AZATHIOPRINE COMPOSITIONS FOR COLONIC ADMINISTRATION

Background of the Invention

Inflammatory bowel disorders or diseases (IBD) encompass a spectrum of overlapping clinical diseases that appear to lack a common etiology. IBD, however, are characterized by chronic inflammation at various sites in the gastrointestinal (GI) tract. Illustrative IBD are regional enteritis (or Crohn's disease), idiopathic ulcerative colitis, idiopathic proctocolitis, and infectious colitis. Most hypotheses regarding the pathogenesis of IBD concern the implication of immunologic, infectious, and dietary factors.

6-mercaptopurine (6MP) and its prodrug azathioprine (AZA) have been used in the treatment of inflammatory bowel disease (IBD) for over 25 years. Multiple controlled trials and a recent meta-analysis support the efficacy of 6MP and AZA in Crohn's disease. See, J.M.T. Willoughby et al., Lancet, ii 944 (1971); J.L. Rosenberg et al., Dig. Dis., 20, 721 (1975). Several controlled trials support the use of AZA in ulcerative colitis, the most recent by Hawthorne and colleagues, in Brit. Med. J., 305, 20 (1992). However, use of 6MP and AZA has been limited by concerns about their toxicities. Dose-related leukopenia is seen in 2-5% of patients treated long-term with 6MP or AZA for IBD. See, for example, D.H. Present et al., Am. Int. Med., 111, 641 (1989); W.R. Connell et al., Gut, 34, 1081 (1993).

Therefore, a need exits for effective, nontoxic therapies for IBD.

Summary of the Invention

The present invention provides a therapeutic method of treating inflammatory bowel disease (IBD) comprising topically administering to the colon of a patient in need of such treatment, an amount of azathioprine (AZA) effective to relieve the symptoms of said IBD. Preferably the azathioprine is administered orally, by means of an enteric-coated unit dosage form that selectively releases AZA in the terminal ileum and/or colon of the patient. The AZA can also be effectively administered to the colon by rectal administration of an enema formulation comprising AZA. Due to poor

absorption of AZA in the bloodstream from the colon, relatively high doses of AZA can be administered to the afflicted tissue, i.e., in the case of Crohn's disease or ulcerative colitis, without inducing systemic toxicities such as leukopenia, Therefore, effective AZA doses of from about 150-1000 mg can be delivered 1-4 times daily to adult patients (150 mg 1 x day - 1000 mg 4 x day) without undue toxicities (about 2-20 mg/kg AZA doses are administered).

As used herein the term "azathioprine" includes the pharmaceutically acceptable salts thereof, as well as functionally equivalent analysis, derivatives, and metabolites, such as 6-MP. For example, see U.S. Patent No. 3,056,785, and W.P. Wilson et al., Anal. Profiles of Drug Substances, 10, 29-53 (1981).

Detailed Description of the Invention

Colonic administration of drugs has been used to reduce the toxicity associated with oral or IV corticosteroids and oral 5-aminosalicyclate in patients with IBD. This decreased toxicity is believed to be due to reduced systemic bioavailability. Several types of colonic drug delivery systems are currently available, including enemas (L. R. Sutherland et al., Med. Clin. North Amer., 74, 119 (1990)); rectal foams (Drug. Ther. Bull., 29, 66 (1991)); and delayed release oral formulations in the form of Eudragit-coated capsules which dissolve at pH 7 in the terminal ileum (K. W. Schroeder et al., New Engl. J. Med., 317, 1625 (1987)).

The effective amount of azathioprine (AZA) can be topically administered to the colon of the patient by oral ingestion of a unit dosage form comprising an effective amount of AZA which is enterically coated so as to be released from the unit dosage form in the lower intestinal tract, e.g., in the terminal portion of the ileum and in the colon of the patient. Microparticles of AZA may be individually coated and delivered as a suspension in a liquid vehicle, may be encapsulated as a powder or may be compressed into a pill or tablet and swallowed. Alternatively, the azathioprine may be combined with adjuvants employed in solid unit dosage forms, such as fillers and binders, compressed into shaped, solid dosage forms such as pills

or tablets, and the pills or tablets treated so as to apply an enteric coating of suitable thickness thereto.

Enteric coatings are those which remain intact in the stomach, but will dissolve and release the contents of the dosage form once it reaches the small intestine. The purpose of an enteric coating is to delay the release of the AZA until it reached the target site of action in the colon. Since the AZA topically-administered to the colonic tissue in this fashion is only about 10% absorbed into the bloodstream, the systemic side-effects of AZA can be avoided or minimized.

