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Research Article

Effect of Food Status on the Gastrointestinal Transit of Amphotericin B-Containing Solid Lipid Nanoparticles in Rats

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Abstract. Amphotericin B (AmB) is poorly absorbed from the gastrointestinal tract. Recent studies have suggested enhanced drug absorption from solid lipid nanoparticles (SLN). Little is known of the fate of AmB absorption within the gastrointestinal tract, and no gastrointestinal transit study has yet been performed on AmB-containing nano-formulations. We aimed to investigate the effect of food on the gastrointestinal transit properties of an AmB-containing SLN in rats. Three SLNs containing AmB, paracetamol, or sulfasalazine were formulated using cocoa butter and beeswax as lipid matrices and simultaneously administered orally to Sprague-Dawley rats. Paracetamol and sulfapyridine were used as marker drugs for estimating gastric emptying and cecal arrival, respectively. The pharmacokinetic data generated for paracetamol and sulfapyridine were used in estimating the absorption of the AmB SLNs in the small and large intestines, respectively. A delayed rate of AmB absorption was observed in the fed state; however, the extent of absorption was not affected by food. Specifically, the percentages of AmB absorption during the fasted state in the stomach, small intestine, and colon were not significantly different from absorption within the respective regions in the fed state. In both states, however, absorption was highest in the colon and appeared to be a combination of absorption from the small intestine plus absorption proper within the colon. The study suggests that AmB SLN, irrespective of food status, is slowly but predominantly taken up by the lymph, making the small intestine the most favorable site for the delivery of the AmB SLNs.

KEY WORDS: amphotericin B; gastrointestinal transit; paracetamol; solid lipid nanoparticles; sulfapyridine.

INTRODUCTION

Amphotericin B (AmB) is a polyene antimycotic agent with broad spectrum activity and is very effective in treating life-threatening fungal infections. Despite its benefits, AmB has poor aqueous solubility and also causes nephrotoxicity, which could lead to permanent renal impairment especially if co-administered with other drugs that cause renal toxicity. Currently, AmB is principally delivered intravenously but this route of administration is associated with adverse effects like fever, chills, rigors, malaise, headache, generalized aches, nausea, vomiting, and hypoxia (1). There is evidence that lipid-based AmB formulations present reduced renal toxicities (2–4) and enhanced bioavailability (compared with AmB suspensions) when delivered orally (3–5). One such lipid-based delivery system is solid lipid nanoparticles (SLNs), which are submicron (10 to 1000 nm) particles formulated from biocompatible solid lipids to produce a matrix within which a drug may be molecularly dispersed. Improved bioavailability, safety, and protection against degradation of the drug payload in the stomach are some of the desirable attributes associated with oral delivery of SLNs. Furthermore, it is possible to tailor formulations to ensure controlled release of the payload from the SLNs (6–10). Uptake of intact SLNs via the lymphatic route into blood has been reported in some studies after oral and duodenal administrations of SLNs to rats. In this regard, the involvement of the Peyer's patches in the uptake of SLN has been indicated (11,12). Prior to their uptake, SLNs may adhere to the gut wall, which prolong their residence time within the gut. An increase in residence time has been linked to improved bioavailability of drugs. SLNs may also be emulsified by lipases to produce surface active mono- and diacylglycerols which can solubilize the incorporated drug prior to uptake and emptying in the lymph, a process which has been reported to augment absorption (13).

The intake of food can considerably alter the bioavailability of orally administered drugs. This alteration may manifest as a result of changes in the drug dissolution process prior to absorption, changes in GI residence time of the dosage form, or modification in membrane permeability of the drug. Furthermore, the type and quantity of food, as well as the time interval between food intake and administration of the dosage form, may reduce, delay, increase, or accelerate drug

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absorption. In some cases, there is no effect on drug absorption. Furthermore, different formulations or dosage forms of the same drug may be affected differently by food (14,15). A study in humans showed that the small intestinal transit time (SITT) of dosage forms is more consistent than the gastric transit time (GTT) and that the former was not affected by the nature (physical state or size) of the administered dosage form or by the presence of food in the stomach. On the other hand, GTT was affected by the aforementioned factors (16). It is therefore necessary that studies involving food status on the absorption of drugs from their various dosage forms are conducted in order to allow appropriate assessment of the resulting pharmacokinetic data, which in turn can be used to optimize the formulation to maximize bioavailability.

