Combinatorial Use of Sodium Laurate with Taurine or L-Glutamine Enhances Colonic Absorption of Rebamipide, Poorly Absorbable Antiulcer Drug, without Any Serious Histopathological Mucosal Damages

MASATERU MIYAKE,[1,2] YOSHIKAZU OKA,[2] TAKANORI MINAMI,[3] HAJIME TOGUCHI,[2] MASAAKI ODOMI,[2] KEN-ICHI OGAWARA,[1] KAZUTAKA HIGAKI,[1] TOSHIKIRO KIMURA[1]

[1]Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka Okayama 700-8530, Japan

[2]Formulation Development 1, Formulation Research Institute, Otsuka Pharmaceutical Co., Ltd., 224-18 Ebino, Hiraishi, Kawauchi-cho, Tokushima 771-0182, Japan

[3]Histopathology Research Office, Department of Toxicology, Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima 771-0192, Japan

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ABSTRACT: We previously reported that the combinatorial use of sodium laurate (C12) with several amino acids such as taurine (Tau) and L-glutamine (L-Gln) enhanced the colonic absorption of phenol red with attenuating the local toxicity caused by C12. However, even these amino acids could not protect epithelial cells from being damaged if the mucosal damage got worse to the coagulation necrosis by an excessive dose of C12. Comparing C12 with sodium caprate (C10), used in drug products marketed, 100 μmol C10 was needed to exert the similar absorption-enhancement of rebamipide, a poorly absorbable antiulcer drug, to that by 10 μmol C12, and 100 μmol C10 was obviously more toxic to the mucosa than 10 μmol C12. The combinatorial use of C12 with Tau or L-Gln enhanced the colonic absorption of rebamipide four to nine times larger in AUC than the control. Histopathologic studies clearly showed that Tau and L-Gln exerted the cytoprotective action on epithelial cells suffering from slight damages such as shrinkage and exfoliation, more articulately at 6 h than at 1.5 h after dosing. In conclusion, the combinatorial use of C12 with Tau or L-Gln could lead to a novel formulation improving the bioavailability of poorly absorbable drugs without any serious local damages.
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Keywords: absorption enhancer; sodium laurate; local toxicity; cytoprotection; taurine; L-glutamine, histopathology

INTRODUCTION

Development of combinatorial chemistry and high-throughput screening technique has made it possible to generate a lot of new drug candidates very rapidly, but which has resulted in a number of poorly soluble and/or poorly absorbable candidates at the same time. This problem must be overcome to develop a new drug having better pharmacological activity. As the improvement of drug solubility or permeability by using an absorption enhancer has been very attractive from the aspects of biopharmaceutics, pharmacology, and economics, many researchers investigated

Correspondence to: Toshikiro Kimura (Telephone: 81 86 251 7948; Fax: 81 86 251 7926;
E-mail: kimura@pheasant.pharm.okayama-u.ac.jp)

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the absorption enhancement using various adjuvants.[1–5] However, it has been very difficult to use those adjuvants for practical formulation, because they possibly cause the local toxicity.[2] Although many compounds have been reported to have the absorption enhancing ability,[6–8] medium-chain fatty acids (MCFA) and medium-chain glycerides are thought to be relatively safe because they are used as nutritional supplementary diets.[9,10] MCFA such as sodium caprate (C10)[5,11,12] and sodium laurate (C12),[5] and medium-chain glycerides[3,13] have been investigated as possible candidates for an absorption enhancer. The potential local toxicity, however, has not been low enough for them to be marketed as an absorption enhancer in pharmaceutical products. That can be the reason why only C10 is used as an absorption enhancer in ampicillin suppository marketed in Japan, Denmark, and Sweden,[5] and in ceftizoxime suppository in Japan.[14] Therefore, the development of the formulation enhancing the drug absorption without any serious local damages, has been very attractive. We have already reported that C12 is a promising absorption enhancer, better than C10 in the enhancing ability,[15] and that its combinatorial use with several amino acids such as L-glutamine (L-Gln), L-arginine, and L-methionine attenuates the local toxicity caused by C12, while keeping the absorption enhancing effect of C12 high.[16] Furthermore, we have clarified some mechanisms behind the cytoprotection by several amino acids as follows: (1) suppression of intracellular Ca^{2+} concentration elevated by C12, (2) inhibition of histamine release induced by C12, and (3) involvement of heat-shock protein 70 (HSP70) only induced by L-Gln.[17] In the present study, first of all, we compared C12 with C10 in the absorption enhancement and the mucosal toxicity, which evidenced that C12 is a more effective and safer adjuvant than C10. Then, employing Tau or L-Gln, which is a promising amino acid for cytoprotective action, the combinatorial use of C12 with Tau or L-Gln was evaluated for the absorption enhancement of rebamipide, a poorly absorbable antiulcer drug,[18] and for the cytoprotective action especially by histopathological studies.

MATERIALS AND METHODS

Materials

C12, Tau and L-Gln were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Phenol red (PR) was purchased from Sigma Chemical Co. (St. Louis, MO). Hydroxypropyl methylcellulose (HPMC) TC-5E (HPMC 2910) was purchased from Shin-etsu Kagaku Co. (Tokyo). Rebamipide and OPC-12853, an internal standard, were obtained from Otsuka Pharmaceutical Co. Ltd. (Tokushima, Japan). All other reagents were analytical grade commercial products.