Thus, a useful enteric coating is one that remains undissociated in the low pH environment of the stomach, but readily ionizes when the pH rises to about 4 or 5. The most effective enteric polymers are polyacids having a pH_a of 3 to 5.

The most extensively use polymer is cellulose acetate phthalate (CAP) which is capable of functioning effectively as an enteric coating. However, a pH greater than 6 usually is required for solubility and thus a delay in drug release may ensue. Another useful polymer is polyvinyl acetate phthalate (PVAP) which is less permeable to moisture and gastric fluid, more stable to hydrolysis and able to ionize at a lower pH, resulting in earlier release of actives in the duodenum.

A more recently available polymer is hydroxypropyl methylcellulose phthalate. This has similar stability to PVAP and dissociates in the same pH range. A further example of currently used polymers are those based on methacrylic acid, e.g., methacrylic acid ester copolymers with acidic ionizable groups, such as Eudragit S-100 (methacrylic acid copolymer). Various systems are available that allow each of these enteric polymers to be applied as aqueous dispersions, thus facilitating the use of aqueous film-coating technology for the enteric coating of pharmaceutical dosage forms.

Another preferred dosage form in the topical administration of AZA to the colon is an enema formulation, which is rectally administered to the lower colon. Useful formulations comprise an effective amount of AZA dissolved or dispersed in a suitable flowable carrier vehicle, such as water,

alcohol or an aqueous-alcoholic fluid. The carrier vehicle is preferably thickened with natural or synthetic thickness such as gums, acrylates or modified celluloses. The formulation can also comprise an effective amount of a lubricant such as a natural or synthetic fat or oil, .i.e., a tris-fatty acid glycerate or lecithin. Nontoxic nonionic surfactants can also be included as wetting agents and dispersants. Unit dosages of enema formulations can be administered from prefilled bags or syringes. The carrier vehicle may also comprise an effective amount of a foaming agent such as n-butane, propane or i-butane. Such formulations can be delivered from a pressurized container, so that the vehicle is delivered to the colon as a foam, which inhibits its release from the target site.

The invention will be further described by reference to the following detailed examples.

Example 1. Preparation of Hydrophilic Rectal AZA Foam

To 948.4 g of purified distilled water containing 1.4 g and 0.14 g of methyl- and propylparaben respectively as preservatives; 2.0 g KELTROL TF (xanthan gum, suspending agent) were added and dispersed. Then 2.0 g of SOYA LECITHIN emulsifying agent (unbleached) were also dispersed as well as 5.0 g of carbomer (CARBOPOL 974 P NF). The carbomer was then neutralized with 2 g of sodium hydroxide dissolved in 20 ml of purified water (pH 7.10). Then 10.0 g of POLYSORBATE 80 (surfactant) were added to the mixture along with 0.250 g CITRAL (perfume) and the two components were dispersed. Finally, 2.366 g of AZA were added and also dispersed. The theoretical content of Azathioprine in this formulation was 50 mg/21 g of mixture. Viscosity of the concentrate measured in Brookfield-viscometer DV-II at 20°C and 12 rpm using spindle 64 was: 29, 700 CPS.

The concentrate was then mixed with n-butane (foaming agent) using standard equipment for the preparation of aerosols. The n-butane content in the aerosol mixture was 2.5%. The mixture was then filled into canisters of the Sepro bag-in-can type (containing a collapsible lamipack bag mounted on a valve and fitted into a monoblock aluminum can: the propellant

was nitrogen present in the monoblock and thus separated from the aerosol concentrate of the bag). The can was equipped with a nozzle and a canula. The delivered amount of concentrate per canister was 21 ± 1 g leading to 200-220 ml of foam at 37°C.

Example 2. Hydrophobic Rectal AZA Foam

To 948.45 g of purified distilled water containing 1.43 g and 0.14 g of methyl- and propylparaben respectively: 2.0 g of KELTROL TF (xanthan gum, suspending agent); 2.0 g of unbleached SOYA LECITHIN (emulsifying agent); 5.0 g of CARBOPOL 974 P NF (carbomer) were added and dispersed with a SILVERSON homogenizer. The carbomer was neutralized with 2.0 g of sodium hydroxide dissolved in 20 ml of purified distilled water, pH 7.05.

Then 47.40 g WITEPSOL H 15 (Hard Fat NF) an hydrophobing agent were added and very efficiently dispersed in the mixture using a SILVERSON homogenizer. A creamy concentrate having an hydrophobic feel was obtained.

