Especially in view of the long-term therapy and prevention which is always necessary in graft-versus-host reactions and host-versus-graft reactions or other autoimmune diseases such as multiple sclerosis, the unexpected effect of the use according to the invention is of the greatest interest. In a combination therapy of cyclosporine with the fumaric acid derivatives, the toxic side effects of the former compounds may be unexpectedly reduced to a substantial degree. In addition, the use according to the invention is also significant in the substitution of the corticosteroid therapy of autoimmune diseases which is known to be accompanied by severe side effects.

That which is claimed is:

1. A pharmaceutical preparation, comprising dimethyl fumarate, wherein the pharmaceutical preparation is in the form of microtablets.

2. The pharmaceutical preparation of claim **1**, wherein the microtablets are enteric coated.

3. The pharmaceutical preparation of claim **2**, wherein the mean diameter of the microtablets ranges from 300 μm to 2,000 μm, exclusive of any coating on the microtablets.

4. The pharmaceutical preparation of claim **3**, wherein the mean diameter of the microtablets is about 2,000 μm, exclusive of any coating on the microtablets.

5. The pharmaceutical preparation of claim **4**, wherein the preparation contains 10 mg to 300 mg of dimethyl fumarate.

6. The pharmaceutical preparation of claim **5**, wherein the preparation contains about 120 mg of dimethyl fumarate.

7. The pharmaceutical preparation of claim **5**, wherein the microtablets are contained in one or more capsules.

8. A pharmaceutical preparation, comprising an active ingredient, wherein the pharmaceutical preparation is in the form of microtablets and the active ingredient consists of dimethyl fumarate.

9. The pharmaceutical preparation of claim **8**, wherein the mean diameter of the microtablets is about 2,000 μm, exclusive of any coating on the microtablets.

10. The pharmaceutical preparation of claim **9**, wherein the preparation contains 10 mg to 300 mg of dimethyl fumarate.

11. The pharmaceutical preparation of claim **10**, wherein the preparation contains about 120 mg of dimethyl fumarate.

12. The pharmaceutical preparation of claim **10**, wherein the microtablets are enteric coated and are contained in one or more capsules.

13. A pharmaceutical preparation consisting essentially of an active ingredient and one or more carriers and excipients, wherein the active ingredient is dimethyl fumarate and the preparation contains 10 mg to 300 mg of dimethyl fumarate, and wherein the pharmaceutical preparation is in the form of microtablets and the mean diameter of the microtablets is about 2,000 μm, exclusive of any coating on the microtablets.

* * * * *

EXHIBIT F

US008399514B2

(12) **United States Patent**
Lukashev et al.

(10) **Patent No.: US 8,399,514 B2**
(45) **Date of Patent: Mar. 19, 2013**

(54) **TREATMENT FOR MULTIPLE SCLEROSIS**

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(65) **Prior Publication Data**

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Related U.S. Application Data

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(60) Provisional application No. 60/888,921, filed on Feb. 8, 2007.

(51) **Int. Cl.**
A61K 31/22 (2006.01)

(52) **U.S. Cl.** **514/549**

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

Provided are certain methods of screening, identifying, and evaluating neuroprotective compounds useful for treatment of neurological diseases, such as, e.g., multiple sclerosis (MS). The compounds described upregulate the cellular cytoprotective pathway regulated by Nrf2. Also provided are certain methods of utilizing such compounds in therapy for neurological disease, particularly, for slowing or reducing demyelination, axonal loss, or neuronal and oligodendrocyte death.

20 Claims, 4 Drawing Sheets

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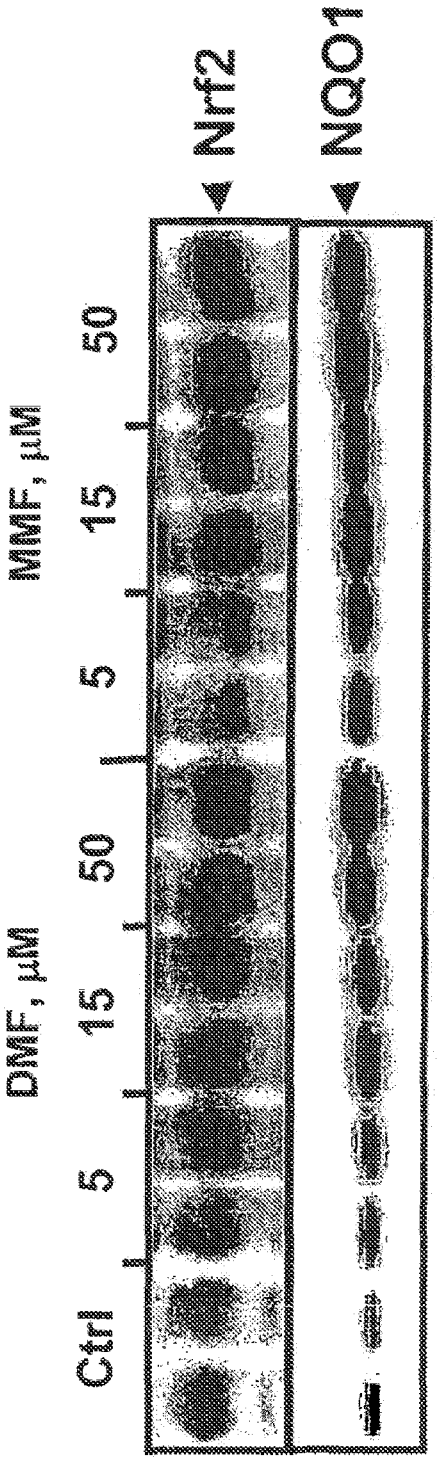


