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Simultaneous Use of Sodium Deoxycholate and Dipotassium Glycyrrhizinate Enhances the Cellular Transport of Poorly Absorbed Compounds Across Caco-2 Cell Monolayers

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Abstract

The absorption-enhancing effect of a combination of sodium deoxycholate and dipotassium glycyrrhizinate in Caco-2 cell monolayers has been compared with that of the enhancers when used alone, and the mechanism of the enhancement was partially elucidated.

The effect of the combined compounds was evaluated by measurement of transepithelial electrical resistance (TEER) and the cellular permeability of the water-soluble model compounds sodium fluorescein (MW 376·3) and fluorescein isothiocyanate dextran (MW 4400). The TEER of the monolayers decreased with increasing concentrations of dipotassium glycyrrhizinate in combination with 0·02% (w/v) sodium deoxycholate for 20 min, and reached a minimum at 1% (w/v) dipotassium glycyrrhizinate. Although a combination of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate enhanced the cellular permeability of sodium fluorescein and fluorescein isothiocyanate dextran, 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate alone had no effect on either the TEER of the monolayers or the cellular transport of the water-soluble compounds. Sequential and separate exposure of the monolayers to each enhancer for 10 min had no effect on the TEER, but a marked decrease in TEER was observed when both compounds were used in combination. The enhancing effect of the combination of sodium deoxycholate and dipotassium glycyrrhizinate was inhibited by H7, a protein kinase C (PKC) inhibitor, suggesting that dipotassium glycyrrhizinate might enhance the activation of PKC via sodium deoxycholate. The combined use of these two enhancers had no toxic effects.

These results provide useful, basic information on the action of these absorption enhancers on drugs for which absorption is limited owing to polarity or molecular size, or both.

In recent years a variety of pharmaceutical absorption enhancers has been developed to improve the absorption characteristics of poorly absorbed drugs whose absorption is limited by polarity, ion charge and molecular size. Because most of these enhancers result in some cytotoxicity while facilitating the absorption of the target drug, it is necessary to use the enhancer at low dose levels.

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In a previous study with Caco-2 cell monolayers the absorption-enhancing effects of several compounds were compared and the minimum effective dose for each enhancer was determined (Sakai et al 1997). For example, concentrations of sodium deoxycholate and sodium caprate in excess of 0·05% and 0·2% (w/v), respectively, were required to increase the permeability of Caco-2 cell monolayers. Dipotassium glycyrrhizinate had no enhancing effect and elicited an increase in TEER when examined in Caco-2 cell monolayers, even though significant enhancing effects have been

reported in in-vivo experiments (Aliverti et al 1989; Mishima et al 1989; Tanaka et al 1992).

This paper describes some attempts to achieve greater enhancement of absorption by use of a combination of two types of absorption enhancer at dose levels lower than when each enhancer is used alone. The absorption-enhancing effect of a combination of sodium deoxycholate and dipotassium glycyrrhizinate was investigated in Caco-2 cell monolayers under conditions where each individual enhancer has minimum absorption-enhancing activity. Additional experiments were performed to enable understanding of the mechanism of the synergistic effect of these two absorption enhancers when used in combination. This is the first report of an evaluation of the combined use of two types of enhancer in conjunction with Caco-2 cell monolayers.

Materials and Methods

Caco-2 cells were purchased from the American Type-Culture Collection (Rockville, MD). Sodium fluorescein (MW 376·3), fluorescein isothiocyanate dextran (MW 4400), Dulbecco's Modified Eagle's Medium (DMEM), non-essential amino acids, benzylpenicillin G, streptomycin sulphate, sodium deoxycholate, 1-(5-isoquinolinesulphonyl)-2-methylpirerazine dihydrochloride (H7), trypsin and disodium ethylenediaminetetraacetate (EDTA) were purchased from Sigma (St Louis, MO). Foetal bovine serum was purchased from Cytosystems (Castle Hill, Australia) and dipotassium glycyrrhizinate was purchased from Alps Pharm. (Tokyo, Japan).

