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Evaluation of oral formulations of gentamicin containing labrasol in beagle dogs

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Abstract

Gentamicin (GM) is a polarized water-soluble compound having very poor intestinal membrane permeability resulting in low oral bioavailability. Labrasol was found to improve the intestinal absorption of GM in rats. In the present study, GM formulations containing labrasol were evaluated in beagle dogs after filling into hydroxypropylmethyl cellulose (HPMC) capsules wrapped with Eudragit L100 (Eud L) and Eudragit S100 (Eud S) films. The results of the in vitro drug release studies could not differentiate between two kinds of enteric capsules and among the three kinds of GM formulations. Oral administration of GM solution at a dose of 50.0 mg per dog of GM and 0.60 ml per dog of labrasol has resulted in C_{max} values of 2.38 ± 0.50 μg/ml and 2.30±0.42 μg/ml with Eud L and Eud S capsules, respectively. The AUC values obtained were also higher at 4.35±1.31 μg h/ml and 5.34±0.95 μg h/ml with Eud L and Eud S capsules, respectively. Formulation of GM as a suspension in labrasol has resulted in the decrease of C_{max} values by two to four times and AUC values by >2.5 times compared to the solution formulation. The above results indicate that solution formulation was better over the suspension. An absorbent, synthetic sponge was used to absorb GM solution formulation and encapsulated with Eud L and Eud S capsules. The C_{max} and AUC values obtained with sponge formulation were higher than those of suspension formulations but were lower than solution formulations. There was no significant difference in the extent of GM absorption between Eud L and Eud S capsules used for encapsulating GM formulations.

Keywords: Gentamicin sulfate; Labrasol; Enteric polymers; Oral delivery; Absorption enhancer

1. Introduction

Gentamicin (GM) is a polarized water-soluble compound having very poor intestinal membrane permeability resulting in low oral bioavailability [BA] (Cox, 1970; Recchia et al., 1995). GM is an aminoglycoside antibiotic widely used in the treatment of a variety of Gram-negative bacilli and Gram-positive cocci infections (Drabu and Blakemore, 1990; Fantin et al., 1991). Because of poor absorption after oral administration, GM is clinically used as parenteral or topical dosage forms. However, parenteral administration is associated with side effects such as nephrotoxicity and ototoxicity (Kaloyandres and Munoz, 1980; Lerner and Matz, 1980). Many studies have been carried out to improve oral absorption of GM (Berkovitch et al., 1993; Constantinides, 1995). A derivative of taurocholic acid, TC002, was reported (Axelrod et al., 1998) as an absorption enhancer for GM. Various non-

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ionic, anionic and cationic surfactants have been investigated as intestinal permeation enhancers. Surfactants that are too hydrophobic to be water-soluble are poor absorption enhancers and surfactants that are very hydrophilic cannot partition into the hydrophobic environment of the lipid bi-layer thereby resulting in poor intestinal absorption promoting effect (Swenson and Curatolo, 1992). For non-ionic surfactants, the hydrophilic–lipophilic balance alone is not a reliable predictor of absorption enhancing capability. The absorption enhancing ability is influenced by the size and shape of both the alkyl chain and the polar group. A medium length alkyl chain surfactant may penetrate the lipid bi-layer easily, and because of its aqueous solubility has a greater monomer concentration and higher critical micellar concentration than a longer alkyl chain surfactant. Labrasol is a surfactant that contains predominantly alkyl chain lengths of C_8 and C_{10} and is having an HLB value of 14. It contains saturated polyglycolyzed C_6–C_{14} glycerides, where C_8 is 58.1% and C_{10} is 39.8%, and its NMR characterization indicated that it is a mixture consisting of 30% mono-, di- and tri-glycerides of C_8 and C_{10} fatty acids, 50% of mono- and di-esters of polyethylene glycol (PEG) and 20% of free PEG 400 (Kreilgaard et al., 2000). The LD_{50} value by oral route in rats was reported to be 22 g/kg and a 13-week oral administration study in dogs has indicated that labrasol was safe without any adverse effect at a dose of 1.0 g/kg per day. Recent studies in our laboratory have indicated that labrasol has a strong absorption enhancing effect on GM (Hu et al., 2001). In situ administration of GM formulations at a dose of 5.0 mg/kg of GM and 1.0 ml/kg of labrasol to the rat colon resulted in improved BA of GM. Hence, in the present study attempts were made to develop oral delivery systems for the delivery of GM formulations to lower small intestine where the absorption enhancing effect of labrasol was found to be more. These delivery systems were evaluated for their in vivo performance by administering to beagle dogs.