Figure 1

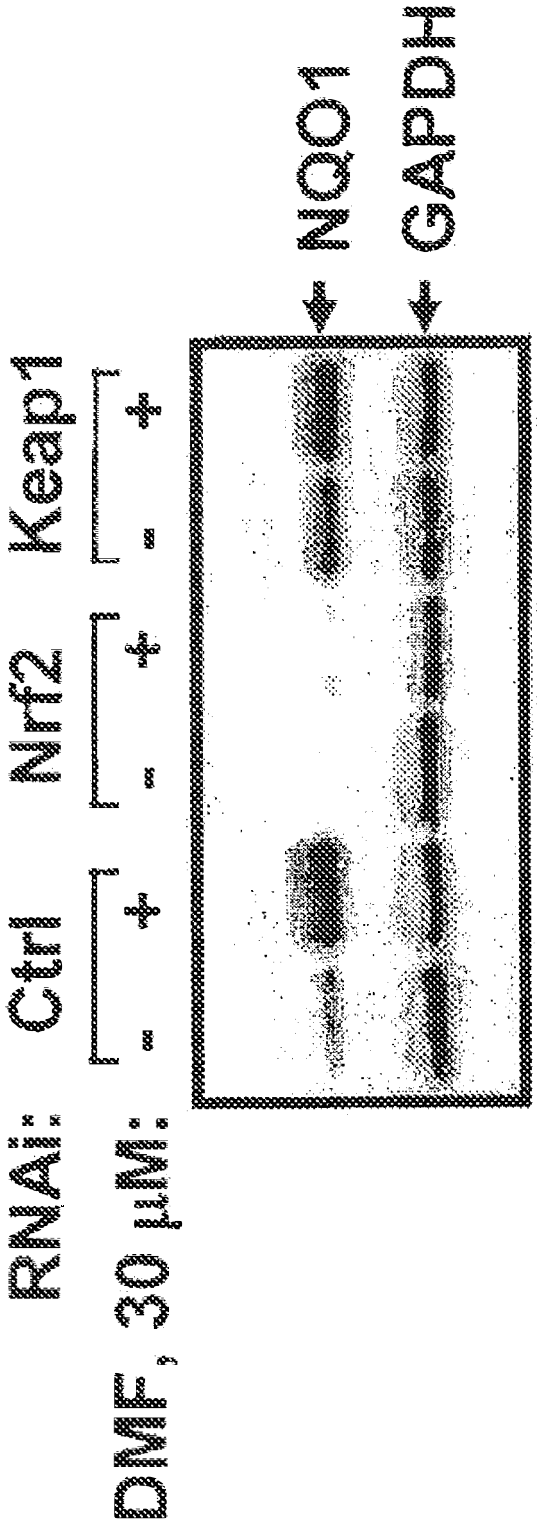


Figure 2

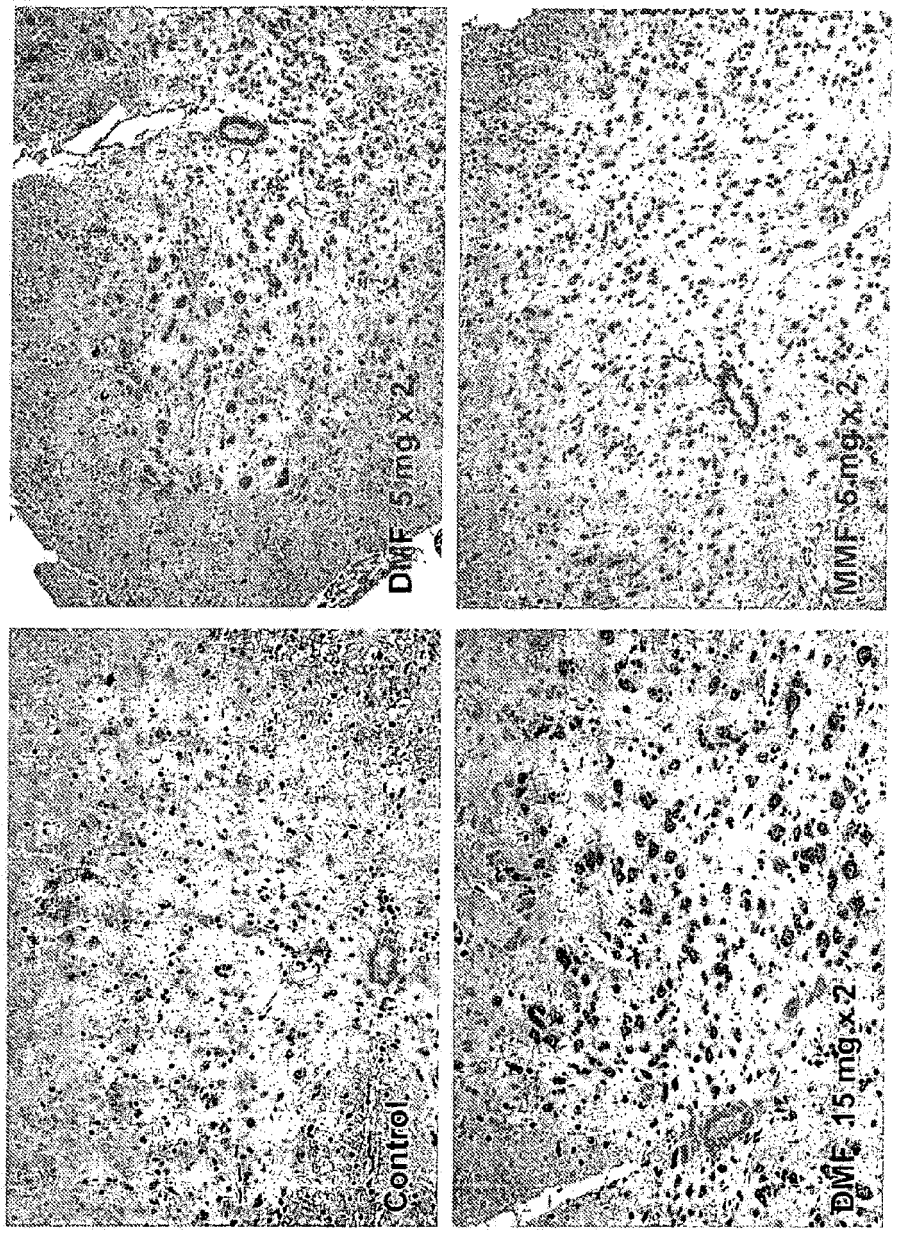
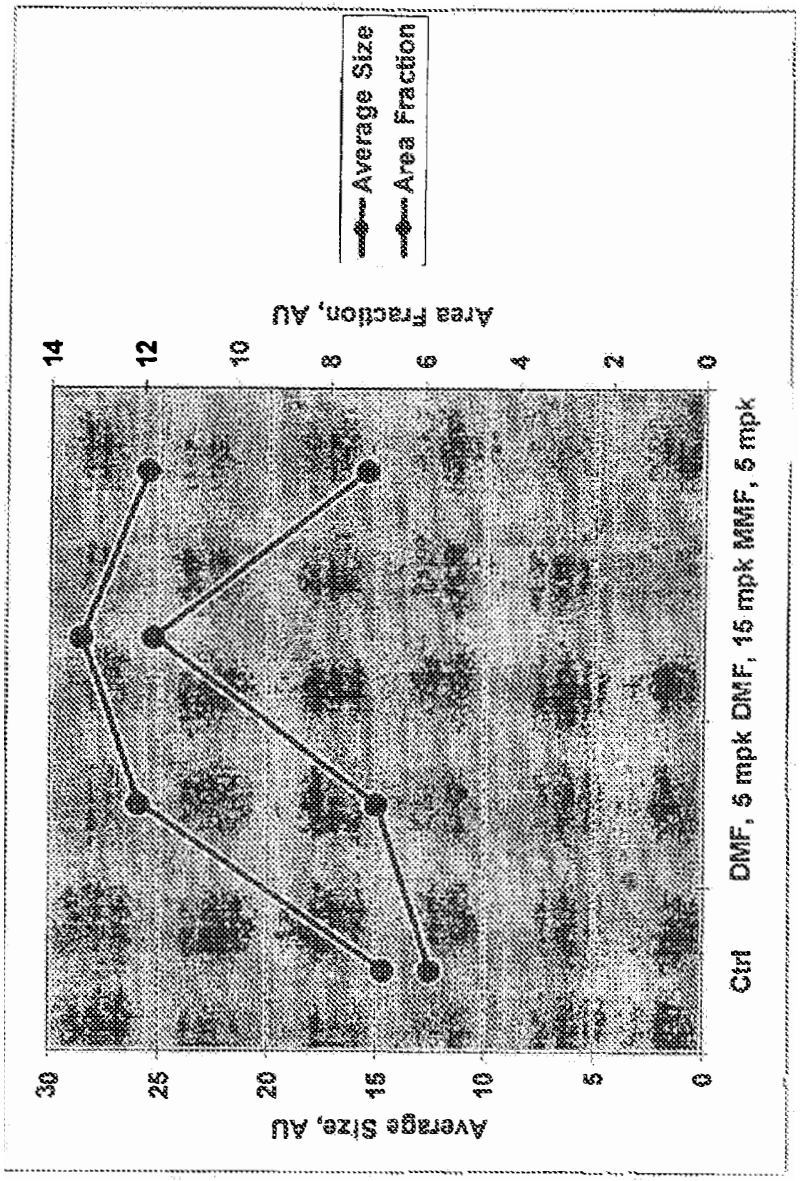


Figure 3


Average Size
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DMF, 5 mpk
DMF, 15 mpk
MMF, 5 mpk

Figure 4

TREATMENT FOR MULTIPLE SCLEROSIS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 12/526,296, §371(c) Date Jan. 13, 2011, now abandoned, which is the U.S. National Phase of International Application No. PCT/US2008/001602, filed Feb. 7, 2008, which claims the benefit of U.S. Provisional Application 60/888,921, filed Feb. 8, 2007.

Provided are certain compounds for treating neurological diseases, including demyelinating neurological diseases, such as, e.g., multiple sclerosis.

Multiple sclerosis (MS) is an autoimmune disease with the autoimmune activity directed against central nervous system (CNS) antigens. The disease is characterized by inflammation in parts of the CNS, leading to the loss of the myelin sheathing around neuronal axons (demyelination), loss of axons, and the eventual death of neurons, oligodenrocytes and glial cells.

An estimated 2,500,000 people in the world suffer from MS. It is one of the most common diseases of the CNS in young adults. MS is a chronic, progressing, disabling disease, which generally strikes its victims some time after adolescence, with diagnosis generally made between 20 and 40 years of age, although onset may occur earlier. The disease is not directly hereditary, although genetic susceptibility plays a part in its development. Relapsing-remitting MS presents in the form of recurrent attacks of focal or multifocal neurologic dysfunction. Attacks may occur, remit, and recur, seemingly randomly over many years. Remission is often incomplete and as one attack follows another, a stepwise downward progression ensues with increasing permanent neurological deficit.

Although various immunotherapeutic drugs can provide relief in patients with MS, none is capable of reversing disease progression, and some can cause serious adverse effects. Most current therapies for MS are aimed at the reduction of inflammation and suppression or modulation of the immune system. As of 2006, the available treatments for MS reduce inflammation and the number of new episodes but not all have an effect on disease progression. A number of clinical trials have shown that the suppression of inflammation in chronic MS rarely significantly limits the accumulation of disability through sustained disease progression, suggesting that neuronal damage and inflammation are independent pathologies. Promoting CNS remyelination as a repair mechanism and otherwise preventing axonal loss and neuronal death are some of the important goals for the treatment of MS. For a comprehensive review of MS and its current therapies, see, e.g., McAlpine's Multiple Sclerosis, by Alastair Compston et al., 4th edition, Churchill Livingstone Elsevier, 2006.

"Phase 2 enzymes" serve as a protection mechanism in mammalian cells against oxygen/nitrogen species (ROS/RNS), electrophiles and xenobiotics. These enzymes are not normally expressed at their maximal levels and, their expression can be induced by a variety of natural and synthetic agents. Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor responsible for the induction of a variety of important antioxidant and detoxification enzymes that coordinate a protective cellular response to metabolic and toxic stress.

ROS/RNS are most damaging in the brain and neuronal tissue, where they attack post-mitotic (i.e., non-dividing) cells such as glial cells, oligodendocytes, and neurons, which are particularly sensitive to free radicals. This process leads to neuronal damage. Oxidative stress has been implicated in the

pathogenesis of a variety of neurodegenerative diseases, including ALS, Alzheimer's disease (AD), and Parkinson's disease (PD). For review, see, e.g., van Muiswinkel et al., Curr. Drug Targets CNS—Neurol. Disord., 2005, 4:267-281. An anti-oxidative enzyme under control of Nrf2, NQO1 (NAD(P)H dehydrogenase, quinone (1), was recently reported to be substantially upregulated in the brain tissues of AD and PD subjects (Muiswinkel et al., Neurobiol. Aging, 2004, 25: 1253). Similarly, increased expression of NQO1 was reported in the ALS subjects' spinal cord (Muiswinkel et al., Curr. Drug Targets—CNS. Neurol. Disord., 2005, 4:267-281) and in active and chronic lesions in the brains of patients suffering from MS (van Horssen et al., Free Radical Biol. & Med., 2006, 41 311-311). These observations indicate that the Nrf2 pathway may be activated in neurodegenerative and neuroinflammatory diseases as an endogenous protective mechanism. Indeed, most recently, it has been reported that induced activation of Nrf2-dependent genes by certain cyclopenanone-based compounds (NEPP) counters the toxic effects of metabolic inhibition and ROS/RNS production in the brain and protects neurons from death in vitro and in vivo (see Satoh et al., PNAS, 2006, 103(3):768-773).

Additionally, many publications have reported neuroprotective effects of compounds in natural plant-derived compounds ("phytochemicals"), including α-tocopherol (vitamin E), lycopene (tomatoes), resveratrol (red grapes), sulforaphane (broccoli), EGCG (green tea), etc. For review, see Mattson and Cheng, Trends in Neurosci., 2006, 29(11):632-639. Originally, the action of these compounds was attributed to their anti-oxidant properties. However, while most antioxidants are effective only at high concentrations, at least some of these compounds appear to exert neuroprotective effects at much lower doses. Emerging evidence suggests that these compounds may exert their neuroprotective effects by activating cellular stress-response pathways, including the Nrf2 pathway, resulting in the upregulation of neuroprotective genes. However, the exact mechanism of action of these compounds remains poorly understood.

