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Improvement in the bioavailability of poorly absorbed glycyrrhizin via various non-vascular administration routes in rats

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Abstract

The purpose of this study was to examine the improvement of the bioavailability of glycyrrhizin (GL) via extra-vascular, i.e. oral, rectal, and nasal routes with or without the aid of an absorption enhancer in place of the vascular intravenous route in rats. Pharmacokinetic behavior following administration via vascular routes, i.e. the intravenous and portal-venous routes was examined in rats. The area under the plasma concentration–time curve (AUC) after administration of GL via the portal vein was decreased slightly, suggesting that the first elimination of GL in the liver may be one of the factors contributing to the low bioavailability after administration via the oral route.

When GL was administered orally as a solution (30 mg/kg), the plasma concentration of GL was extremely low. However, after rectal or nasal administration of GL solution (30 mg/kg) with or without sodium caprate, the mean AUC value was remarkably increased compared with oral administration. In particular, the absolute bioavailability of GL after nasal administration was estimated to be approximately 20%, which was approximately 80-fold greater compared with after oral administration despite of the absence of an enhancer. Furthermore, the fatty acids co-administered orally with GL produced an increase in GL absorption in the following order: sodium caprate > sodium laurate > sodium caprylate > sodium oleate. These results indicate that the rectum and nasal cavity are useful administration routes for systemic delivery of GL. It was also found that the fatty acids were enhancers for the absorption of GL.

Keywords: Glycyrrhizin; Absorption enhancer; Fatty acids; Sodium caprate

1. Introduction

Glycyrrhizin (GL), one of the main components extracted from *Glycyrriza glabra* L., is a glucuronide consisting of two molecules of glucuronic acid and one molecule of glycyrrhetinic acid (GA) and has been widely used as an additive in pharmaceutical and food products. In recent studies, the in vitro and in vivo enhancing activity of GL and its derivatives in drug absorption has been reported (Tanaka et al., 1992; Imai et al., 1999). Furthermore, GL is a pharmaceutically active ingredient known to be a therapeutic agent for hepatic diseases (Van Rossum et al., 1998; Iino et al., 2001), allergy and inflammation (Amagaya

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et al., 1984), and is a medically essential drug due to its excellent ability to improve hepatic functions, especially in hepatic diseases. At present, two kinds of used clinically GL-containing preparations, i.e. parenteral injections and oral tablets are in the hepatic disease therapy. In terms of efficacy, the use of injections is superior to the oral tablets. In the case of tablets, the transfer of unchanged GL having a high pharmacological activity into the blood is hardly observed and accordingly its effect is far poorer compared with injections (Yano et al., 1989). The reason, the bioavailability of GL administered via the oral route is extremely low, has been ascribed to its poor absorption through the gastrointestinal tracts resulting from its low membrane permeability and/or hydrolysis to its metabolite GA by gastric fluid and β-glucuronidases in the gastrointestinal flora (Nakano et al., 1980; Terasawa et al., 1986). As mentioned above, the pharmacokinetics and absorption mechanisms of GL following oral and intravenous administration have been investigated in detail in the experiments using animals and humans. However, few studies have focused on the improvement of bioavailability of GL via non-vascular routes, which may provide patients with a convenient therapy in place of injections.

In this study, therefore, we briefly examined the pharmacokinetic behavior of GL and attempted two pharmaceutical approaches to improve the incomplete bioavailability of GL, i.e. one approach examined the possibility of rectal and nasal absorption via non-oral routes, and a separate approach utilized absorption enhancers of various fatty acids classified as medium-chain (sodium caprylate, sodium caprate, sodium laurate) and long-chain (sodium oleate) fatty acids in order to improve the poor permeability of GL, since these have been widely used and accepted to improve the bioavailability of poorly absorbed drugs (Hussain et al., 1981; Tomita et al., 1988a; Yamamoto et al., 2001).