2. Materials and methods

2.1. Materials

Gentamicin sulfate (Content of GM: 673 μg/mg), 1-pentanesulfonate, sodium sulfate, boric acid, PEG 400 and acetic acid were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Labrasol (Gattefösse, France) was a gift from CBC Co., Ltd. (Tokyo, Japan). Hydroxypropylmethyl cellulose (HPMC) capsules were gratis sample from Shionogi Qualicaps Co., Ltd. (Yamatokoriyama, Japan). *O*-Phthalaldehyde (OPA) was procured from Ishizu Seyaku, Ltd. (Osaka, Japan). Eudragit® S100 (Eud S) and Eudragit® L100 (Eud L) were obtained through Higuchi Inc. (Tokyo, Japan) from Röhm GmbH (Darmstadt, Germany). Triethyl citrate was purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Male beagle dogs used in the study and standard solid commercial food (Labo D Stock®) were obtained from Nippon Nousan Co., Ltd. (Yokohama, Japan). Sponge was obtained commercially in the local retail market. All other materials used were of reagent grade and were used as received.

2.2. Preparation of enteric polymer films

Two types of enteric polymer films, Eud L and Eud S, were used in the study. Eud S films were prepared by dissolving the Eud S in acetone to get a 25% (w/v) solution containing 12.5% (w/v) of Triethyl citrate as plasticiser. Then the solution was casted on a Teflon plate, $10 \times 10\ cm^2$, and the solvent was evaporated at 10 °C for 12 h. The dried films were removed from the plates and their thickness was measured. The average thickness of the films was found to be 45 ± 2.31 μm. Eud L films were prepared by dissolving the Eud L in a mixture of methylene chloride and methanol (1:1) to get a 30% (w/v) solution containing 7.5% (w/v) of PEG 400 as plasticiser. Then, the solution was casted on Teflon plates and allowed to dry as described above. The average thickness of the dried films was found to be 53 ± 3.12 μm.

2.3. GM formulations

The composition of the formulations used in this study is given in Table 1. The solution formulation was prepared by dissolving GM in deionized water followed by the addition of labrasol. The resulting transparent solution was equilibrated at ambient temperature overnight and was filled into size '00' HPMC capsules. The capsules were wrapped with enteric polymer films (Eud S or Eud L, 3.5 cm × 5.5 cm) and

Table 1
Gentamicin formulations used in the studies

Formulation	Gentamicin sulfate[a] (mg)	Water (ml)	Labrasol (ml)
Solution	74.29	0.20	0.60
Suspension	74.29	0.00	0.60
Sponge[b]	74.29	0.20	0.60

The doses of GM and labrasol were 50.0 mg per dog and 0.60 ml per dog, respectively, in all the experiments.

[a] 74.29 mg of gentamicin sulfate = 50.0 mg of gentamicin.

[b] The solution formulation was absorbed on to synthetic sponge.

sealed with a heat sealer. The suspension formulation was prepared by suspending GM (particle size: <177 μm) in labrasol through vortex mixing. The suspension was filled into size '00' HPMC capsules, wrapped with Eud L and Eud S films as described above. GM solution formulation was also absorbed onto a hollow cylindrical sponge piece (length: 2.0 cm, diameter: 2.5 cm, wall thickness: 0.6 cm). Initially, the sponge piece was put into size '00' HPMC capsules and sealed. Then, a small hole was made in the capsules using a 21G needle. Then, required volume of the GM solution was added onto the sponge through the hole using a syringe fitted with 23G needle. The pore of the capsules was closed with synthetic glue and the capsules were wrapped with enteric polymer films as described above. All the enteric capsules were checked for the presence of pinholes in the enteric wrappings by placing them in 0.1 N HCl for 30 min under mild mixing conditions. The capsules into which the acid solution was penetrated were excluded from the study.