To date, more than 10 different chemical classes of inducers of Nrf2 pathway have been identified including isothiocyanates and their thiol addition products, dithiocarbamates, as well as 1,2-dithiole-3-thiones, trivalent arsenic derivatives (e.g., phenyl arsenoxide), heavy metals, certain conjugated cyclic and acyclic polyenes (including porphyrins, chlorophyllins, and chlorophyll), and vicinal dimercaptans. These inducers have few structural similarities. They are mostly electrophiles, and all can react chemically with thiol groups by alkylation, oxidation, or reduction, suggesting that the intracellular sensor for inducers is likely to contain very highly reactive (cysteine) thiols. The inducers can modify thiol groups by a variety of mechanisms including: alkylation (Michael addition acceptors, isothiocyanates, quinones); oxidation (e.g., peroxides and hydroperoxides); and direct reaction with thiol/disulfide linkages (e.g., vicinal dithiols such as 1,2-dimercaptopropanol, lipoic acid). These diverse response mechanisms provide plasticity for cellular responses to a variety of electrophilic and oxidant stressors.

Provided are methods that comprise at least one of the following methods:

1) methods of screening for at least one new candidate compound for treating a neurological disease;
2) methods of evaluating neuroprotective properties of at least one drug candidate for treating a neurological disease;
3) methods of comparing (e.g., for bioequivalence) at least two pharmaceutical compositions which comprise fumaric acid derivatives;

4) methods of treating a neurological disease by administering to the subject in need thereof at least one compound that is partially structurally similar to DMF or MMF; and

5) methods of treating a neurological disease by a combination therapy that comprises administration of at least one first compound that upregulates the Nrf2 pathway and at least one second compound that does not upregulate the Nrf2 pathway.

In some embodiments, the neurological disease is a neurodegenerative disease such as, for example, ALS, Parkinson's disease, Alzheimer's disease, and Huntington's disease. In some embodiments the neurological disease is MS or another demyelinating neurological disease.

In some embodiments, the methods 1-3 further comprise:

a) contacting a cell with the test compound, and

b) determining whether the Nrf2 pathway is upregulated in the cell.

In some embodiments, the methods may further comprise:

c) determining whether the test compound slows or prevents demyelination, axonal loss, and/or neuronal death, and/or

d) selecting the test compound as a candidate for treating neurodegeneration in a neurological disease if 1) the Nrf2 pathway is upregulated and 2) demyelination, axonal loss, and/or neuronal death are/is prevented or slowed.

In some embodiments, the methods 1-3 comprise contacting a cell with at least one test compound and determining whether the Nrf2 pathway is upregulated in the cell. In such methods, an upregulation of the Nrf2 pathway above a threshold (e.g., by at least 30% over a control) indicates that the at least one compound has at least one biological property beneficial in treating a neurological disease (e.g., neuroprotective properties). In some embodiments, the upregulation of the Nrf2 pathway is assessed (in vivo and/or in vitro) by at least one of the following:

i) expression levels of endogenously produced and/or exogenously introduced Nrf2;

ii) subcellular localization and/or nuclear translocation of Nrf2;

iii) expression levels and/or activity of one or more genes under control of Nrf2 (e.g., endogenous NQO1) or an Nrf2-regulated reporter gene in an artificial reporter construct;

iv) levels of Nrf2 binding to the Nrf2-binding DNA element ARE;

v) stability of Nrf2/Keap1 complexes; and

vi) modification (e.g., alkylation) levels of Keap1 and/or at least one other Nrf2/Keap1-associated proteins.

In some embodiments of methods 1-3, the compounds that are being screened, evaluated, or compared comprise at least one member of at least one of the following classes of compounds: mild alkylating agents, Michael addition acceptors, and compounds that are metabolized upon administration to Michael addition acceptors. In some embodiments, the Michael addition acceptor has the structure of Formula I, II, III, or IV set forth below.

In some embodiments method 1 comprises:

a) contacting a cell with a plurality of test compounds,

b) determining whether the Nrf2 pathway is upregulated in the cell, and

c) selecting from the plurality of compounds at least one compound that upregulates the Nrf2 pathway,

wherein an upregulation of the Nrf2 pathway by the selected at least one compound indicates that the selected at least one compound may be useful for treating a neurological disease.

The plurality of compounds may be represented, e.g., by a combinatorial chemical library, and the method may be performed, e.g., by high-throughput screening.

In some embodiments method 2 comprises:

a) contacting a cell with the at least one drug or drug candidate, and

b) determining whether the Nrf2 pathway is upregulated in the cell,

wherein an upregulation of the Nrf2 pathway by the at least one drug or drug candidate indicates that the at least one drug or drug candidate is useful for neuroprotection in treating a human having a neurological disease.

In some embodiments method 3 comprises:

a) contacting a cell with a first composition comprising at least one test compound, and

b) comparing the level of Nrf2 pathway upregulation in the cell by the at least one test compound to the corresponding level of the Nrf2 pathway upregulation in a control cell treated with a second composition comprising at least one of DMF and MMF.

In some embodiments of method 3, the test compound is fumaric acid, a salt thereof, or a fumaric acid derivative. In some embodiments, the first composition comprises DMF, MMF, or both. In some embodiments, the dose and/or the formulation of the first composition differs from the dose and/or the formulation of the second composition.

In some embodiments, method 3 further comprises:

c) comparing at least one pharmacokinetic parameter (e.g., serum-half-life) of the first and the second compositions.

In some embodiments method 4 comprises administering to the mammal a therapeutically effective amount of at least one neuroprotective compound having Formula I, II, III, or IV, e.g., a fumaric acid derivative (e.g., DMF or MMF).

In some embodiments method 4 provides a method of slowing or preventing neurodegeneration in a patient in need thereof, by administering the compound in an amount and for a period of time sufficient to slow or prevent demyelination, axonal loss, and/or neuronal death, e.g., by at least 30% relative to a control.

In some embodiments method 5 comprises:

a) administering to the mammal a therapeutically effective amount of at least one first compound that upregulates the Nrf2 pathway, and

b) administering a therapeutically effective amount of at least one second compound that does not upregulate the Nrf2 pathway.

In some embodiments of method 5, the at least one first compound, used in step (a), is a compound of Formula I, II, III, or IV, e.g., a fumaric acid derivative (e.g., DMF or MMF); and the at least one second compound, which is used in step (b), is an immunosuppressive or an immunomodulatory compound that does not upregulate the Nrf2 pathway (e.g., by more than 30% over a control).

In some embodiments method 5 comprises administering to the mammal a therapeutically effective amount of a compound of Formula I, II, III, or IV.

In some embodiments of methods 1-5, the at least onecompound being screened, identified, evaluated, or used for treating a neurological disorder is not fumaric acid or its salt, or a fumaric acid derivative (e.g., DMF or MMF).

Other features and embodiments of the invention will be apparent from the following description and the claims.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** demonstrates that DMF and MMF are activators of Nrf2 at concentrations within clinical exposure range (cells in culture).

FIG. **2** shows results of RNAi experiments.

FIG. **3** shows evidence of Nrf2 activation by DMF and MMF In vivo.

FIG. **4** shows evidence of Nrf2 activation by DMF and MMF In vivo.

Fumaric acid esters, such as DMF, have been proposed for treatment of MS (see, e.g., Schimrigk et al., Eur. J. Neurol., 2006, 13(6):604-10; Drugs R&D, 2005, 6(4):229-30).

Provided are, among other things, means for identifying compounds with a new therapeutic modality useful in at least one of multiple neurological indications and, optionally, complementary to other drugs for the treatment of a neurological disease, including a number of currently used immunomodulators.

DMF is a member of a large group of anti-oxidant molecules known for their cytoprotective and anti-inflammatory properties. These molecules also share the property of the Nrf2 pathway activation. Thus, the finding that DMF activates the Nrf2 pathway in conjunction with the neuroprotective effects of DMF further offers a rationale for identification of structurally and/or mechanistically related molecules that would be expected to be therapeutically effective for the treatment of neurological disorders, such as, e.g., MS.

Certain terms are defined in this section; additional definitions are provided throughout the description.

The terms "activation" and "upregulation," when used in reference to the Nrf2 pathway, are used interchangeably herein.

The terms "disease" and "disorder" are used interchangeably herein.

The term "a drug for treating a neurological disease" refers to a compound that has a therapeutic benefit in a specified neurological disease as shown in at least one animal model of a neurological disease or in human clinical trials for the treatment of a neurological disease.

The term "neuroprotection" and its cognates refer to prevention or a slowing in neuronal degeneration, including, for example, demyelination and/or axonal loss, and/or, neuronal and/or oligodendrocyte death. Neuroprotection may occur through several mechanisms, e.g., through reducing inflammation, providing neurotrophic factors, scavenging free radicals, etc. As used herein, a compound is considered neuroprotective if it (1) upregulates the Nrf2 pathway above a certain threshold and (2) provides neuroprotection, regardless of possible other mechanisms of action.

The terms "treatment," "therapeutic method," "therapeutic benefits," and the like refer to therapeutic as well as prophylactic/preventative measures. Thus, those in need of treatment may include individuals already having a specified disease and those who are at risk for acquiring that disease.

The terms "therapeutically effective dose" and "therapeutically effective amount" refer to that amount of a compound which results in at least one of prevention or delay of onset or amelioration of symptoms of a neurological disorder in a subject or an attainment of a desired biological outcome, such as reduced neurodegeneration (e.g., demyelination, axonal loss, and neuronal death) or reduced inflammation of the cells of the CNS.

In one aspect, provided are methods of evaluating neuroprotective properties of test compounds, including the following methods:

1) methods of screening for new candidate compounds that may be useful for treating a neurological disease;

2) methods of evaluating neuroprotective properties of drugs and candidates that are used or proposed for treating a neurological disease;

3) methods of comparing (e.g., for bioequivalence) two or more pharmaceutical compositions which contain fumaric acid derivatives;

In some embodiments, methods 1-3 may comprise:

a) contacting a cell with the test compound,

b) determining whether the Nrf2 pathway is upregulated in the cell, and, in some embodiments, additionally performing the following step(s):

c) determining whether the test compound slows or prevents demyelination, axonal loss, and/or neuronal death, and/or

d) selecting the test compound as a candidate for treating neurodegeneration in a neurological disease if 1) the Nrf2 pathway is upregulated and 2) demyelination, axonal loss, and/or neuronal death are/is prevented or slowed.