Animals

Male SD rats (Japan SLC, Hamamatsu, Japan), maintained at 23°C and 60% humidity, were allowed free access to standard laboratory chow (Oriental Yeast Co. Ltd., Tokyo) and water prior to the experiments. Rats weighing about 220 g were assigned randomly to each experimental group. Our investigations were performed after approval by our local ethical committee at Otsuka Pharmaceutical Co. Ltd. and Okayama University and in accordance with "Principles of Laboratory Animal Care (NIH publication # 85-23)."

Absorption Study

The absorption of PR, which is often used as an unabsorbable marker,[4,13] or rebamipide was estimated by performing the *in situ* colon loop study, because the intracolonic administration of drugs and/or delivery agent combination is a good model for oral administration in rats,[19] and because the development of a colon-target formulation or suppository is under consideration. In the present study, the descending colon was chosen as a typical segment of colon to be examined. Briefly, after rats were anesthetized by intraperitoneal injection of pentobarbital (1 g/kg), the descending colonic lumen was washed out by saline, and 2.5 mL of drug solution was introduced into the 5-cm length loop of the colon. The volume of 2.5 mL is usually too much for the 5-cm length lumen, but this large volume of drug solution was intentionally introduced to the colonic loop, because we wanted to make the solution evenly contact with the mucosal cells for the subsequent histopathological study. After 1.5 or 6 h, the remaining solution in the loop and the washings of the lumen with Tris/HCl buffer (pH 7.4) were combined and used for the assay of chemicals. In the case of rebamipide absorption study, blood samples were periodically taken from the jugular vein. PR solution was prepared by dissolving C12, an amino acid and 100-μM PR in Tris/HCl buffer (pH 7.4). Rebamipide solution was prepared by

dissolving 2 mg/mL rebamipide, 1% (w/v %) HPMC and a proper amount of 2N NaOH in Tris/HCl buffer (pH 7.4) with or without C12 and/or an amino acid. AUC and MRT from 0 to 6 h were calculated according to the trapezoidal rule.

Estimation of Local Toxicity by C12

After 1.5 or 6 h of *in situ* absorption study described above, the remaining solution in the loop and the washings of the lumen with Tris/HCl buffer (pH 7.4) were combined and used for the assay of total protein. In the present study, the elution of total proteins was only employed as a biochemical marker for the local toxicity, because our previous studies clearly showed that it could be a representative marker among phospholipids, total proteins and lactate dehydrogenase.[16,17]

In Vitro Membrane Transport Study

To estimate the apparent permeabilities of rebamipide and PR, *in vitro* membrane transport studies were performed using the rat colon. The contents in the colon were washed out by introducing slowly ice-cold Ringer's solution (pH 7.4), containing 1.2 mM NaH_2PO_4, 125 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 10 mM $NaHCO_3$, and 2 mg/mL D-glucose. The 3-cm segment of the colon was isolated and opened and then immediately the muscularis propria was stripped off. The resulting colon sheet was mounted in a diffusion chamber with 1.25 cm^2 of exposed area (Corning Costar Japan, Tokyo). Both sides of the tissue were bathed with 6 mL of Ringer's solution gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. After 10-min preincubation, the bathing solution was replaced with rebamipide solution (2 mg/mL, pH 7.4) or PR solution (1 μmol/mL, pH 7.4) on the apical side and fresh Ringer's solution on the basal side. Samples of 1 mL were drawn out of the receptor side at 10-min intervals to 90 min. An equal volume of Ringer's solution was immediately added to the receptor side after each sampling. Apparent permeability coefficient (P_{app}) was calculated according to the following equation (1):