2.4. Drug release studies

Both Eud S and Eud L capsules of all the three formulation were evaluated for their drug release characteristics. The studies were carried out using USP dissolution rate test apparatus (Apparatus 1 at 100 rpm) in 900 ml of pH 7.4 phosphate buffer solution maintained at 37 °C. Samples of 5 ml each were collected at 0.25, 0.5, 1, 1.5, 2 and 3 h intervals and replaced with fresh pH 7.4 phosphate buffer solution pre-warmed to 37 °C. The samples were diluted, filtered through 0.45 μm filters and analyzed for their drug content using HPLC post column labeling method that was developed by Hitachi Co., Ltd. A model LC-10AS pump (Shimadzu Corp.) was used to deliver the mobile phase (1.0 ml/min) containing 0.02 M 1-pentanesulfonate, 0.05 M sodium sulfate and 0.1% acetic acid in water–acetonitrile mixture (98:2). A Hitachi Gel #3056 column (C_{18}-ODS, 5 μm, 15 cm × 4.6 mm i.d.) was used for the analysis. The OPA reagent (6.0 mM), which contains 0.35 M boric acid, 0.30 M sodium hydroxide and 0.03 M 2-mercaptoethanol, was delivered at a flow-rate of 0.5 ml/min to the column effluent via a mixing T-piece with a LC-10AS pump (Shimadzu Corp.). A reaction coil consisting of a Teflon tube (0.33 μm × 7 m) was placed between the mixing T-piece and a fluorescence detector (Shimadzu RF-10AXL fluorometer) set at an excitation wavelength of 360 nm and an emission wavelength of 450 nm. The calibration of GM was linear over 0.05–20.0 μg/ml. The detection limit for GM was 0.05 μg/ml.

2.5. GM absorption studies in beagle dogs

Pharmacokinetic studies were carried out after oral administration of enteric capsules containing three kinds of GM formulations viz. solution, suspension and sponge to dogs. Three adult male beagle dogs (weighing 10.0–12.1 kg) were fasted overnight for at least 12 h with free access to water. The dogs received each of the test preparations in a crossover fashion with a washout period of one week. All experiments were carried out at the same time of the day to exclude the influences by circadian rhythm. At 15 min before administration, a control blood sample (1.0 ml) was taken from the jugular vein. Each dog was orally administered with a test capsule containing 50.0 mg of GM at 9.30 a.m. with 10.0 ml of 0.1% citric acid solution and 30 ml of water. After oral administration of the test preparation, 1.0 ml blood samples were collected from the jugular vein at 0.5, 1, 2, 3, 4, 5, 6 and 8 h. The plasma fraction used for GM assay was obtained by centrifuging the blood samples at 12,000 rpm for 5 min using Kubota 1720 centrifuge (Tokyo, Japan). These plasma samples were immediately frozen at −80 °C until analysis. For the determination of absolute BA of GM following the administration of different formulations, GM solution in sterile saline (2.5 mg/ml) was administered intravenously at a dose of 5.0 mg per dog and blood samples were collected at different time intervals. All the

animal experiments were carried out in accordance with the Guidelines for Animal Experimentation in Kyoto Pharmaceutical University.

2.6. Estimation of GM in plasma samples

GM in plasma was purified according to Anhalt's method (Anhalt, 1977; Tawa et al., 1998). A column was prepared from CM-Sephadex (C25) (Muromachi Kagaku Kogyo Kaisha, Ltd., Tokyo, Japan) with a bed volume of 1.0 ml. The column was washed with 1.0 ml of 0.2 M sodium sulfate solution (rinse buffer). A 100 μl volume of plasma was applied to the column followed by 1.0 ml of the rinse buffer. Thereafter, 1.0 ml of the rinse buffer was added to the column twice to wash out protein adulterant. After the column was drained completely, 500 μl of an alkaline buffer containing 10 mM of sodium hydroxide in 0.2 M sodium sulfate solution (elution buffer) was added and all the eluted solution was collected as HPLC injection sample. The drug content of the samples was analyzed by HPLC as described above in the drug release studies.