Method 1

In some embodiments the methods of screening for a candidate compound for treating a neurological disease comprise:

a) contacting a cell with a plurality of test compounds,

b) determining whether the Nrf2 pathway is upregulated in the cell, and

c) selecting from the plurality of compounds at least one compound that upregulates the Nrf2 pathway,

wherein an upregulation of the Nrf2 pathway by the selected at least one compound indicates that the selected at least one compound may be useful for treating a neurological disease. For example, the plurality of compounds may be represented by a combinatorial chemical library, and the screening method may be performed by a high-throughput screening as described in, e.g., High-Throughput Screening in Drug Discovery (Methods and Principles in Medicinal Chemistry), by Jörg Hüser (ed.), John Wiley & Sons (2006).

Combinatorial libraries of compounds are also described in, e.g., Solid-Supported Combinatorial and Parallel Synthesis of Small-Molecular-Weight Compound Libraries (Tetrahedron Organic Chemistry) Ian Salusbury (ed.), Elsevier (1998); Combinatorial Libraries: Synthesis, Screening and Application Potential (Library Binding), by Riccardo Cortese (ed.), Walter de Gruyter (1995). The libraries of compounds may be, for example, quinone libraries and other libraries as described in Mittoo, Comb. Chem. & High Throughput Screen, 2006, 9:421-423.

In some embodiments, the at least one compound or plurality of compounds being screened and/or selected comprises at least one compound selected from at least one of the following groups of compounds: mild alkylating agents, Michael addition acceptors or compounds that are metabolized to Michael addition acceptors, including compounds of Formulas I, II, III, or IV.

In some of the embodiments, the at least one compound is selected from fumaric acid, its salts, and fumaric acid derivatives.

Method 2

Also provided are methods of evaluating neuroprotective properties of at least one drug or drug candidate for treating at least one neurological disease. Such methods comprise:

a) contacting a cell with the at least one drug or drug candidate, and

b) determining whether the Nrf2 pathway is upregulated in the cell,

wherein the upregulation of the Nrf2 pathway by the at least one drug or drug candidate indicates that the at least one drug or drug candidate is neuroprotective in treating a human having a neurological disease.

In some embodiments, the upregulation of the Nrf2 pathway by the at least one drug or drug candidate indicates that the at least one drug or drug candidate has at least one activity selected from slowing demyelination, slowing the loss of axons, and slowing the rate of neuronal death.

In some embodiments, the method of evaluating at least one drug or drug candidate comprises an additional step:

c) evaluating demyelination, loss of axons, and/or neuronal death.

In some embodiments, steps a) and c) are performed in vivo in at least one model of a neurological disease, e.g., as described below.

In other embodiments, particularly those in which the neurological disease is multiple sclerosis or another demyelinating disease, the evaluated at least one drug or drug candidate for a neurological disease is chosen from the following: FTY720 (2-(4-octylphenethyl)-2-aminopropane-1,3-diol; Novartis); anti-IL12 antibody (e.g., ABT-874; Abbott Laboratories); GSK683699 (GSK/Tanabe); NeuroVax (Immune Response Corp.; Darlington, Curr. Opin. Mol. Ther., 2005, 7(6):598-603); anti-CCR2 antibody (e.g., MLN 1202; Millennium); interferon β-1a (e.g., Avonex®; Biogen Idec); anti-α4-integrin antibody (e.g., Tysabri®; Biogen Idec/Elan); anti-CD20 antibody (e.g., Rituxan® (Biogen Idec/Genentech); TV 5010 (Teva); NBI-788 (Neurocrine); MBP8298 (BioMS (see Warren et al., Eur. J. Neurol., 2006, 13(8):887-95); Mylinax (Oral Cladribine; 2-chlorodeoxyadenosine; Serono/IVAX); Teriflunomide ((Z)-2-cyano-N-(4-(trifluoromethyl)phenyl)-3-hydroxybut-2-enamide; Sanofi-Aventis); Temsirolimus (Wyeth); Laquinimod (5-chloro-N-ethyl-1,2-dihydro-4-hydroxy-1-methyl-2-oxo-N-phenylquinoline-3-carboxamide; Active Biotech/Teva); and interferon tau (Tauferon; Pepgen).

In some embodiments, the at least one drug or drug candidate being evaluated is at least one compound selected from at least one class selected from a mild alkylating agent, a Michael addition acceptor, and a compound that is metabolized to a Michael addition acceptor, including compounds of Formulas I, II, III, or IV.

In some of the embodiments, the compound is fumaric acid, its salt, or a fumaric acid derivative.

Method 3

Also provided are methods of comparing (e.g., for bioequivalence) at least two pharmaceutical compositions. Such methods comprise:

a) contacting a cell with at least one first composition comprising a test compound, and

b) comparing the level of the Nrf2 pathway upregulation in the cell by the test compound to the corresponding level of the Nrf2 pathway upregulation in a cell treated with at least one second composition ("comparator composition") comprising DMF, MMF, or both.

In some embodiments, substantially dissimilar levels of upregulation by the at least one first and at least one second compositions indicate that the compositions are not bioequivalent.

In some embodiments, the test compound is fumaric acid, its salt thereof, a fumaric acid derivative, or mixtures thereof. In some embodiments, the first composition comprises at least one of DMF, MMF, and both DMF and MMF. In some embodiments, the dose and/or the formulation of the at least one first composition differs from the dose and/or the formulation of the at least one second composition. The at least one first composition may be a controlled release composition such as, e.g., compositions described in WO 2006/037342.

In some embodiments, the method further comprises and additional step:

c) comparing at least one pharmacokinetic parameter of the at least one first and the at least one second compositions.

Pharmacokinetic parameters and methods for evaluating the same are well known and are described in, e.g., Pharmacokinetics, Second Edition (Drugs and the Pharmaceutical Sciences) by Milo Gibaldi et al. (eds.), Informa Healthcare (1982). Examples of such pharmacokinetic parameters that can be evaluated include serum half-life, clearance, and volume distribution.

In some embodiments, substantially dissimilar pharmacokinetic parameter(s) of the a least one first and at least one second compositions indicate that the compositions are not bioequivalent.

In some embodiments, the test compound being evaluated is a mild alkylating agent, and more specifically, a Michael addition acceptor, or a compound that is metabolized to a Michael addition acceptor.

In some of the embodiments, the test compound is fumaric acid or its salt, or a fumaric acid derivative.

Also provided are methods of treating a mammal who has or is at risk for developing a neurological disease, including the following methods:

4) methods of treating a neurological disease by administering to the subject in need thereof at least one compound that is partially structurally similar to DMF or MMF (including compounds selected using methods 1-3 described above); and

5) methods of treating a neurological disorder by a combination therapy that includes administration of a first compound that does not upregulate the Nrf2 pathway and a second compound that upregulates the Nrf2 pathway.

Method 4

Also provided are methods of treating a neurological disease by administering to the subject in need thereof at least one compound that is at least partially structurally similar to DMF and/or MMF.

In some embodiments of method 4, a method of treating a mammal who has or is at risk for a neurological disease is provided. The methods comprises administering to the mammal a therapeutically effective amount of at least one neuroprotective compound which has Formula I, II, III, or IV, e.g., a fumaric acid derivative (e.g., DMF or MMF).

In some embodiments of method 4, a method of slowing or preventing neurodegeneration (more specifically, e.g., demyelination, axonal loss, and/or neuronal death) in a subject in need thereof, by administering the at least one compound in an amount and for a period of time sufficient to do at least one of slow or prevent demyelination, slow or prevent axonal loss, and slow or prevent neuronal death, e.g., by at least 30%, 50%, 100% or higher over a control over a period of at least 5, 10, 12, 20, 40, 52, 100, or 200 weeks, or more.

Method 5

Also provided are methods of treating a mammal having a neurological disease by combination therapy. In some embodiments such methods comprise:

a) administering to the mammal a therapeutically effective amount of at least one first compound that upregulates the Nrf2 pathway, and

b) administering a therapeutically effective amount of at least one second compound that does not upregulate the Nrf2 pathway.

In some of embodiments of method 5, the at least one first compound, used in step (a), is a compound of Formula I, II, III, or IV, e.g., DMF or MMF; and the at least one second compound, which is used in step (b), is an immunosuppres-

sive or an immunomodulatory compound that does not upregulate the Nrf2 pathway (e.g., by more than 30%, 50%, 100% over a control).

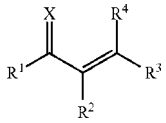
In some embodiments of method 5, the method comprises administering to the mammal a therapeutically effective amount of a compound of Formula I, II, III, or IV.

In method 5, the at least one first compound and the at least one second compound may be administered concurrently (as separate compositions or a mixed composition) or consecutively over overlapping or non-overlapping intervals. In the sequential administration, the at least one first compound and the at least one second compound can be administered in any order. In some embodiments, the length of an overlapping interval is more than 2, 4, 6, 12, 24, or 48 weeks, for example.

Michael addition acceptors generally include olefins or acetylenes conjugated to an electron withdrawing group, such as carbonyl containing groups, thiocarbonyl containing groups, cyano, sulfonyl, sulfonamido, amido, formyl, keto, and nitro. Exemplary carbonyl groups include carboxylic acid esters and carboxylic acid.

In some embodiments of methods 1-5, the at least one compound being screened, identified, evaluated, or used for treating a neurological disorder is selected from a mild alkylating agent, a Michael addition acceptor, and a compound that is metabolized to a Michael addition acceptor.

In some embodiments, the Michael addition acceptor has the structure of Formula I:



(I)

or a pharmaceutically acceptable salt thereof, wherein:

X is O; S; C(R)(C_{1-12})alkyl; or C(R)(C_{2-12})alkenyl, wherein R is H, (C_{1-12})alkyl or (C_{2-12})alkenyl;

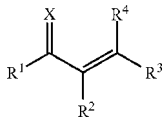
R^1, R^2, R^3 and R^4 are independently selected from: H; OH; O^-; CO_2H, CO_2^-; SH; S^-; SO_2H, SO_2^-; (C_{1-24})alkyl; (C_{1-24})alkenyl; ($C_{6-5\bullet}$)aryl, CO_2(C_{1-24})alkyl; SO_2(C_{1-24})alkyl; CO_2(C_{1-24})alkenyl; SO_2(C_{1-24})alkenyl; CO_2Y, wherein Y is psoralen-9-yl, retinyl, alpha-tocopherol, calciferyl, corticostreoid-21-yl or monosaccarid-ω-yl; (C_{1-24})alkoxy; (C_{1-24})alkenyloxy; ($C_{6-5\bullet}$)aryloxy; (C_{1-24})alkylthio; (C_{1-24})alkenylthio; ($C_{6-5\bullet}$)arylthio, amino; amido; arylalkyl; cyano; nitro; sulfonyl; sulfoxido; sulfonamido; formyl; keto; and D and L natural or unnatural amino acids; or any two of X, R^1, R^2 and R^3, and R^4 may be joined together to form a cyclic moiety; and wherein the alkyl, alkoxy, alkenyl, alkenyloxy, aryl and aryloxy groups may be optionally substituted with at least one group chosen from halogen (F, Cl, Br, or I), OH, (C_{1-4})alkoxy, nitro and cyano.