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{A \cdot C_0} \quad (1)$$

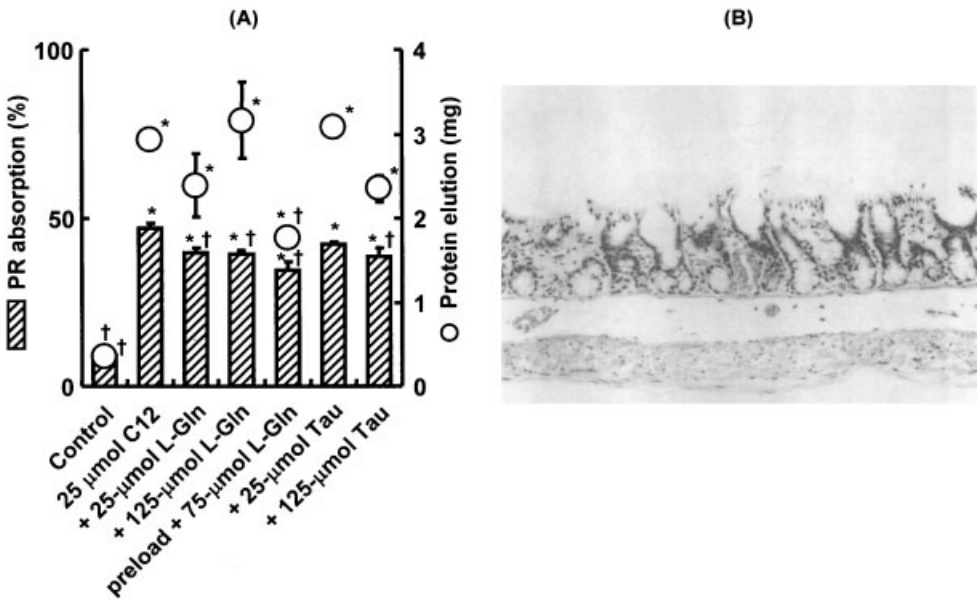

Figure 1. Effect of high dose of C12 on absorption enhancement of PR and local toxicity in rat colon. (A) PR absorption and protein elution. An *in situ* colon loop study was performed for 1.5 h. C12 was administered at a dose of 25 μmol. L-Gln was coadministered at 25 or 125 μmol, or preloaded and coadministered at 75 μmol. Tau was applied at 25 or 125 μmol. Results are expressed as the mean with the bar showing SE value of four or five experiments. $^*p < 0.05$ compared with control; †$p < 0.05$ compared with C12; Key: PR absorption, striped; open circles, protein elution. (B) Light micrograph of coagulation of rat colonic epithelia. Light micrograph was taken after 1.5-h *in situ* closed-loop study with PR and 25 μmol C12 (77.5 × magnification).

Table 1. No Cytoprotective Action by L-Gln or Tau after 1.5 h *In Situ* Colonic Loop Study for PR with a High Dose of C12

Findings for Epithelial Cells	C12 Only					C12 + 25 μmol L-Gln					C12 + 125 μmol L-Gln					C12 + Preload 75 μmol L-Gln[a]					C12 + 25 μmol Tau						C12 + 125 μmol Tau				
	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	5	Mean	1	2	3	4	Mean	1	2	3	4	5	Mean	1	2	3	4	Mean
Shrinkage	×	×	×	×	×	2	2	×	×	×	×	×	×	×	×	×	1	1	2	2	×	×	×	×	×	×	×	2	2	2	2	2.00
Exfoliation	2	2	2	2	2.00	2	2	2	2	2.00	2	2	2	2	2	2.00	2	2	2	2	2.00	2	2	2	2	2	2.00	2	2	2	2	2.00
Coagulation necrosis	2	2	1	1	1.50	0	0	1	1	0.50	3	1	3	1	2	2.00	0	1	2	0	0.75	2	3	1	1	2	1.80	0	1	2	1	1.00

[Score] 0, no change; 1, very slight; 2, slight; 3, moderate; 4, severe; ×, unable to estimate.
Doses of PR and C12 were 250 nmol and 25 μmol, respectively. Histopathologic examination was performed by an experienced veterinary histopathologist according to the procedure described in Materials and Methods.
[a] 75 μmol L-Gln was preloaded 1 h before dosing of rebamipide and was also coadministered with C12.

where dQ/dt, A, and C_0 reveal the initial permeation rate, the exposing surface area and the initial concentration of rebamipide or PR, respectively.

Analytical Method

Rebamipide in Serum

Serum was separated using Separapid tube S (Sekisui Medical, Osaka, Japan) from blood. HPLC-grade acetonitrile (0.1 mL) including OPC-12853 was added to 0.1 mL of serum. After vortex mixing and left for 30 min, 0.1 mL of distilled water was added to the mixture. Following centrifugation at 14,000 rpm for 5 min (HIMAC centrifuge, HITACHI, Tokyo), the supernatant was filtrated with a microfilter (pore size 0.5 μm, Millipore, Tokyo) and introduced into HPLC system, which consisted of a model of CCPM HPLC pump (Tosoh, Tokyo) and a fluorescence detector (SF-8010; Tosoh) set at Ex. 330 nm and Em. 370 nm. Precolumn and analytical column were BSA-ODS (35 × 4.6 mm i.d., Tosoh) and ODS-80 TM (150 × 6.0 mm i.d., Tosoh), respectively. The mobile phase, acetonitrile:5 mM Na_2SO_4 (3:5, v/v) including 1% acetic acid and 0.3% tetrahydrofuran, was delivered at 1 mL/min. The retention times of rebamipide and OPC-12853 in this system were 9.5 and 11.5 min, respectively. Coefficient of variation of the standard curve ranged from 0.1 to 20.0% and the correlation coefficient was over 0.980.

PR Assay

PR was determined by a spectrophotometer (Shimazu UV-260, Shimazu, Kyoto, Japan) at 560 nm after alkalinization by 0.02N NaOH including 1 mM EDTA-2Na.