In a previous work, we have successfully developed an AmB-containing SLN formulation, subjected the same to extensive characterization (17–19) and we have performed a pilot GI transit study of the formulation in fasted rats (5). In the present paper, we describe the pharmacokinetics of AmB as a consequence of food status and the GI transit of AmB-containing SLNs using an indirect estimation method (20–22). Paracetamol (PAR) was used to estimate the GTT of the SLNs based on the rate of its appearance in the blood following oral administration of PAR-containing SLNs (23) similarly formulated as the AmB and sharing identical physical characteristics (5). Sulfapyridine (SP) is a metabolic product from the activity of colonic flora on sulfasalazine (SSZ) and is very quickly and almost completely absorbed from the colon into the blood and, thus, served as an estimate of the arrival time of the SSZ-containing formulation at the cecum (24,25), also similarly formulated as AmB SLNs (5).

PAR and SP have been used as marker drugs to estimate the gastric emptying (GE) and orocecal transit times of pellet-filled capsules in humans (20,26) and liquids in monkeys (21,22), as well as in our previous pilot study (5). These marker drugs provide a cheaper, safer, and more appropriate alternative to the use of gamma scintigraphy for evaluating GI transit of pharmaceutical formulations in experimental animals like rats.

MATERIALS AND METHODS

Materials

Cocoa butter (JB Cocoa Sdn Bhd, Johor, Malaysia), beeswax (Acros Organic, New Jersey, USA), amphotericin B (Nacalai Tesque, Inc., Kyoto, Japan), and sulfasalazine (Tokyo Chemical Industry Co. Ltd, Japan) were purchased from the respective manufacturers. Paracetamol and sulfapyridine were purchased from Sigma-Aldrich (Sigma-Aldrich Co. LLC., Missouri, USA), lecithin soy and sodium cholate were obtained from MP Biomedicals (Illkirch, France). Chloroform, ethyl acetate, and methanol were purchased from Fisher Scientific (Loughborough, UK). All other reagents and chemicals used were of analytical grade or high-performance liquid chromatography (HPLC) grade.

Preparation of SLNs

The three types of SLNs (containing AmB, PAR, or SSZ) were prepared as previously described (5,17). Briefly, 50 mg of drug (AmB, PAR, or SSZ) and 120 mg of lecithin were initially dissolved in a 40-mL mixture of chloroform and methanol at a 1:1 ratio, along with 200 mg each of cocoa butter and beeswax. The solvent was evaporated off using Rotavapor® R-200/205 (Büchi, Switzerland) at 50°C. The resulting drug-lipid matrix was melted in 20 mL of ethyl acetate at 70°C and added to 40 mL of 2.5% *w/v* sodium cholate solution at the same temperature. The mixture was homogenized at 10,000 rpm using an IKA T 25® homogenizer (IKA, Germany) for 6 min. A total of 60 mL of water at 70°C was then added slowly to the mixture with continuous stirring for 20 min after which the organic solvent was evaporated off using the Rotavapor at 70°C.

Animals

Six male Sprague-Dawley rats weighing 250±20 g obtained from the Animal Holding Unit of the University of Science, Malaysia, were used for the study. The study protocol complied with the recommendations of and was approved by the Animal Ethics Committee of the University of Science, Malaysia. The animals were maintained under a 12/12 h dark/light cycle.

The rats were randomly divided into fasted and fed groups and were allowed free access to food and water prior to commencement of the study. Water was withdrawn from both groups until 2 h post dose administration. The fasted group was fasted overnight and allowed access to food 8 h after dosing with the SLNs.

Drug Administration and Blood Sampling

All the animals were given a single-dose oral gavage containing 10 mg/kg of each SLN (AmB, PAR, and SSZ) dispersed in distilled water. Blood samples (300 μL) were collected from the tail end of the rats before dosing and at 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, 24, and 30 h post dose administration into heparinized microcentrifuge tubes. Plasma was immediately separated from the blood samples by centrifugation and then frozen until analysis.