10.50 g of POLYSORBATE 80 (surfactant) and 0.25 g CITRAL (perfume) were added and also dispersed with the homogenizer. To stabilize this emulsion and particularly the corresponding foam, 20.80 g of POLAWAX NF (as non-ionic emulsifying wax) were added and dispersed in the mixture. Finally 1.93 g of AZA was added and also dispersed. The theoretical content of the Azathioprine in the formulation was 50 mg in 27.5 g of mixture. Viscosity of the concentrate measured as for AZA-1:41,000 CPS. The concentrate was then mixed with n-butane (foaming agent) as for sample AZA-1, n-butane content: 2.5%. The mixture was then filled into canisters of the Sepro bag-in-can type. The delivered amount of concentrate per canister was 27.5 g ± 1 g leading to 205-220 ml of foam at 37°C. The hydrophobic foam had a lower expansion ratio than the hydrophilic foam.

Example 3. Preparation of AZA Enteric-Coated Capsules

1 IMURAN tablet (50 mg Azathioprine tablet containing 50 mg AZA (Burroughs Wellcome Co.) was crushed in a mortar; and the powder

was filled in a size 2 hard gelatin capsules. The capsules were closed manually. Each capsule was weighed individually (IPC)

The capsules were banded on a laboratory type banding machine with a 50 wt% solution of gelatin in water (1% polysorbate 80). The capsule was finally coated in a laboratory coating machine by the spraying with an aqueous solution of Eudragit S-100 (1N NH_4OH). The finished capsules (12 wt-% Eudragit) were resistant to artificial gastric juice (pH 1.2) for 2 hours, but disintegrated in artificial gut juice (pH 7.2) in no more than 60 minutes.

Example 4. Topical Colonic Administration of AZA

A. Subjects: Twenty-four healthy human volunteers were recruited and screening physical exam and laboratory studies (complete blood count, chemistry panel, and urinalysis) were performed. Prior to study entry, the erythrocyte thiopurine methyltransferase (TPMT) activity was determined in all patients and subjects with homozygous or heterozygous low TPMT activity were excluded from the study because of prior reports of severe neutropenia associated with AZA or 6MP use in this population. Subjects were screened for medical conditions or a surgical history which could impact AZA absorption or its metabolism. Demographics of subject group are summarized in Table 1. There were no statistically significant differences between the groups for the parameters indicated.

Table 1

Group	Number	Age (yr)	Weight (kg)	TPMT level (Units/ml RBC)
Oral	6	27.7 ±7.5	80.3 ± 20.0	21.9 ± 2.3
DRO	6	36.7 ± 18.5	87.9 ± 44.0	21.2 ± 2.6
HBF	6	29.2 ± 5.8	83.7 ± 11.6	23.5 ± 4.0
HPF	6	33.2 ± 8.2	71.7 ± 10.0	19.5 ± 1.8

* mean ± SD

B. Study Design: The 24 healthy human subjects were randomly assigned to receive 50 mg of one of four AZA dosage formulations (each n=6): oral: delayed-release oral (DRO); hydrophobic rectal foam (HBF); and hydrophilic rectal foam (HPF). All subjects also received a 50 mg does of intravenous (IV) AZA. The two doses of AZA were separated by at least 3 weeks and the order of dosing (IV vs. non-IV) was randomly determined. Subjects fasted after midnight the night prior to the study. If rectal foam were to be administered, subjects were given a Fleet's enema if they had not had a bowel movement the morning of the study.

The intravenous dose (see below) was administered as an infusion diluted in 20 ml of normal saline and delivered over 5 minutes. The oral preparations were administered with a sip of water. Rectal formulations were administered by a nurse. Subjects remained fasting for three hours following dose administration. A time zero blood sample was obtained prior to the study. After dose administration or after initiation of the IV infusion, blood samples were drawn in 7 ml EDTA (K_3)-containing vacuum tubes (Sherwood Medical, St. Louis, Mo.) at the following time intervals: 5, 10, 15, 30 minutes and 1, 1.5, 2, 3, 4, 5, 6, and 8 hours.

The AZA pharmacokinetics were studied by determining 6MP bioavailability rather than AZA bioavailability because of the availability of reliable techniques for measuring plasma 6MP levels. In addition, 6MP is the

more biologically relevant molecule, as AZA functions as a prodrug for 6MP. Following absorption, AZA is quickly converted to 6MP via non-enzymatic attack on the bond between the imidazole ring and the 6MP molecule by sulfhydril-containing compounds such as glutathione. 6MP is then metabolized to compounds with immunomodulatory activity, the 6-thioguanine nucleotides (6TGN).

