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Sequential group trial to determine gastrointestinal site of absorption and systemic exposure of azathioprine

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Abstract

Azathioprine (AZA) is used in the treatment of patients with refractory inflammatory bowel disease; however, its use is limited because of systemic toxicity associated with long-term use. Ileocecal delivery of AZA might be advantageous if local intestinal therapeutic effects could be provided with decreased systemic side effects. Decreased cecal systemic absorption would allow higher dosages of AZA to be administered. A two-phase study was performed to compare the systemic exposure of AZA and 6-mercaptopurine (6-MP) following administration of AZA into the stomach, jejunum, and cecum and to compare the systemic exposure to AZA and 6-MP following administration of three different dosages of AZA into the cecum. In phase I, six healthy male volunteers received three 50 mg sequential doses of AZA via an oral tube directly placed into the stomach, jejunum, and cecum, respectively. In phase II, six healthy male volunteers received three different dosages (50, 300, 600 mg of AZA) into the cecum. Plasma concentrations of AZA and 6-MP at various times were quantified and area under the plasma concentration-time curve (AUC) and mean residence time (MRT) were determined. No significant differences in the AUC of AZA were seen at the different sites. The AUC of 6-MP following administration of AZA into the jejunum (67.0 ± 30.1 ng × hr/ml) was higher compared to the stomach (39.9 ± 38.1 ng/hr/ml) and cecum (29.2 ± 10.9 ng × hr/ml). Jejunal absorption was 68% higher than absorption from the stomach and 129% higher than that of the cecum. Gastric absorption was 27% higher than that of the cecum. Increased dosages given into the cecum resulted in increased AUCs of AZA and 6-MP. The AUCs of AZA following 50, 300, and 600 mg dosages were 16.9 ± 7.4, 52.3 ± 67.2, and 132 ± 151 ng × hr/ml, respectively, and the AUCs of 6-MP were 22.2 ± 14.9, 63.4 ± 50.6, and 104 ± 115 ng × hr/ml, respectively. Systemic exposure to 6-MP is reduced following administration of AZA into the cecum, most likely secondary to reduced absorption of 6-MP from the colon. Higher dosages of AZA presented to the cecum do result in increased systemic absorption, but may still allow more drug to be administered with less toxicity than the same dose received orally.

Azathioprine (AZA) and 6-mercaptopurine (6-MP) are used in the treatment of patients with inflammatory bowel disease (IBD).[1] While AZA and 6-MP are not first line agents in IBD, several studies have demonstrated benefit in patients with refractory Crohn's disease[2-4] and ulcerative colitis[5-8]. Administration of AZA and 6-MP has been shown to reduce the relapse rate,[9] induce remission quicker and more frequently, and decrease the dose of steroid needed.[3] Unfortunately, because of the dose-dependent, systemic toxicity associated with use of AZA, its use in IBD is limited to select patients.[10] Toxicities that are dose- and concentration-dependent include leukopenia in 2–5% of patients,[10-12] opportunistic infection,[10,11,13] and lymphoma.[10]

Colonic drug delivery of AZA might be advantageous if local intestinal therapeutic effects could be provided with reduced systemic absorption. Local intestinal drug delivery is already used with mesalamine and budesonide in patients with IBD.[14-16] It has previously been demonstrated that ileocolonic delivery of AZA via a delayed-release oral formulation significantly decreases systemic bioavailability of 6-MP in comparison with AZA administered as a standard oral tablet in healthy human subjects.[17]

With decreased bioavailability, delivery of higher doses could possibly be used and the likelihood of response to AZA or 6-MP would increase.[4] Increased colonic dosages of AZA may increase efficacy and decrease the time to response.

Previous studies have investigated the pharmacokinetics of oral, intravenous, and colonic administration of AZA[17-19] but to our knowledge, no study has determined the gastrointestinal site of absorption. Hence, the objective of this study was, first, to compare the systemic exposure to 6-MP following administration of AZA into the stomach, jejunum, and cecum and, second, to compare the systemic exposure to 6-MP following administration of 50, 300, and 600 mg of AZA into the cecum.