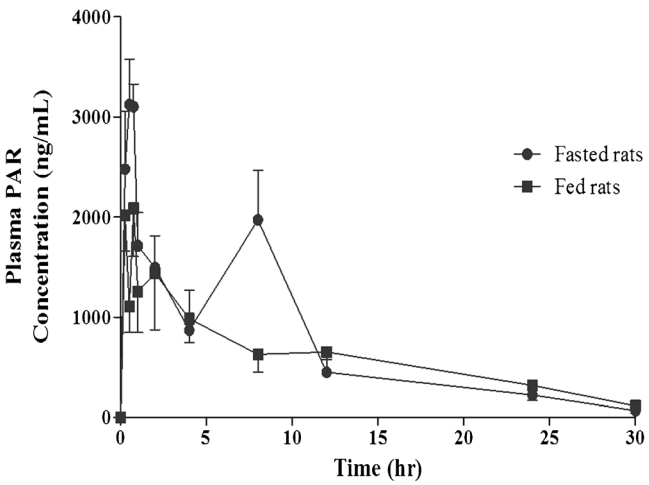

Fig. 1. Effect of food on absorption of PAR SLN in rat GI tract

Table I. Mean Pharmacokinetic Parameters for PAR After Simultaneous Oral Gavage of SLNs Containing AmB, PAR, and SSZ Each at an Equivalent Dose of 10 mg/kg (mean±SD, *n*=3)

Group	Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-30} (ng·hr/mL)
Fasted	10	0.5±0.25	3616.3±68.4*	21,786±5879.8
Fed	10	0.75±0.29	2383.3±747.9	18,135.7±5712.2

AUC area under the curve
*$p<0.05$—the difference between fasted and fed groups is statistically significant

Plasma Sample Treatment

A 150 μL aliquot of internal standard (IS) solution (piroxicam in a methanol/ethanol (1:1) mixture) was added to 100 μL of rat plasma. The methanol/ethanol mixture served as a solvent for the IS and a deproteinizing agent for the plasma. The samples were then vortex-mixed for 5 min and centrifuged at 14,000 rpm for 10 min. The supernatants obtained were filtered through a 0.20-μm filter and then analyzed for drug (AmB, PAR, and SP) content using a validated HPLC method (27).

Estimation of Gastric Transit Time

The parameters for estimating the time elapsed for GE or GTT were obtained from the plasma PAR absorption-time profiles and based on the assumption that the percentage of PAR absorbed was directly related to the percentage of SLNs emptied from the stomach to the duodenum (20). This served as a fairly good estimate since all three types of SLNs shared identical physical characteristics and therefore the assumption was that all three SLNs would transit similarly within the GI tract (5). The time for complete emptying of the SLNs (T_{90P}) from the stomach was estimated using the time for 90% of PAR absorption in the small intestine, which was also considered to be the GTT. Another parameter obtained from the plasma PAR absorption-time profile was the time for 10% PAR absorption (T_{10P}), which signaled the arrival of the SLNs at the small intestine.

Estimation of Small Intestine Transit Time

The SITT was estimated as the time difference between the cecal arrival of the SLNs and the start of emptying of the SLNs into the small intestines (T_{10P}). SITT was therefore approximately the difference between T_{10S} (the time taken for 10% SP absorption in the cecum) and T_{10P}.

Table II. Individual Values for T_{10P}, T_{90P}, and GTT Estimated from Plasma PAR Profile (*n*=3)

Rat no.	Fasted		Fed	
	T_{10P} (h)	T_{90P} (GTT) (h)	T_{10P} (h)	T_{90P} (GTT) (h)
1	0.16	1.56	0.08	1.74
2	0.18	2.38	0.16	2.38
3	0.09	1.19	0.10	2.63
Mean	0.14	1.71	0.11	2.25
SD	0.04	0.61	0.04	0.46

GTT gastric transit time, *SD* standard deviation

Estimation of Cecal Arrival Time and Colonic Transit Time

Data obtained from the plasma SP concentration-time curves were used to determine the cecal arrival time (CAT) of the AmB SLNs. The CAT by definition is the time taken for the SLNs to arrive at the cecum and has been estimated as the time for the initial detection of SP in the plasma using the indirect method of estimation (25). Due to the time lapse in SP production from SSZ released from SSZ SLNs reaching the cecum, CAT was estimated to be approximately T_{10S}. T_{10S} serves as a better estimate for CAT of the SLNs than the first SP detection in plasma as the latter may be mainly due to free SSZ released from the SLN rather than released SSZ from the intact SSZ SLNs within the cecum. The colonic transit time (CTT) was estimated as the time for 90% SP absorption (T_{90S}).