Protein Assay

Protein was assayed utilizing a kit (DC Protein assay) supplied by Bio Rad Laboratories (Hercules, CA) according to a modified method reported by Lowly et al.[20]

Histopathologic Evaluation

After *in situ* rat colon loop studies were carried out for 1.5 or 6 h as described above, the colonic lumen was washed out with 10 mM Tris/HCl buffer (pH 7.4) and was filled with 10% neutral formalin buffer, and was fixed *in situ*. The loop segment was then removed and immersed in the

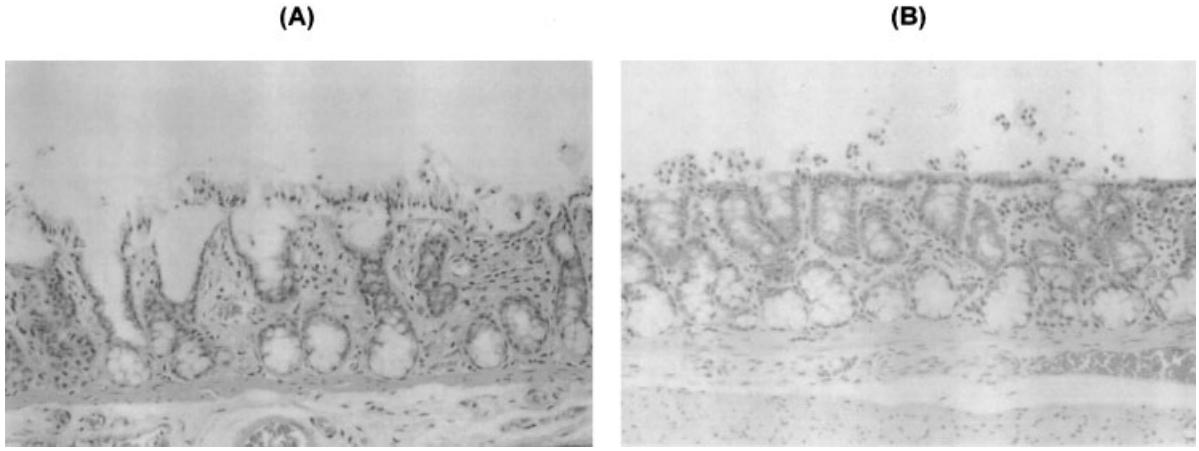

Figure 2. Histopathologic comparison between C10 (A) and C12 (B) in local toxicity. Light micrographs were taken at 1.5 h after starting an *in situ* colon loop study (A) with 100 μmol C10 and (B) with 10 μmol C12, respectively (93 × magnification).

same fixative. Cross-sections were prepared and stained with hematoxylin-eosin, and were examined by light microscopy. Various measures of histopathologic abnormality were quantified on an arbitrary scale of 0–4, with 0 indicating no effect and 4 indicating a sever effect. This evaluation was carried out by an experienced veterinary histopathologist in a blinded fashion.

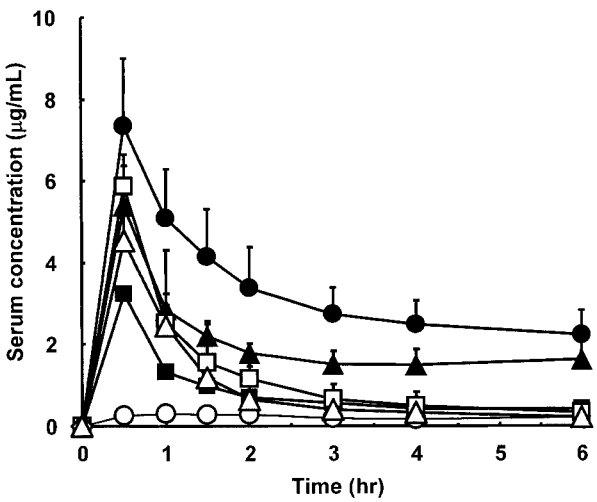

Figure 3. Effect of combinatorial use of C12 and amino acids on absorption of rebamipide in *in situ* colon loop study. C12 was administered at a dose of 10 μmol. 75 μmol L-Gln was preloaded 1 h before dosing of rebamipide and was also coadministered with C12. Results are expressed as the mean with the bar showing SE value of three or four experiments. Key: ○, control; ●, C12; △, C12 + 25 μmol Tau; ▲, C12 + 50 μmol Tau; □, C12 + 125 μmol Tau; ■, C12 + preload and coadministration of 75 μmol L-Gln.

Statistical Analysis

Results are expressed as mean ± SE of more than three experiments. Analysis of variance (ANOVA) was used to test the statistical significance of differences among groups. Statistical significance in the differences of the means was determined by Dunnet's method or Student's *t*-test.

RESULTS

Using C12 at a higher dose (25 μmol) than used in the previous study (10 μmol),[16,17] PR absorption and the cytoprotective action of Tau and L-Gln were investigated in an *in situ* colon loop study (Figure 1A and Table 1). Although PR absorption was enhanced extensively, the protein elution could not be attenuated either by Tau or L-Gln (Figure 1A). Only the preload and coadministration of 75 μmol L-Gln tended to decrease the elution of protein. Histopathologic examination also showed that the local toxicity caused by 25 μmol C12 was severe and that the addition of either Tau or L-Gln could not protect the epithelial cells from being damaged (Table 1). Figure 1B is a typical picture of epithelial cells after an *in situ* loop study with 25 μmol C12, where the coagulation necrosis of epithelial cells was observed, meaning that the epithelial cells kept necrosing continuously. These results suggest that even Tau and L-Gln cannot protect the epithelial cells from being damaged, if the local damages caused by C12 are very severe such as the coagulation necrosis.

