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Enhanced intestinal absorption of vancomycin with Labrasol and D-α-tocopheryl PEG 1000 succinate in rats

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Abstract

Vancomycin hydrochloride (VCM) is a glycopeptide antibiotic used for the treatment of infections caused by methicillin-resistant staphylococci. It is water soluble, having a high molecular weight, and poorly absorbed from the gastrointestinal tract. Mixtures of VCM with Labrasol and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) were prepared to improve oral absorption of VCM. Administration of VCM solution to rat ileum at a dose of 20 mg/kg did not result in detectable plasma VCM concentration. Formulation containing 50% of Labrasol resulted in a Cmax value of 5.86 ± 0.97 μg/ml and an AUC_{0-6h} value of 16.06 ± 1.78 μg h/ml. Addition of TPGS to VCM solution at 12.5% concentration also increased the plasma VCM concentration with a Cmax value of 4.98 ± 0.45 μg/ml. But the AUC_{0-6h} (9.87 ± 1.90 μg h/ml) was significantly lower than that obtained with Labrasol. The addition of 5.0 and 25.0% TPGS to solutions of VCM containing 50% of Labrasol did not result in any significant increase either in Cmax or AUC_{0-6h} of VCM. Whereas the addition of 12.5% of TPGS has resulted in an increase in Cmax and AUC_{0-6h} by 2.2 and 2.4 times, respectively, suggesting that this concentration of 50% Labrasol and 12.5% TPGS (1:0.25) was optimum for improving intestinal absorption of VCM. A dose dependent decrease in the Cmax and AUC_{0-6h} values was observed when the dose of absorption enhancers was decreased by 50% with formulation containing Labrasol and TPGS in 1:0.25 ratio. The results of the study indicate that formulations containing Labrasol and TPGS improve intestinal absorption of hydrophilic macromolecular drug, VCM.

Keywords: Vancomycin hydrochloride; Glycopeptides; Antibiotic; Methicillin-resistant staphylococci; Labrasol; Vitamin E TPGS

1. Introduction

The oral absorption of highly polar and macromolecular drugs is frequently limited by poor intestinal wall permeability. Some physicochemical properties that have been associated with poor membrane permeability are low octanol/aqueous partitioning, the presence of strongly charged

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functional groups, high molecular weight, a substantial number of hydrogen-bonding functional groups and high polar surface area (Aungst, 2000). For some compounds, permeation through the intestinal epithelium is hindered by their active transport from the enterocyte back into the intestinal lumen. The secretory transporters involved may include *P*-glycoprotein (Pgp), the family of multidrug resistance-associated proteins, and possibly others. There are many reports of using absorption enhancers such as surfactants, bile acids, fatty acids and chelating agents to improve absorption of polar molecules across the intestinal wall (Swenson and Curatolo, 1992). Many therapeutic compounds such as antibiotics and peptide and protein drugs require the use of some kind of absorption enhancer to obtain reasonable plasma concentrations.

Vancomycin hydrochloride (VCM) is a glycopeptide antibiotic used for the treatment of infections caused by methicillin-resistant staphylococci. It has a molecular weight of approximately 1500 Da, water soluble, and poorly absorbed from the gastrointestinal (GI) tract (Lucas et al., 1987). Different strategies for improving the intestinal absorption of VCM are under investigation such as liposomes (Anderson et al., 2001), multiple emulsions (Kajita et al., 2000), and using sodium glycocholate as absorption promoter (Geary and Schlameus, 1993). In our earlier studies, Labrasol, caprylocaproyl macrogolglycerides, enhanced the intestinal absorption of gentamicin (GM) (Hu et al., 2001, 2002) and insulin (Eaimtrakarn et al., 2002). A self-microemulsion system with Labrasol was developed in the earlier studies to improve the GI absorption of extremely water-soluble drug, GM. Microemulsions of GM administered to rat colon achieved an absolute bioavailability (BA) of 54.2% at a dose of 1.0 ml/kg of Labrasol and 5.0 mg/kg of GM.

