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(54) **ANTIFOLATE COMBINATION THERAPIES**

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See application file for complete search history.

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(57) **ABSTRACT**

A method of administering an antifolate to a mammal in need thereof, comprising administering an effective amount of said antifolate in combination with a methylmalonic acid lowering agent.

22 Claims, No Drawings

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ANTIFOLATE COMBINATION THERAPIES

This application is a divisional of application Ser. No. 11/288,807, filed 29 Nov., 2005 now abandoned, which is a divisional of application Ser. No. 10/297,821 filed 12 May, 2002, now U.S. Pat. No. 7,053,065, which claims priority under 35 USC 371, for PCT/US01/14860, filed 15 Jun., 2001, which claims the priority of U.S. provisional applications No. 60/215,310, filed 30 Jun., 2000, No. 60/235,859, filed 27 Sep., 2000, and No. 60/284,448, filed 18 Apr., 2001.

Potentially, life-threatening toxicity remains a major limitation to the optimal administration of antifolates. (see, generally, *Antifolate Drugs in Cancer Therapy*, edited by Jackman, Ann L., Humana Press, Totowa, N.J., 1999.) In some cases, a supportive intervention is routinely used to permit safe, maximal dosing. For example, steroids, such as dexamethone, can be used to prevent the formation of skin rashes caused by the antifolate. (*Antifolate*, pg 197.)

Antifolates represent one of the most thoroughly studied classes of antineoplastic agents, with aminopterin initially demonstrating clinical activity approximately 50 years ago. Methotrexate was developed shortly thereafter, and today is a standard component of effective chemotherapeutic regimens for malignancies such as lymphoma, breast cancer, and head and neck cancer. (Bonnadonna G, Zambetti M, Valagussa P. Sequential or alternating doxorubicin and CMF regimens in breast cancer with more than three positive nodes: Ten year results. JAMA 1995;273(7):542-547; Bonnadonna G, Valagussa P, Moliterni A, Zambetti M, Brambilla C. Adjuvant cyclophosphamide, methotrexate, and fluorouracil in node-positive breast cancer: The results of 20 years of follow-up. N Engl J Med 1995; 332(14):901-906; and Hong W K, Schaefer S, Issell B, et al. A prospective randomized trial of methotrexate versus cisplatin in the treatment of recurrent squamous cell carcinoma of the head and neck. Cancer 1983; 52:206-210.) Antifolates inhibit one or several key folate-requiring enzymes of the thymidine and purine biosynthetic pathways, in particular, thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT), by competing with reduced folates for binding sites of these enzymes. (Shih C, Habeck L L, Mendelsohn L G, Chen V J, Schultz R M. Multiple folate enzyme inhibition: Mechanism of a novel pyrrolopyrimidine-based antifolate LY231514 (MTA). Advan Enzyme Regul, 1998; 38:135-152 and Shih C, Chen V J, Gossett L S, et al. LY231514, a pyrrolo[2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes. Cancer Res 1997; 57:1116-1123.) Several antifolate drugs are currently in development. Examples of antifolates that have thymidylate synthase inhibiting ("TSI") characteristics include 5-fluorouracil and Tomudex®. An example of an antifolate that has dihydrofolate reductase inhibiting ("DHFRI") characteristic is Methotrexate®. An example of an antifolate that has glycinamide ribonucleotide formyltransferase inhibiting ("GARFTI") characteristics is Lometrexol. Many of these antifolate drugs inhibit more than one biosynthetic pathway. For example Lometrexol is also an inhibitor of dihydrofolate reductase and pemetrexed disodium (Alimta®, Eli Lilly and Company, Indianapolis, Ind.) has demonstrated thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase inhibition.

A limitation to the development of these drugs is that the cytotoxic activity and subsequent effectiveness of antifolates may be associated with substantial toxicity for some patients. Additionally antifolates as a class are associated with sporadic severe mylosuppression with gastrointestinal toxicity which, though infrequent, carries a high risk of mortality. The inability to control these toxicities led to the abandonment of clinical development of some antifolates and has complicated the clinical development of others, such as Lometrexol and raltitrexed. (Jackman A L, Calvert A H Folate-Based Thymidylate Synthase Inhibitors as Anticancer Drugs. Ann Oncol 1995; 6(9):871-881; Laohavinij S, Wedge S R, Lind M J, et al. A phase I clinical study of the antipurine antifolate Lometrexol (DDATHF) given with oral folic acid. Invest New Drugs 1996; 14:325-335; and Maughan T S, James R D, Kerr D, et al., on behalf of the British MRC Colorectal Cancer Working Party. Preliminary results of a multicenter randomized trial comparing 3 chemotherapy regimens (deGramont, Lokich, and raltitrexed) in metastatic colorectal cancer. Proc ASCO 1999; 18:Abst 1007.) Initially, folic acid was used as a treatment for toxicities associated with GARFTI see, e.g. U.S. Pat. No. 5,217,974. Folic acid has been shown to lower homocysteine levels (see e.g. Homocysteine Lowering Trialist's Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomized trials. BMJ 1998; 316:894-898 and Naurath H J, Joosten E, Riezler R, Stabler S P, Allen R H, Lindenbaum J. Effects of vitamin B 12, folate and vitamin B6 supplements in elderly people with normal serum vitamin concentrations. Lancet 1995; 346:85-89), and homocysteine levels have been shown to be a predictor of cytotoxic events related to the use of GARFT inhibitors, see e.g. U.S. Pat. No. 5,217,974. However, even with this treatment, cytotoxic activity of GARFT inhibitors and antifolates as a class remains a serious concern in the development of antifolates as pharmaceutical drugs. The ability to lower cytotoxic activity would represent an important advance in the use of these agents.