2. Materials and methods

2.1. Materials

Dipotassium glycyrrhizinate was obtained from Tokiwa Photochemical Co. (Tokyo, Japan). Sodium caprylate (C8), and sodium caprate (C10) were obtained from Tokyo Kasei Industries Co. (Tokyo, Japan). Sodium laurate (C12) and sodium oleate (C18) were obtained from Wako Pure Chemical Industries Co. (Tokyo, Japan). All other materials were commercially available and of analytical grade.

2.2. Animals

Male Sprague–Dawley rats weighing more than 250 g (Japan SLC Inc., Tokyo, Japan) were used. The animals were fed standard laboratory chow and had free access to water. They were fasted overnight (16–20 h) with access to water prior to the peroral and rectal administration studies. In some studies, rats were anesthetized lightly with diethyl ether or anesthetized with sodium pentobarbital for surgical operation and administration of the drug.

2.3. Systemic intravenous and portal-venous administration study

In the case of portal-venous administration, the rats were anesthetized lightly with diethyl ether and the portal vein was surgically exposed. GL dissolved in sterile saline was injected at a dose of 5 mg/kg (dose volume: 1 ml/kg) into the systemic vein and portal vein over 20 s. After dosing, blood samples (approximately 0.25 ml) were taken from the tail vein at 0.08, 0.17, 0.25, 0.5, 1.0, 2.0, 4.0, and 6.0 h. Plasma samples were obtained by centrifugation at 3000 rpm for 10 min after the blood collection and stored at −30 °C until analysis. The plasma concentration of GL was determined by HPLC.

2.4. Peroral administration study

GL dissolved in distilled water was administered perorally at a dose of 30 mg/kg (dose volume: 5 ml/kg). In experiments to determine enhancers of the gastrointestinal absorption of GL, various fatty acids (C8, C10, C12, and C18) as absorption enhancers were added to the dosing solutions. GL solution including the absorption enhancers (0.2 M) was administered perorally at a dose of 100 mg/kg (dose volume: 5 ml/kg). Furthermore, GL solution containing varying concentrations of C10 (0–2%, w/v) was administered perorally at a dose of 100 mg/kg (dose

volume: 5 ml/kg) to examine the influence of varying concentrations. After dosing, blood samples (approximately 0.25 ml) were taken periodically from the tail vein.

2.5. Rectal administration study

GL dissolved in sterile saline was administered under diethyl ether anesthesia at a dose of 30 mg/kg (dose volume: 250 μl/kg) with or without C10 (1.0%, w/v) into the rectum through the anus, which was closed immediately after administration with an adhesive agent (Aron Alpha® A, Sankyo Co., Tokyo, Japan) to prevent expulsion. After dosing, blood samples (approximately 0.25 ml) were taken from the tail vein at 0.25, 0.5, 1.0, 2.0, and 4.0 h.

2.6. Nasal administration study

An operation for the nasal absorption study was performed prior to the administration by a method (Hussain et al., 1981) reported previously. After the operation, GL dissolved in sterile saline was administered at a dose of 30 mg/kg (dose volume: 250 μl/kg) into the nasal cavity by means of a micropipette through the nostril, which was closed immediately after administration with an adhesive agent (Aron Alpha® A, Sankyo Co., Tokyo, Japan) to prevent expulsion. After the administration, blood samples (approximately 0.25 ml) were taken from the tail vein at 0.25, 0.5, 1.0, 2.0, and 4.0 h.

2.7. Analytical methods

One hundred microliters of plasma was deproteinized by the addition of 500 μl of methanol containing propyl benzoate as an internal standard. Then the mixture was vortexed and centrifuged for 10 min at 3000 rpm and the supernatant layer was removed and evaporated to dryness under reduced pressure. The residues were dissolved with 200 μl of a mobile phase (acetonitrile:0.3% trifluoroacetic acid solution, 35:65, v/v) and 50–100 μl of the solution was injected into a HPLC reverse phase column (TSK-Gel ODS-80TM, Tosoh Co., Tokyo, Japan). Flow rate of the mobile phase was 1 ml/min and GL was monitored at a wavelength of 254 nm.