Cell culture

Caco-2 cells between passages 75 and 90 were routinely cultured in DMEM (pH 7·4) supplemented with a 1% (w/v) solution of non-essential amino acids, 10% (v/v) heat-denatured foetal bovine serum, benzylpenicillin G (100 units mL^{-1}) and streptomycin sulphate (100 $\mu g\,mL^{-1}$) at 37°C under an atmosphere of 95% air and 5% CO_2. Cells were harvested by treatment with 0·05% (w/v) trypsin-0·53 mM EDTA for 10 min at 37°C before reaching confluence, resuspended in DMEM and then seeded at a density of (approx.) 1×10^4 cells cm^{-2} on Transwell inserted filters (Costar, Cambridge, MA) with a surface area of 1 cm^2 and a 3 μm pore size. Culture medium was added to the apical (0·5 mL) and basal (1 mL) chambers. Cells were left to reach confluence and to differentiate for 3 weeks before use. The transepithelial electrical resistance (TEER) of the filter-grown monolayers reached a value of at least 600 $\Omega\,cm^2$ when the monolayers were used for the experiment.

Measurement of TEER

A Caco-2 monolayer was equilibrated for 1 h in fresh calcium- and magnesium-free Hanks' balanced salt solution (HBSS-CMF) and DMEM on the apical and basal sides, respectively. For the inhibition experiments, 200 μM H7 was added to the HBSS-CMF. The TEER of cell monolayers at $t = 0$ was measured with the Millicell electrical resistance system (Millipore Corporation, Bedford, MA) and taken as the initial value. HBSS-CMF in the apical chamber was replaced with 0·5 mL HBSS-CMF with or without enhancers. After 20 min treatment, the apical and basal chambers were gently washed twice with fresh HBSS-CMF and DMEM, respectively, to wash out any residual enhancer. Fresh HBSS-CMF (0·5 mL) and DMEM (1 mL) were then placed in the apical and basal chambers, respectively. The TEER of the Caco-2 cell monolayers was measured and expressed as a percentage of the initial ($t = 0$) value for the same monolayer.

Transport experiment

Test solutions of sodium fluorescein and fluorescein isothiocyanate dextran were prepared in HBSS-CMF at final concentrations of 100 $\mu g\,mL^{-1}$ and 1 $mg\,mL^{-1}$, respectively. After treatment of a monolayer with the enhancers and H7 in the same manner as for the TEER measurement, test solution (0·5 mL) was added to the apical chamber ($t = 0$). The basal chamber was then bathed with 1 mL of DMEM. Samples (50 μL) were collected from the basal chamber at appropriate time points up to 3 h and were analysed by high-performance liquid chromatography (HPLC). The apparent permeability coefficient (P_{app}, $cm\,s^{-1}$) was calculated by use of the equation $P_{app} = (dQ/dt)/(A \cdot C_0)$, where dQ/dt is the permeation rate (steady-state transport rate, $\mu g\,s^{-1}$) obtained from the profile of the amount of fluorescent model compound transported against time, C_0 ($\mu g\,mL^{-1}$) is the initial concentration of fluorescent model compound in the apical chamber, and A (cm^2) is the surface area of the membrane.

HPLC analysis

The HPLC system consisted of a pump (L-6000, Hitachi, Tokyo, Japan), a fluorescence detector (F-1050, Hitachi), a Chromato-integrator (D-2500, Hitachi) and a 4·5 mm i.d. × 10 cm LiChrospher

RP-18 column (Cila-Merck, Darmstadt, Germany). The mobile phase used was 5 mM phosphate buffer (pH 7·4)–acetonitrile, 88 : 12, at a flow rate of 1 mL min^{-1}. The excitation and the emission wavelengths of the fluorescence detector were set at the established wavelengths of maximum absorption of sodium fluorescein and fluorescein isothiocyanate dextran, i.e. 494 and 518 nm, respectively.

Statistical analysis

Depending on the groups, Student's *t*-test or Tukey's multiple rank test was used to compare data. P values < 0.05 were considered indicative of significance. Results are expressed as means ± standard deviations.

