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Absorption of m-AMSA and Its Biliary Metabolites from the Rat Small Intestine

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Abstract—*The ability of orally administered m-AMSA (NSC-249992) to be absorbed was examined in the rat. Studies using isolated* in situ *intestinal loops showed that m-AMSA* (25 μM) *was absorbed equally well in jejunum and ileum and that absorption was* 50% *complete in* 5–10 min *and* 90% *complete by* 60 min. *In the intact rat, orally administered m-AMSA was found to be* 93% *absorbed at a dose of* 50 mg/kg *and* 82% *absorbed at a dose of* 200 mg/kg. *Absorption was measured* 8 hr *after dosing. m-AMSA biliary metabolites were found to be poorly absorbed by the gut. Following a dose of m-AMSA* (10 mg/kg, *i.v.*), *rats were observed for* 4 hr *and less than* 10% *of the available m-AMSA biliary metabolite was absorbed. Enterohepatic circulation of m-AMSA appears to be insignificant.*

INTRODUCTION

m-AMSA (NCS-249992) [4′-(9-acridinylamino)methanesulfon-m-anisidide] is an acridine derivative that has generated considerable clinical interest because of its demonstrated activity against leukemias and selected solid tumors [1, 2]. The drug has been given intravenously in most of the reported Phase I and Phase II trials but oral administration has also been clinically explored [3]. Earlier work in our laboratory has shown that blood levels in mice are much lower for an oral as compared to an intravenous dose [4]. Since m-AMSA has an extensive biliary excretion, the lower blood concentrations may arise either from a simple failure of gut absorption or from absorption of the drug followed by rapid removal of the drug from the portal circulation through biliary excretion. This paper examines the absorption of m-AMSA from the rat gut in some detail and also provides information on the existence and extent of m-AMSA enterohepatic recirculation.

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MATERIALS AND METHODS

Materials

m-AMSA·HCl (9-[^{14}C]-labeled, 11.4 mCi/mmol) was obtained from Stanford Research Institute, Menlo Park, CA. ACS Scintillation fluid and NCS Tissue Solubilizer were products of Amersham Corp., Arlington Heights, IL. All experimental animals were Sprague–Dawley rate (300–500 g) obtained from the Small Animal Facilities, NCI, NIH and maintained on Purina Rat Chow and water.

Intestinal loop studies

Rats were anesthetized with pentobarbital sodium (45 mg/kg, i.p.). The intestines were exposed through a ventral mid-line incision and the flexura duodenojejunalis and valvula ileocoecalis were identified. A cannula was inserted into the jejunum approximately 2 cm caudal to the flexura and tied in place. The intestine was cut approximately 12 cm caudal to the cannula insertion and the segment of gut gently flushed clear of fecal matter with normal saline phosphate buffer, pH 7.4. The cut end was ligated and [^{14}C]-labeled m-AMSA in phosphate-buffered normal saline was introduced through the cannula in a volume sufficient to slightly distend the entire jejunal

segment. The concentration of the infused m-AMSA was 25 μM, a value just below the solubility limit of m-AMSA in phosphate-buffered normal saline, pH 7.4. A 12 cm segment of ileum just rostral to the valvula ileocoecalis was similarly prepared. After a specified drug dwell time, the gut segment was gently stripped from the mesentery, one end cut and lumen contents drained by gravity into 5 ml of normal saline. The gut was then cut open and dropped into the saline. After a 15–20 sec wash the gut was removed and washed in 50 ml of fresh buffer, blotted dry and homogenized in 3 ml of distilled water. Aliquots of the saline wash and of the gut homogenate were mixed with ACS scintillation fluid and counted in a Beckman 9000 scintillation counter. Prior to addition of scintillation fluid the gut homogenate was dissolved in NCS.

Intact gut

For 24 hr prior to use in the absorption studies rats were restricted to water alone. Rats were anesthetized with pentobarbital sodium (45 mg/kg, i.p.) and the intestines exposed through a ventral incision. The bile duct was cannulated with PE-10 tubing and bile collected over the duration of the experiment. Cannulas were placed for blood sampling. The drug [(^{14}C]-labeled) was then introduced into the small intestine by syringe just caudal to the flexura. The m-AMSA was administered as a suspension in 0.5 ml of 0.5% carboxymethylcellulose. The abdomen was closed and the rat held in a restraining cage until the termination of the experiment. After 8 hr the rat was killed and the entire gut removed, cut open along its entire length and washed extensively. The washes were combined and aliquots counted on a Beckman 9000 scintillation counter.