2.8. Calculation of pharmacokinetic parameters

The area under the plasma concentration–time curve up to the terminal measurement time (AUC_{0-T}) was calculated by the trapezoidal method and the $AUC_{0-\infty}$ was expressed as the sum of the AUC_{0-T} and the remaining area was calculated by an elimination rate constant. The mean residence time (MRT) was also calculated according to the trapezoidal method. The total body clearance (CL_{tot}) was calculated from the ratio of the dose to $AUC_{0-\infty}$, and the steady-state volume of distribution (Vd_{ss}) was calculated as the product of CL_{tot} and MRT. The absolute bioavailability (F) of GL was calculated by the ratio of AUC after administration via various routes to that for intravenous administration. The gastrointestinal absorption (FA) of GL was calculated by the ratio of AUC after administration via various routes to that after portal-venous administration. The results are expressed as the mean ± S.E. and statistical analysis was performed by the Student's t-test with $P = 0.05$ as the minimal level of significance.

3. Results and discussion

3.1. Pharmacokinetic behavior of GL after intravenous and portal-venous administration in rats

Several studies that estimated the pharmacokinetic behavior and the billiary elimination of GL after peroral and intravenous administration have been reported (Nakano et al., 1980; Terasawa et al., 1986; Ichikawa et al., 1986). However, a hepatic first-pass effect for GL administration has not been adequately studied. Therefore, as a first step, to examine whether the hepatic first-pass effect for GL is attributed to the low bioavailability or not, we studied the pharmacokinetic behavior of GL after systemic intravenous and portal-venous administration. The mean plasma concentration of GL after each administration of GL solution (5 mg/kg) to rats is shown in Fig. 1, and the calculated pharmacokinetic parameters are summarized in Table 1. The plasma concentration of GL administrated via each route declined rapidly, and the mean $AUC_{0-\infty}$ values were 45.7 ± 11.4 μg h/ml and 35.0 ± 2.5 μg h/ml for intravenous and portal-venous administration, respectively. The mean $C_{(0)}$ values

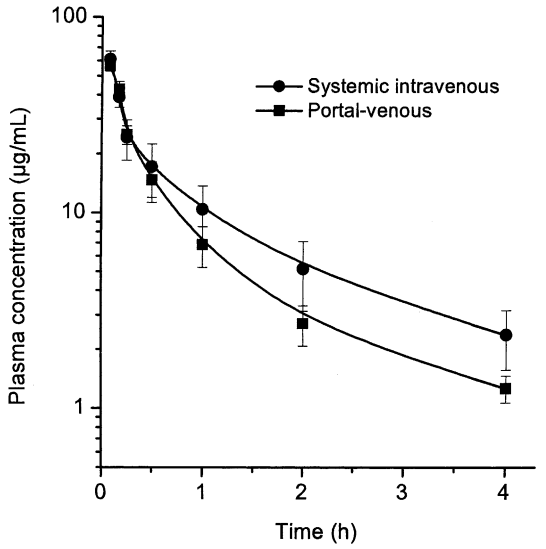

Fig. 1. Plasma concentration of GL after systemic intravenous and portal-venous administration of GL solution at a dose of 5 mg/kg in rats. Each value represents mean ± S.E. of three or four animals.

were 83.8 ± 2.9 μg/ml and 78.0 ± 4.5 μg/ml, respectively. In the case of the portal-venous route, the mean $AUC_{0-\infty}$ and $C_{(0)}$ values decreased approximately 23 and 7%, respectively, compared with those after intravenous administration. Although these differences were not significant, the finding that the pharmacokinetic parameters after portal-vein administration showed a tendency to decrease suggested that a part of the administrated GL is eliminated during first passing through a liver. It has been reported that most GL administered intravenously was quickly excreted into the bile in rats (Terasawa et al., 1986; Ichikawa et al., 1986). These results suggest that GL is hardly hydrolyzed in the liver or the systemic circulation. Thus, it is assumed that the potential reason for the hepatic first-pass extraction of GL is not due to its metabolisms, but its elimination into the bile. Additionally, this first elimination of GL in the liver may be one of the factors contributing to the low bioavailability after oral administration. However, it is not considered a principal factor because the degree of reduction was slight on the whole. On the other hand, there was also no significant difference in the other parameters, MRT, Vd_{ss}, and CL_{tot} between both administration routes.