2.7. Pharmacokinetic analysis

The time to reach maximum GM concentration, T_{max}, and the maximum plasma GM concentration, C_{max}, were determined from the authentic plasma GM concentration versus time data. The area under the plasma GM concentration versus time curve (AUC) and the area under the first-moment curve (AUMC) after administration of the test preparations were calculated using the linear trapezoidal rule up to the last measured GM plasma concentration. The mean residence time (MRT) was calculated by $AUMC_{0-last}/AUC_{0-last}$. The absolute BA values were calculated using the following formula:

$$\text{BA}\ (\%) = \frac{\text{AUC}_{\text{oral}}}{\text{AUC}_{\text{i.v.}}} \times \frac{\text{Dose}_{\text{i.v.}}}{\text{Dose}_{\text{oral}}} \times 100$$

2.8. Statistical analysis

All values are expressed as their mean ± S.E. Means of two groups were compared using non-paired Student's *t*-tests. A value of $P < 0.05$ was considered statistically significant.

3. Results

To study the effect of the type of enteric coating polymers on the delivery and absorption of GM in GI tract, two types of HPMC capsules, wrapped with Eud L and Eud S films, containing three kinds of formulations were prepared and subjected to in vitro drug release studies in pH 7.4 phosphate buffer and in vivo pharmacokinetic studies in beagle dogs. The cumulative mean percent drug released at different time intervals from capsules coated with Eud L and Eud S polymers is shown in Fig. 1. The percent drug released in the first half-an-hour was significantly different among the three kinds of formulations coated with Eud S (Fig. 1b) polymer. However, the total

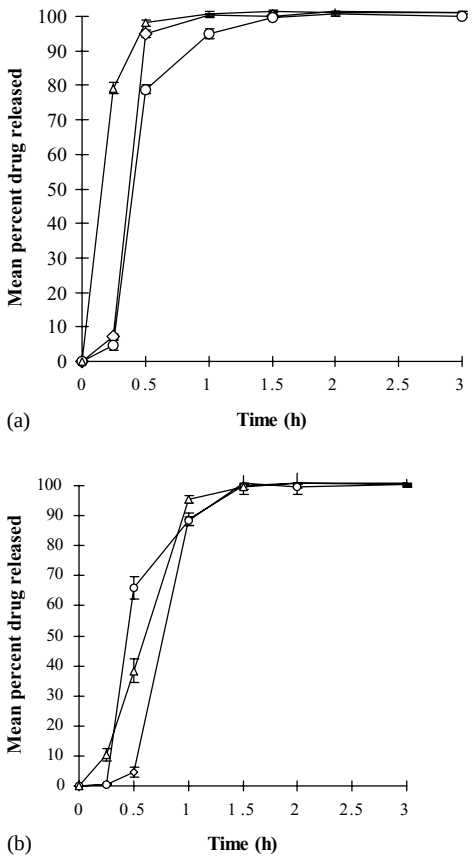

Fig. 1. Mean percent drug released from (a) Eud L and (b) Eud S capsules containing solution (◇), suspension (○) and sponge (△) formulations in pH 7.4 phosphate buffer. Values are the mean±S.E. of three experiments.

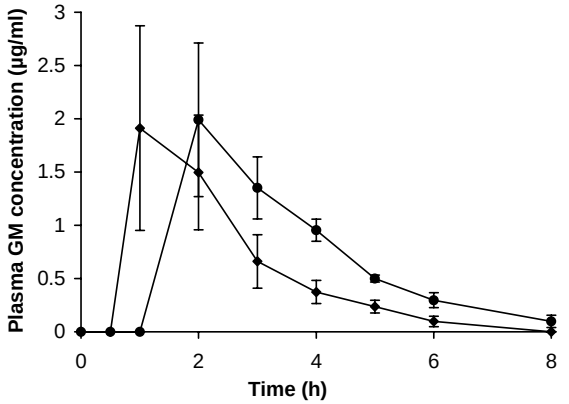

Fig. 2. Plasma GM concentration vs. time profiles following oral administration of solution formulation to beagle dogs. GM was dissolved in water and labrasol was added and the solution was filled in HPMC capsules wrapped with Eud L (◆) or Eud S (●). The dose of GM was 50.0 mg per dog and that of labrasol was 0.60 ml per dog. Values are the mean ± S.E. of three dogs.

