Poor and Unusually Prolonged Oral Absorption of Amphotericin B in Rats

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INTRODUCTION

Amphotericin B is the drug of choice in the treatment of systemic fungal infections (1). It is usually administered as short (4–6 h) intravenous infusion because of its poor (5–9%) oral bioavailability (1,2). However, no reason for the low bioavailability has been reported. Moreover, oral absorption and bioavailability data of amphotericin B in other species is unknown.

The purpose of this study was to determine the pharmacokinetics of amphotericin B following oral administration in conscious rats. Potential reasons for the low bioavailability and unusually prolonged absorption (up to 96 h) were explored. In addition, the contribution of mean hepatic transit time (MTT_h) to overall mean absorption time (MAT) was also determined.

METHODS

Materials

Amphotericin B for injection USP, abbreviated as Am-B (each vial contains a lyophilized mixture of 50 mg amphotericin B, 41 mg sodium desoxycholate, buffered with 20.2 mg of sodium phosphates; Pharmacia Inc., Albuquerque, NM), was reconstituted with either 5% dextrose injection USP (Baxter, Deerfield, IL) or distilled water. Amphotericin B powder and 2,4-dinitrophenol (DNP) were purchased from Sigma (St. Louis, MO). Sprague Dawley rats from Harlan Sprague Dawley Inc. (Indianapolis, IN) were used in all studies.

Oral Absorption Studies

Am-B in 3-ml distilled water was administered as single oral gavage (1.6 to 16.7 mg/kg) to ten conscious male rats (270–400 g) and 0.25-ml blood samples were collected from an exteriorized artery (3,4) at various time intervals for 5 days. All blood samples, in this and subsequent experiments, were immediately centrifuged and the separated plasma was analyzed for amphotericin B by HPLC.

In Situ Closed Intestinal Loop Study

Fasted male rats (n = 6; 240–340 g) were anesthetized with urethane and a total of five 10-cm loops, 3 jejunal and 2 ileal loops, were made in each rat (5). Following dosing with Am-B in 5% dextrose (0.5 ml of 1 mg/ml), the amount remaining unabsorbed in the loop after 3, 6 and 8 h was estimated as the sum of amounts recovered from the lumen and mucus.

Gastrointestinal Recovery After Oral Dosing

A single oral dose of Am-B (5 mg) in 3-ml water was administered to conscious rats (n = 7) via oral gavage. The rats were sacrificed by excessive ether inhalation at 9, 24, 48 and 72 hours post dosing and the entire rat gut (except esophagus) was isolated and the amount of drug remaining in the stomach, duodenum, jejunum, ileum, cecum and large intestine were quantified (4,6).

Hepatic Mean Transit Time

The right jugular vein and carotid artery of seven male rats (222–440 g) were cannulated for intravenous infusion and blood sampling, respectively (4). Three rats (# 34–36) were infused with Am-B in 5% dextrose (100 μg/ml) at 0.008 ml/min for 24 hours and thereafter the flow rate was reduced to 0.0040 ml/min for Rat # 34 and 35, and 0.0027 ml/min for Rat # 36, and infusion continued for an additional 24 hours. Rat # 37–40 were infused with Am-B in 5% dextrose (300 μg/ml) at a flow rate of 0.026 ml/min for 40 min and thereafter a concentration of 60 μg/ml was infused at 0.0043 ml/min for 32 hours. After cessation of infusion, the rats were sacrificed and residual blood in the liver was depleted by flushing 20 ml of saline through the portal vein (7). The liver samples were homogenized and analyzed using HPLC. Liver concentrations in Rat # 39 and 40 were not analyzed due to incomplete flushing of blood from the liver.

High Performance Liquid Chromatographic Assay

The slightly modified HPLC method of Golas *et al.* (8) which was previously described (3,9) was employed for analysis of amphotericin B concentrations in rat intestinal lumen, mucus, tissue, and, bile, liver and plasma.