Table 1
Pharmacokinetic parameters of GL after systemic intravenous and portal-venous administration of GL solution at a dose of 5 mg/kg in rats

Parameters	Dosing routes	
	Intravenous	Portal-venous
$C_{(0)}$ (μg/ml)	83.8 ± 2.9	78.0 ± 14.5
$AUC_{0-\infty}$ (μg h/ml)	45.7 ± 11.4	35.0 ± 2.5
MRT (h)	1.5 ± 0.5	1.4 ± 0.3
Vd_{ss} (ml/kg)	159.4 ± 56.9	166.2 ± 24.3
CL_{tot} (ml/h/kg)	128.4 ± 38.7	108.1 ± 5.8
F (%)	(100)	76.6

Each value represents the mean ± S.E. of three or four.

3.2. The bioavailability of GL after oral administration

After oral administration of GL solution (30 mg/kg), the plasma concentration of GL was extremely low, i.e. the mean C_{max} and $AUC_{0-\infty}$ values were 0.3 ± 0.1 μg/ml and 0.7 ± 0.1 μg h/ml, respectively (Table 2). The F value for GL was estimated to be approximately 0.25%, which was similar to the low bioavailability reported previously in rats (Nakano et al., 1980; Wang et al., 1996). The FA value for GL was estimated to be only 0.33%, suggesting that GL hardly passes from the gastrointestinal tract into the portal vein. Several papers reported that GL was poorly absorbed from any gastrointestinal tracts in rats. Additionally, GL was hydrolyzed to its metabolite GA by bacterial β-glucuronidases in the gastrointestinal tracts, especially in the large intestine, but its degree was negligible (Ichikawa et al., 1986; Hattori et al., 1985). In addition, GL was stable in the supernatant fraction of the mucosa (Imai et al., 1999). These findings suggest that the low bioavailability after administration via the oral route is mainly attributed to the impermeability of GL across the gastrointestinal mucosa, even though many complex factors may be related to this. Accordingly, in order for the bioavailability of GL to be improved, other sites having potential high permeability for GL need to be utilized and/or the membrane permeability needs to be increased by the use of an absorption enhancer. In this context, we secondly examined the possibility of non-oral routes, i.e. rectal and nasal absorption for the systemic delivery of GL, because these have been investigated as useful delivery routes for drugs that are

Table 2
Pharmacokinetic parameters of GL after oral, rectal, and nasal administration of GL solution (30 mg/kg) with or without C10 in rats

Parameters	Dosing routes			
	Peroral	Rectal	Rectal + C10	Nasal
C_{max} (μg/ml)	0.3 ± 0.1	7.9 ± 1.9	21.2 ± 6.3	26.8 ± 4.8
$AUC_{0-\infty}$ (μg h/ml)	0.7 ± 0.1	6.7 ± 1.2	18.2 ± 5.9	55.4 ± 9.5
MRT (h)	7.9 ± 1.9	1.4 ± 0.4	1.9 ± 0.2	2.5 ± 0.1
F (FA) (%)	0.25 (0.33)	2.4	7.7	20.2

Each value represents the mean ± S.E. of three or four animals.

extensively metabolized in the gastrointestinal tracts and the liver (Nishihata et al., 1982). These routes also have many advantages for drugs with low membrane permeability because of their large molecular weight and or low lipophilic character (Hussain et al., 1981).

3.3. The absorption of GL after non-oral administration via rectal and nasal routes

In the case of the rectal administration study, for more GL to be absorbed, we selected C10 among the many absorption enhancers and estimated its enhancing effect on GL absorption, since the effects and mechanisms of the enhancer action of C10 have been studied in detail and also it has the advantage of already being used as an enhancer in a sodium ampicillin suppository as an enhancer in clinical use in Japan and Sweden (Lindmark et al., 1997).