Labrasol is obtained from coconut oil and shows high tolerance and low toxicity. The LD_{50} is 22 g/kg for rats. Labrasol is a surfactant that contains saturated polyglycolysed 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 triglycerides of C_8 and C_{10} fatty acids, 50% of mono- and di-esters of poly (ethylene glycol) (PEG) and 20% of free PEG 400 (Kreilgaard et al., 2000). It was originally developed as a pharmaceutical additive for the solubilization of hydrophobic drugs. Labrasol was investigated as a component of microemulsions for enhancing the transdermal absorption of drugs (Tran et al., 1999; Kreilgaard et al., 2000; Rhee et al., 2001). It was found to improve intestinal absorption of drugs after oral administration (Kommuru et al., 2001; Shibata et al., 2001). Labrasol is also in use as a main component of self-microemulsifying drug delivery system (Kim et al., 2000). D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) is a derivative of vitamin E consisting of a hydrophilic polar head group and a lipophilic alkyl tail resulting in amphiphillic properties. TPGS has a relatively low critical micelle concentration, 0.02% w/w, and has a hydrophile/lipophile balance value of 13.2 (Wu and Hopkins, 1999). It is reported to be nontoxic with an acute LD_{50} of >7 g/kg for young adult rats of both sexes (Krasavage and Terhaar, 1977). TPGS has been found to increase the oral absorption of cyclosporin through micelle formation (Boudreaux et al., 1993; Sokol et al., 1991). It is also found to have intestinal Pgp inhibitory activity (Dintaman and Silverman, 1999; Chang et al., 1996). In the present study, mixtures of VCM with Labrasol and TPGS were prepared and evaluated in rats by administering to the lower small intestine i.e. ileum.

2. Materials and methods

2.1. Materials

VCM was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Labrasol (Gattefösse, France) and TPGS were obtained from Chugai Boyeki Co., Ltd. (Tokyo, Japan) and Peboc Division of Eastman Chemical (UK) Limited (United Kingdom), respectively. Acetonitrile and methanol were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Trifluoroacetic acid (TFA) and acetic acid were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Male Wistar rats used in the study were obtained from Nippon

SLC Company (Hamamatsu, Japan) and standard solid meal of commercial food (LabDiet®) was purchased from Nippon Nousan Co., Ltd. (Yokohama, Japan). All other materials used were of reagent grade and were used as received.

2.2. VCM preparations

The composition of formulations used in the present study is shown in Table 1. VCM was initially dissolved in saline and Labrasol was added and mixed well. To this, weighed quantity of melted TPGS was added, and upon mixing, transparent formulations were obtained. Formulations containing VCM were equilibrated at ambient temperature overnight and then used in animal experiments.

2.3. Absorption studies

Male Wistar rats (300–350 g) fasted overnight for at least 12 h were used in the study. The rats were anaesthetized by intraperitoneal administration of sodium pentobarbital solution (50 mg/kg). The abdominal cavity was cut opened and lower small intestine was isolated. A small pore was made in the ileum with 23G needle, 15 cm from ileo-caecal junction, and the VCM formulations were administered at a dose of 20 mg/kg of VCM. The pore was sealed with synthetic glue. The dose of Labrasol was maintained at 1.0 ml/kg in all the experiments, which was found to be optimum in our earlier studies with GM (Hu et al., 2001) and insulin (Eaimtrakarn et al., 2002). Blood samples of 0.3 ml each were collected from the right jugular vein at 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h intervals. The blank blood sample was taken at 5 min prior to the administration of test preparations. Plasma was obtained from whole blood by centrifugation at 14 000 rpm for 5 min using Kubota 1720 centrifuge (Tokyo, Japan), and then stored at −80 °C until analysis. All the animal experiments were carried out in accordance with the Guidelines for Animal Experimentation in Kyoto Pharmaceutical University.