Estimation of AmB Absorption in the Stomach, Small Intestine, and Colon

The percentages of AmB SLNs absorbed in the stomach, small intestine, and the colon were estimated from the plasma AmB absorption-time profiles using the respective transit times (GTT and SITT). The percentage of AmB absorbed during gastric transit or GTT was estimated from the time of dose administration to T_{10P}. The time from T_{10P} to T_{10S} or SITT was used to estimate the percentage absorption in the small intestine while the remaining percentage of AmB SLNs absorbed after this point was considered as the summation of both colonic and progressed lymphatic absorption.

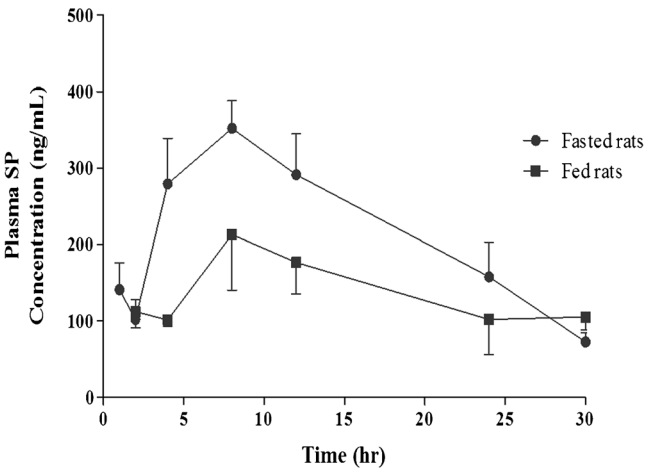

Fig. 2. Effect of food on absorption of SP (from SSZ) in rat colon

Table III. Individual Values of T_{max}, T_{10S}, CAT, and SITT Under Fasted and Fed States Estimated from SP Plasma Profiles (n=3)

Rat no.	Fasted				Fed			
	T_{max} (h)	T_{10S} (CAT) (h)	SITT (h)	CTT (h)	T_{max} (h)	T_{10S} (CAT) (h)	SITT (h)	CTT (h)
1	8	1.92	1.76	19.9	8	0.92	0.84	23.6
2	12	0.93	0.75	13.9	12	1.42	1.26	22.7
3	8	2.54	2.45	18.8	12	3.36	3.26	27.5
Mean	9.3	1.80	1.65	15.5*	10.7	1.90	1.79	24.6
SD	2.3	0.81	0.86	3.2	2.3	1.29	1.29	2.6

CAT cecal arrival time, *SITT* small intestinal transit time, *CTT* colonic transit time, *SD* standard deviation
*p<0.05—the difference between fasted and fed groups is statistically significant

Statistical Analysis

The data have been presented as mean±SD where indicated. Statistical analyses were performed using GraphPad Prism 5 software. A statistically significant difference was considered when the p value <0.05, using paired t test for fasted and fed groups.