The results after rectal and nasal administration of GL solution (30 mg/kg) are shown in Fig. 2 and Table 2. After rectal administration of GL, the mean $AUC_{0-\infty}$ and C_{max} values, 6.7 ± 1.2 μg h/ml and 7.9 ± 1.9 μg/ml, respectively, were greater than those after oral administration. Furthermore, the addition of C10 (1.0%, w/v) resulted in a remarkable increase in the rectal absorption of GL, i.e. the mean $AUC_{0-\infty}$ and C_{max} values after co-administration with C10 were 18.2 ± 5.9 μg h/ml and 21.2 ± 6.3 μg/ml, respectively, which were approximately 2.7-fold greater than administration of GL alone. The F values for each rectal formula were estimated to be approximately 2.5 and 7.7%, respectively. These values were greater than those after oral administration, especially in the presence of C10, where the F value was approximately 30-fold greater. From these results, it was found that the rectal absorption of GL was superior to the peroral absorption and C10 worked efficiently when used as an absorption enhancer for GL. On the other hand, the absorption of GL at the nasal mucosa in rats was much better than the oral absorption as with the rectal absorption, and the mean $AUC_{0-\infty}$ and C_{max} values for GL were 55.4 ± 9.5 μg h/ml and 26.8 ± 4.8 μg/ml, respectively. The F value was estimated to be approximately 20%, which was approximately 80-fold greater than oral administration despite the absence of an enhancer.

The reason for the superior absorption upon rectal and nasal administration may be because the GL solution at the high concentration was directly delivered to specific small areas, i.e. the rectum and ligated nasal cavity. Additionally, these routes are not affected by interference such as spreading and dilution by gastrointestinal fluid and contents (Sutton et al., 1992),

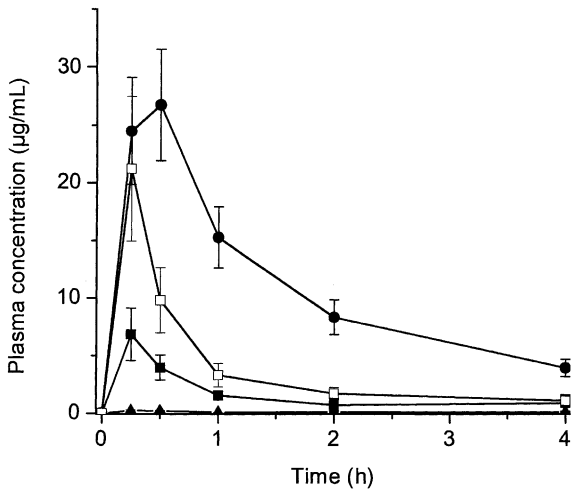

Fig. 2. Plasma concentration of GL after oral, rectal and nasal administration of GL (30 mg/kg) with or without C10 in rats. (▲) Oral; (■) rectal; (□) rectal + C10 (1.0%, w/v); (●) nasal. Each value represents mean ± S.E. of three or four animals.

resulting in the retention of a high concentration of GL at the absorption sites. Consequently, these differences in absorption among the dosing routes observed in this study may be partially explained by the different degrees of dilution of GL at the absorption sites. Additionally, it could be ascribed to morphological differences in the mucosal membranes.

From these findings, it became evident that the rectum and the nasal cavity are very useful administration routes for systemic delivery of GL. It was also found that C10 was a suitable enhancer for absorption of GL.