Materials and Methods

This study was an open-label, repeat-dose, two-phase trial to compare the absorption and bioavailability characteristics of AZA (Imuran, Glaxo Wellcome Inc., Research Triangle Park, North Carolina) and 6-MP. Both the university institutional review board and the clinical research center approved the study. Healthy male volunteers between the ages of 18 and 50 were evaluated for enrollment into the study. Subjects had to be within 20% of their ideal body weight, have clinically acceptable vital signs and laboratory values, be able to fast for 12 hr, and abstain from ingesting alcohol, drugs, and caffeine while enrolled in the study. Subjects were excluded from the study that had any predisposing conditions that would interfere with the delivery, absorption, distribution, metabolism, or excretion of AZA or any abnormal laboratory values, including a low homozygous or low heterozygous thiopurine methyltransferase (TPMT) activity.

The screening phase included a history and physical examination, laboratory blood screening of electrolytes, liver enzymes, complete blood cell count, urinalysis, and a urine drug screen. Each subject's erythrocyte TPMT activity was determined. This enzyme is important in the metabolism of 6-MP to inactive metabolites. There is a trimodal distribution of TPMT enzyme activity in the general population. Homozygous low TPMT (<5.0 units/ml erythrocyte) occurs at a frequency of 0.3%; heterozygous low TPMT activity (5.0–13.7 units/ml erythrocyte) occurs at a frequency of 11.1%; and normal TPMT activity (13.8–25.1 units/ml erythrocyte) occurs at a frequency of 88.6%.[20] Homozygous or heterozygous low TPMT activity is associated with increased likelihood of severe neutropenia; therefore any subject with low activity was excluded.[21,22]

Subjects were instructed to abstain from ingestion of alcohol, drugs, or caffeine for 24 hr prior to and during the study. They received a low-fat, caffeine-free diet throughout the protocol. Subjects fasted overnight prior to each drug dosing and received a meal 4 hr following drug dosing, an evening meal, and a nightly snack.

Phase I

Phase I of the study was an open-label, repeat-dose trial to determine the absorption and bioavailability characteristics of AZA in the stomach, proximal jejunum, and colon. A 50 mg dose of AZA was directly administered via an oral tube into the stomach, the proximal jejunum, and the cecum. Each dose was separated by approximately 24 hr.

The design and placement of the oral tube was done based on a similar pharmacokinetics study by Williams et al.[23] On the morning of day 1 of the study, a three-lumen, 4.5-m-long enteric tube was inserted orally and advanced until the tip reached the stomach. One lumen of this tube was fitted with two pH probes, one at the level of the drug delivery port near the tip of the tube, and one 35 cm proximal to the first probe. A second lumen terminated at the distal pH probe and served as the drug administration port. The tip of the tube was fitted with a weight and a balloon that could be inflated or deflated with room air via the third lumen. Positioning of the drug administration port in the stomach was assessed by pH monitoring (distal probe indicating acidic pH, proximal probe indicating acidic or neutral pH), auscultation during air insufflation, and fluoroscopy.

A 50 mg AZA dose was administered at 8:00 AM on day 1. Azathioprine for injection (100 mg/vial, lympholized powder, Glaxo-Wellcome) was reconstituted with 10 ml of normal saline. Five milliliters of that solution was given to equal the 50 mg dose. The oral tube was flushed with 5 ml normal saline before and immediately following the dose. The volume was sufficient to assure that all of the drug was flushed from the tubing (lumen volume = 2 ml). The subjects remained in a semireclining position for 4 hr after dosing. Blood samples were collected into ethylene diamine tetraacetic acid-containing glass vacuum tubes at the following time intervals: 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, and 10 hr after dosing. Blood samples were immediately placed in an ice-water slurry. Within 30 min the blood samples were centrifuged for 10 min at 1000g and 4°C. Plasma was transferred to plastic cryotubes and stored at -70°C until analyzed.

Four hours after drug administration, the tube was allowed to advance 35 cm, with the goal to have the drug administration port in the proximal jejunum (approximately 10 cm beyond the ligament of Treitz) by the next morning. Location of the tube was assessed by pH monitoring (both probes neutral).