2.4. LC/MS/MS analysis of plasma samples

The plasma samples (100 μl) were treated with 300 μl of 10% TFA: methanol (2:1, v/v), the mixture was vortexed for 30 s and centrifuged at 12 000 rpm for 10 min. Then the supernatant was decanted to a clean test tube, and diluted with 300 μl of distilled water. The mixture was passed through a Millex®-LG filter (0.2 μm, Millipore Corp., MA, USA) and the filtrate was placed into a clean HPLC vial, and then 20 μl of sample was injected into a LC/MS/MS system. The LC/MS/MS system consisted of a PE-Sciex API 365 triple quadrupole mass spectrometer equipped with turbo ion spray sample inlet and Analyst™ workstation (Perkin-Elmer-Sciex, Ontario, Canada), a LC-10AD micropump (Shimadzu, Kyoto, Japan) and an AS8020 automatic injector (Tosoh Co., Ltd., Tokyo, Japan). The mobile phase of water/acetonitrile (9:1, v/v) containing 0.1% acetic

Table 1
Vancomycin formulations used in absorption studies

Formulation	Labrasol:TPGS:saline	Volume of solution (ml/kg)	Dose (g/kg)	
			Labrasol	TPGS
A	0:0:2	2.0	0.00	0.00
B	1:0:1	2.0	1.06	0.00
C	0:0.25:1.75	2.0	0.00	0.26
D	1:0.1:0.9	2.0	1.06	0.11
E	1:0.25:0.75	2.0	1.06	0.26
F	1:0.5:0.5	2.0	1.06	0.53
E/2[a]	0.5:0.125:0.375	1.0	0.53	0.13

The dose of VCM was 20.0 mg/kg in all the experiments.
[a] VCM dose of 20.0 mg/kg was present in 1.0 ml compared to 2.0 ml of other formulations.

acid was degassed and pumped through an Inertsil ODS-3 column (2.1 mm i.d. × 100 mm, GL Science Inc., Tokyo, Japan) at a flow rate of 0.2 ml/min, and the column temperature was maintained at 40 °C. The ionization was carried out via the turbo ion spray inlet in the positive ion mode. The flow rates of nebulizer gas, curtain gas and collision gas were set at 8, 8 and 2 l/min, respectively. The ion spray voltage and temperature were set at 4500 V and 425 °C, respectively. The declustering potential, the focusing potential, the entrance potential, the collision energy and the collision cell exit potential were set at 20, 200, −10, 30 and 6 V, respectively. Since VCM changes as a precursor ion in the bivalent proton addition ion, the mass-scanning mode by multiple reactions monitoring with a precursor/product ion for VCM was set at 725.2/143.8 m/z. A calibration curve was prepared with each assay at a concentration range of 0.01–20 μg/ml, and the linear regression line was passed through the origin with a correlation coefficient of 0.999 or better. The limit of detection was 0.01 μg/ml.

2.5. Pharmacokinetic analysis

Pharmacokinetic (PK) parameters were calculated by a non-compartmental PK analysis method using WINHARMONY software (Yoshikawa et al., 1998). The time to reach maximum VCM concentration, Tmax, and the maximum plasma concentration of VCM, Cmax, were determined from the authentic plasma VCM concentration vs. time data. The area under the plasma VCM concentration vs. time curve up to 6 h (AUC_{0-6h})) and the area under the first-moment curve up to 6 h ($AUMC_{0-6h}$) after administration of the test preparations were calculated using the linear trapezoidal rule up to the last measured VCM plasma concentration. The mean residence time (MRT) was calculated by $AUMC_{0-6h}/AUC_{0-6h}$.