DATA ANALYSIS

The total area under the curve (AUC_{oral}) was calculated using the trapezoidal rule; the extrapolated area to infinity was estimated as the ratio of the last concentration to the mean terminal first-order rate constant obtained from an earlier intravenous bolus study using 10 rats (3,9). The absolute oral bioavailability (F) was calculated by (4,10)

$$F = \frac{AUC_{oral} \cdot CL}{\text{Oral Dose}} \quad (1)$$

where CL is the mean total plasma clearance (116 ml/h/kg)

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obtained earlier in ten rats after intravenous administration (3,9). The mean residence time after oral administration (MRT_{oral}) was calculated as the ratio of the area under the first moment curve (AUMC) to AUCoral (10).

The MAT was calculated by (10)

$$MAT = MRT_{oral} - MRT_{iv} \quad (2)$$

where MRT_{iv} is the mean residence time (20.1 h) following intravenous dosing to ten rats (3,9).

Total plasma clearance from the infusion study was estimated as the ratio of infusion rate to steady-state plasma concentration (Cpss) (10). A blood:plasma ratio of 1.36 (3) was used for the estimation of hepatic/blood partition ratio (Rh).

The MTT_h was determined by (11 and references therein)

$$MTT_h = \frac{Vss}{Q_H} \quad (3)$$

where Vss is the product of Rh and the hepatic tissue volume and, Q_H, the hepatic blood flow rate, was assumed to be 11.8 ml/min/250 g (12) and scaled by allometry using an exponent of 0.75 (13) for individual rats.

RESULTS AND DISCUSSION

Amphotericin B plasma concentrations following single oral administration of Am-B in water (dose 1.6 to 16.7 mg/kg) in individual rats are depicted in Fig. 1 and the estimated pharmacokinetic parameters are listed in Table I. Following oral gavage, plasma concentrations increased to a maximum by 5 to 9.5 h, and remained fairly constant thereafter for prolonged periods of time (up to 96 h) in most rats. The mean F ± S.D. was found to be only 4.8 ± 3.8 % in the present study (Table I) that is similar to the low bioavailabilities reported in humans (1,2). A preliminary study (n = 3; 232–270 g) involving oral dosing with freshly prepared amphotericin B suspension in water (5 mg in 3 ml) yielded a similar mean F (5%) and similar plasma profiles as Am-B in water, indicating an apparent lack

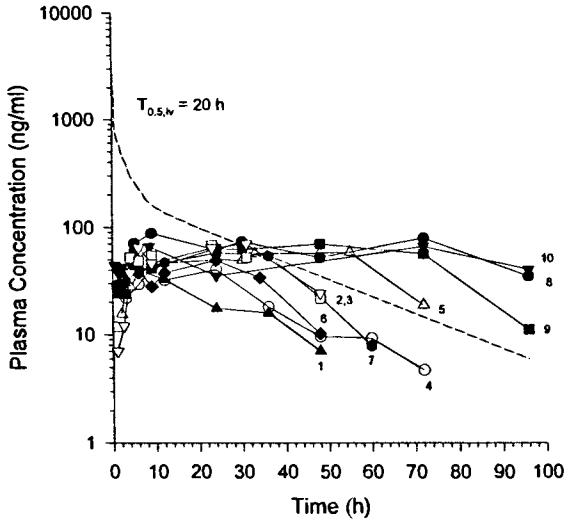

Fig. 1. Amphotericin B plasma concentrations following single oral gavage of Am-B in water (1.6 to 16.7 mg/kg) to ten rats. Dashed line: typical intravenous plasma concentration profile following 1 mg/kg bolus dose in rats.

Table I. Pharmacokinetic Parameters of Amphotericin B Obtained After Oral Administration to Ten Rats

Rat #	Body weight (g)	Dose (mg)	%F	MRT_{oral} (h)	MRT_{oral}-$MRT_{i.v}$ (h)
1	310	0.5	9	27	7
2	325	1.0	13	39	19
3	330	2.2	5	34	14
4	400	3.5	2	32	12
5	320	3.5	2	36	16
6	340	3.5	2	31	11
7	340	3.5	3	33	13
8	280	3.8	6	57	37
9	270	3.8	5	49	29
10	300	5.0	1	45	25

of formulation effect on oral bioavailability in the present study. Since, first-pass hepatic metabolism of amphotericin B is anticipated to be negligible (theoretical hepatic extraction ratio = 0.035) in rats (3,4), potential reasons for the low F such as instability or metabolism of drug in gut or poor permeability were investigated using the in situ intestinal closed loop method.