In some embodiments, the at least one Michael addition acceptor has the structure of Formula I, with the following provisos:

R^1 is selected from: H; OH; O^-; CO_2H, CO_2; SH; S^-; SO_2H, SO_2^-; (C_{1-24})alkyl; (C_{1-24})alkenyl; ($C_{6-5\bullet}$)aryl; CO_2(C_{1-24})alkyl; SO_2(C_{1-24})alkyl; CO_2(C_{1-24})alkenyl; SO_2(C_{1-24})alkenyl; CO_2Y, wherein Y is psoralen-9-yl, retinyl, alpha-tocopherol, calciferyl, corticostreoid-21-yl or monosaccarid-ω-yl; (C_{1-24})alkoxy; (C_{1-24})alkenyloxy; ($C_{6-5\bullet}$)aryloxy; (C_{1-24})alkylthio; (C_{1-24})alkenylthio; ($C_{6-5\bullet}$)arylthio; arylalkyl; amino; amido; cyano; nitro; sulfonyl; sulfoxido; sulfonamido; formyl, keto; and D or L natural or unnatural amino acids; and wherein the alkyl, alkoxy, alkenyl, alkyenyloxy, aryl and aryloxy groups may be optionally substituted with at least one group chosen from halogen (F, Cl, Br, or I), OH, (C_{1-4})alkoxy, nitro and cyano;

R^2 is selected from: H; CO_2H; CO_2^-; SO_2H; SO_2^-; (C_{1-24})alkyl; (C_{1-24})alkenyl; ($C_{6-5\bullet}$)aryl; CO_2(C_{1-24})alkyl; SO_2(C_{1-24})alkyl; CO_2(C_{1-24})alkenyl; SO_2(C_{1-24})alkenyl; CO_2Y, wherein Y is psoralen-9-yl, retinyl, alpha-tocopherol, calciferyl, corticostreoid-21-yl or monosaccarid-ω-yl; (C_{1-24})alkoxy; (C_{1-24})alkenyloxy; ($C_{6-5\bullet}$)aryloxy; (C_{1-24})alkylthio; (C_{1-24})alkenylthio; ($C_{6-5\bullet}$)arylthio; amido; arylalkyl; cyano; nitro; cyano; nitro; sulfonyl; sulfoxido; sulfonamido; formyl; and keto; wherein the alkyl, alkoxy, alkenyl, alkyenyloxy, aryl and aryloxy groups may be optionally substituted with at least one group chosen from halogen (F, Cl, Br, or I), OH, (C_{1-4})alkoxy, nitro and cyano.

In some embodiments, the at least one Michael addition acceptor has the structure of Formula II:



(II)

or a pharmaceutically acceptable salt thereof, wherein:

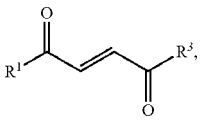
X is selected from O; S; C(R)(C_{1-12})alkyl; and C(R)(C_{2-12})alkenyl, wherein R is selected from H; (C_{1-12})alkyl; and (C_{2-12})alkenyl; and R^1, R^2, R^3, and R^4 are independently selected from: H; OH; O^-; CO_2H; CO_2^-; (C_{1-12})alkyl; (C_{1-12})alkenyl; and CO_2(C_{1-12})alkyl;

or any two of X, R^1, R^2 and R^3 may be joined together to form a cyclic moiety.

In some embodiments of the compounds of Formulae I-IV, the pharmaceutically acceptable salt is a salt of a metal (M) cation, wherein M can be an alkali, alkaline earth, or transition metal such as Li, Na, K, Ca, Zn, Sr, Mg, Fe, or Mn.

In some embodiments of methods 1-5, the compounds of Formula I include fumaric acid, its salts, and fumaric acid derivatives.

In some embodiments, the at least one compound of Formula I has the structure of Formula III:



(III)

or a pharmaceutically acceptable salt thereof, wherein:

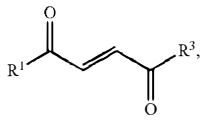
R^1 and R^3 are independently selected from OH; O^-; (C_{1-24})alkoxy; (C_{1-24})alkenyloxy; ($C_{6-5\bullet}$)aryloxy; psoralen-9-yloxy; retinyloxy; alpha-tocopheroloxy; calciferyloxy; corticostreoid-21-yloxy; monosaccarid-ω-yloxy; amino; and

a D or L natural or unnatural amino acid; and wherein at least one of the (C_{1-24})alkoxy; (C_{1-24})alkenyloxy; and (C_{6-50})aryloxy groups may be optionally substituted with at least one group chosen from halogen (F, Cl, Br, or I), OH, (C_{1-4})alkoxy, nitro and cyano.

Compounds wherein at least one of R^1 and R^3 is derived from a natural or unnatural D or L amino acid are described in U.S. application Ser. Nos. 10/433,295, paragraphs 10 to 11 and 18-28, and 11/421,083, which are incorporated herein by reference.

In some embodiments, the compound of formula (I) has the structure of Formula IV:

(IV)



or a pharmaceutically acceptable salt thereof., wherein:

R^1 and R^3 are independently selected from OH; O^-; (C_{1-24})alkoxy; allyloxy; vinyloxy; (C_{6-50})aryloxy; psoralen-9-yloxy; retinyloxy; alpha-tocopheroloxy; calciferyloxy; corticostreoid-21-yloxy; monosaccarid-ω-yloxy; amino; and a D or L natural or unnatural amino acid; and wherein at least one of the (C_{1-24})alkoxy, allyloxy, vinyloxy, and (C_{6-50})aryloxy may be optionally substituted with at least one group chosen from Cl, F, I, Br, OH, (C_{1-4})alkoxy, nitro, and cyano.

In some embodiments, the "fumaric acid derivative" is chosen from the compounds of Formula III, compounds of Formula IV and the following:

1) fumaric acid amides derived from natural and unnatural amino D or L acids, as described in U.S. patent application Ser. Nos. 10/433,295, paragraphs 10 to 11 and 18-28, and 11/421,083.

2) a carbocyclic or oxacyclic fumaric acid oligomer as described in U.S. patent application Ser. No. 10/511,564, paragraphs 15-44; and

3) a glycerol or alkane diol or polyol derivative of fumaric acid as described in U.S. Pat. Nos. 4,851,439, 5,149,695, 5,451,667, at cols. 2-4.

In some embodiments, "fumaric acid derivative" is one or more dialkyl fumarates (e.g., DMF), mono alkyl fumarates (MMF) or salts thereof.

In some of the embodiments of methods 1-5, the at least one compound being screened, evaluated, compared or used for treating a neurological disorder is not fumaric acid or its salt, or a fumaric acid derivative (e.g., DMF or MMF).

Nrf2 (Nuclear Factor-E2-related factor 2; for sequence of the Nrf2, see Accession No. AAB32188) is a transcription factor that, upon activation by oxidative stress, binds to the antioxidant response element (ARE), and activates transcription of Nrf2-regulated genes. This pathway has been well characterized for its role in hepatic detoxification and chemoprevention through the activation of phase II gene expression. ARE-regulated genes may also contribute to the maintenance of redox homeostasis by serving as endogenous anti-oxidant systems. At present, the list of Nfr2-regulated genes contains over 200 genes encoding proteins and enzymes involved in detoxification and antioxidant response (Kwak et al., J. Biol. Chem., 2003, 278:8135) such as, e.g., HO-1, ferritin, glutathione peroxidase, glutathione-S-transferases (GSTs), NAD(P)H:quinone oxidoreductases, now commonly known as nicotinamide quinone oxidoreductase 1 (NQO1; EC 1.6.99.2; also known as DT diaphorase and menadione reductase), NQO2, g-glutamylcysteine synthase (g-GCS), glucuronosyltransferase, ferritin, and heme oxygenase-1 (HO-1), as well as any one of the enzymes proteins listed in Table 1 in Chen & Kunsch, Curr. Pharm. Designs, 2004, 10:879-891; Lee et al., J. Biol. Chem., 2003, 278(14):12029-38, and Kwak, supra.

Accordingly, in some embodiments, the at least one Nrf2-regulated gene which is used to assess the activation of the Nrf2 pathway is selected from a phase II detoxification enzyme, an anti-oxidant enzyme, an enzyme of the NADPH generating system, and Nrf2 itself. Examples of the phase II detoxification enzymes include NQO1, NQO2, GST-Ya, GST-pi, GST-theta 2, GST-mu (1,2,3), microsomal GST 3, catalytic γ-GCS, regulatory-GCS, microsomal epoxide hydrolase, UDP-glucuronosyltransferase, transaldolase, transketolase, and drug-metabolizing enzyme. Examples of the anti-oxidant enzymes include HO-1, ferritin (L), glutathione reductase, glutathione peroxidase, metallothionein I, thioredoxin, thioredoxin reductase, peroxiredoxin MSP23, Cu/Zn superoxide dismutase, and catalase. Examples of the enzymes of the NADPH generating system include malic enzyme, UDP-glucose dehydrogenase, malate oxidoreductase, and glucose-6-phosphate dehydrogenase.

The antioxidant response element (ARE, also referred to as the electrophile response element (EpRE), GRE1, ARE4, and StREb) is a cis-acting DNA regulatory element with a core nucleotide sequence of 5'-TGA(C/T/G)NNNGC-3' (SEQ ID NO:1) (Rushmore et al., J. Biol. Chem., 1991, 266(18):11632-9; see also Nioi et al., Mutation Res., 2004, 555:14-171).