On the morning of day 2, with the two pH probes both recording the relatively alkaline pH of the small bowel, the tube was slowly withdrawn until the proximal pH probe displayed an acidic reading. This indicated that the proximal probe was in the stomach and that the distal probe and drug administration port were 35 cm distal to the stomach and in the proximal jejunum. Fluoroscopy was used to confirm correct positioning of the tube in the jejunum. A second 50 mg

AZA dose was administered via the oral tube and the tube was flushed as previously described. Blood samples were collected as before.

Four hours after drug administration, the balloon at the tip of the tube was inflated with 10 ml of air. Inflation of the balloon allowed the propulsive effects of gastrointestinal peristalsis to facilitate successful movement of the tube tip into the cecum, usually within 18 hr.

During the morning of day 3, fluoroscopy assured correct positioning of the tube tip in the cecum. When the drug administration port was at the desired position in the cecum, a 50 mg AZA dose was administered, and the tube was flushed. Blood samples were collected as before. At midday, the tube was withdrawn. An exit physical exam, including laboratory blood screening of electrolytes, liver enzymes, complete blood count, and urinalysis were performed on the subjects.

Phase II

Phase II of the study was an open-label comparison of the bioavailability and pharmacokinetics of AZA using three different dosages (50, 300, and 600 mg) placed directly into the cecum via an oral tube. Each dose was separated by at least 24 hr.

A three-lumen, 4.5-m-long enteric tube (described above) was inserted orally at admission, and the tube tip was advanced into the cecum over 20–48 hr. Methods previously described were used to facilitate successful movement of the tube tip into the cecum, and fluoroscopy confirmed placement.

On the morning of the first day of drug dosing, fluoroscopy was used to assure correct positioning of the tube tip in the cecum. If the position of the balloon was determined to be in the terminal ileum or cecum, the balloon was deflated and the tube secured to prevent further movement. A 50 mg AZA dose was administered. The oral tube was flushed with 5 ml normal saline before and immediately following the dose. The subjects remained in a semireclin- ing position for 4 hr after dosing. Blood samples were collected into ethylene diamine tetraacetic acid-containing vacuum tubes at the following time intervals: 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, and 6 hr after dosing. Blood samples were immediately placed in an ice-water slurry. Within 30 min the blood samples were centrifuged for 10 min at 1000g and 4°C. Plasma was transferred to plastic cryotubes and stored at -70°C until analyzed.

On the morning of the second and third day of dosing, fluoroscopy was again used to assure that the tube had not dislodged and positioning was still in the terminal ileum or cecum. Azathioprine, 300 mg and 600 mg, were given on day 2 and day 3 of drug dosing, respectively, and blood was drawn in a similar manner to that stated above. Four hours after the last dose was given on day 3, the tube was re- moved. An exit physical exam was performed on the subjects. Because of the higher dosages used in phase II, an exit red blood cell–6-thioguanine concentration was determined. Elevation in the red blood cell–6-thioguanine concentration is

associated with nonallergic type reactions such as leukopenia, opportunistic infection, and lymphoma and are dose-dependent.

Statistics

Continuous data were measured using one- way analysis of variance. To compare between-group differences, t tests were used. Simple correlation analysis was used to compare plasma concentration–time curves of AZA and 6-MP. All data are presented as means ± standard deviation. $P < 0.05$ was considered statistically significant.

Pharmacokinetic Evaluations

The Division of Bioanalysis Drug Metabolism at Glaxo Wellcome Inc. analyzed plasma samples for AZA and 6-MP using an HPLC procedure. The bioanalytical method for the determination of azathioprine and 6-mercaptopurine in human plasma employed automated solid-phase extraction of the analytes and mass-labeled internal standards from plasma using a 96-well format solid-phase extraction block with robotic liquid handling. The solid-phase extraction eluent was then injected into a liquid chromatography–mass spectrometry system that employed a reverse-phase chromatography column. The mass spectrometer produced ions via a Turbo ionspray interface, and the ions produced were subjected to multiple reaction monitoring.

Systemic exposure of 6-MP following administration of AZA was assessed by determining the area under the plasma concentration–time curve (AUC) and mean residence time (MRT) of 6-MP and AZA. AUC and MRT were determined using either one of two methods: (1) fitting the sum of coefficients and exponents with either instantaneous first-order input to the plasma concentration-time data or (2) using the trapezoidal rule.