2.6. Statistical analysis

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

3. Results

The plasma VCM concentration vs. time profiles following administration of VCM solution (control) and formulations containing Labrasol and TPGS to rat ileum are shown in Fig. 1. There was no VCM absorption in the control experiment without any absorption enhancer, as the drug was not detected in the plasma samples at any time points. The PK parameters obtained Tmax, Cmax, MRT and AUC_{0-6h} following administration of different formulations are given in Table 2. The Tmax and Cmax values given in Table 2 were the mean of Tmax and Cmax values of individual rats ($n = 3$–4). The addition of Labrasol at 1:1 ratio of saline (formulation B) has resulted in increased absorption of VCM with Cmax and AUC_{0-6h} values of 5.86 ± 0.97 μg/ml and 16.06 ± 1.78 μg h/ml, respectively. Addition of TPGS at 0.25:1.75 ratio to saline also increased the intestinal absorption of VCM with a Cmax value of 4.98 ± 0.45 μg/ml. But the AUC_{0-6h} (9.87 ± 1.90 μg h/ml) was

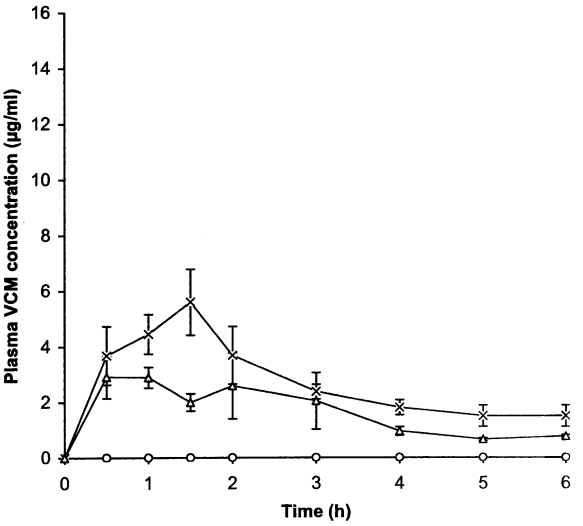

Fig. 1. Plasma VCM concentration vs. time profiles following small intestinal administration in rats. VCM was dissolved in saline without Labrasol (○) and with Labrasol (×) or TPGS (△). The VCM dose was 20.0 mg/2.0 ml/kg. The concentration of Labrasol in the formulation was 50% and that of TPGS was 12.5%. As control, VCM saline solution was administrated into rat ileum without any absorption enhancer. Values are the mean ± SE of 4 animals.

Table 2
Pharmacokinetic parameters of VCM after administration of test preparations to rat ileum

Formulation	Cmax (μg/ml)	Tmax (h)	MRT[a](h)	AUC_{0-6h} (μg h/ml)
A	NC	NC	NC	NC
B	5.86±0.97	1.62±0.12	2.44±0.18	16.06±1.78*
C	4.98±0.45	1.00±0.35	2.28±0.11	9.87±1.90
D	6.42±1.01	0.75±0.14	2.47±0.16	17.62±1.97
E	12.94±1.26**	1.25±0.14	2.08±0.30	39.17±6.30***
F	5.80±1.25	1.37±0.24	2.35±0.19	14.35±2.40
E/2[b]	7.39±0.41	1.67±0.14	2.32±0.12	21.32±2.01

NC, not calculated. VCM was dissolved in saline with or without surfactants.
[a] $MRT = AUMC_{0-6h}/AUC_{0-6h}$.
[b] Administered at 50% volume of E.
* Significantly different from formulation C, $P < 0.05$.
** Significantly different from B, C, D, E/2 and F, $P < 0.05$.
*** Significantly different from B, C, D and F, $P < 0.05$.

significantly lower than that of formulation B containing Labrasol.