Accordingly, in some embodiments, the DNA sequence of the ARE element, to which Nrf2 binds (whether the former is a part of an endogenous gene or an artificial construct), comprises the core ARE sequence TGA(C/T/G)NNNGC (SEQ ID NO:2) or the ARE consensus sequence (G/A)TGA(C/T/G)NNNGC(A/G) (SEQ ID NO:3). In further specific embodiments, the ARE sequence comprises any one of the "minimal enhancer" sequences shown in Table 1.

In some embodiments, the ARE sequence further comprises at least one of corresponding 5'- and 3'-USR sequences as shown in Table 1. In some embodiments, the ARE sequence comprises the sequence GTGANNNNGCA (SEQ ID NO:4), or more particularly, the mouse (NNNN=gtcg) or human (NNNN=ctca) versions thereof.

TABLE 1

Species	Gene	Element	5'-USR	Minimal enhancer	3'-USR	SEQ ID NO
mouse	nqo1	ARE	agTCAca	GTGAgtcgGCA	aaattt	SEQ ID NO: 5
rat	NQO1	ARE	agTCAca	GTGACttgGCA	aaatct	SEQ ID NO: 6
human	NQO1	ARE	agTCAca	GTGACtcaGCA	gaatct	SEQ ID NO: 7
mouse	gsta1	EpRE	gcTAAtg	GTGACaaaGCA	actttc	SEQ ID NO: 8

TABLE 1-continued

Species	Gene	Element	5'-USR	Minimal enhancer	3'-USR	SEQ ID NO
rat	GSTA2	ARE	gcTAAtg	GTGACaaaGCA	actttc	SEQ ID NO: 9
mouse	gsta3	ARE	ctcAggc	ATGACattGCA	tttttc	SEQ ID NO: 10
rat	GSTP1	GPE1	agTCAct	ATGATtcaGCA	acaaaa	SEQ ID NO: 11
human	GCLC	ARE4	ccTCccc	GTGACtcaGCG	ctttgt	SEQ ID NO: 12
human	GCLM	EpRE	gaagAca	ATGACtaaGCA	gaaatc	SEQ ID NO: 13
mouse	ho1	StREb	cccAAcc	ATGACacaGCA	taaaag	SEQ ID NO: 14
ARE 'core' . . .				TGACnnnGC		SEQ ID NO: 15
ARE consensus . . .			T<u>A</u>Ann C	<u>A</u>TGA<u>C</u>nnnGC<u>A</u> G T G	<u>aaaa</u> tttt	SEQ ID NO: 16

A current model of Nrf2 function is as follows. Under basal conditions, Nrf2 is sequestered in the cytoplasm to the actin-bound Kelch-like ECH-associated protein 1 (Keap1; Accession No. NP_987096 for human Keap1), a Cullin3 ubiquitin ligase adaptor protein. More specifically, the N-terminal domain of Nrf2, known as Neh2 domain, is thought to interact with the C-terminal Kelch-like domain of Keap1. In response to xenobiotics or oxidative stress, Nrf2 is released from the Keap1/Nrf2 complex, thereby promoting nuclear translocation of Nrf2 and concomitant activation of ARE-mediated gene transcription. Keap1 function, in turn, requires association with Cullin3, a scaffold protein that positions Keap1 and its substrate in proximity to the E3 ligase Rbx1, allowing the substrate (Nrf2) to be polyubiquitinated and thus targeted for degradation. The exact mechanism of how the Keap1/Nrf2 complex senses oxidative stress is not fully understood. Human Keap1 contains 25 cysteine residues that were hypothesized to function as sensors of oxidative stress; 9 of the cysteines are thought to be highly reactive (Dinkova-Kostova et al., PNAS, 2005, 102(12):4584-9). It was theorized but is not relied on for the purposes of this invention that alkylation of cysteins leads to a conformational change, resulting in the liberation of Nrf2 from Nrf2/Keap1/Cullin3 complexes, followed by nuclear translocation of the liberated Nrf2.

In some embodiments, methods 1-3 described herein comprise contacting a cell with at least one test compound and determining whether the Nrf2 pathway is upregulated in the cell. In such methods, an upregulation of the Nrf2 pathway above a threshold (e.g., by at least 30%, 50%, 100%, 200%, 500% over a control) indicates that the at least one compound has certain biological properties beneficial in treating a neurological disease (e.g., neuroprotective properties).

The ability of a compound to activate the Nrf2 pathway can be determined by one or more in vitro and in vivo assays, including, e.g., the following assays described below.

i) Expression levels of Nrf2—The sequence of the promoter region of the nrf2 gene (−1065 to −35) has been published, for example, in Chan et al., PNAS, 1996, 93:13943-13948. One may use an artificially constructed expression construct containing the Nrf2 promoter element and an artificial reporter gene. Alternatively, one may use PCR or Northern blotting to determine expression levels of Nrf2 mRNA, or Western blotting to determine Nrf2 protein levels. Exemplary procedures for determining expression levels of Nrf2 are described in Kwak et al., Mol. Cell. Biol. 2002, 22(9):2883-2892 and Kwak et al., Mol. Med., 2001, 7:135-145. Antibodies against Nrf2 are can be produced by methods known in the art and are commercially available from, for example, Stress-Gen. Accordingly, in some embodiments, the Nrf2 pathway is activated so that the expression levels of Nrf2 are increased by, for example, at least 30%, 50%, 100%, 200%, 500% or more as compared to the non-activated state.

ii) Subcellular localization and/or nuclear translocation of Nrf2—Such assays include cell staining, or analysis of cytoplasmic versus nuclear cell extracts. For example, a Nrf2-green fluorescence protein (GFP) fusion protein construct can be made and introduced into cells and visualized as described in, e.g., Kraft et al., J. Neurosci., 2004, 24, 1101-1112; and Satoh et al., PNAS, 2006, 103(3):768-773. Accordingly, in some embodiments, the Nrf2 pathway is activated so that the ratio between cytomplasmic and nuclear Nrf2 is elevated by, for example, at least 30%, 50%, 100%, 200%, 500% or more as compared to the non-activated state.

iii) Expression levels and/or activity of one or more genes under the control of Nrf2—Such genes under the control of Nrf2 include endogenous or artificially introduced reporter genes in reporter constructs introduced into cells. For example, expression levels of endogenous or exogenously introduced NQO1 may be determined as described in the Examples. Alternatively, a reporter gene construct with one or more ARE sites operably linked to a reporter gene (e.g., luceferase or GFP) can be made, as described in, e.g., Satoh et al., PNAS, 2006, 103(3):768-773. Expression levels of an Nrf-2 induced gene product can be measured at the protein (e.g., by Western blotting or enzymatic activity assays) or at the mRNA levels (e.g., by PCR). Methods for performing RT-PCT are described in, e.g., Calabrese et al., J. Neurosci. Res., 2005, 79:509-521 for HO-1, in Wierinckx et al., J. Neuroimmunology, 2005, 166:132-143 for NQO1. Methods for measuring enzymatic activity of NQO1, using for example, menadione as a substrate, are described in Dinkova-Kostova et al., PNAS, 2001, 98:3404-09 or by Prochaska et al., Anal. Biochem., 1988, 169:328-336. Methods for measuring GST activity, using for example, 1-chloro-2,4-dinitrobenzene as a substrate, are described in Ramos-Gomez et al., J. Neurosci., 2004, 24(5):1101-1112 and Habig et al., 1974, J. Biol. Chem., 219, 7130-7139. Methods for measuring HO-1 activity are described in, e.g., in Calabrese et al., 2005, J. Neurosci. Res., 79:509-521. Accordingly, in some embodiments, the Nrf2 pathway is activated so that the expression levels and/or activity of the gene produced are increased by, for example, at least 30%, 50%, 100%, 200%, 500% or more as compared to the non-activated state.

iv) Levels of Nrf2 binding to ARE—For example, such assays may utilize electromobility shift assays (EMSA) and Chromatin Immununoprecipitation (ChIP) assay, as described in, e.g., Satoh et al., PNAS, 2006, 103(3):768-773 and Kwak et al., Mol. Cell. Biol., 2002, 22(9):2883-2892. Accordingly, in some embodiments, the Nrf2 pathway is activated so that the level of Nrf2 binding to ARE is increased by, for example, at least 30%, 50%, 100%, 200%, 500% or more as compared to the non-activated state.

v) The stability of Nrf2/Keap1 complexes—Such assay may include analysis of immunoprecipitated complexes with Nrf2 and/or Keap1 or other Nrf2/Keap1-associated proteins as described in, e.g., Satoh et al., PNAS, 2006, 103(3):768-773. Anti-Keap1 antibodies can be produced using methods known in the art and are available commercially from, for example, Santa Cruz Biotechnology. Accordingly, in some embodiments, the Nrf-2 pathway is activated so that the stability of Nrf2/Keap1 complexes is increased by, for example, at least 30%, 50%, 100%, 200%, 500% or more as compared to the non-activated state.

vi) Modification (e.g., alkylation levels) of Keap1 and other Nrf2/Keap1-associated proteins—Such assays may include mass spectrometric analysis of immunoprecipitated Keap1, using techniques as described in, e.g., Dinkova-Kostova et al., PNAS, 2005, 102(12):4584-9 and Gao et al., J. Biol. Chem., on-line pub. Manuscript M607622200. In some embodiments, the Nrf-2 pathway is activated so that the level of Keap1 and other Nrf2/Keap1-associated proteins is increased by, for example, at least 30%, 50%, 100%, 200%, 500% or more as compared to the non-activated state.

Alkylating capacity of a compound can be assessed using recombinant Keap1, by a competition reaction with 5,5'-dithiobis(2-nitrobezoic acid) (DTNB) as described in, e.g., Gao et al., J. Biol. Chem., on-line pub. Manuscript M607622200.

In some embodiments, the cell being contacted with at least one test compound is a neuron or a neuronal cell line. In some embodiments, the cell being contacted with the at least one test compound is selected from a colon carcinoma cell line (e.g., DLD1), a neuroblastoma cell line (e.g., SkNSH or IMR32), and a primary monocyte. The cell may be a cell in culture (in vitro) or be inside of an animal (in vivo).