Table 2. Effect of Combinatorial Use of C12 and L-Gln or Tau on Absorption of Rebamipide and Local Toxicity Induced by 10 μmol C12 in an *In Situ* Rat Colon Loop Study

Adjuvants	C_{max} (μg/mL)	T_{max} (h)	MRT (h)	AUC (μg · h/mL)	Protein Elution (mg)
Control	0.31 ± 0.03^{b}	2.7 ± 1.7	2.49 ± 0.26	1.26 ± 0.07^{b}	3.25 ± 0.09^{b}
C12	7.35 ± 1.71^{a}	0.5 ± 0.0	2.92 ± 0.18	19.47 ± 4.28^{a}	4.95 ± 0.15^{a}
C12 + 75 μmol L-Gln[c]	3.23 ± 0.18^{b}	0.5 ± 0.0	1.97 ± 0.25^{a}	$4.84 \pm 0.49^{a,b}$	3.82 ± 0.21^{b}
C12 + 25 μmol Tau	4.74 ± 1.14^{a}	0.5 ± 0.0	1.63 ± 0.04^{a}	$6.98 \pm 1.91^{a,b}$	3.63 ± 0.20^{b}
C12 + 50 μmol Tau	5.37 ± 0.50^{a}	0.5 ± 0.0	2.49 ± 0.28	11.88 ± 1.73^{a}	3.71 ± 0.11^{b}
C12 + 125 μmol Tau	5.55 ± 0.50^{a}	0.5 ± 0.0	1.56 ± 0.33^{a}	$7.23 \pm 1.59^{a,b}$	$4.11 \pm 0.17^{a,b}$

Doses of rebamipide and C12 were 5 mg and 10 μmol, respectively. AUC and MRT mean the area under the serum concentration–time curve and the mean residence time of rebamipide from 0 to 6 h obtained following the trapezoidal rule, respectively. Each value represents the mean ± SE of more than three experiments.

[a] $p < 0.05$ compared with control.

[b] $p < 0.05$ compared with 10 μmol C12.

[c] 75 μmol L-Gln was preloaded 1 h before dosing of rebamipide and was also coadministered with C12.

To make it clear that C12 and its combinatorial use with Tau or L-Gln should be promising, we compared C12 with C10 in the absorption enhancing ability and local toxicity. In an *in situ* colon loop study, 10 μmol of C12 enhanced the absorption of rebamipide about 16 times larger in AUC (19.15 ± 1.61 μg · h/mL; $n = 4$; $p < 0.05$ versus control) than the control (1.17 ± 0.16 μg · h/mL; $n = 4$) and 100 μmol of C10 was needed to exert the similar effect (22.87 ± 1.09 μg · h/mL; $n = 4$; $p < 0.05$ versus control) to that by 10 μmol of C12. Ten micromoles of C10 could not improve the absorption of rebamipide at all (0.61 ± 0.12 μg · h/mL; $n = 4$). The amount of protein elution caused by 100 μmol C10 (5.58 ± 0.22 mg; $n = 4$; $p < 0.05$ versus 10 μmol C12) was significantly greater than that by 10 μmol C12 (3.17 ± 0.06 mg; $n = 4$). Furthermore, as shown in Figure 2, 100 μmol C10 brought about the coagulation necrosis of epithelial cells (Figure 2A), while 10 μmol C12 did not (Figure 2B). These results suggest that 10-μmol C12 should be a more effective and safer than 100 μmol C10.

Then, we examined the effect of the combinatorial use of C12 with Tau or L-Gln on the absorption of rebamipide, a poorly absorbable antiulcer drug,[18] and on the colonic mucosa. First of all, an *in vitro* membrane transport studies for rebamipide and PR were performed to estimate the apparent permeability (P_{app}) using an isolated colon sheet. P_{app} of rebamipide is $2.93 \pm 0.35 \times 10^{-6}$ cm/s ($n = 4$), which is significantly smaller than that for PR ($p < 0.001$, P_{app} is $5.06 \pm 0.35 \times 10^{-6}$ cm/s, $n = 6$). This result clearly shows that the membrane permeability of rebamipide is very low.

Figure 3 shows the serum concentration–time profile of rebamipide in an *in situ* colon loop study and the pharmacokinetic parameters are summarized in Table 2. Ten micromoles of C12 enhanced the absorption of rebamipide about 15 times larger in AUC than the control. Although the addition of Tau or L-Gln attenuated the enhancing effect of C12, the combinatorial use could enhance the absorption four to nine times larger in AUC than the control. Protein elution from the mucosa, a biochemical marker for the mucosal damage, is also shown in Table 2. Ten micromles of C12 increased significantly the protein elution comparing with the control. Its combinatorial use with Tau or L-Gln significantly attenuated protein elution from the colonic mucosa, and there was not any significant difference between control and 75 μmol L-Gln, 25 μmol Tau, or 50 μmol Tau.