In the subsequent studies, both Labrasol and TPGS were added to the VCM solution to find out their synergistic effect on VCM absorption from the rat ileum. Fig. 2 shows the plasma concentration vs. time profiles following administration of formulations containing different quantities of TPGS in Labrasol and saline mixture. When 5.0% of TPGS was added (formulation D), there was no significant difference either in the AUC_{0-6h} or in the Cmax values of VCM compared to preparation containing no TPGS (formulation B). The MRT values were also the same at 2.44±0.18 h and 2.47±0.16 h without and with TPGS, respectively. Since the addition of 5.0% of TPGS has no effect on the absorption enhancing activity of Labrasol, the amount of TPGS was increased to 12.5% (formulation E). The Cmax and AUC_{0-6h} values of VCM were found to be 12.94±1.26 μg/ml and 39.17±6.30 μg h/ml, respectively. The AUC_{0-6h} was increased by about 2.4 times with the addition of 12.5% of TPGS compared with 5.0% of TPGS. Further increase in the quantity of TPGS to 25.0% (formulation F) did not improve VCM absorption over 12.5% TPGS concentration. The Cmax (5.80±1.25 μg/ml) and AUC_{0-6h} (14.35±2.40 μg h/ml) values obtained were almost close to those obtained without any TPGS (formulation B).

As the increase in TPGS content to 25.0% has resulted in decreased VCM absorption, further studies with increased quantities of TPGS were not carried out. Instead, studies were carried out with a lower dose of formulation containing 12.5% of TPGS, which has shown the highest VCM absorption. The dose of the surfactants was decreased by 50% (formulation E/2) as the volume of solution administered was decreased from 2.0 ml/kg to 1.0 ml/kg. The Cmax and AUC_{0-6h} values obtained were 7.39±0.41 μg/ml and 21.32±2.01 μg h/ml, respectively. A dose dependent response was obtained when the quantity of surfactants was decreased by 50% as the AUC_{0-6h} was also decreased by about 50%. However, the AUC_{0-6h} value obtained (21.32±2.01 μg h/ml) was slightly higher than that obtained with formulations containing 0% (16.06±1.78 μg h/ml), 5.0% (17.62±1.97 μg h/ml) and 25.0% (14.35±2.40 μg h/ml) of TPGS, which were administered at higher dose (2.0 ml/kg) of surfactants. Interestingly, the MRT values of VCM were almost the same with all the formulations (Table 2). However, the Tmax values were varying, being the lowest at 0.75±0.14 h with formulation containing 5.0% of TPGS (formulation D) and was increased to more than twice (1.67±0.14 h) when the surfactant quantity was decreased by 50% (formulation E/2). The effect of the ratio of Labrasol and TPGS in the formulation on the AUC_{0-6h} of VCM is shown in Fig. 3. The