Cell viability, and in particular, neuronal viability can be assessed in vivo or in vitro using any suitable method, including methods as described in the Examples. For example, neuronal viability can be assessed using an MTT assay after exposure of neuronal cell cultures to cytotoxic levels of glutamate as described in, e.g., Shih et al., J. Neurosci., 2005, 25(44):10321-35. Additionally, cell viability may also be assessed in assays in which cell death is induced by oxidative damage, for example, by the addition of glucose oxidase to astrocyte cell cultures, as described in, e.g., Calabrese et al., J. Neurosci. Res., 2005, 79:509-521. In vivo assays may be performed as described in, e.g., Misgeld, Histochem. Cell Biol., 2005, 124:189-196.

The amount of the reporter gene expressed can be determined by any suitable method. Expression levels, at the RNA or the protein level, can be determined using routine methods. Expression levels are usually scaled and/or normalized per total amount of RNA or protein in the sample and/or a control, which is typically a housekeeping gene such as actin or GAPDH. RNA levels are determined by quantitative PCR (e.g., RT-PCR), Northern blotting, or any other method for determining RNA levels, e.g., as described in Cloning: A Laboratory Manual, by Sambrook et al. (eds.), 2nd ed., Cold Spring Harbor Laboratory Press, 1989; Lodie et al., Tissue Eng., 2002, 8(5):739-751); or as described in the Examples. Protein levels are determined using, Western blotting, ELISA, enzymatic activity assays, or any other method for determining protein levels as described in, e.g., Current Protocols in Molecular Biology, by Ausubel et al. (eds.), John Wiley and Sons, 1998.

Expression levels may also be determined using reporter gene assays in cell/tissue extracts or by tissue or whole-animal imaging. In addition to MRI, tissue imaging on living animals can be performed by fluorescence detection (Hoffman Lancet Oncol., 2002 3:546-556; Tung et al., Cancer Res., 2000, 60:4953-4958), bioluminescence detection (Shi et al., PNAS, 2001, 98:12754-12759; Luke et al., J. Virol., 2002, 76:12149-12161; and U.S. Pat. Nos. 5,650,135 and 6,217,847), positron emission tomography (Liang et al., Mol. Ther., 2002, 6:73-82, near-infrared fluorescence (Tung et al., Cancer Res., 2000, 60:4953-4958), or X-ray imaging (Hemminki et al., J. Nat. Cancer Inst., 2002, 94:741-749).

A neurological disease in methods 1-5 above can be a neurodegenerative disease such as, for example, ALS, Parkinson's disease, Alzheimer's disease, and Huntington's disease. The neurological disease can also be multiple sclerosis (MS), or other demyelinating diseases of the central or peripheral nervous system. In some embodiments the form of MS in methods 1-5 is selected from: relapsing remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), and malignant MS (Marburg Variant).

The subject being treated or administered the compound as per methods described herein, is a mammal in need thereof, such as a subject in need of neuroprotection, including a subject who has or is at risk for developing a demyelinating and another specified neurodegenerative disease. The subject is mammalian, and can be a rodent or another laboratory animal, e.g., a non-human primate. In some embodiments, the subject is human.

Neurodegenerative diseases are described in, for example, Neurodegenerative Diseases: Neurobiology, Pathogenesis and Therapeutics, M. Flint Beal, Anthony E. Lang, Albert C. Ludolph, Cambridge University Press (Jul. 11, 2005). Examples of neurological diseases suitable for the methods described herein include neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, and Huntington's disease. Other examples include demyelinating neurological disease including, in addition to MS, the following diseases: acute haemorrhagic leucoencephalomyelitis, Hurst's disease, acute disseminated encephalomyelitis, optic neuritis, Devic's disease, spinal cord lesions, acute necrotizing myelitis, transverse myelitis, chronic progressive myelopathy, progressive multifocal leukoencephalopathy (PML), radiation myelopathy, HTLV-1 associated myelopathy, monophasic isolated demyelination, central pontine myelinolysis, and leucodystrophy (e.g., adrenoleucodystrophy, metachromatic leucodystrophy, Krabbe's disease, Canavan's disease, Alexander's disease, Pelizaeus-Merbacher disease, vanishing white matter disease, oculodentodigital syndrome, Zellweger's syndrome), chronic inflammatory demyelinating polyneuropathy (CIDP), acute inflammatory demyelinating polyneuropathy (AIDP), Leber's optic atrophy, and Charcot-Marie-Tooth disease.

Additional examples of diseases suitable for the methods described herein include polyneuritis and mitochondrial disorders with demyelination. These disorders may be co-presented with, and possibly aggravated by diabetes, e.g., insulin-dependent diabetes mellitus (IDDM; type I diabetes), or other diseases.

A test compound may be further assayed in an animal model of MS, known as Experimental Autoimmune Encepha-

lomyelitis (EAE) (Tuohy et al., J. Immunol., 1988, 141:1126-1130, Sobel et al. J. Immunol., 1984, 132:2393-2401, and Traugott, Cell Immunol., 1989 119:114-129). Chronic relapsing EAE provides a well established experimental model for testing agents that would be useful for the treatment of MS. The mouse EAE is an induced autoimmune demyelinating disease with many similarities to human MS in its clinical manifestations. In both EAE and MS, clinical disease is associated with blood-brain barrier (BBB) dysfunction, infiltration of central nervous system by mononuclear cells (mainly macrophages and T lymphocytes, and serum products), and demyelination (Baker et al. J. Neuroimmunol., 1990, 28:261; Butter et al., J. Neurol. Sci., 1991, 104:9; Harris et al., Ann. Neurol., 1991, 29:548; Kermonde et al., Brain, 1990, 113:1477).

Clinical signs of MS and demyelinating pathology in EAE result from immunization with CNS myelin proteins or peptides (e.g., MBP, PLP, and MOG) under Th1 conditions (direct immunization model), or by adoptive transfer of CNS antigen-specific Th1 cells (adoptive transfer model) (Ben-Nun et al., Eur. J. Immunol., 1981, 11:195-199; Ando et al., Cell Immunol., 1989, 124:132-143; Zamvil et al., Nature, 1985, 317:355-358; Zamvil et al., Ann. Rev. Immunol., 1990, 8:579-621). For example, in the SJL mouse model of EAE, immunization with the CNS peptide PLP 139-151 or adoptive transfer of PLP-specific Th1 cells results in a disease course consisting of an acute phase with loss of tail tone on day 10 to day 12, followed by hind limb paralysis and CNS mononuclear cell infiltration (Tuohy et al., J. Immunol., 1988, 141:1126-1130, Sobel et al., J. Immunol., 1984, 132:2393-2401, and Traugott, Cell Immunol., 1989, 119:114-129). Resolution of clinical signs and recovery occurs on day 20 to day 25 and the animals may undergo several more relapses less severe than the initial phase. EAE has been used to evaluate new therapeutic approaches to T-cell-mediated autoimmune disease because of the clinical and histopathological similarities to the human demyelinating MS.

The ability of a compound to slow or prevent neurodegeneration (including demyelination and neuronal death) can be assessed in the EAE model or another animal model, including for example, Thieler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease, murine hepatitis virus (MHV), Semliki Forest Virus, or Sindbis virus as described in, e.g., Ercoli et al., J. immunol., 2006, 175:3293-3298.

A compound may be optionally tested in at least one additional animal model (see, generally, Immunologic Defects in Laboratory Animals, eds. Gershwin et al., Plenum Press, 1981), for example, such as the following: the SWR×NZB (SNF1) mouse model (Uner et al., J. Autoimmune Disease, 1998, 11(3):233-240), the KRN transgenic mouse (K/B×N) model (Ji et al., Immunol. Rev., 1999, 69:139); NZB×NZW (B/W) mice, a model for SLE (Riemekasten et al., Arthritis Rheum., 2001, 44(10):2435-2445); the NOD mouse model of diabetes (Baxter et al., Autoimmunity, 1991, 9(1):61-67), etc.); or mouse models of multiple sclerosis (see, e.g., Linker et al., Eur. J. Immunol., 2002, 8(6):620-624, and Eugster et al., Nat. Med., 1999, 29:626-632; and Gold et al., Brain, 2006, 129:1953-1971).

Preliminary doses, for example, as determined in animal tests, and the scaling of dosages for human administration is performed according to art-accepted practices. Toxicity and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. In some embodiments compositions that exhibit large therapeutic indices are used.

The therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the therapeutic compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture assays or animal models. Levels in plasma may be measured, for example, by ELISA or HPLC. The effects of any particular dosage can be monitored by a suitable bioassay. Examples of dosages are: about $0.1\times IC_{50}$, about $0.5\times IC_{50}$, about $1\times IC_{50}$, about $5\times IC_{50}$, $10\times IC_{50}$, about $50\times IC_{50}$, and about $100\times IC_{50}$.

The data obtained from the in vitro assays or animal studies can be used in formulating a range of dosages for use in humans. Therapeutically effective dosages achieved in one animal model can be converted for use in another animal, including humans, using conversion factors known in the art (see, e.g., Freireich et al., Cancer Chemother. Reports, 1966, 50(4):219-244 and Table 2 for Equivalent Surface Area Dosage Factors).

TABLE 2

	To:				
From:	Mouse (20 g)	Rat (150 g)	Monkey (3.5 kg)	Dog (8 kg)	Human (60 kg)
Mouse	1	1/2	1/4	1/6	1/12
Rat	2	1	1/2	1/4	1/7
Monkey	4	2	1	3/5	1/3
Dog	6	4	3/5	1	1/2
Human	12	7	3	2	1

In some embodiments the dosage of such compounds lies within a range of circulating concentrations that include the ED_{50} with little or no toxicity. In some embodiments the dosage varies within this range depending upon the dosage form employed and the route of administration utilized. Generally, a therapeutically effective amount may vary with the subject's age, condition, and sex, as well as the severity of the medical condition in the subject. Examples of pharmaceutically acceptable dosages for compounds described herein are from 1 μg/kg to 25 mg/kg, depending on the compounds, severity of the symptoms and the progression of the disease. The appropriate therapeutically effective doses can be selected by a treating clinician and in some embodiments range approximately from 1 μg/kg to 20 mg/kg, from 1 μg/kg to 10 mg/kg, from 1 μg/kg to 1 mg/kg, from 10 μg/kg to 1 mg/kg, from 10 μg/kg to 100 μg/kg, from 100 μg to 1 mg/kg. Additionally, certain specific dosages are indicated in the Examples.