Surprisingly and unexpectedly, we have now discovered that certain toxic effects such as mortality and nonhematologic events, such as skin rashes and fatigue, caused by antifolates, as a class, can be significantly reduced by the presence of a methylmalonic acid lowering agent, without adversely affecting therapeutic efficacy. The present invention thus provides a method for improving the therapeutic utility of antifolate drugs by administering to the host undergoing treatment with a methylmalonic acid lowering agent. We have discovered that increased levels of methylmalonic acid is a predictor of toxic events in patients that receive an antifolate drug and that treatment for the increased methylmalonic acid, such as treatment with vitamin B12, reduces mortality and nonhematologic events, such as skin rashes and fatigue events previously associated with the antifolate drugs.

Additionally, we have discovered that the combination of a methylmalonic acid lowering agent and folic acid synergistically reduces the toxic events associated with the administration of antifolate drugs. Although, the treatment and prevention of cardiovascular disease with folic acid in combination with vitamin B12 is known, the use of the combination for the treatment of toxicity associated with the administration of antifolate drugs was unknown heretofore.

The present invention relates to a method of administering an antifolate to a mammal in need thereof, comprising administering an effective amount of said antifolate in combination with a methylmalonic acid lowering agent.

Furthermore, the present invention relates to a method of reducing the toxicity associated with the administration of an antifolate to a mammal comprising administering to said mammal an effective amount of said antifolate in combination with a methylmalonic acid lowering agent.

Furthermore, the present invention relates to a method of inhibiting tumor growth in mammals comprising administering to said mammals an effective amount of an antifolate in combination with a methylmalonic acid lowering agent.

Furthermore, the present invention relates to a method of administering an antifolate to a mammal in need thereof, comprising administering an effective amount of said antifolate in combination with a methylmalonic acid lowering agent and a FBP binding agent. A preferred FBP binding agent is folic acid.

Furthermore, the present invention relates to a method of reducing the toxicity associated with the administration of an antifolate to a mammal comprising administering to said mammal an effective amount of said antifolate in combination with a methylmalonic acid lowering agent and a FBP binding agent. A preferred FBP binding agent is folic acid.

Furthermore, the present invention relates to a method of inhibiting tumor growth in mammals comprising administering to said mammals an effective amount of an antifolate in combination with a methylmalonic acid lowering agent and a FBP binding agent. A preferred FBP binding agent is folic acid.

Furthermore, the present invention relates to the use of a methylmalonic acid lowering agent, alone or in combination with a FBP binding agent, in the preparation of a medicament useful in lowering the mammalian toxicity of an antifolate. A preferred FBP binding agent is folic acid.

Furthermore, the present invention relates to the use of a methylmalonic acid lowering agent in the preparation of a medicament useful in lowering the mammalian toxicity associated with an antifolate, and the medicament is administered in combination with an antifolate.

Furthermore, the present invention relates to the use of a methylmalonic acid lowering agent in the preparation of a medicament useful in lowering the mammalian toxicity associated with an antifolate, and the medicament is administered in combination with an antifolate and a FBP binding agent.

Furthermore, the present invention relates to the use of a methylmalonic acid lowering agent in the manufacture of a medicament for use in a method of inhibiting tumor growth in mammals, which method comprises administering said methylmalonic acid lowering agent in combination with an antifolate.

Furthermore, the present invention relates to a product containing a methylmalonic acid lowering agent, an antifolate and optionally a FBP binding agent as a combined preparation for the simultaneous, separate or sequential use in inhibiting tumour growth.

The current invention concerns the discovery that administration of a methylmalonic acid lowering agent in combination with an antifolate drug reduces the toxicity of the said antifolate drug.

The term "inhibit" as it relates to antifolate drugs refers to prohibiting, alleviating, ameliorating, halting, restraining, slowing or reversing the progression of, or reducing tumor growth.