Results

Fourteen healthy male subjects were enrolled into this study, seven subjects in each phase of the study. Two subjects, one in each phase of the study, withdrew due to inability to tolerate the oral tube. The 12 remaining subjects completed the study without difficulty. All subjects experienced local throat irritation from the oral tube, and one subject had an episode of diarrhea and mild flatulence relieved by simethicone. Subject characteristics are listed in Table 1. All TPMT concentrations were within the normal activity range.

Phase I

Six subjects received 50 mg AZA at three different absorption sites: stomach, jejunum, and cecum. No AZA or 6-MP concentration–time data were detected in one subject following administration of AZA into the stomach.

No significant difference in the AUCs and MRTs of AZA and the MRTs of 6-MP were observed following administration of AZA to the three absorption sites (Table 2), suggesting similar absorption characteristics at the three sites. The AUC of 6-MP following administration of AZA into the jejunum was significantly different when compared to the AUC of 6-MP into the cecum

Table 1. Subject characteristics

Patient	Age (yr)	Height (cm)	Weight (kg)	TPMT (units/ml)
Phase I				
1	27	184	70.5	16.1
2	28	165	70.8	15.0
3	32	190	82.3	16.4
4	28	180	80.0	17.3
5	27	185	100.3	17.3
6	26	177	85.4	18.6
Phase II				
7	26	169	85.0	16.3
8	36	178	76.2	15.5
9	23	180	95.0	19.0
10	23	181	77.3	15.2
11	21	180	76.4	16.5
12	28	180	93.3	16.7
Mean ± SD	27.1 ± 3.9	179.1 ± 6.4	82.7 ± 9.1	16.7 ± 1.2

Table 2. Comparison of AUC and MRT of AZA and 6-MP following administration of 50 mg of AZA into stomach, jejunum, and cecum

	AZA		6-MP	
Site	AUC (ng × hr/ml)	MRT (hr)	AUC (ng × hr/ml)	MRT (hr)
Stomach	22.3 ± 7.3	0.9 ± 0.4	39.9 ± 38.1	2.6 ± 0.9
Jejunum	22.5 ± 10.4	0.6 ± 0.2	67.0 ± 30.1	1.6 ± 1.1
Cecum	18.4 ± 6.7	0.6 ± 0.1	29.2 ± 10.9*	2.5 ± 2.0

*$P < 0.05$ cecum vs jejunum.

(Table 2). The AUC of 6-MP into the stomach was also lower than that of the jejunum, but did not reach statistical difference. These data support the idea that the bioavailability of 6-MP is higher if AZA is administered into the jejunum as compared to the stomach and cecum. Azathioprine absorption from the jejunum was 1% higher than absorption from the stomach and 22% higher than absorption from the cecum. Gastric absorption of AZA was 17% higher than absorption from the cecum. Likewise, 6-MP bioavailability from the jejunum was 68% higher than from the stomach and 129% higher than from the cecum. Gastric bioavailability of 6-MP was 27% higher than absorption from the cecum.

Phase II

Six subjects were to receive 50, 500, and 600 mg of AZA into the cecum, resulting in 18 drug concentration-versus-time profiles for each drug. Two subjects did not receive a 50-mg dose in phase II. Azathioprine and 6-MP concentrations in two subjects receiving the 50 mg dose, and one receiving the 300 mg dose were too low to detect. One patient's AZA concentration after the 300 mg dose could be analyzed, but the 6-MP concentration was too low to detect. Thus, 13 AZA and 12 6-MP concentration-time profiles were analyzed.