RESULTS AND DISCUSSION

Gastric Transit of the AmB SLNs

The mean plasma PAR concentration-time profiles in both fasted and fed rats are presented in Fig. 1, and the pharmacokinetic data obtained from the profiles are presented in Table I. In both the fasted and fed states, we observed a rapid PAR absorption albeit slower rate in the fed state. Furthermore, multiple peaks were observed within the first 4 h of the PAR concentration time profile for the fed rats, possibly due to the effect of food. A later peak occurred at 8 h post dose administration in the fasted rats and can be attributed to the delayed appearance of PAR in the plasma due to possible uptake of intact SLNs by Peyer's patches via the lymph (11,12) followed by assimilation and PAR release into the blood. This is a slow process and therefore expected to manifest late. In the fed rats, however, no such sharp peak at a later time is observed as the presence of food slowed the absorption rate of the intact SLNs. PAR has better aqueous solubility as compared with AmB or SSZ hence PAR favorably partitions out of the hydrophobic lipid matrix of the SLNs into the more aqueous GI fluids leaving most of the absorbed SLNs in the fed rats containing very minimal amounts of the drug. The presence of food caused an insignificant reduction in C_{max} (p=0.05) and area under the curve $(AUC)_{0-30}$ (p=0.484) and a delay in T_{max} (p=0.725) of PAR absorption. It is clear from the mean values that a delayed T_{max} was caused by the presence of food through a slower gastric emptying process of the SLNs. In both fasted and fed groups, no lag time in PAR absorption was observed; however, this phenomenon is most likely a result of the rapid emptying of PAR solution released from the SLN within the administered dose and subsequent absorption in the small intestine. The absorption of this PAR solution therefore occurred almost instantly after dose administration to the rats and emptying of this solution into the small intestines occurred unobstructed. PAR absorption in humans has been found to be largely hindered in patients with pyloric stenosis and may cause a delay in GE (23); however, the data obtained in the present study suggest unimpeded emptying into the small intestine. Magnetic resonance monitoring was used in a study to estimate GE time in rats (equivalent to T_{90P}) and was found to be approximately 1.67 and 2.34 h after liquid (ferrofluid) and solid (ferrite powder pellets and laboratory chow) meals were orally administered, respectively (28). These values are comparable with those observed in the present study which are 1.71 and 2.25 h for the fasted and fed rats, respectively, as presented in Table II.

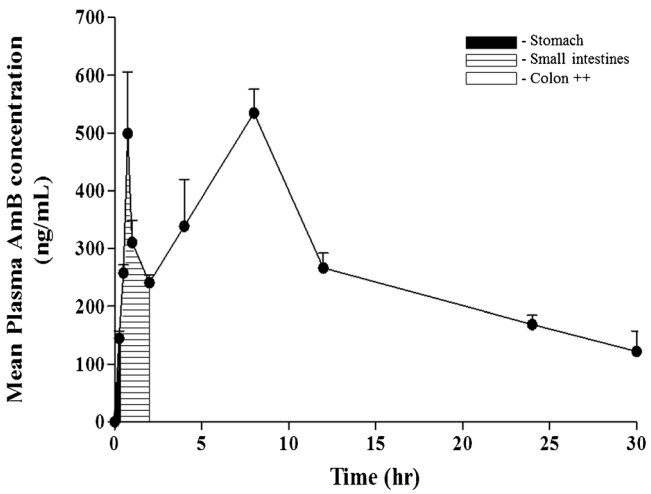

Fig. 3. Absorption of AmB SLNs in the fasted rats

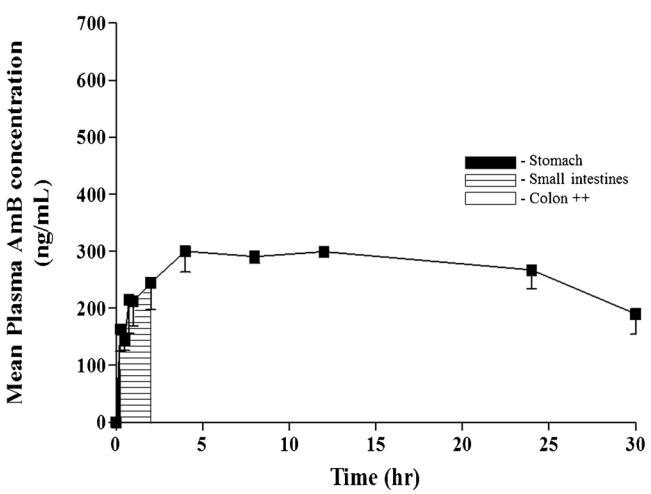

Fig. 4. Absorption of AmB SLNs in the fed rats

Table IV. Pharmacokinetic Parameters for AmB After Simultaneous Oral Gavage of SLNs Containing AmB, PAR, and SSZ Each at an Equivalent AmB Dose of 10 mg/kg (mean±SD, n=3)