As used herein, the term "effective amount" refers to an amount of a compound or drug, which is capable of performing the intended result. For example, an effective amount of an antifolate drug that is administered in an effort to reduce tumor growth is that amount which is required to reduce tumor growth.

As used herein, the term "toxicity" refers to a toxic event associated with the administration on an antifolate. Such events include, but are not limited to, neutropenia, thrombopenia, toxic death, fatigue, anorexia, nausea, skin rash, infection, diarrhea, mucositis, and anemia. For further explanation of the types of toxicity experienced by patients receiving antifolates, see, generally, *Antifolate Drugs in Cancer Therapy*. Preferably, toxicity refers to toxic death, fatigue, neutropenia, thrombopenia, and mucositis.

As used herein, the term "nonhematologic event" refers to the occurrence of skin rash or fatigue due to the administration of an antifolate.

As used herein, the term "in combination with" refers to the administration of the methylmalonic acid lowering agent, the antifolate drug, and optionally the folic acid; in any order such that sufficient levels of methylmalonic acid lowering agent and optionally folic acid are present to reduce the toxicity of an antifolate in a mammal. The administration of the compounds maybe simultaneous as a single composition or as two separate compositions or can be administered sequentially as separate compositions such that an effective amount of the agent first administered is in the patient's body when the second and/or third agent is administered. The antifolate drug may be administered to the mammal first, followed by treatment with the methylmalonic acid lowering agent. Alternatively, the mammal may be administered the antifolate drug simultaneously with the methylmalonic acid lowering agent. Preferably, the mammal is pretreated with the methylmalonic acid lowering agent and then treated with the antifolate. If folic acid is to be administered in addition to the methylmalonic acid lowering agent, the folic acid may be administered at any time prior, post, or simultaneously to the administration of either the methylmalonic acid lowering agent or the antifolate. Preferably, the mammal is pretreated with the methylmalonic acid, and then treated with folic acid, followed by treatment with the antifolate compound.

The terms "antifolate" and "antifolate drug" refer to a chemical compound which inhibits at least one key folate-requiring enzyme of the thymidine or purine biosynthetic pathways, preferably thymidylate synthase ("TS"), dihydrofolate reductase ("DHFR"), or glycinamide ribonucleotide formyltransferase ("GARFT"), by competing with reduced folates for binding sites of these enzymes. Preferred examples of antifolates include Tomudex®, as manufactured by Zeneca; Methotrexate®, as manufactured by Lederle; Lometrexol®, as manufactured by Tularik; pyrido[2,3-d]pyrimidine derivatives described by Taylor et al in U.S. Pat. Nos. 4,684,653, 4,833,145, 4,902,796, 4,871,743, and 4,882,334; derivatives described by Akimoto in U.S. Pat. No. 4,997,838; thymidylate synthase inhibitors as found in EPO application 239,362; and most preferred, Pemetrexed Disodium (ALIMTA), as manufactured by Eli Lilly & Co.

The terms "methylmalonic acid" and "MMA" refer to a structural isomer of succinic acid present in minute amounts in healthy human urine.

The term "methylmalonic acid lowering agent" refers to a substrate, which lowers the concentration of methylmalonic acid in a mammal. A preferred example of such a substrate is vitamin B12. For methods of determining methylmalonic acid and substrates therefore, see, e.g., Matchar D B, Feussner J R, Millington D S, et al. Isotope dilution assay for urinary methylmalonic acid in the diagnosis of vitamin B12 deficiency. A prospective clinical evaluation. Ann Intern Med 1987; 106: 707-710; Norman E J, Morrison J A. Screening elderly populations for cobalamin (vitamin B12) deficiency using the urinary methylmalonic acid assay by gas chromatography mass spectrometry. Am J Med 1993; 94: 589-594; Norman E J. Gas Chromatography mass spectrometry screening of urinary methylmalonic acid: early detection of vitamin B12 (cobalamin) deficiency to prevent permanent neurologic disability. GC/MS News 1984; 12:120-129; Martin D C, Francis J, Protetch J, Huff F J. Time dependency of cognitive recovery with cobalamin replacement: report of a pilot study. JAGS 1992; 40: 168-172; Norman E J, Cronin C. Cobalamin deficiency. Neurol 1996; 47: 310-311; Rasmussen K, Moelby I, Jensen M K. Studies on methylmalonic acid

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The term "vitamin B12" refers to vitamin B12 and its pharmaceutical derivatives, such as hydroxocobalamin, cyano-10-chlorocobalamin, aquocobalamin perchlorate, aquo-10-chlorocobalamin perchlorate, azidocobalamin, chlorocobalamin, and cobalamin. Preferably the term refers to vitamin B12, cobalamin, and chlorocobalamin.

The dosage generally will be provided in the form of a vitamin supplement, namely as a tablet administered orally, such as a sustained release formulation, as an aqueous solution added to drinking water, or as an aqueous parenteral formulation. Preferably the methylmalonic acid lowering agent is administered as an intramuscular injection formulation. Such formulations are known in the art and are commercially available.