Results

The effect on TEER of simultaneous treatment with sodium deoxycholate and dipotassium glycyrrhizinate

Figure 1A shows the effect of each enhancer on TEER when used alone and Figure 1B shows the effect of dipotassium glycyrrhizinate at concentrations ranging from 0·02 to 2% (w/v) in the presence of 0·02% (w/v) sodium deoxycholate on the TEER of Caco-2 cell monolayers. TEER was taken as an indicator of the opening and closing of the paracellular space in the cell monolayers. In a previous report (Sakai et al 1997), treatment with sodium deoxycholate induced a dose-dependent TEER-reducing effect in Caco-2 cell monolayers; no effect on TEER was observed for concentrations of sodium deoxycholate up to 0·02% (w/v). TEER was, however, reduced dose-dependently by concentrations ranging from 0·02 to 0·1% (w/v); at concentrations above 0·1% (w/v) sodium deoxycholate induced a complete and immediate lowering effect. In contrast, treatment with dipotassium glycyrrhizinate resulted in a slight change of TEER in the range 0·02 to 2% (w/v). Therefore, the concentration of sodium deoxycholate was fixed at 0·02% (w/v) and the concentration of dipotassium glycyrrhizinate was varied, to determine the effect of combined dipotassium glycyrrhizinate and sodium deoxycholate. As shown in Figure 1A, treatment with 1 and 2 % (w/v) dipotassium glycyrrhizinate and 0·02% (w/v) sodium deoxycholate had a negligible effect on TEER; reproducibility was good, as has been shown previously. In contrast, combined treatment with sodium deoxycholate and dipotassium glycyrrhizinate had a TEER-reducing effect which was dependent on the dose of dipotassium glycyrrhizinate and reached a maximum in the presence of 1% (w/v) of the compound (Figure 1B). This suggests that the absorption-enhancing effect was increased as a result of the combined use of sodium deoxycholate and dipotassium glycyrrhizinate and that the optimum combination is 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate.

To examine the effects of combined sodium deoxycholate and dipotassium glycyrrhizinate more closely, the Caco-2 cell monolayer was