C. Determination of 6MP concentration in Plasma:

Immediately after collection, blood samples were placed in an ice-water slurry. Within 30 minutes the blood sample was centrifuged for 10 minutes at 1000 g, 4°C. Plasma was then transferred to plastic cryotubes (Nunc Inc., Naperville, IL) and stored at -70°C. until analysis. 6MP concentrations were determined by HPLC using the technique of Zimm (20) with modifications. Solid phase extraction columns (C18 Sep-Paks, Waters, Inc., Millford, MA) were sequentially pre-rinsed with 2.5 ml of methanol and 5 ml of 0.2% acetic acid. One ml of plasma was loaded onto the Sep-Pak following addition of 0.04 ml of saturated EDTA•2Na (Aldrich, Milwaukee, Wisconsin) solution to the plasma. Dithiothreitol (DTT) was not included because AZA is immediately converted to 6MP in the presence of DTT. The cartridges were rinsed with 2 ml of 0.2% acetic acid and then centrifuged at 3200 rpm for 5 minutes to remove excess water. The samples were eluted from the cartridges with 2 ml of methanol and evaporated to dryness under a stream of nitrogen at 37°C. Samples were then reconstituted in 200 uL of mobile phase, vortexed for 30 seconds, transferred to 1.5 ml conical microfuge tubes and centrifuged in a microcentrifuge for 5 minutes. A portion (0.175 ml) of supernatant was transferred to HPLC vials.

6MP levels were then determined by HPLC. The analytical column was a Hewlett-Packard (Rockville, Maryland) octadecylsilane (ODS) Hypersil, 200 x 4.6 mm, 5 μm particle size. It was protected by a Zorbax (Mac-mod Analytical, Chadds Ford, Pennsylvania) ODS 4 x 12.5 mm guard column. The mobile phase was 0.8% acetonitrile in 1 mM triethylamine, adjusted to pH 3.2 with phosphoric acid. Absorbance was monitored at 340 nm. The injection volume was 80 uL. Unknowns were determined by

comparing them to a standard curve constructed the same day by adding known quantities of 6MP to blank plasma. The lower limit of quantification of 6MP was 2 ng/ml. The mean calculated concentration ± coefficient of variation for the 2 ng/ml and 50 ng/ml standards were 2.0 ng/ml ± 18% and 50.2 ng/ml ± 3.4%, respectively.

D. Study Medications: 1) Intravenous AZA, lyophilized as the sodium salt (Burroughs Wellcome, Research Triangle Park, North Carolina). 2) Standard 50 mg oral tablet (Burroughs Wellcome, Research Triangle Park, North Carolina). 3) Delayed-release 50 mg oral tablet (DRO). 4) Hydrophobic rectal form (HBF). Administered rectally via a pressurized foam canister. 50 mg of AZA (Fermion/Orion Corporation, Espoo, Finland) is dissolved in a dose of foam containing witepsol H15 (an oleagenous base) to make it hydrophobic. 5) Hydrophilic rectal foam. Administered rectally via a pressurized foam canister. 50 mg of AZA (Fermion/Orion Corporation, Espoo, Finland) is dissolved in a foam. The delayed-release oral and rectal foam forms of AZA were prepared by Tillotts Pharma A.G. (Ziefen, Switzerland).

E. Results:

1. Pharmacokinetic Parameters for IV Dosing:

The AUC, CL, V_{ds}, and $T_{1/2}$ (mean ± SD) for all 24 subjects following 50 mg AZA administered intravenously are listed in Table 2.

TABLE 2: PHARMACOKINETIC PARAMETERS FOR 6MP FOLLOWING 50 MG OF AZA ADMINISTERED INTRAVENOUSLY TO 24 HEALTHY VOLUNTEERS*

AUC	CL	V_{ds}	$T_{1/2}$
(ng.hr/ml)	L/kg.hr)	(L/kg)	(hr)
100.7 ± 30.5	3.8 ± 1.2	6.7 ± 3.1	1.2 ± 0.37

* mean ± SD

2. Pharmacokinetic Parameters for Colonic Delivery:

The AUC, F, T_{MAX}, and C_{MAX} (mean ± SE) for each of the non-IV delivery routes are listed in Table 3. The mean bioavailability of the oral

preparation was significantly greater than for the other preparations, and the mean T_{MAX} of the DRO formulation was significantly greater than for the other formulations. None of the other differences were statistically significant.