The skilled artisan will appreciate that the methylmalonic lowering agents are effective over a wide dosage range. For example, when cobalamin is used as the methylmalonic lowering agent, the dosage of cobalamin may fall within the range of about 0.2 μg to about 3000 μg of cobalamin from once daily for a month to once every nine weeks for a year. Preferably, cobalamin will be dosed as an intramuscular injection of about 500 μg to about 1500 μg administered from about every 24 hours to about every 1680 hours. Preferably, it is an intramuscular injection of about 1000 μg administered initially from about 1 to about 3 weeks prior to administration of the antifolate and repeated from about every 24 hours to about every 1680 hours, regardless of when treatment with the antifolate is started and continued until the administration of the antifolate is discontinued. Most preferred is an intramuscular injection of about 1000 μg administered initially from about 1 to about 3 weeks prior to the first administration of the antifolate and repeated every 6 to 12 weeks, preferably about every 9 weeks, and continued until the discontinuation of the antifolate administrations. However, it will be understood that the amount of the methylmalonic acid lowering agent actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual agent administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect.

The term "FBP binding agent" as used herein refers to a folic binding protein binding agent which includes folic acid, (6R)-5-methyl-5,6,7,8-tetrahydrofolic acid, and (6R)-5-formyl-5,6,7,8-tetrahydrofolic acid, or a physiologically-available salt or ester thereof. This latter compound is the (6R)-isomer of leucovorin as disclosed in J. Am. Chem. Soc., 74, 4215 (1952). Both of the tetrahydrofolic acid compounds are in the unnatural configuration at the 6-position. They are 10-20 fold more efficient in binding the folate binding protein compared with their respective (6S)-isomer, see Ratnam, et. al., Folate and Antifolate Transport in Mammalian Cells Symposium, Mar. 21-22, 1991, Bethesda, Md. These compounds are usually prepared as a mixture with their natural form (6S) of diastereomers by non-stereoselective reduction from the corresponding dehydro precursors followed by separation through chromatographic or enzymatic techniques. See e.g. PCT Patent Application Publication WO 880844 (also Derwent Abstract 88-368464/51) and Canadian Patent 1093554. See, e.g. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline (2000), 8 Folate, pp. 196-305.

"Physiologically-available salt" refers to potassium, sodium, lithium, magnesium, or preferably a calcium salt of the FBP binding agent. "Physiologically-available . . . ester" refers to esters which are easily hydrolyzed upon administration to a mammal to provide the corresponding FBP binding agent free acid, such as C_1-C_4 alkyl esters, mixed anhydrides, and the like.

The FBP binding agent to be utilized according to this invention can be in its free acid form, or can be in the form of a physiologically-acceptable salt or ester which is converted to the parent acid in a biological system. The dosage generally will be provided in the form of a vitamin supplement, namely as a tablet administered orally, preferably as a sustained release formulation, as an aqueous solution added to drinking water, an aqueous parenteral formulation, e.g., an intravenous formulation, or the like.

The FBP binding agent is usually administered to the subject mammal prior to treatment with the antifolate. Pretreatment with the suitable amount of FBP binding agent from about 1 to about 24 hours is usually sufficient to substantially bind to and block the folate binding protein prior to administration of the antifolate. Although one single dose of the FBP binding agent, preferably an oral administration of folic acid, should be sufficient to load the folate binding protein, multiple dosing of the FBP binding agent can be employed for periods up to weeks before treatment with the active agent to ensure that the folate binding protein is sufficiently bound in order to maximize the benefit derived from such pretreatment.

In the especially preferred embodiment of this invention, about 0.1 mg to about 30 mg, most preferably about 0.3 mg to about 5 mg, of folic acid is administered orally to a mammal about 1 to 3 weeks post administration of the methylmalonic acid lowering agent and about 1 to about 24 hours prior to the parenteral administration of the amount of an antifolate. However, it will be understood that the amount of the methylmalonic acid lowering agent actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual agent administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect.

In general, the term "pharmaceutical" when used as an adjective means substantially non-toxic to living organisms.

Methods

To assess the effect of a methylmalonic acid lowering agent, alone or in combination with folic acid on the antitumor efficacy of an antifolate in a human tumor xenograft model, female nude mice bearing human MX-1 breast carcinoma were treated with ALIMTA alone or along with superphysiologic doses of folic acid or vitamin B12 (cobalamin).

The animals were maintained on sterilized standard lab chow ad libitum and sterilized water ad libitum. The human MX-1 tumor cells (5×10^6) obtained from donor tumors were implanted subcutaneously in a thigh of female nude mice 8- to 10-weeks old. Beginning on day 7 post tumor cell implanta-

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