3.4. Enhancing effects of various sodium fatty acids on the oral absorption of GL

From the results described above, it can be expected that fatty acids such as C10 are useful as absorption enhancers to increase the oral absorption of GL. Thus, the enhancing effects of several fatty acids including C10 were examined on the oral absorption of GL in rats. The mean plasma concentration of GL after oral administration of GL solution (100 mg/kg) containing each enhancer (0.2 M) in rats is shown in Fig. 3 and the calculated pharmacokinetic parameters are summarized in Table 3. The C_{max} values were seen at the first sampling time (15 min), and then the plasma concentration of GL declined rapidly. The mean C_{max} values were 0.4 ± 0.0 μg h/ml, 12.2 ± 2.2 μg h/ml, 24.1 ± 4.5 μg h/ml, 17.4 ± 3.5 μg h/ml, and 6.3 ± 1.1 μg h/ml for control, C8, C10, C12, and C18, respectively. The mean $AUC_{0-4\,h}$ values were 0.3 ± 0.0 μg h/ml, 7.6 ± 1.6 μg h/ml, 19.0 ± 3.7 μg h/ml, 15.6 ± 2.1 μg h/ml, and 5.6 ± 1.1 μg h/ml, respectively. Thus, these fatty acids remarkably increased the digestive absorption of GL in the following order: C10 > C12 > C8 > C18, indicating that fatty acids would be suitable as absorption enhancers when used to improve the oral absorption of GL. Among the fatty acids used, C10 was the most effective for its absorption at the same concentration (0.2 M), and its F value was approximately 60-fold higher than control.

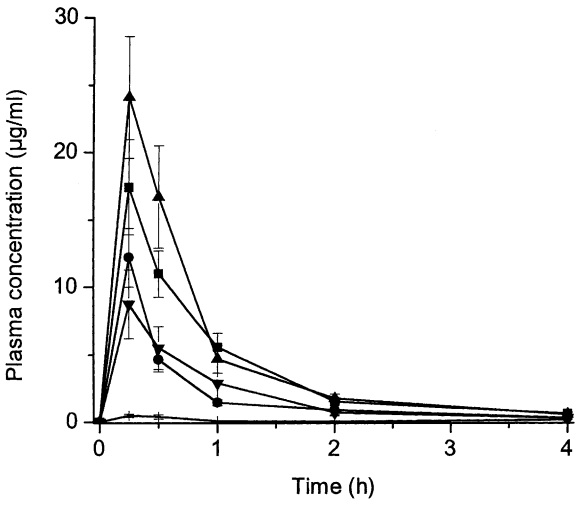

Fig. 3. Enhancing effects of various 0.2 M fatty acids on the oral absorption of GL in rats. GL at a dose of 100 mg/kg was administered as a solution. (●) C8; (▲) C10; (■) C12; (▼) C18; (+) GL alone. Each value represents mean ± S.E. of three animals.

Table 3
Pharmacokinetic parameters of GL after oral administration of GL solution (100 mg/kg) containing various sodium fatty acids (0.2 M)

Enhancers	C_{max} (μg/ml)	$AUC_{0-4\,h}$ (μg h/ml)
Control	0.4 ± 0.0	0.3 ± 0.0
C8	12.2 ± 2.2**	7.6 ± 1.6*
C10	24.1 ± 4.5**	19.0 ± 3.7**
C12	17.4 ± 3.5**	15.6 ± 2.1*
C18	6.3 ± 1.1**	5.6 ± 1.1**

*$P < 0.05$, **$P < 0.01$, compared with the control. Each value represents the mean ± S.E. of three animals.

It has been reported that a high concentration of an enhancer at the absorption site was a principal factor in inducing the ability of enhancers such as C10 (Yoshitomi et al., 1987), and saturation of the enhancing effects of the enhancers at the high concentration was observed in in vitro and in vivo studies (Sekine et al., 1997; Shima et al., 1997). Therefore, the influence of varying concentrations of C10 on the oral absorption of GL was investigated in rats.

The mean $AUC_{0-4\,h}$ value was increased in a concentration-dependent manner by co-administration of C10 (Table 4). Although the trend was toward a leveling off at the concentration of 1.0% (w/v), a similar tendency was observed in the mean C_{max} value, as shown in Fig. 4. Furthermore, it seems that the plasma concentration of GL was sustained in the early period at the concentration of 2.0% (w/v).