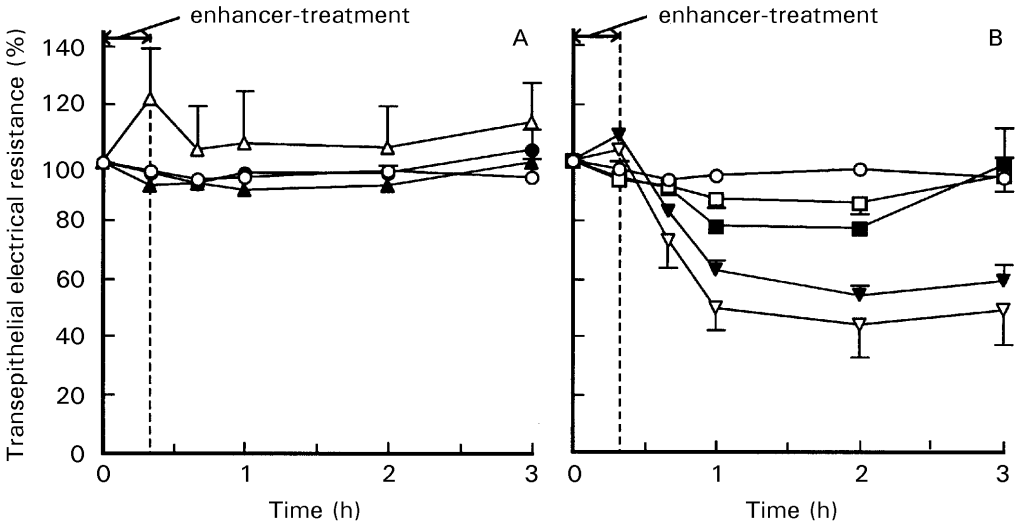

Figure 1. Effects of different concentrations of dipotassium glycyrrhizinate in combination with 0·02% (w/v) sodium deoxycholate on transepithelial electrical resistance in Caco-2 cell monolayers. The treatment period for the combined absorption enhancers was 0 to 20 min: A. control (calcium- and magnesium-free Hanks' balanced salt solution) (○); 1% (w/v) dipotassium glycyrrhizinate alone (●), 2% (w/v) dipotassium glycyrrhizinate alone (△), 0·02% (w/v) sodium deoxycholate alone (▲), B. control (○), 0·02% (w/v) sodium deoxycholate combined with 0·02% (w/v) (□), 0·2% (w/v) (■), 1% (w/v) (▽) and 2% (w/v) (▼) dipotassium glycyrrhizinate. Data are the means ± standard deviation of three TEER measurements for each monolayer.

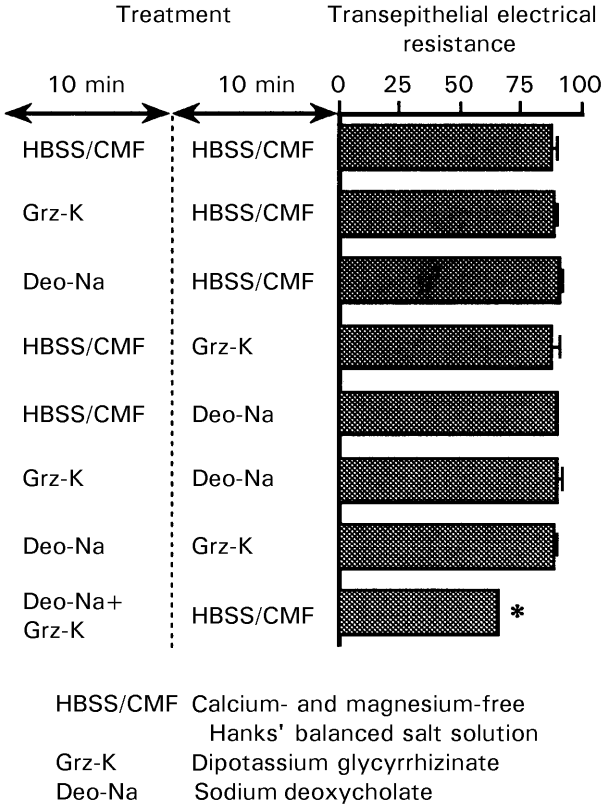

Figure 2. Interdependency and combination effects of sodium deoxycholate (Deo-Na) and dipotassium glycyrrhizinate (Gr2-K) on TEER in Caco-2 cell monolayers. The first treatment of the monolayers with enhancers was for 10 min, and the second for 10 min after washing out the retained enhancer with calcium- and magnesium-free Hanks' balanced salt solution (HBSS/CMF). The concentrations of sodium deoxycholate and dipotassium glycyrrhizinate were 0·02% (w/v) and 1% (w/v), respectively. Data are the means ± standard deviation of three TEER measurements for each monolayer 1 h after treatment. $*P < 0.05$, significantly different from control result (Tukey's multiple rank test).

sequentially exposed to each enhancer for 10 min. Figure 2 shows the TEER values 1 h after the onset of each treatment. The enhancement was negligible irrespective of the order of treatment. However, a marked decrease in TEER was observed for a combination of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate.

Effect of simultaneous treatment with sodium deoxycholate and dipotassium glycyrrhizinate on the permeation of sodium fluorescein and fluorescein isothiocyanate dextran

To verify the absorption-enhancing effect of the combined use of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate, cellular permeabilities were compared for individual enhancers and for a combination of the two enhancers for the water-soluble model compounds sodium fluorescein and fluorescein isothiocyanate dextran. The results are shown in Figure 3 and Table 1. Figure 3 shows the cumulative permeation of the model compounds per unit area ($\mu g\,cm^{-2}$) with time and Table 1 lists the apparent permeability coefficients (P_{app}) calculated from the time course in Figure 3. The cumulative permeability of the model compounds was increased by use of a combination of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate, compared with the effect of the individual compounds. The P_{app} values of sodium fluorescein and fluorescein isothiocyanate dextran in the monolayers treated with the combination increased significantly by (approx.) 1·7- and 7·1-fold, respectively, compared with that of the control, and these values were significantly greater than after the use of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate individually.