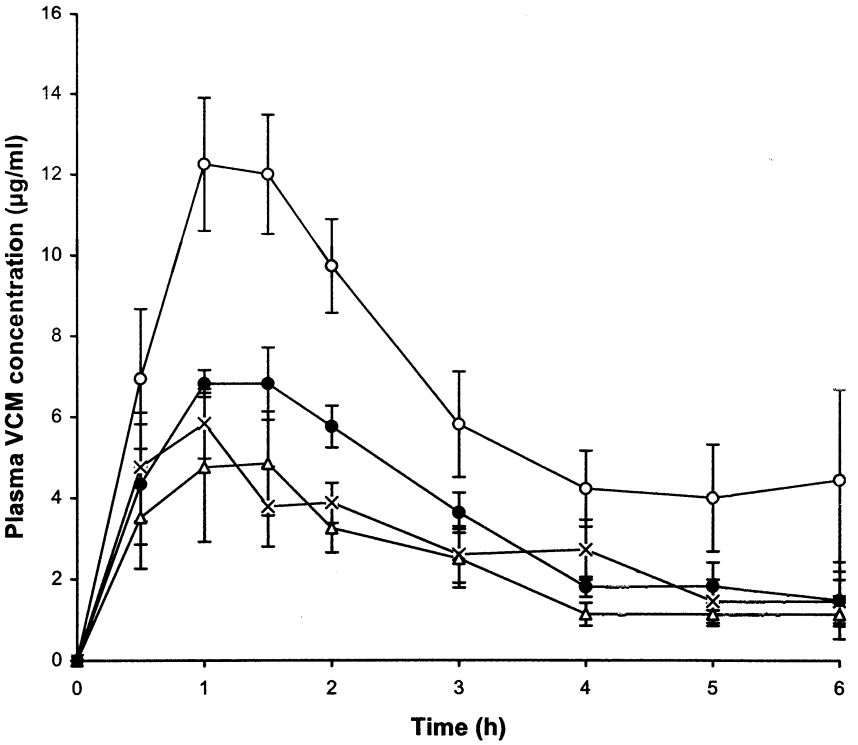

Fig. 2. Plasma VCM concentration vs. time profiles in rats following small intestinal administration of formulations of Labrasol and VCM containing 5.0% (×), 12.5% (○) and 25.0% (△) of TPGS. VCM was dissolved in saline with Labrasol and TPGS and the final VCM dose was 20.0 mg/2.0 ml/kg. The concentration of Labrasol in the formulation was 50%. Formulations containing 12.5% TPGS were also administered at a dose of 20.0 mg/1.0 ml/kg (●). Values are the mean ± SE of 3–4 animals.

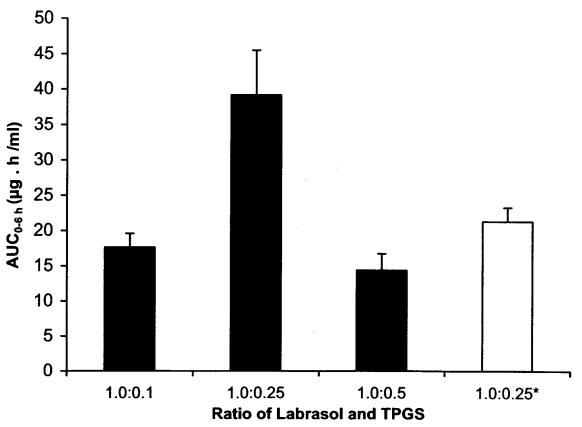

Fig. 3. The effect of the ratio of Labrasol and TPGS on AUC_{0-6h} obtained following small intestinal administration of formulations of VCM. Values are the mean ± SE of 3–4 animals. *Administered 1.0 ml/kg of formulation containing Labrasol and TPGS at 1:0.25 ratio and VCM 20.0 mg/ml.

results indicate that formulation containing Labrasol and TPGS at 1:0.25 ratio produce significantly ($P < 0.05$) higher AUC_{0-6h} value compared to formulations containing Labrasol and TPGS in 1:0.1 and 1:0.5 ratios.

4. Discussion

There are several barriers for the oral delivery of macromolecular drugs such as recombinant protein drugs. The luminal enzymatic hydrolysis and low membrane permeability are the major causes of low oral BA of these drugs. Drugs, which are presented in the lumen, can enter the blood stream through three processes, active or facilitated transport, passive transcellular transport and passive paracellular transport. Transcellular transport is limited to relatively small hydrophobic com-

pounds (Jackson, 1987). 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). The GI membrane consists of a bimolecular layer of lipids, which is covered on the surface by a mucus layer. 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. Drugs with high water-solubility and low molecular weight can cross this barrier by a filtration process through membrane pores. However, VCM has a relatively large molecular weight and it cannot pass through the membrane pores. One approach to overcome the restriction to paracellular transport of drugs is to co-administer drugs with absorption enhancing agents. Generally, the effects of an absorption enhancing agent are related to its concentration at the site of drug absorption, and the drug and the absorption promoter must be delivered to the absorption site simultaneously. Therefore, the present work was designed to study whether the membrane permeability of a highly water-soluble drug such as VCM can be modified through the use of surfactants as absorption promoters.