Group	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-30} (ng·hr/mL)
Fasted	10	8±0	564.7±122.46*	7953±551.23
Fed	10	9.33±4.62	323.2±43.96	7565.33±1390.64

*p<0.05—the difference between fasted and fed groups is statistically significant

Small Intestinal Transit Time of AmB SLNs

The mean SITT of the SLNs in the fasted rats was 1.65 and 1.79 h in the fed group, giving a time difference of only about 0.14 h. This shows that transit of the SLNs in the small intestines was similar in both food states. In a study conducted by Quini *et al*. (28), an identical mean value of approximately 1.7 h was obtained as SITT in both liquid meal- and solid meal-fed rats using magnetic resonance monitoring in the estimation. In humans, the same observation was made, where food did not significantly affect the SITT of orally administered dosage forms, whether a solution, pellets, or a single unit dosage form was administered (16,20,29).

Cecal Arrival and Colonic Transit Times of the AmB SLNs

A number of studies on pellets or suspensions have estimated cecal arrival time as the time for the initial detection of SP in plasma (20–22,26). In the present study, T_{10S} was used to estimate CAT of the AmB SLNs instead of the time for the first appearance of SP in plasma which with most certainty is a result of SSZ released from the SLN as solution and arriving at the cecum ahead of the SLNs. Such an earlier arrival of the solution SSZ would lead to the hydrolysis of free SSZ and not SSZ released from intact SLNs reaching the cecum and therefore will underestimate the CAT. A more accurate CAT estimation using the first SP detection in plasma requires more frequent blood sampling, which is a constraint in the use of this indirect approach. Figure 2 and Table III show the mean SP concentration-time profiles in plasma and the individual pharmacokinetic data obtained from the profiles, respectively. Both curves showed a similar absorption pattern. Initially, there was a slight drop in plasma SP concentrations in both food statuses after which the plasma SP level rose and attained a T_{max} at 8 h. The first concentration point in each food status is due to the absorption of SP after colonic bacterial activity on released SSZ which arrives at the colon relatively earlier than the SSZ SLNs. The slight drop in SP concentration thereafter can be attributed to a depleted source of this dissolved SSZ that arrived ahead of the SLNs due to microbial action. On the other hand, the slow rise in SP absorption in both food states was due to the time lapse required for the release of SSZ from the SSZ SLNs and microbial action by colonic bacteria when the latter was resident in the colon. The mean CAT was found to be only 0.1 h longer in the fed rats as compared with the fasted group (p=0.912).

Methods employed in measuring CTT in humans include the use of radio-opaque markers or gamma scintigraphy and involve tracking the time it takes for ingested markers to travel along the large bowel. The mean CTT obtained in one human study with and without the use of barium paste were 74.9±49.3 and 85.7±46.1 h, respectively (30). In a study by Enck *et al*. (31), a mean CTT value of 15.5 h was obtained in rats. In that study, a carmine red solution was infused into the cecum of the rats and the time for the first discharge of a red-colored fecal pellet was considered as CTT. Usually in CTT determination, subjects or animals are allowed to maintain their usual dietary intake and medications if any, provided the latter does not interfere with intestinal motility. In the present study, the mean CTT in the fasted rats was found to be 15.5 h, which was statistically shorter (p=0.04) than that observed for the fed rats (24.6 h). The difference in the values obtained in the present study and that from the study by Enck *et al*. (31) is due to the methods employed by both groups. The CTT estimation by Enck *et al*. (31) was based on infusing of the marker solution directly into the cecum, which has the potential of staining the fecal matter already present before the study commenced.

Estimated AmB Absorption in the Stomach, Small Intestine, and Colon

All three types of particles were spherical in shape and appeared to have somewhat rough surfaces as previously reported (5). In a previous study, we have shown that AmB is molecularly dispersed with the lipid matrix of the SLNs (19)

Table V. Estimated Percentage Absorption of AmB from SLNs in the Stomach, Small Intestines, and Colon (n=3)