To confirm the cytoprotective action by the combinatorial use of C12 with Tau or L-Gln, the histopathologic examination was performed. Table 3 shows the scoring results obtained by histopathologic observation at 1.5 h after starting an *in situ* loop study. The results clearly reveal that the preload and coadministration of 75 μmol L-Gln or 125 μmol Tau exerted the cytoprotective action on the colonic mucosa. Figure 4 shows the typical picture of the colonic mucosa in the case of the combinatorial use of C12 with 125 μmol Tau. As already shown in Figure 2B, 10 μmol C12 caused the shrinkage and exfoliation for colonic epithelial cells, although the coagulation necrosis was not observed. On the other hand, its combinatorial use with 125 μmol Tau did attenuate mucosal damages such as the shrinkage and exfoliation (Figure 4).

Table 4 and Figure 5 show the results of the histopathologic examination after 6-h loop study. It was also clearly shown that a using Tau or L-Gln

Table 3. Cytoprotective Action by L-Gln and Tau after 1.5 h *In Situ* Colonic Loop Study for Rebamipide with 10 μmol C12

Findings for Epithelial Cells	Control					C12 Only					C12 + 125 μmol L-Gln					C12 + Preload[a] 75 μmol L-Gln					C12 + 125 μmol Tau				
	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean
Shrinkage	0	0	0	0	0.00	2	2	2	1	1.75	2	1	2	1	1.50	1	0	1	1	0.75	1	1	1	2	1.25
Exfoliation	0	0	0	0	0.00	2	2	2	2	2.00	1	2	2	2	1.75	0	0	0	1	0.25	0	1	2	1	1.00
Coagulation necrosis	0	0	0	0	0.00	0	0	0	0	0.00	0	0	0	0	0.00	0	0	0	0	0.00	0	0	0	0	0.00

[Score] 0, no change; 1, very slight; 2, slight; 3, moderate; 4, severe; ×, unable to estimate.
Doses of rebamipide and C12 were 5 mg and 10 μmol, respectively. Histopathologic examination was performed by an experienced veterinary histopathologist according to the procedure described in Materials and Methods.
[a]75 μmol L-Gln was preloaded 1 h before dosing of rebamipide and was also coadministered with C12.

could protect the mucosal cells from being damaged from the scoring results (Table 4). Furthermore, Figure 5 evidences more explicitly that there was not any mucosal damages after a 6-h loop study with the combinatorial use of C12 with 125 μmol Tau (Figure 5A), while the exfoliation of the epithelial cells were still observed in the use of C12 only (Figure 5B).

DISCUSSION

We have already reported that C12 could be one of the potent absorption enhancers[15] and that the combinatorial use of C12 with some amino acids could be useful to develop a new dosage form which can enhance the oral absorption of poorly absorbable drugs safely.[16,17] For the practical use of this combinatorial regimen, first of all, we investigated the effect of high dose of C12 on the colonic mucosa (Figure 1 and Table 1). Twenty-five micromoles of C12 caused severe mucosal damages including coagulation necrosis and could not be recovered even by either Tau or L-Gln, although the absorption of PR was enhanced extensively. The scoring results of mucosal damages show that L-Gln (25 μmol and preload 75 μmol) and Tau (125 μmol) tend to decrease the score in coagulation necrosis, but there must not be even a single observation of coagulation necrosis from the aspect of histopathology. So, we judged that the mucosal damages were not attenuated here. By contrast, the mucosal damages caused by 10 μmol C12 were only the shrinkage and exfoliation of epithelia (Figure 2B and Table 3), which were attenuated by Tau or L-Gln (Figure 4 and Table 3). Especially, the cytoprotective action of 125 μmol Tau was remarkable at 6 h after dosing (Figure 5A and Table 4). Considering these results, neither Tau nor L-Gln can protect the epithelial cells from being damaged at the severe degree where coagulation necrosis is observed. Turnover of intestinal epithelial cells is well known to be very fast,[4] and the shrinkage and exfoliation of epithelia are usually observed during the cycle of turnover. However, the epithelial cells obviously suffered from both of them much more severely when C12 was applied than the control (Figure 2B and Table 3), indicating that C12 actually caused some damages on the mucosa.

In the present study, rebamipide, an antiulcer drug, was chosen as a model drug, because rebamipide is a poorly water soluble and poorly absorbable drug. This drug has been marketed in

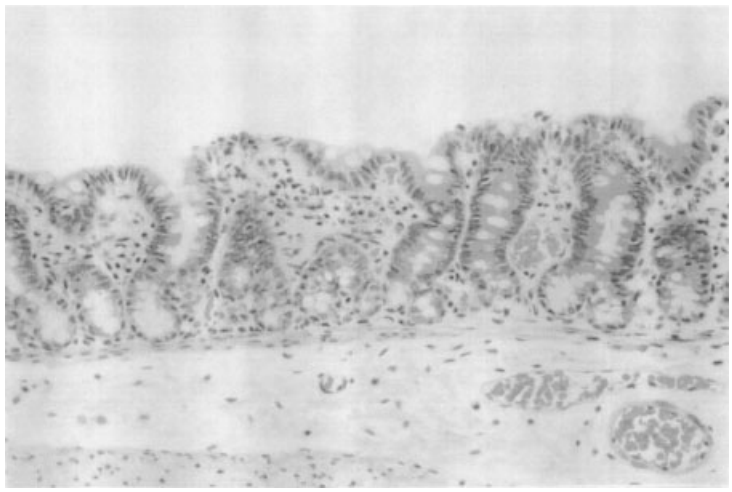

Figure 4. Histopathologic observation of cytoprotective action by Tau after 1.5-h *in situ* colon loop study with 10 μmol C12. Light micrographs were taken after 1.5-h *in situ* colon loop study with 125 μmol Tau (93 × magnification).