Surfactants that are too hydrophobic are poor enhancers and surfactants that are very hydrophilic cannot partition into the hydrophobic environment of the lipid bi-layer (Swenson and Curatolo, 1992). 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}. Previous studies in our laboratory have indicated that Labrasol improve intestinal absorption of macromolecular polar drug, insulin (Eaimtrakarn et al., 2002). Earlier studies have suggested that the local toxicity caused by surfactants decreases with increasing polarity (Bryan et al., 1980). TPGS is a water soluble derivative of natural source vitamin E and was reported to be non-toxic even at a dose of 1.0 g/kg/day (Wu and Hopkins, 1999). Hence, in the present study Labrasol and TPGS were used as absorption enhancers for hydrophilic drug, VCM.

Different formulations were administered to ileum, as an earlier report has indicated that VCM absorption was better from lower small intestine (ileum) and colon and the stability of VCM was more in ileum compared to duodenum and colon (Geary and Schlameus, 1993). Because of the barrier function of the intestinal epithelium, VCM was not absorbed from the rat ileum from saline solution (Fig. 1). Addition of Labrasol at 50% concentration as absorption enhancer has resulted in significant increase in plasma VCM concentration. The precise reason for obtaining a higher AUC_{0-6h} value (16.06 ± 1.78 μg h/ml) with the addition of Labrasol is not known. 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). But this surfactant-induced intestinal permeation enhancement is correlated with acute epithelial damage. There were reports of intestinal damage caused by surfactants resulting in the release of lactate dehydrogenase, phospholipids and proteins. The extent of damage depends upon the type of surfactant, the quantity of surfactant and the time of exposure. However, there were many reports of rapid reversibility in the acute damage caused by different surfactants immediately after their removal from the intestinal site (Swenson and Curatolo, 1992; Erickson, 1988; Nakanishi et al., 1983). These studies suggested that this rapid repair involves (a) villus shortening which reduces the surface area of injury and (b) epithelial cell migration to cover the injured area. The lipid nature of the intestinal mucosa results in high interfacial tension between the aqueous VCM solution and the absorption membrane resulting in less or no contact between the formulation and the intestinal epithelium. The presence of surfactants reduces this interfacial tension resulting in improved contact between the formulation and the absorbing membrane. This increased contact area facilitates improved absorption when surfactant induced changes in the structure and fluidity of the intestinal membranes occurred. Clearly, a precise understanding of the biophysical characteristics of

the epithelial membranes and enhancer-membrane interactions are necessary to elucidate the precise mechanism by which water-soluble drugs cross the mucosal membranes.

The intestinal epithelium has specialized transport systems that can secrete drugs in the serosa-to-mucosa direction, and these provide a barrier function against drug absorption. Secretory transport is expected to have a significant role on compounds with low or moderate passive permeability such as more hydrophilic compounds, peptides and peptidomimetics (Aungst and Saitoh, 1996). Pgp, a membrane transporter, is over expressed in multidrug-resistant (MRD) tumor cells, and is also expressed in normal rat intestinal epithelium and human intestinal cell cultures like Caco-2 cells (Hunter et al., 1993). Pgp works as a pump in an energy dependent manner and facilitates the secretion of drugs from cells. Our earlier studies have indicated that Labrasol also possess inhibitory action on secretory transporters such as Pgp (Hu et al., 2001). However, the role of secretory transporters in the intestinal absorption of VCM, a glycopeptide, is not clear. The increase in VCM absorption may also involve the inhibition of secretory transporters. However, this hypothesis is to be established through further studies.

The addition of TPGS at 12.5% concentration to VCM solution in saline (formulation C) has also resulted in increased VCM plasma concentration (Fig. 1). But the AUC_{0-6h} obtained was significantly ($P < 0.05$) lower than with Labrasol (Table 2). TPGS has very low critical micelle concentration of 0.02% w/w (Wu and Hopkins, 1999), and the micelles formed of TPGS can cross from the intestinal lumen through the unstirred water layer to the enterocytes (Traber et al., 1986). It was reported to improve BA of cyclosporine via enhanced solubilization, inhibition of Pgp or protection from intestinal metabolism (Chang et al., 1996). By imparting some lipophilicity to the hydrophilic VCM through micelle formation, TPGS might have improved the intestinal absorption of VCM. TPGS was found to inhibit Pgp mediated drug transport in Caco-2 cells suggesting that enhanced oral BA of drugs co-administered with TPGS may, in part, be due to inhibition of Pgp in the intestine (Dintaman and Silverman, 1999). It may, hence, be considered that the increase in intestinal absorption of hydrophilic VCM with the addition of TPGS may be due to both micelle formation and Pgp inhibition.