The enhancing effect of C10 was reportedly observed while C10 itself was absorbed from the digestive tract by an in situ loop method using phenolsulfonphthalein as a model drug, i.e. it correlated to the disappearance of C10 from the absorption site (Takahashi

Table 4
Pharmacokinetic parameters of GL after oral administration of GL solution (100 mg/kg) containing C10

C10 concentration (w/v, %)	T_{max} (h)	C_{max} (μg/ml)	$AUC_{0-4\,h}$ (μg h/ml)
Control	0.60 ± 0.2	0.4 ± 0.0	0.3 ± 0.0
0.5	0.08 ± 0.0	9.7 ± 0.9**	6.4 ± 0.4**
1.0	0.08 ± 0.0	18.4 ± 4.2*	11.1 ± 2.3*
2.0	0.21 ± 0.0	20.1 ± 2.0**	15.0 ± 2.3**

*$P < 0.05$, **$P < 0.01$, compared with the control. Each value represents the mean ± S.E. of four animals.

et al., 1994). Thus, it is considered that the absorption behavior of GL in this study may be related to the behavior of C10 at the digestive site.

The enhancing effects, action mechanisms and safety of various fatty acids have been investigated physiologically and biochemically. As an example, the sodium fatty acids including C10 increased the absorption of poorly absorbed drugs such as cefmetazole by enhancing the paracellular permeability by some structural change in the tight junction (Tomita et al., 1988a) and altering the transcellular permeability by membrane perturbation arising from the interaction between an enhancer and membrane proteins or lipids (Tomita et al., 1988b). In a recent report, it was confirmed visually that C10 enhanced fluorescein isothiocyanate-dextran 4000 of a hydrophilic compound via the paracellular route and rhodamine 123 hydrate of a hydrophobic compound via the transcellular route in an assessment using confocal laser scanning of Caco-2 cell monolayers (Sakai et al., 1997). In these studies, the enhancing effect of C10 was greater than other fatty acids, and also the degree of the enhancing effect of various fatty acids in this study was nearly in accordance with the results reported previously using drugs without a specific transport system. From these viewpoints, GL may be transported via the paracellular or transcellular route. However, further study will be necessary to elucidate the transport mechanism of GL.

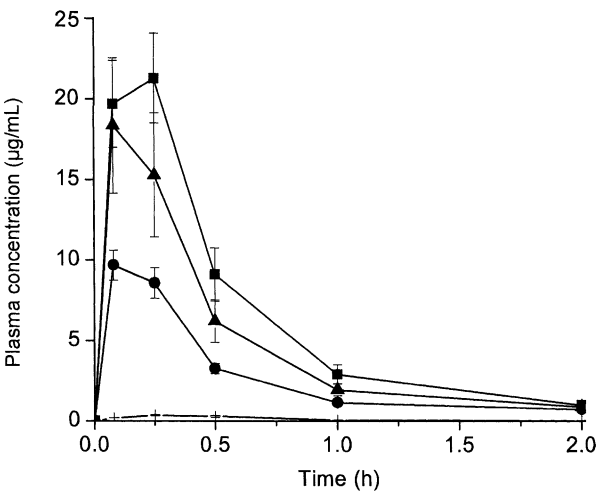

Fig. 4. Enhancing effects of the various concentration of C10 on the oral absorption of GL in rats. GL at a dose of 100 mg/kg was administrated as a solution. (+) Control; (●) 0.5%; (▲) 1.0%; (■) 2.0%. Each value represents mean ± S.E. of four animals.

On the other hand, it has been reported that some fatty acids damaged Caco-2 cell monolayers in in vitro study (Shima et al., 1997), whereas the addition of C10 reduced the damages at the human rectal mucosa caused by the triglyceride suppository base in rectal drug product in Sweden, Doktacillin™ suppository (Lindmark et al., 1997). Therefore, further study will be also necessary to clear these different findings on safety of various fatty acids.

In conclusion, it became evident that the rectum and the nasal cavity are very useful administration routes for the systemic delivery of GL. In addition, it was shown that when used to improve the oral bioavailability of GL, fatty acids were useful absorption enhancers. These results are expected to support the possibility of new dosage forms for non-intravenous GL therapies.

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