Table 3. Comparison of AUC and MRT of AZA and 6-MP following administration of different doses of AZA into cecum (Phase II)

	AZA		6-MP	
Dose [mg (N)]	AUC (ng × hr/ml)	MRT (hr)	AUC (ng × hr/ml)	MRT (hr)
50 (4)	16.9 ± 7.4	0.7 ± 0.6	22.2 ± 14.9	2.5 ± 2.0
300 (5)	52.3 ± 67.2	0.5 ± 0.3	63.4 ± 50.6	2.7 ± 1.8
600 (6)	132 ± 151	0.7 ± 0.9	104 ± 115	1.4 ± 0.2

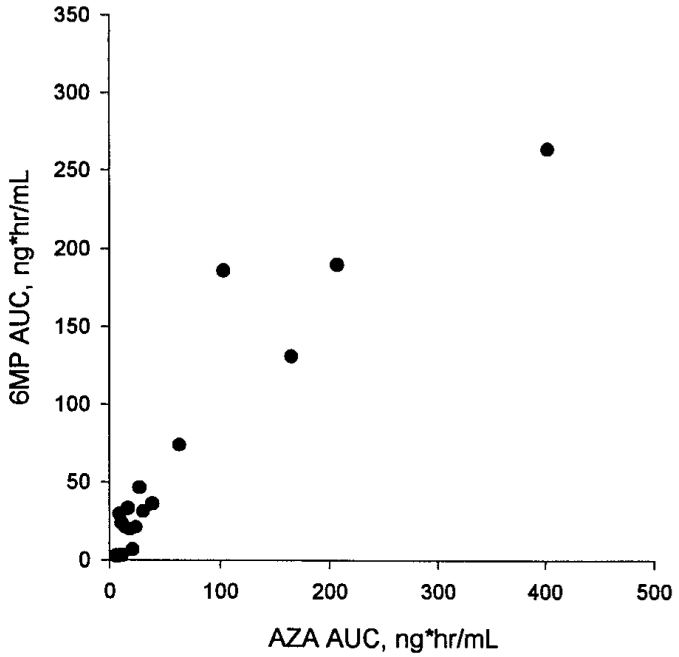

Figure 1. Correlation of individual subject's azathioprine area under the plasma concentration-time curve (*x* axis) to 6-mercaptopurine area under the plasma concentration-time curve (*y* axis). $r^2 = 0.83$, $P < 0.0001$.

The MRTs following administration of 50, 300, and 600 mg into the cecum were similar and AUCs of AZA and 6-MP increased with increasing doses (Table 3). There was approximately a three- and eight-fold increase in AZA AUC when the 50-mg dose was compared to the 300-mg and 600-mg doses. Likewise, there was a three- and five-fold increase in 6-MP AUC from the 50 mg dose compared to the 300 mg and 600 mg doses. At doses administered in this study, a saturable conversion of AZA to 6-MP was not observed (Figure 1).

In both phases, the relationship between AZA dose and AUC varied considerably from subject to subject. Although considerable interpatient variability was seen, a statistically significant correlation between individual AZA AUC and 6-MP AUC was found (Figure 1).

Discussion

AZA is a prodrug that undergoes approximately 88% conversion to 6-MP.[24] The conversion of AZA to 6-MP has been reported to occur rapidly via a nonenzymatic nucleophilic action by sulfhydryl-containing compounds such as glutathione present in red blood cells (RBCs) and other tissues.[25] Neither parent AZA nor 6-MP have immune modifier activity.[26] Activity has been associated with 6-MP metabolites, 6-thioguanine nucleotides. The 6-thioguanine nucleotides apparently act by inhibiting the synthesis of proteins, DNA, and RNA.[26] Because glutathione is present in every mammalian cell, including colonic epithelial cells and lymphocytes, it may be expected that local conversion of AZA to 6-MP would occur in these cells. When mice were give oral AZA, very high concentrations of 6-MP were found in intestinal mucosa and intermediate levels of 6-thioguanine necleotides were also present.[29] Because many drugs are poorly absorbed from the colon, it is reasonable to postulate that if AZA was not well absorbed into the systemic circulation, then local generation of 6-MP and 6-thioguanine necleotides would result in local immunomodulatory effects.

In this study, both AZA and 6-MP plasma concentrations were analyzed. No significant differences were seen comparing the AUC and MRT of AZA between the stomach, jejunum, and cecum. The AUC of 6-MP between the stomach, jejunum, and cecum were substantially different. There was a 67% increase in AUC when jejunal administration was compared with gastric administration. AUC decreased by 29% when cecal administration was compared with gastric administration. These data support that the bioavailability of 6-MP is higher if AZA is administered into the jejunum as compared to the stomach or cecum. This interpretation assumes that the AUC of 6-MP is determined by the fraction of AZA converted to 6-MP, the fraction of formed 6-MP available for absorption, and the clearance of 6-MP.