In our earlier studies Labrasol was used for improving the absorption of a protein drug, insulin. By formulating with Labrasol, extensive hypoglycemic effect was obtained in rats. However, the BA of insulin measured by ELISA assay method was 2.0% only (Eaimtrakarn et al., 2002). This value is too low for the development of oral insulin preparation. Therefore, it is required to look for a stronger absorption enhancer that can improve drug absorption even at low dose and has comprehensive applicability. Fractionation of Labrasol was carried out to improve absorption enhancing effect of Labrasol so that the dose of absorption enhancer required can be decreased. Diethyl ether fraction of Labrasol was found to increase the BA of GM by about 2.5 times at a dose of 0.1 ml/kg compared to Labrasol at a dose of 0.2 ml/kg (Hu et al., 2002). In the present study, TPGS was added as a co-surfactant to improve the absorption enhancing effect of Labrasol on VCM.

Different quantities of TPGS were added to VCM formulations containing 50% Labrasol (formulations D–F). Addition of 5.0 and 25.0% of TPGS did not result in any increment in VCM absorption compared to Labrasol alone. Whereas, when TPGS was added at 12.5% concentration there was about 2.2 times increase in Cmax value and 2.4 times increase in AUC_{0-6h} value of VCM over formulation without TPGS. The absence of any increase in VCM absorption with lowest amount (5.0%) of TPGS might be due to insufficient quantity to elicit its absorption promoting effect. In the case of highest concentration (25.0%), TPGS might have formed viscous liquid crystalline phases, which were reported to form at $>20\%$ of TPGS concentration (Wu and Hopkins, 1999). These highly viscous liquid phases might have decreased the mobility of the formulation resulting in impaired diffusion of the drug and absorption enhancers from the formulation and through the epithelial membrane of the intestinal wall. The ratio of Labrasol to TPGS may also be another important factor as there was a great

increase in AUC_{0-6h} value of VCM (Fig. 3) with 1:0.25 ratio (formulation E) compared to 1:0.1 (formulation D) and 1:0.5 (formulation F) ratios. The decrease in the amount of absorption enhancers to 50% by administering 1.0 ml/kg of formulation E (1:0.25) has resulted in the decrease of AUC_{0-6h} value by about 50% compared to formulations administered at 2.0 ml/kg. But the AUC_{0-6h} was higher than that obtained by administering 2.0 ml/kg of formulations containing Labrasol and TPGS at 1:0.1 and 1:0.5 ratios. It indicates the presence of an optimum ratio between Labrasol and TPGS for improving the intestinal absorption of VCM and this optimum ratio appears to be 1:0.25 as per the results of the present study. A clear dose dependence in AUC_{0-6h} was also observed between the 2 doses (2.0 ml/kg vs. 1.0 ml/kg) of surfactants (1:0.25) as the AUC_{0-6h} was decreased from 39.17 ± 6.30 to 21.32 ± 2.01 μg h/ml when the quantity of solution administered was decreased from 2.0 to 1.0 ml/kg with the same dose of VCM i.e. 20 mg/kg.

In conclusion, Labrasol increased the intestinal absorption of hydrophilic macromolecular drug, VCM. Increased intestinal VCM absorption was also obtained with TPGS, but it was less than that obtained with Labrasol. Addition of TPGS to Labrasol at a ratio of 1:0.25 has resulted in the highest plasma VCM concentration. Further studies are needed to establish the precise mechanism my which Labrasol and TPGS increased intestinal VCM absorption and their toxicity on the intestinal mucosa.

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