TABLE 3: PHARMACOKINETIC PARAMETERS FOR 6MP FOLLOWING 50 MG AZA ADMINISTERED VIA ORAL, DRO, HBF AND HPF*

Group	AUC	F	T_{MAX}	C_{MAX}
(n=6)	(ng.hr/ml)	(%)	(hr)	(ng/ml)
Oral	55 ± 27	52 ± 26	1.8 ± 0.8	21 ± 13
DRO	11 ± 9	13 ± 15	5.4 ± 0.2[c]	5 ± 5[c]
HBF	5 ± 4	5 ± 4	2.1 ± 0.3[d]	2 ± 1[d]
HPF	3 ± 4	2 ± 3	2.0 ± 0.0[e]	1 ± 1[e]
P value	0.01[a]	0.01[a]	0.01[b]	0.01[a]

[a] P values derived by analysis of covarience. Post-hoc tests (Duncan's): a-oral>DRO=HBF=HPF; b-DRO>oral=HBF=HPF. c-mean ± SE of the 5 subjects with detectable concentrations of 6MP. d-mean ± SE of the 4 subjects with detectable concentrations of 6MP. e-mean ± SE of the 3 subjects with detectable concentrations of 6MP.

* mean ± SE

3. Rectal Administration

The rectal foam preparations were well-tolerated. Twenty-two of the subjects retained the foam for greater than 6 hours. One subject reported expelling the hydrophobic foam after one hour and another reported expelling the hydrophobic foam after two hours. The subject who reported expelling the foam after one hour did not have detectable absorbtion. The subject who reported expelling the foam after 2 hours had a C_{MAX} of 2.35 ng/ml at 3 hours. No adverse reactions to the foam preparations were reported.

F. Discussion: This example demonstrates that the systemic bioavailability of 6MP following dosing with delayed-release oral and rectal

delivery formulations of AZA is significantly lower than the bioavailability of 6MP after standard oral AZA. There are several potential factors contributing to this observation. The most likely is that the absorbtion of AZA across the colonic mucosa is reduced compared to absorbtion across gastric and small intestinal mucosa due to absence of specific transport mechanisms or differing rates of passive absorbtion. Reduced colonic absorbtion compared to jejunal absorbtion has been demonstrated for 5-aminosalicylate (5-ASA) (S. Bondesen et al., Br. J. Clin. Pharm., 25, 269 (1988)). AZA and 6MP may be more completely metabolized in the colonic mucosa of the more proximal GI tract. In addition, some AZA may be lost in the stool. Fecal excretion of 5-ASA following administration of Eudragit-coated tablets is approximately 25% (B. Norlander et al., Aliment. Pharmacol. Therap., 4, 497 (1990)).

Following absorbtion, AZA is quickly converted to 6MP in plasma. This conversion occurs as the result of non-enzymatic attack by sulfhydril-containing compounds such as glutathione on the bond between the 6MP molecule and the imidazole ring of AZA (L. Lennard et al., Clin. Pharmacol. Ther., 46, 149 (1989)). Glutathione is present in every mammalian cell, including colonic epithelial cells and lymphocytes, and prior studies have shown that lymphocytes contain the enzymes necessary to convert 6MP to the active metabolites, the 6TGNs (B. Bostrom et al., Am. J. Ped. Hem./Onc., 15, 80 (1993)). It is, therefore, reasonable to expect that topical delivery of AZA will result in local immunosuppressive effects on colonic lymphocytes.

This example demonstrates that colonic delivery of AZA results in significantly less 6MP bioavailability that standard oral AZA. It is believed that colonic delivery of AZA can reduce the drug's toxicity by reducing systemic exposure to 6MP. In addition, this topical form of AZA administration can allow delivery of higher, locally concentrated doses to intraepithelial and lamina propria colonic lymphocytes.

The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be

understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

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WHAT IS CLAIMED IS:

1. A method of preparing a medicament for treating inflammatory bowel disease comprising an amount of azathioprine adapted for topical administration to the colon of a patient in need of such treatment, wherein said amount is effective to relieve the symptoms of said inflammatory bowel disease.

2. The method of claim 1 wherein the inflammatory bowel disease is ulcerative colitis.

3. The method of claim 2 wherein the inflammatory bowel disease is Crohn's disease.

4. The method of claim 1 wherein the medicament is adapted to be administered to the lower colon of said patient by rectal enema.

5. The method of claim 1 wherein the medicament is a unit dosage form adapted for oral administration, which comprises an effective amount of enterically coated azathioprine which is released from the dosage form in the terminal portion of the ileum and in the colon of said patient.

6. The method of claim 1 wherein the unit dosage form comprises an enteric coating which disintegrates at about pH 7.

7. The method of claim 1 wherein the dosage form delivers a total daily dosage of about 3-75 mg/kg of azathioprine is administered.

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