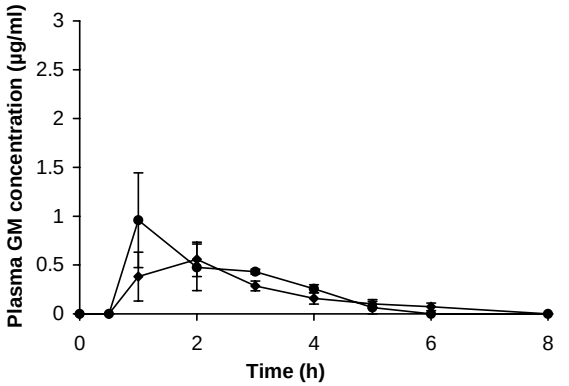

Fig. 3. Plasma GM concentration vs. time profiles following oral administration of suspension formulation to beagle dogs. GM was suspended in labrasol and the suspension was filled in HPMC capsules wrapped with Eud L (◆) or Eud S (●). The dose of GM was 50.0 mg per dog and that of labrasol was 0.60 ml per dog. Values are the mean ± S.E. of three dogs.

drug load was released from all the formulations by 1.5 h.

The plasma GM concentration versus time profiles following oral administration of GM solution formulation in HPMC capsules wrapped with Eud L or Eud S films are shown in Fig. 2. The plasma GM levels with Eud L capsules were detected after 0.5 h and reached maximum value (C_{max}) at 1 h and then decreased. The pharmacokinetic parameters, T_{max}, C_{max}, MRT and AUC, obtained following administration of different formulations of GM are given in Table 2. The T_{max} and C_{max} values given in Table 2 are the mean of individual values of three dogs. The administration of Eud L capsules containing GM solution has resulted in C_{max} and AUC values of 2.38 ± 0.50 μg/ml and 4.35 ± 1.31 μg h/ml, respectively. Administration of GM solution in Eud S capsules has delayed the T_{max} value (2.33 ± 0.33 h) by 1 h compared to that observed with Eud L capsules (1.33 ± 0.33 h). However, the C_{max} and AUC values obtained, 2.30 ± 0.42 μg/ml and 5.34 ± 0.95 μg h/ml, respectively, were not significantly different from those obtained with Eud L capsules.

Fig. 3 shows the plasma GM concentration versus time profiles obtained following administration of GM suspension formulation in Eud L and Eud S capsules to beagle dogs. The C_{max} values obtained with Eud L capsules and AUC values obtained with Eud S

Table 2
Pharmacokinetic parameters of GM after oral administration of test preparations to beagle dogs

Formulation	Capsule	C_{max} (μg/ml)	T_{max} (h)	MRT[a] (h)	AUC (μg h/ml)	BA (%)
Solution	Eud L	2.38 ± 0.50*	1.33 ± 0.33	2.29 ± 0.39	4.35 ± 1.31	18.2
	Eud S	2.30 ± 0.42	2.33 ± 0.33	3.33 ± 0.18	5.34 ± 0.95*	22.4
Suspension	Eud L	0.58 ± 0.19	1.67 ± 0.33	2.70 ± 0.18	1.50 ± 0.52	6.3
	Eud S	1.12 ± 0.33	1.67 ± 0.67	2.22 ± 0.60	1.95 ± 0.48	8.2
Sponge	Eud L	0.82 ± 0.11	2.67 ± 0.33	3.45 ± 0.32	1.76 ± 0.33	7.4
	Eud S	1.02 ± 0.42	3.33 ± 0.33	4.10 ± 0.29	2.94 ± 1.29	12.3

Values are the mean ± S.E. of three dogs.
[a] $MRT = AUMC_{0-last}/AUC_{0-last}$.
* Significantly different from suspension formulation, $P < 0.05$.