Japan since 1990 as a therapeutic product for the gastric ulcer and acute and/or chronic gastritis,[21–24] and its solubility is quite low as follows: 0.0001 or 0.013%(w/v) at pH 3 or 7, respectively. Although the solubilization by HPMC enhanced the absorption of rebamipide (AUC for rebamipide suspension, 0.50 ± 0.10 μg · h/mL; $n = 4$), AUC is only 1.26 ± 0.07 μg · h/mL (Table 2). P_{app} of this drug obtained in an *in vitro* transport study using an isolated colon sheet is significantly smaller than that for PR. Considering the low solubility and low permeability, rebamipide should be classified into Class IV in Biopharmaceutics Classification System (BCS).[25] Drugs categorized into BCS Class IV such as rebamipide need some formulation that can solubilize them and enhance their membrane permeability for the improvement in the therapeutic efficacy and in the economics. Compared with C10 in the absorption enhancement and the mucosal damages, 100 μmol of C10, 10 times of C12, was needed to exert the similar enhancing effect for the absorption of rebamipide to that by 10 μmol of C12 and the use of 100 μmol C10 led to the coagulation necrosis (Figure 2A), which would not be recovered by either Tau or L-Gln. These results indicate that the smaller amount of C12 can enhance the drug absorption effectively and cause more slight damages to the mucosa than C10, which has already been used in marketed drug products.[5,14] These results mean that C12 is a safer and more effective absorption enhancer than C10. However, even using the smaller amount of C12 (10 μmol), the slight mucosal damages that should be prevented for the practical use was observed from the histopathological aspect (Figure 2B and Table 3).

We focused on Tau or L-Gln here, because they are unique among amino acids examined previously.[17] L-Gln is only an amino acid that was evidenced to be related with heat-shock protein 70 (HSP70) in terms of its cytoprotective action and Tau was suggested to have the most effective cytoprotective activity among them.[17] As shown in Table 3, the cytoprotective action by L-Gln was not as clear as observed before.[16,17] However, the combination of the preload with coadministration of 75 μmol L-Gln obviously functions as a cytoprotective adjuvant as well as the coadministration of Tau (Tables 3 and 4). Because quercetin, an inhibitor for the biosynthesis of HSP70, canceled out the cytoprotective action by L-Gln, the induction of HSP70 expression was suggested to be closely related with it.[17] Although we indicated that the combinatorial use of C12 with L-Gln extensively induced HSP70 expression during a 1.5-h loop study by Western blot analysis,[17] the longer exposure to L-Gln might allow HSP70 to be biosynthesized much more, leading to the more effective cytoprotection. On the other hand, Tau showed the magnificent cytoprotection by its coadministration (Figures 4 and 5, Tables 3 and 4). As reported previously, Tau can suppress most effectively the elution of phospholipids and of proteins, and the secretion of histamine from rat colon and the cytosolic concentration of Ca^{2+}, which were enhanced by C12.[17] The present study confirmed that Tau should be one of the most promising adjuvants from the aspect of cytoprotection.

Table 4. Cytoprotective Action by L-Gln and Tau after 6 h *In Situ* Colonic Loop Study for Rebamipide with 10 μmol C12

Findings for Epithelial Cells	Control				C12 Only				C12 + Preload[a] 75 μmol L-Gln				C12 + 50 μmol Tau				C12 + 125 μmol Tau			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
Shrinkage	0	1	1	0.67	0	0	0	0.00	0	0	0	0.00	0	0	0	0.00	0	0	0	0.00
Exfoliation	1	1	1	1.00	2	2	2	2.00	0	0	2	0.67	0	0	2	0.67	0	0	0	0.00
Coagulation necrosis	0	0	0	0.00	0	0	0	0.00	0	0	0	0.00	0	0	0	0.00	0	0	0	0.00

[Score] 0, no change; 1, very slight; 2, slight; 3, moderate; 4, severe; ×, unable to estimate.
Doses of rebamipide and C12 were 5 mg and 10 μmol, respectively. Histopathologic examination was performed by an experienced veterinary histopathologist according to the procedure described in Materials and Methods.
[a]75 μmol L-Gln was preloaded 1 h before dosing of rebamipide and was also coadministered with C12.