Effect of H7 on TEER and on fluorescein isothiocyanate dextran permeability after treatment with sodium deoxycholate and dipotassium glycyrrhizinate

The inhibitory effect of H7, a protein kinase C (PKC) inhibitor, on the absorption-enhancing activity of the combination was evaluated; the results are shown in Figure 4 and Table 2. Measurement of TEER and the fluorescein isothiocyanate dextran transport experiment were performed after exposure of the apical side of each monolayer to 200 μM H7 for 1 h. The effect of H7 on the TEER of the control was negligible. After H7 pretreatment the decrease in TEER induced by a combination of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate reverted from 35% to 75% of the initial TEER value. A positive control, 0·3% (w/v) EDTA, which has previously been reported to have PKC-mediated absorption-enhancing activity (Tomita et al 1996), induced a 30% decrease in TEER. The TEER-reducing activity of 0·3% (w/v) EDTA was completely inhibited by pretreatment with H7.

In the transport of fluorescein isothiocyanate dextran, treatment with H7 resulted in no difference from control monolayers. The enhanced transport of fluorescein isothiocyanate dextran as the result of combined treatment with 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate was significantly reduced by H7 (Table 2).

Discussion

This study shows the potential of the use of a combination of absorption enhancers and their

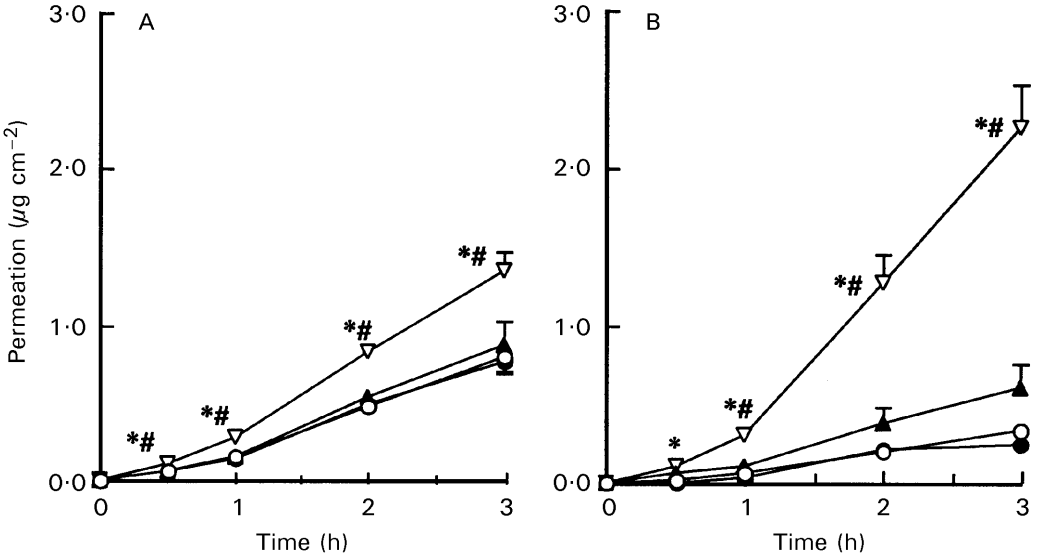

Figure 3. Effect of the combined use of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate on the transport of sodium fluorescein (A) and fluorescein isothiocyanate dextran (B) in Caco-2 cell monolayers. Fluorescent model compounds (sodium fluorescein (100 μg mL^{-1}) and fluorescein isothiocyanate dextran (1 mg mL^{-1}) were applied to the apical side followed by treatment with absorption enhancers for 20 min: control (calcium- and magnesium-free Hanks' balanced salt solution) (○), 0·02% (w/v) sodium deoxycholate alone (▲), 1% (w/v) dipotassium glycyrrhizinate alone (●), the combined use of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate (▽). Data are the means ± standard deviation of the cumulative permeation per unit area (n = 3). *,#$P < 0{\cdot}05$, significantly different from the control and 0·02% (w/v) sodium deoxycholate, respectively (Tukey's multiple rank test).