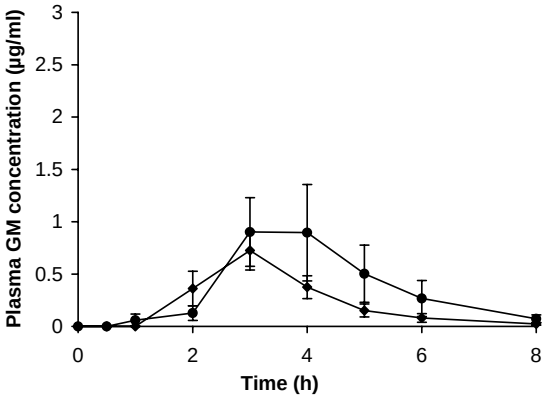

Fig. 4. Plasma GM concentration vs. time profiles following oral administration of sponge formulation to beagle dogs. GM was dissolved in water and labrasol was added to it. The solution was absorbed onto sponge present in HPMC capsules wrapped with Eud L (◆) or Eud S (●) films. The dose of GM was 50.0 mg per dog and that of labrasol was 0.60 ml per dog. Values are the mean ± S.E. of three dogs.

capsules of suspension formulation were significantly lower than their solution counterparts ($P < 0.05$). Interestingly, the T_{max} values of both the capsules of suspension formulations were almost the same at about 1.67 h. The AUC values obtained with both type of capsules (Eud L and Eud S) were also not significantly different. But, the C_{max} value obtained with Eud S capsules (1.12 ± 0.33 μg/ml) was almost two times more than that of Eud L capsules (0.58 ± 0.19 μg/ml).

The plasma GM concentration versus time profiles obtained after administration of GM sponge formulations in Eud L and Eud S capsules are shown in Fig. 4. There was about 1.3 and 1 h increase in T_{max} values with Eud L (2.67 ± 0.33 h) and Eud S (3.33 ± 0.33 h) capsules, respectively, compared to their solution counterparts. But the C_{max} and AUC values of both Eud L (0.82 ± 0.11 μg/ml and 1.76 ± 0.33 μg h/ml) and Eud S (1.02 ± 0.42 μg/ml and 2.94 ± 1.29 μg h/ml) capsules were lower than those of solution formulations. However, the C_{max} and AUC values of sponge formulations were comparable or more than those of suspension formulations. The BA obtained with solution formulation of both Eud L (18.2%) and Eud S (22.4%) capsules was higher compared to sponge and suspension formulations. There was no significant difference in the BA of GM obtained with Eud L and Eud S capsules of all the three formulations.

4. Discussion

Several barriers limit the oral delivery of polar and macromolecular drugs such as GM and recombinant proteins. The major causes of low oral BA of these drugs are the luminal enzymatic hydrolysis and low membrane permeability. Absorption of large and more hydrophilic drugs is mostly limited to the paracellular pathway. Entry of molecules through the paracellular pathway is primarily restricted through the tight junction (Madara, 1989). Drugs with high water-solubility and low molecular weight can cross the barrier of bimolecular lipid layer of GI tract by a filtration process through membrane pores. The membrane is not continuous, but is interrupted by aqueous pores, the diameter of which allows small molecules such as water or urea to pass freely. But, the high water-solubility and relatively large molecular size of GM did not allow it to pass through the membrane pores. One approach to improve the membrane permeability of drugs is to co-administer drugs with absorption enhancing agent. In the earlier studies (Hu et al., 2001), labrasol was found to increase the intestinal absorption of GM and the absorption enhancing effect was more prominent in the rat ileum and colon. The plasma GM concentration was maintained at more than 2.5 μg/ml during the entire 6 h study period, which is higher than the minimum inhibitory concentration (MIC; 0.03–2.0 μg/ml) of GM (Sweetman, 2002). But, the dose of labrasol used in the study was high (1.0 ml/kg), which is difficult to formulate for a big animal like dog. Hence, in the present study the dose of labrasol was decreased to 0.6 ml per dog (10–12.1 kg), while maintaining the GM dose at about 5.0 mg/kg (50.0 mg per dog).