Oral toxicity studies

Rats were fasted for 24 hr prior to use in the study. A stomach intubation cannula attached to a 1 ml syringe was used to administer the drug directly into the stomach of the rat. Three rats were used at each dose. The drug was given as a suspension in 0.5% carboxymethylcellulose with m-AMSA concentration adjusted to keep administered volume to 0.5 ml. Rats alive 28 days following the dose of m-AMSA were defined as having received a non-lethal dose. The toxic end-point of the experiment was death.

Bile metabolite absorption

The following method was used to determine if biliary metabolites are reabsorbed by the gut. Two rats were used for each experiment. The rats were anesthetized; each rat had a iliac artery cannulated with polyethylene tubing; each rat had its bile duct cannulated with PE-10 tubing; and one rat (the primary rat) had the free end of its bile duct cannula introduced into the intestine of the other (secondary) rat just caudal to the flexura and tied in place. The primary rat was given intravenous m-AMSA (10 mg/kg dissolved in water). Blood samples from both rats as well as bile samples from the secondary rat were collected for the next 4 hr, after which time both rats were killed. The entire gut of the secondary rat was removed and saved separately from the remainder of the rat. The primary rat, the secondary rat and the gut from the secondary rat were separately homogenized and aliquots combusted on a Packard Sample Oxidizer and counted in a Beckman scintillation counter.

RESULTS

Intestinal loop studies

The results of the intestinal loop studies are shown in Fig. 1. There appears to be no difference between the rate of absorption of m-AMSA in jejunum and ileum. The initial uptake of the drug was rapid, with 50% absorption from the lumen in the first 5–10 min. As the drug was absorbed and the lumenal concentration of m-AMSA fell, the rate of absorption slowed. By the 60-min time point

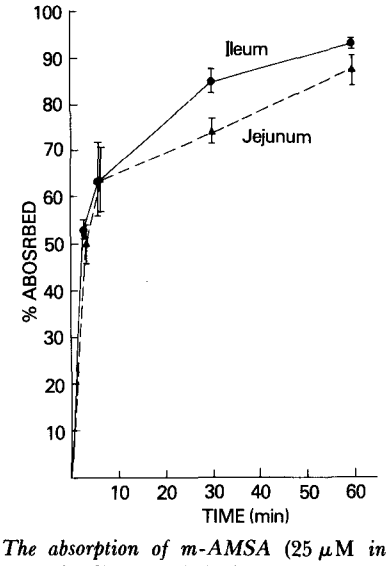

Fig. 1. The absorption of m-AMSA (25 μM in phosphate-buffered normal saline, pH 7.4) from regions of the rat small intestine. (●) Ileum and (▲) jejunum. Each point is the average of 4 rats and error bars show ± S.D.

absorption was 90% complete. An analysis of drug retained in gut tissue revealed that 40% of the absorbed dose was still present in gut tissue at early time points; however, by 60 min the percentage retained by the gut fell to only 10%.

Oral toxicity and absorption

In the intact animal, drug was absorbed rather well at non-lethal doses. The dose–toxicity relationship for oral m-AMSA treated rats is presented in Table 1. No animal death occurred below 200 mg/kg but, as the m-AMSA dose was increased from 200 mg/kg to 250 mg/kg, striking changes occurred and the drug progressed from being non-lethal to being nearly uniformly lethal. The same table presents data from absorption studies. The highest non-lethal dose (200 mg/kg) showed 80% drug absorption at 8 hr. This percentage is only slightly less than the 90+% observed at the lower doses. Plasma concentrations of m-AMSA were low in all orally dosed animals, with maximum concentrations of less than 0.2 μg/ml detected at doses of 200 mg/kg. Bile excretion was rapid and dose-dependent. At a dose of 25 mg/kg, the 8 hr biliary excretion was 23.5 ± 6.2 (S.D.)% of the dose, while at the highest dose of 200 mg/kg, 8 hr biliary excretion was 47.2 ± 8 (S.D.)% of the dose.

Enterohepatic reabsorption

Because the hepatic clearance of m-AMSA is substantial, the potential exists for significant enterohepatic circulation of parent drug or metabolites. Four dual-rat studies were performed, as described in "Methods", and the results are shown in Table 2. When the experiment was terminated, the carcass of the primary rat was found to contain only half of the administered dose (44 ± 18%). Thus, the bile which was infused into the gut of the secondary rat contained 56 ± 18% of the administered dose of radioactivity in the form of bile excretory products. Only 9.3 ± 5.6% of this available [^{14}C]-label was absorbed. Most (90.7%) of the counts in the infused bile remained in the gut of the secondary rat. Attempts to monitor radioactive label in the blood of the secondary rat failed to detect any change from background.