Very high mean total recoveries of administered dose, such as 95.2, 91.0 and 87.5% from the jejunal loops and, 95.0, 92.4 and 94.4% from the ileal loops at 3, 6 and 8 h, respectively,

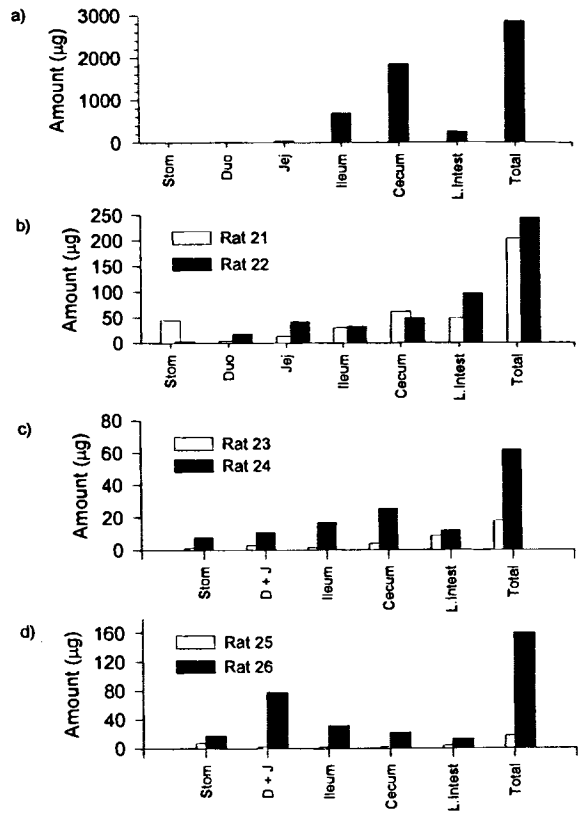

Fig. 2. Amounts of amphotericin B recovered from different regions of rat gut at a) 9 h; b) 24 h; c) 48 h and d) 72 h following a single oral dose (4.5 mg) of Am-B in water. Stom: stomach; Duo: duodenum; Jej: jejunum; D + J: duodenum and jejunum; L.Intest: large intestine.

indicated poor absorption and good stability of drug in the gut. The recovery from the gut tissue was low at all the times studied with a mean recovery of 4.2 and 2.8% from the jejunal and ileal loops, respectively. The similar percent of dose remaining in the jejunal and ileal loops also indicated the lack of existence of practically significant absorption window. The poor bioavailability is thus hypothesized to be mainly due to poor GI permeability. Poor solubility and hence slow dissolution from precipitated amphotericin in the lumen may also be a potential factor contributing to the low bioavailability.

Based on our previous intravenous studies in rats, a mean terminal-phase amphotericin B plasma half-life of only 20 h was estimated (3,9). In light of this, the unusually prolonged plasma concentrations up to 96 h obtained following a single oral administration is very interesting because this implies continuous absorption of drug from the GI tract for very extended periods of time. This is completely unexpected and exceeds gastric emptying time which is in the order of minutes (14) and small intestinal transit time of 2–3 h (5) in rats. The steady plasma concentrations do not seem to be due to active transport since similar average amphotericin B plasma concentrations of 15 ng/ml and 18 ng/ml were observed following introduction of 1 ml of 1 mg/ml Am-B dose into a ligated loop of rats (n = 3) containing 1 mM of 2,4-dinitrophenol, an active transport inhibitor (15,16), and that of control rats (n = 4), respectively. Additionally, during steady-state intravenous infusion, <3% of the amount eliminated from the body was collected in the diverted bile of rats. Therefore, enterohepatic circulation was expected to contribute minimally to the prolongation of the steady plasma levels. This was subsequently confirmed in a bile duct diverted rat whose plasma concentrations were prolonged up to 96 hours following a 15 mg/kg oral dose. Although, prolonged oral absorption up to 18–24 h has been previously reported (5) for chlorothiazide in rats, prolonged absorption of amphotericin B found in the present study may be the longest reported thus far.

Since an approximate absorption rate of only 2–3 μg/h, estimated as the product of the steady plasma concentration and CL (10), was required to maintain a typical steady plasma concentration of 70 ng/ml seen after oral dosing. It is therefore hypothesized that residual amounts of the slowly-dissolving drug (drug particles were seen in the stomach) remaining in the GI tract from a single oral dose may contribute to the prolonged absorption observed.