The use of pH-sensitive polymers is one of the approaches for the site-specific delivery of drugs to the different parts of small or large intestines (Peeters and Kinget, 1993). The threshold pH for the dissolution of these polymers is below 7.0, for example it is 6.0 for Eud L and 6.8 for Eud S (Hardy, 1989). These polymers would dissolve in duodenum (Eud L) and middle part of jejunum or upper part of ileum (Eud S) resulting in the release of their drug load. However, the drug release studies did not indicate any significant difference between two kinds of enteric polymers as well as among the solution, suspension and sponge formulations. Except some difference in the percent drug released in the first half-an-hour (Fig. 1b),

almost the total drug load was released within 1.5 h from all the formulations. These results indicate that the type of enteric polymer has no effect on the drug release in the simulated intestinal fluids and formulation of GM either as solution or suspension or sponge would not affect the solubility or release of GM.

The in vivo performance of GM formulations was evaluated by oral administration in beagle dogs. As the GM solution formulation contains water, which would dissolve the HPMC capsules on long run, the formulation was encapsulated on the day of administration and stored in the refrigerator at 4–10 °C until they are used. Following oral administration, the plasma GM concentrations increased sharply, reached to C_{max} within 0.5–1 h after the dissolution of the enteric polymers. The plasma GM levels obtained were within the MIC range (0.03–2.0 μg/ml) with both types of capsules and were maintained at >0.5 μg/ml for about 3–4 h after the dissolution of the enteric capsules. The sharp rise in plasma GM levels indicates an immediate absorption of GM following release of GM solution from the capsules. This pattern of sharp increase in the plasma GM levels was also observed in in situ absorption studies carried out in rats where C_{max} was observed by about 0.25 h following in situ administration. Earlier permeability studies (Hu et al., 2001) on colonic mucosa have indicated the involvement of P-glycoprotein (P-gp) inhibition in the promotion of GM absorption. As a class, it has been speculated that surfactants increase the permeability of drugs via disruption or fluidization of the cell membrane and subsequently increase transcellular transport (Liu et al., 1999). The combined effect of inhibition of P-gp and increased transcellular transport by labrasol might be responsible for increased intestinal absorption of GM.

It appears that labrasol-mediated absorption of GM from solution formulation was instant and was affected by the dissolution rate of the capsules. But, the in vitro drug release studies did not indicate any significant difference in the drug release rate among the formulations. This may be because of the difference in the volume of fluids available in the small intestine and the volume of dissolution medium used (900 ml of pH 7.4 buffer) in the drug release studies. Also, the intensity of peristaltic movements in the small intestine may be lesser than the intensity of agitation used (Apparatus 1, 100 rpm) in the in vitro studies. The scantly available fluids in the small intestine might have taken a longer time to dissolve the enteric layer resulting in delayed and slow release of GM solution. The site of dissolution of the two enteric polymers, middle or lower part of the small intestine, seems to have no influence on the extent of GM absorption, as the AUC values were not significantly different between Eud L and Eud S capsules. Similar findings were observed in the in vitro studies as well. However, it is significant to note that C_{max} values of >2.0 μg/ml were obtained even with a very low dose of labrasol (0.6 ml per dog) used in this study.

Studies were also carried out with GM suspension formulation in labrasol. The absence of water would impart stability to the HPMC capsules. Since GM is freely soluble in water, it was assumed that exclusion of water from the formulations would not affect the GM dissolution and absorption. After dissolution of the enteric capsules, the released GM was thought to dissolve immediately and the presence of labrasol would facilitate GM absorption. The results of the in vitro drug release studies have indicated the absence of significant effect of the suspension formulation on the drug release as the total drug load was released within 1.5 h as in solution formulations. The plasma GM concentration versus time profiles (Fig. 3) have shown decreased drug absorption with suspension formulations. Both C_{max} and AUC values were lower than those of solution formulations of both Eud L and S capsules. The decrease in GM absorption with suspension formulation may be because of lag time required for dissolution of the enteric capsules and the suspended drug particles in the scantly available digestive fluids of the middle or lower part of the small intestine. By the time the drug was dissolved, labrasol might have moved away and got diluted in the intestinal fluids. As the dose of labrasol used was less, the transient absorption promoting effect of labrasol might have lost to some extent resulting in decreased GM absorption. Generally, the effect of an absorption enhancer is related to its concentration at the site of drug absorption. The drug and the absorption promoter must be delivered to the absorption site simultaneously, and a sufficient concentration of the absorption enhancer must be achieved and maintained there (Aungst, 2000). Interestingly, the T_{max} value of suspension formulation