Table 1. Oral absorption and oral toxicity of m-AMSA in rats

m-AMSA dose (mg/kg)	Rats alive day 28	Percent m-AMSA absorbed in 8 hr ± S.D.
25	5/5	93.0 ± 0.8
50	5/5	92.5 ± 3.8
100	5/5	77.9 ± 11.0
150	5/5	N.D.
200	4/5	81.8 ± 3.9
250	0/5	N.D.

DISCUSSION

Although the gut absorbs substances through a variety of mechanisms, including active transport, facilitated diffusion, and simple diffusion, most organic bases appear to be absorbed by simple diffusion [5]. Since simple diffusion from the gut requires drug passage through lipid membranes, the rate of diffusion is dependent on the ionization state of the drug. For weak organic bases, a general requirement for rapid absorption is $pK_a < 9.0$ [5]. m-AMSA, a weak organic base with a $pK_a = 7.4$ and only moderate lipid solubility [6], marginally satisfies this requirement; thus, its ability to be absorbed is not obvious from physicochemical considerations alone. The intestinal loop studies presented in this paper demonstrate that solutions of m-AMSA are well absorbed and that absorption occurs throughout the small intestine and is not restricted to jejunum or ileum.

Interestingly, the *in vivo* absorption of m-AMSA is slower than would be expected from the studies of the intestinal loop. In the isolated loop, 50% of the drug was absorbed in 10 min while, in the highest oral dosing

Table 2. Extent of enterhepatic circulation as shown by dual-rat experiments—distribution of [^{14}C]-label 4 hr after administration (as a percentage)

Experiment	Retained in primary rat	Secondary rat Absorbed	Secondary rat Non-absorbed	Secondary rat Absorption of bile radioactivity
1	48	4	48	7.6
2	68	2	30	6.2
3	30	4	66	5.7
4	38	11	51	17.7
Mean ± S.D.	44 ± 18	5.3 ± 3.9	49 ± 14.8	9.3 ± 5.6

experiments, 80% absorption required 8 hr. An explanation of this difference may rest in the poor aqueous solubility of m-AMSA. When a 300 g rat was dosed at 200 mg/kg it received 60 mg of drug. At saturation, approximately 6 l of gut fluid would be required to completely dissolve the 60 mg of m-AMSA. Since the rat clearly does not have 6 l of lumenal fluid, solid drug must be continually dissolving as solubilized drug is absorbed. While the percentage rate of solubilized drug absorption is high (50% in 10 min), the absolute absorption rate is slow compared to the drug dose to be absorbed. Alternatively, some of the delay may be caused by binding of drug to retained fecal material. The rats were fasted prior to use to reduce the amount of fecal material in the gut, but the larger amount of fecal material in the fasted gut as compared to washed gut may play a role in the prolonged absorption seen *in vivo*. Regardless, m-AMSA absorption, up to uniformly lethal doses, was substantially complete in 8 hr.

Once absorbed, m-AMSA is quickly metabolized by the liver and the metabolic products, containing less than 5% parent compound, are secreted into the bile [7]. The presence of these metabolites in the bile raises the possibility of an m-AMSA enterohepatic recirculation. The dual-rat studies were designed to answer the simple question of whether such a recirculation exists. The finding, at 4 hr, of a 10% absorption of radioactivity from the bile of m-AMSA treated rats establishes the existence of an enterohepatic recirculation. However, since there is only one primary bile metabolite (90% of bile radioactivity) [7], the low enterohepatic recirculation percentage precludes good absorption of the primary metabolite. This metabolite has been identified as an AMSA-glutathione conjugate and would not be expected to be well absorbed [7]. The absence of a large enterohepatic recirculation is consistent with the pharmacokinetic patterns seen in the blood of both laboratory animals and man. With extensive enterohepatic recirculation, a rise in blood concentrations should be seen when the biliary products are secondarily absorbed; but, in contrast, published plasma pharmacokinetic curves have all been monotonically decreasing [2, 8].

These results have certain implications for clinical studies. Because of hepatic sequestering and biliary excretion, a percentage of absorbed m-AMSA will never clear the liver and enter the systemic circulation. Any estimate of oral absorption based on pharmacokinetic analysis of blood concentrations will underestimate the percentage of drug absorbed. For most purposes this consideration is academic, but it could be important in evaluating the response of hepatic disease.

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