Considering the process of causing mucosal damages, the shrinkage of epithelial cells comes about at the beginning and the exfoliation is at the next stage. As shown in Table 3, the preload and coadministration of 75 μmol L-Gln or 125 μmol Tau attenuated the shrinkage as well as the exfoliation of epithelia at 1.5 h after dosing, suggesting that the exfoliation that will be caused at later periods could be attenuated. Actually, Table 4 shows that the combinatorial use of C12 with Tau or L-Gln, especially 125 μmol Tau, completely prevented the epithelia from being damaged at 6 h after dosing. Comparing with the epithelia applied with C12 only, the cytoprotective effect by 125 μmol Tau is clearly significant (Figure 5). Histopathologic examination at 6 h after dosing also reveals that no shrinkage was observed even in the rat colonic tissue applied with C12 only (Figure 5 and Table 4), suggesting that mucosal damages caused by 10 μmol C12 themselves would be slight enough to be recovered spontaneously after 6 h and more. This might be partly attributed to the rapid absorption of medium-chain fatty acids.[26] Although the remaining amount of C12 in the colonic lumen was not monitored, C12 dosed must have almost completely been absorbed for 6 h by considering that the dose of C12 was quite small, and that there could be a close relationship between its enhancing effect and its absorption kinetics.[13,15]

Rebamipide has also been reported to have a cytoprotective action against the gastric ulcer by enhancing the level of endogenous prostaglandins and so on.[21,27] Therefore, rebamipide may partly play a role in the cytoprotection in the present study, but the difference in the mucosal damages between the use of C12 only and the combinatorial use indicated clearly the cytoprotective action by Tau and L-Gln (Figures 4 and 5, Tables 3 and 4).

When it comes to the absorption-enhancing ability, the combinatorial use with Tau or L-Gln decreased the enhancing effect by C12 itself (Figure 3 and Table 2). However, the coadministration of 50 or 125 μmol Tau enhanced the absorption of rebamipide about nine or six times larger than the control, respectively. Considering that there were not any damages in the colonic tissue from the viewpoint of histopathology at 6 h after dosing, this combinatorial use should lead to a quite safe and effective absorption-enhancing formulation for poorly absorbable drugs. To fix the optimal formulation, further studies in detail must be needed, but the combinatorial use of 10 μmol C12 with 50 μmol or 125 μmol Tau must be a standard formulation. Mechanisms of the absorption

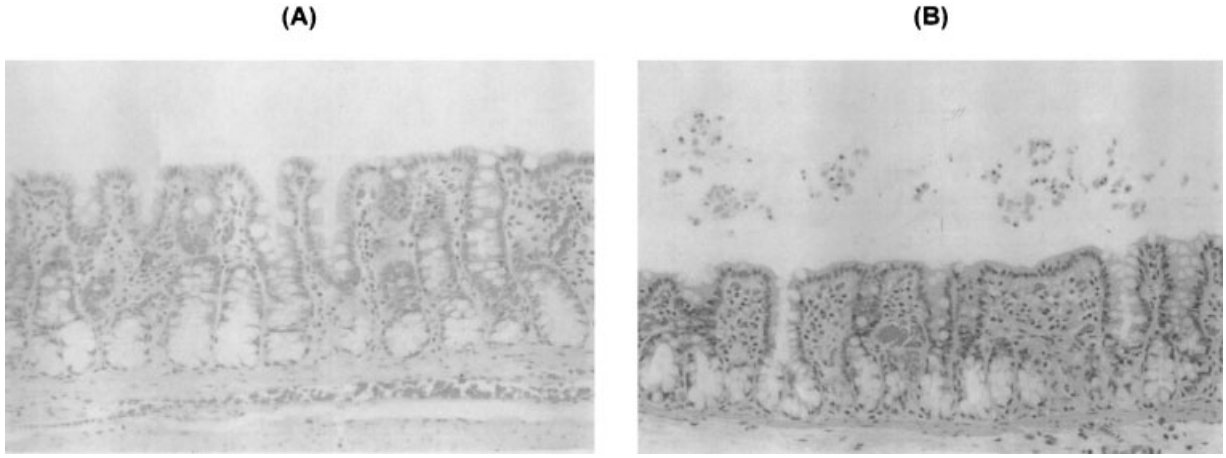

Figure 5. . Histopathologic observation of cytoprotective action by Tau after 6-h *in situ* colon loop study with 10 μmol C12. Light micrographs were taken after 6-h *in situ* colon loop study (A) with 125 μmol Tau and (B) without Tau, respectively (93 × magnification).

enhancement by C12 itself and those of change in the enhancing ability by using Tau or L-Gln remain to be clarified, but medium-chain fatty acids including C10 and C12 have been reported to enhance drug absorption via both paracellular[5,12] and transcellular routes.[28,29] Several amino acids including Tau and L-Gln attenuated the decrease in transepithelial electric resistance caused by C12, which is a kind of criteria for the paracellular transport of drugs.[17] Therefore, the absorption of rebamipide via paracellular route could partly be decreased. On the other hand, the absorption enhancement via transcellular route might be kept to some extent.

In conclusion, C12 is a safer and more effective absorption-enhancing adjuvant than C10 used in drug products marketed, and its combinatorial use with Tau or L-Gln can enhance significantly and safely the absorption of a poorly soluble and poorly absorbable drug such as rebamipide. Further studies based on the present results would lead to the development of the safe and effective practical formulation enhancing the absorption of drugs categorized into Class IV of BCS.

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