Rat no.	Fasted			Fed		
	Stomach (%)	Small intestines (%)	$Colon^{++}$ (%)	Stomach (%)	Small intestines (%)	$Colon^{++}$ (%)
1	3.4	53.5	43.1	1.9	19.8	78.3
2	3.4	32.4	64.2	3.8	40.4	55.8
3	1.5	46.4	52.1	4.0	51.3	44.7
Mean	2.8	44.1	53.1	3.2	37.2	59.6
SD	1.1	10.7	10.6	1.2	16.0	17.1

SD standard deviation

and that all three SLNs shared identical physical characteristics (5) and therefore expected to respond to the hydrodynamics of the GI similarly. A molecularly dispersed arrangement of AmB within the lipid matrix favors prior interaction of the lipid with the intestinal epithelia and assimilation of the SLNs in Peyer's patches (10,11,13). The mean plasma AmB concentration-time profiles in the fasted and fed rats are depicted in Figs. 3 and 4 respectively, and the pharmacokinetic data obtained from them are shown in Table IV. The first and second boundaries demarcated in Figs. 3 and 4 represent T_{10P} and T_{10S}, respectively, and the various regions within the profiles correlate the extent of absorption of AmB within these respective regions.

As shown in Figs. 3 and 4, the plasma concentration *versus* time curves obtained for the absorption of AmB from the AmB SLNs under fasted and fed conditions presented two peaks, with those from the fasted rats being more prominent and occurring at 0.75 and 8 h while in the fed group, 4 and 12 h, respectively. The presence of food slowed down the rate of AmB absorption, similarly to that observed in the absorption of PAR from SLNs (Fig. 1). Using TEM analysis, intact SLNs have been observed circulating the lymph and blood 30 min post duodenal administration of SLNs to rats (11). We believe that the appearance of the second peak is attributed to the process of SLN uptake by Peyer's patches and then assimilation of AmB in the lymph, prior to emptying in the blood. This process is thus slow, and hence, AmB only appears in blood after a finite time. The latter is the basis for "colon^{++}" designated in Figs. 3 and 4. Absorption of the AmB SLN in the colon^{++} regions is attributable to a combination of absorption processes in the colon *per se* as well as the continued absorption process via the lymph in the small intestines explained earlier. This means that plasma AmB concentration post CAT is not attributed solely to colonic drug absorption. At the present time, we have not established the proportion of colonic only AmB absorption against the same via the lymph. However, we believe that the incidence of the second peak culminates from prolonged absorption of AmB due to slowed transit SLNs within the gut (7).

The C_{max} for AmB obtained from the fasted rats is well defined and significantly higher (p=0.03) than that in the fed group, signifying a more rapid rate of absorption of AmB in the fasted state. There was no significant difference (p=0.854) between the AUC_{0-30} in the two groups. The absence of sharp peaks in the AmB absorption profile in the fed rats (Fig. 4) and relatively steady plasma concentration during the study period is a feature that can be exploited for controlled AmB delivery.

Table V shows the estimated percentage absorptions of AmB from the SLNs in the various GI regions during the fasted and fed conditions. In both cases, the highest percentage absorption occurred in the colon^{++} region, indicating major colonic and lymphatic absorption. There was no statistically significant difference (p>0.05) between the fasted and fed groups with regard to percentage absorptions in the respective GI regions (pair-wise comparisons), confirming that although the presence of food slowed the rates of absorption of AmB SLNs, the extent of absorption of AmB remained unchanged under either food status. The stomach represented the poorest percentage absorption of AmB albeit slightly higher in the fasted state. A significantly higher level of absorption occurred in the small intestines compared to the stomach and slightly higher in the fasted state (44.1 and 37.2% obtained for the fasted and fed rats, respectively). These findings agree with those obtained by Li *et al*. 2009 who showed that the ileum and colon were the main segments of absorption of quercetin-containing SLNs.

CONCLUSIONS

An indirect method was used to study the GI transit of AmB SLN, and the data obtained indicate that the presence of food slowed the rate of absorption of the AmB from the SLNs, but the extent of absorption remained essentially unchanged. Furthermore, the percentage AmB absorption was lowest in the stomach region but significantly higher in the small intestine and this process was slow to manifest and continued post cecal arrival of the SLNs. Some absorption of AmB appears to occur in the colon; however, this amount is masked by the continued lymphatic absorption process in the small intestine resulting in late AmB appearance in the plasma.

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