The distribution and amounts recovered from various regions of gut at 9, 24, 48 and 72 h are presented in Fig. 2. At 9 and 24 h post dosing a total of 64% and 5% of the administered dose were recovered, respectively. However, the recovery of detectable levels of drug at 48 and 72 h post dosing (Fig. 2) from the gut was surprising. Although very small percentages of the total dose were recovered, 0.4–1.3% and 0.4–3.5%, after 2 and 3 days, respectively, the actual amounts of 18–62 μg and 18–160 μg, after 2 and 3 days, respectively, exceed the amount needed to sustain plasma concentrations. This may also explain the apparent increase in MRT_{oral} (Table I) seen with an increase in dose; a small percentage of a larger dose is probably capable of sustaining plasma concentrations for longer periods of time. The variability in amount recovered could explain the variability in the length of time for which plasma levels are prolonged in different rats. For example, # 26 is more likely to exhibit steady plasma concentrations up to 96 h because of a higher amount recovered (160 μg) in contrast to Rat # 25 whose total amount recovered was only 17.5 μg at the end of 72 h post dosing (Fig. 2).

The presence of drug in stomach and upper small intestine at 72 h in conscious unfasted rats was completely unexpected (Fig. 2) and may represent the first report of its kind in rats. Perry *et al.* (17) have shown little contractile activity in the orad region compared to the aborad portion of the stomach following administration of barium suspension in rats. Therefore, a possible explanation for the prolonged presence of amphotericin B in the stomach could be retention of a portion of precipitate in the orad portion of the stomach and its subsequent slow emptying into the intestine.

Chiou (11) has previously shown that the conventional method of estimation of MAT (i.e., $MRT_{oral} - MRT_{iv}$) may be incorrect when the contributions of MTT_h and MTT_{gw} to MRT_{oral} are significant, such as seen with chloroquine and hydroxychloroquine in rats whose MTT_h were 16 and 13 h, respectively. In the present oral absorption study, the difference between MRT_{oral} and MRT_{iv} was unexpectedly large, as high as 37 hours for a 14 mg/kg dose (Table I); which is much greater than the reported rat small intestinal MTT of 2 to 3 hours (5). Therefore, the MTT_h of amphotericin B at steady-state was determined to estimate its contribution to MRT_{oral}.

As listed in Table II, an average MTT_h of only 1 hour was estimated for amphotericin B at steady-state. It is also of interest

Table II. Summary of Relevant Information on Mean Hepatic Transit Time Study of Amphotericin B in Seven Rats

Rat #	B.wt. (g)	Inf. rate (μg/h/kg)	Css (ng/ml)	CL (ml/h/kg)	Liver conc. (ng/g)	Rh	Vss (ml)	MTT (min)
34	410	61.9	447.5	138.4	36.4	59.8	1054	61.6
35	390	62.8	444.2	141.3	34.1	56.4	952	57.8
36	440	34.8	274.9	126.6	24.9	66.5	1249	69.2
37	340	44.6	364.5	122.5	29.0	58.5	873	58.8
38	380	38.1	253.4	150.3	14.9	43.1	710	44.0
39	340	47.0	315.0	149.2				
40	220	61.6	529.2	116.5				
Mean	360			135.0		56.9	968	58.3
SD	71			13.3		8.6	201	9.1

to note that the steady-state concentrations observed in conscious rats in the present study were close to the plasma levels estimated based on the mean CL of 121.9 ml/h/kg obtained following bolus administration in male rats in our previous study (3,9).

In summary, the poor bioavailability of amphotericin B is mainly due to poor GI permeability. The prolonged absorption beyond mean rat GI transit time is probably due to the presence of residual amounts of drug in the gut, which exceeds the calculated absorption rate required to maintain the observed steady plasma concentration. The presence of drug in the stomach for extended times after dosing can be rationalized to be due to precipitation and to an apparent "reservoir effect" of the orad region of the stomach due to reduced contractility. The results of the present rat study is consistent with the hypothesis that man and rat are alike in the extent of oral absorption of drugs (18).

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