A previous study by Van Os et al[17] analyzing the pharmacokinetics of AZA also showed decreased bioavailability when the drug was administered in the cecal area. Azathioprine 50 mg was given orally and in three different colonic delivery forms to healthy male volunteers. Significantly lower bioavailability of 6-MP was seen after cecal AZA administration via delayed release oral, hydrophobic rectal foam, and hydrophilic rectal foam (7%, 5%, 1%; respectively) than that of oral AZA administration (47%). Van Os et al[17] showed an 85% decrease in bioavailability between oral and delayed-release oral capsules and a 99% reduction in bioavailability from hydrophilic rectal foam. The decreased colonic bioavailability between the stomach and colon reported in their study was greater than the decrease reported in our study. One could speculate that the injectable solution we used, instilled directly into the cecum, was better absorbed by the colonic absorptive tissue than the delayed-release products used in the previous study. The delayed-release oral capsule used by Van Os et al[17] was a crushed AZA tablet placed in a capsule and coated with Eudragit-S, a polymer used to delay the dissolution of the capsule to allow it to reach the colonic area. This enteric-coated capsule may not have totally dissolved in the cecal area, which may have resulted in the capsule dissolving somewhere in the large bowel providing less absorptive area for the drug. In support of this theory is the

differences reported between the rectal foams and the enteric-coated capsule. The rectum probably has poorer absorption of 6-MP than the colon.

Additionally, the differences reported may in part be due to the complexity of the metabolism of AZA and interpatient variability.[27] Previous pharmacokinetic studies have reported wide interindividual variation in AUC and bioavailability with oral AZA and 6-MP.[17,19,27] In our study, interpatient variability in bioavailability was also considerable, particularly when comparing bioavailability of 6-MP from AZA administered into the cecum.

In the second phase, larger doses of AZA into the cecum resulted in increased AZA and 6-MP AUCs but these increases were not proportional to the dose increase and the percentage increase between individual patient's AZA or 6-MP AUCs also was not proportional.

In a similar study by Zins et al[28], doses of 200, 400, and 600 mg were given into the cecum via a delayed-release capsule. The 6-MP AUCs following those doses were 34.2 ± 27.7, 72.4 ± 68.5, and 74.4 ± 74.3 ng × hr/ml, respectively. Almost identical mean 6-MP AUC values for the 400 mg and 600 mg delayed-release oral AZA doses were observed, suggesting saturation of a specific transport mechanism for AZA or saturable conversion of AZA to 6-MP in the terminal ileum for doses 400 mg or greater. In our study, a saturable process was not confirmed, although the mean 6-MP AUC for the 600-mg dose was greater than that seen in the previous study.[28] The differences in AUC may again be largely due to the differences in colonic delivery as well as interpatient variability.

Whether ileocolonic delivery of AZA in IBD would be successful is not known. The indication for ileocolonic delivery of AZA in IBD is based on an assumption that local intestinal delivery of AZA may result in local immunomodulatory effects on intestinal leukocytes. Glutathione present in the colonic epithelial cells and lymphocytes is thought to convert the AZA to 6-MP, similar to systemic conversion. In a study by Kurowski and Iven,[29] mice dosed with oral AZA (50 mg/kg) showed high concentrations of 6-MP in the intestinal mucosa, suggesting conversion of AZA to 6-MP in the gut wall. Furthermore, Erdmann et al[30] have shown that lymphocytes do contain the enzymes necessary to convert 6-MP to 6-thioguanine nucleotides. To our knowledge, no study has investigated whether ileocolonic delivery of AZA is effective in IBD.

From our study a decrease in bioavailability of 6-MP was shown after cecal administration compared to jejunal and gastric administration. Higher dosages of AZA presented to the cecum do result in increased systemic absorption, but may still allow more drug to be administered with less toxicity than the same dose received orally. The effect of ileocecal delivery of AZA in patients with IBD should be investigated.

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