in Eud S capsules (1.67 ± 0.67 h) was lower than the solution formulation in Eud S capsules (2.33 ± 0.33 h) and same as that of suspension formulation in Eud L capsules (1.67 ± 0.67 h). This may possibly due to fast gastric emptying of the Eud S capsules of suspension formulation on the day of the study as the gastric emptying times in the dogs are known to show high inter- and intra-subject variations.

Comparison of the pharmacokinetic parameters clearly indicates that the solution formulation of GM was better than that of suspension formulation. But the presence of water in solution formulation would result in long-term stability problems for HPMC capsules. Since the present study was a limited feasibility study to find out the possibility of oral delivery of GM in bigger animals like dog, a non-absorbable and indigestible synthetic sponge was used for absorbing GM solution. At the end of the experiment, the undigested cylindrical sponge pieces were found excreted into the faeces. After dissolution of the enteric capsules, the sponge was expected to unfold and come in contact with the intestinal wall. Since the sponge has lot of pores, free diffusion of fluids into and out of sponge would have taken place resulting in the release of GM and labrasol simultaneously and directly onto the intestinal absorbing surface. This conclusion was arrived on the basis of the in vitro drug release studies wherein the percent drug released from sponge formulation was similar or relatively faster than solution formulation.

The C_{max} value obtained with Eud L capsules (0.82 ± 0.11 μg/ml), and AUC value obtained with Eud S capsules (2.94 ± 1.29 μg h/ml) of sponge formulations were found to be more than Eud L (0.58 ± 0.19 μg/ml) and Eud S (1.95 ± 0.48 μg h/ml) capsules of suspension formulations. The results of the above study indicate that Eud S capsules of sponge formulations were better as they have maintained plasma GM concentration above 0.5 μg/ml for about 2 h compared to 0 h observed with Eud L capsules of sponge formulations and 1 h observed with Eud S capsules of suspension formulation. However, the T_{max} values were higher than those of solution formulation. Also, BA and the C_{max} values were lower than those obtained with solution formulation. This is again possibly because of slow diffusion of scantly available small intestinal fluids into and out of sponge formulation resulting in slow release of GM and labrasol compared to instant and complete release from solution formulation. Further studies are going on to find a pharmaceutically acceptable excepient, which could absorb or adsorb the GM solution and enables encapsulation without any problem. In our earlier studies in rats (Hu et al., 2001), an absolute BA of about 54% was obtained with GM formulation containing labrasol as absorption enhancer. However, in the present study the highest BA observed was only 22% with solution formulations (Eud S) and was still less (12%) with sponge formulation (Eud S). This may be because of use of less quantity of labrasol in the present study (about 0.06 ml/kg) compared to the earlier studies (1.0 ml/kg). Also, in the earlier studies the formulations were directly administered to the site of absorption, whereas in the present study the formulations were administered orally in the form of enteric capsules, which involves dissolution of capsules and release of the formulations. Moreover, the difference in the physiology of the small intestines of the dogs from that of the rats might have also contributed for the difference in the BA values observed between these studies.

The results of the in vitro drug release studies could not differentiate between two kinds of enteric capsules and among the three kinds of GM formulations. Oral administration of enteric capsules containing GM solution formulation has resulted in higher plasma GM levels compared to suspension formulations containing same dose of labrasol and GM. Results of the studies with sponge formulations have indicated the possibility of absorbing GM solution onto an absorbent while maintaining enhanced GM plasma levels. There was no significant difference in the extent of GM absorption with Eud L and Eud S capsules used for encapsulating GM formulations.

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