For DMF or MMF, an effective amount can range from 1 mg/kg to 50 mg/kg (e.g., from 2.5 mg/kg to 20 mg/kg or from 2.5 mg/kg to 15 mg/kg). Effective doses will also vary, as recognized by those skilled in the art, dependent on route of administration, excipient usage, and the possibility of co-usage with other therapeutic treatments including use of other therapeutic agents. For example, an effective dose of DMF or MMR to be administered to a subject orally can be from about 0.1 g to 1 g per pay, 200 mg to about 800 mg per day (e.g., from about 240 mg to about 720 mg per day; or from about 480 mg to about 720 mg per day; or about 720 mg per day). For example, the 720 mg per day may be administered in separate administrations of 2, 3, 4, or 6 equal doses.

The dosage may be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment. The compositions may be given as a bolus dose, to

maximize the circulating levels for the greatest length of time after the dose. Continuous infusion may also be used after the bolus dose.

In some embodiments, compositions used in the methods described herein further comprise a pharmaceutically acceptable excipient. As used herein, the phrase "pharmaceutically acceptable excipient" refers to any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. The compositions may also contain other active compounds providing supplemental, additional, or enhanced therapeutic functions. The pharmaceutical compositions may also be included in a container, pack, or dispenser together with instructions for administration.

A pharmaceutical composition is formulated to be compatible with its intended route of administration. Methods to accomplish the administration are known in the art. "Administration" is not limited to any particular delivery system and may include, without limitation, parenteral (including subcutaneous, intravenous, intramedullary, intraarticular, intramuscular, or intraperitoneal injection), rectal, topical, transdermal, or oral (for example, in capsules (e.g., as, poweder, granules, microtablet, micropellets, etc.), suspensions, or tablets). Examples of some of formulations containing DMF and/or MMF are given in, e.g., U.S. Pat. Nos. 6,509,376, and 6,436,992.

Administration to an individual may occur in a single dose or in repeat administrations, and in any of a variety of physiologically acceptable salt forms, and/or with an acceptable pharmaceutical carrier and/or additive as part of a pharmaceutical composition. Physiologically acceptable salt forms and standard pharmaceutical formulation techniques and excipients are well known to persons skilled in the art.

The following Examples are intended for illustrative purposes and do not limit the inventions claimed.

EXAMPLES

Example 1

Human colon carcinoma DLD1 cells were treated with DMF or MMF at indicated concentrations (5, 15, or 50 μM) for 16 hours, rinsed with PBS, and harvested into reducing SDS sample buffer. The lysates were subjected to SDS PAGE and the separated proteins were electrophoretically transferred onto nitrocellulose membranes for Western blot analysis. To detect Nrf2 and NQO1, the membranes were incubated with the respective primary antibodies overnight at 4° C., washed, and incubated with peroxidase-conjugated secondary antibodies followed by the chemiluminescent peroxidase substrate. Detection of the target protein band luminescence and image acquisition were done using CCD-equipped imaging station Kodak2000R. The results shown in FIG. 1, demonstrate that DMF and MMF are potent activators of Nrf2 at concentrations within clinical exposure range.

Example 2

DLD1 cells were grown in MEM supplemented with 10% fetal bovine serum. The cells were transfected with the indicated siRNA's using the Lipofectamine reagent (Invitrogen) according to the manufacturer's instructions and 30 hrs later stimulated with 30 μM DMF for 40 hours. The cells were harvested and processed for Western blotting analysis of Nrf2 and NQO1 levels as described in Example 1. Sources and the identity of reagents used in Examples 1 and 2 are specified Table 3 below:

	Target	Reagent	Source/Sequence	Vendor
Primary Antibody	Nrf2	Nrf2 (T-19)	goat polyclonal antibody	Santa Cruz Biotechnology
	Keap1	Keap1 (E-20)	goat polyclonal antibody	Santa Cruz Biotechnology
	NQO1	NQO1 (A180)	mouse monoclonal antibody	Santa Cruz Biotechnology
	GAPDH	Anti-GAPDH	mouse monoclonal antibody	Ambion
Secondary antibody	anti-mouse	HRP-Mouse IgG	sheep	Amersham Biosciences
	anti-rabbit	HRP-Rabbit IgG	donkey	Amersham Biosciences
	anti-goat	HRP-Goat IgG	Bovine	Santa Cruz Biotechnology
siRNA	Nrf2	Nrf2-2	UCAUUGAACUGCUCUUUGGUU (antisense) (SEQ ID NO: 17)	Dharmacon
	Keap1	Keap1-1	GAAUUAAGGCGGUUUGUCCUU (antisense) (SEQ ID NO: 18)	Dharmacon

The results are shown in FIG. 2 (for ease of representation, the image of the Western blot is turned upside down). The results demonstrate that DMF-induced upregulation of NQO1 requires Nrf2 and can be mimicked by activation of Nrf2 through repression of Keap1. Therefore, DMF acts as an Nrf2 agonist causing cellular accumulation of Nrf2 and Nrf2 target gene expression.

Example 3

For induction of EAE, mice received s.c. injections in the flanks and tail base of 50 μg MOG 35-55 peptide in PBS emulsified in an equal volume of complete Freund's adjuvant (CFA) containing *Mycobacterium tuberculosis* H37RA (Difco, Detroit Mich., USA) at a final concentration of 0.5

mg/ml. Two injections of pertussis toxin (List Biological Laboratories Inc., California, USA; 200 μg per mouse i.p) were given on days 0 and 2.

DMF and MMF was diluted in 200 μl 0.08% Methocel/H_2O as vehicle and administered by oral gavage starting from day 3 post immunization (p.i) until termination. Each treatment group consisted of 8 animals: vehicle alone as a negative control, 5 mg/kg body weight DMF twice a day, 15 mg/kg body weight DMF twice a day, 15 mg/kg body weight MMF twice a day. The compounds were obtained via Fumapharm AG. Oral gavage was used to ensure exact dosing and to avoid compound degradation.

Spinal cord tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Slides were deparaffinized and rehydrated in graded alcohol solutions. Antigen retreival was performed by immersing the slides in 10 mM Citrate, pH 6.0 for 20 minutes in a pressure cooker at 120 C (Pascal, Dako Cytomation).

Immunohistochemistry was performed using the Dako autostainer as follows. Endogenous peroxidase was quenched by a 10 minute incubation in 3% H_2O_2/Methanol. The rabbit anti Nrf2 antibody C-20 (sc-722, Santa Cruz Biotechnology) was added at a 1:250 dilution in Dako Diluent with Background Reducing Components (Dako # S3022) C-20 antibody was detected using the Envision anti rabbit labeled polymer-HRP (Dako #K4003) and DAB (Vector Labs #SK-4100) was used as the chromogenic substrate. Morphometric analysis of Nrf2 immunostaining was performed using ImageJ software from NIH.

The results, shown in FIGS. **3** and **4**, demonstrate MMF and DMF activation of Nrf2 in vivo.

All publications and patent documents cited herein are incorporated by reference in their entirety. To the extent the material incorporated by reference contradicts or is inconsistent with the present specification, the present specification will supersede any such material.

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The invention claimed is:

1. A method of treating a subject in need of treatment for multiple sclerosis comprising orally administering to the subject in need thereof a pharmaceutical composition consisting essentially of (a) a therapeutically effective amount of dimethyl fumarate, monomethyl fumarate, or a combination thereof, and (b) one or more pharmaceutically acceptable excipients, wherein the therapeutically effective amount of dimethyl fumarate, monomethyl fumarate, or a combination thereof is about 480 mg per day.

2. The method of claim **1**, wherein the pharmaceutical composition is administered in the form of a tablet, a suspension, or a capsule.

3. The method of claim **1**, wherein the therapeutically effective amount is administered in separate administrations of 2, 3, 4, or 6 equal doses.

4. The method of claim **3**, wherein the therapeutically effective amount is administered in separate administrations of 2 equal doses.

5. The method of claim 3, wherein the therapeutically effective amount is administered in separate administrations of 3 equal doses.

6. The method of claim 1, wherein the pharmaceutical composition consists essentially of dimethyl fumarate and one or more pharmaceutically acceptable excipients.

7. The method of claim 1, wherein the pharmaceutical composition consists essentially of monomethyl fumarate and one or more pharmaceutically acceptable excipients.

8. The method of claim 1, wherein the pharmaceutical composition is administered to the subject for at least 12 weeks.

9. The method of claim 6, wherein the therapeutically effective amount is administered to the subject in 2 equal doses.

10. The method of claim 9, wherein the therapeutically effective amount is administered to the subject for at least 12 weeks.

11. A method of treating a subject in need of treatment for multiple sclerosis consisting essentially of orally administering to the subject about 480 mg per day of dimethyl fumarate, monomethyl fumarate, or a combination thereof.

12. The method of claim 11, wherein about 480 mg of dimethyl fumarate per day is administered to the subject.

13. The method of claim 12, wherein the dimethyl fumarate is administered in separate administrations of 2 equal doses.

14. The method of claim 12, wherein the dimethyl fumarate is administered in separate administrations of 3 equal doses.

15. A method of treating a subject in need of treatment for multiple sclerosis comprising orally administering to the subject pharmaceutical composition consisting essentially of (a) a therapeutically effective amount of dimethyl fumarate and (b) one or more pharmaceutically acceptable excipients, wherein the therapeutically effective amount of dimethyl fumarate is about 480 mg per day.

16. The method of claim 15, wherein the dimethyl fumarate is administered in separate administrations of 2 equal doses.

17. The method of claim 1, wherein the expression level of NQO1 in the subject is elevated after administering to the subject the therapeutically effective amount of dimethyl fumarate, monomethyl fumarate, or a combination thereof.

18. The method of claim 11, wherein the expression level of NQO1 in the subject is elevated after administering to the subject about 480 mg per day of dimethyl fumarate, monomethyl fumarate, or a combination thereof.

19. The method of claim 15, wherein the expression level of NQO1 in the subject is elevated after administering to the subject the therapeutically effective amount of dimethyl fumarate.

20. A method of treating a subject in need of treatment for multiple sclerosis comprising treating the subject in need thereof with a therapeutically effective amount of dimethyl fumarate, monomethyl fumarate, or a combination thereof, wherein the therapeutically effective amount of dimethyl fumarate, monomethyl fumarate, or a combination thereof is about 480 mg per day.

* * * * *