Table 1. Apparent permeability coefficients for the transport of sodium fluorescein and fluorescein isothiocyanate dextran in Caco-2 cell monolayers.

Enhancer	Apparent permeability coefficient (10^{-7} cm s^{-1})	
	Sodium fluorescein	Fluorescein isothiocyanate dextran
Control*	8·37 ± 0·80	0·35 ± 0·03
Sodium deoxycholate, 0·02% (w/v)	9·23 ± 1·64	0·65 ± 0·19
Dipotassium glycyrrhizinate, 1% (w/v)	8·17 ± 0·81	0·29 ± 0·08
Sodium deoxycholate, 0·02% (w/v) + dipotassium glycyrrhizinate 1% (w/v)	14·16 ± 1·27†‡	2·47 ± 0·29†‡

Data were calculated from the results of transport experiments (Figure 3) and are means ± standard deviation. *Calcium- and magnesium-free Hanks' balanced salt solution. †,‡ $P < 0{\cdot}05$, significantly different from control buffer result and that from 0·02% (w/v) sodium deoxycholate, respectively (Tukey's multiple rank test).

application to the future pharmaceutical design of oral preparations of poorly absorbed drugs. A combination of sodium deoxycholate and dipotassium glycyrrhizinate was selected as the absorption enhancer for the purposes of this study. Although both TEER and permeability were not affected by treatment with 0·02% (w/v) sodium deoxycholate alone or with 0·1–2% (w/v) dipotassium glycyrrhizinate alone, the TEER in the presence of 0·02% (w/v) sodium deoxycholate decreased with increasing concentration of dipotassium glycyrrhizinate, the effect being greatest for 1% (w/v) dipotassium glycyrrhizinate (Figure 1). In addition, the combination of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate clearly enhanced the permeability of the water-soluble model compounds sodium fluorescein and fluorescein isothiocyanate dextran, as shown in Figure 3 and Table 1.

We have previously shown, by use of confocal laser-scanning microscopy techniques, that sodium fluorescein and fluorescein isothiocyanate dextran were mainly transported through the paracellular route in the absence or presence of sodium deoxycholate and sodium caprate. The enhancing effect of combined treatment with sodium deoxycholate

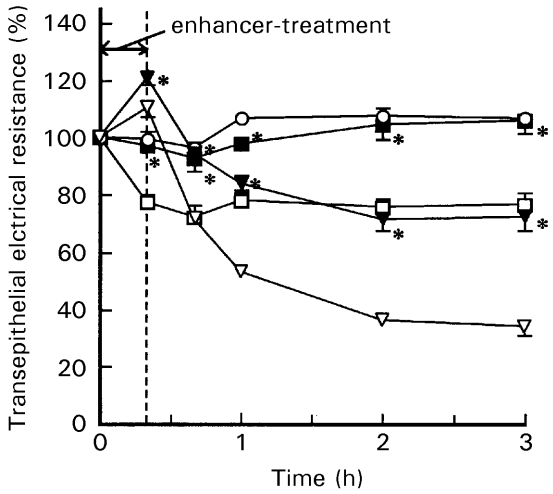

Figure 4. Effect of H7 on TEER in Caco-2 cell monolayers treated with a combination of 0·02% (w/v) sodium deoxycholate, 1% (w/v) dipotassium glycyrrhizinate and 0·3% (w/v) EDTA. The treatment period for the combined absorption enhancers was 0 to 20 min: 200 μM H7 alone (○), the combination (▽), the combination with H7 (▼), 0·3% (w/v) EDTA (□), and 0·3% (w/v) EDTA with H7 (■). Data are means ± standard deviation of three TEER measurements for each monolayer. $*P < 0{\cdot}05$, significantly different from result obtained without H7 (Student's *t*-test).

Table 2. Effect of H7 on the apparent permeability coefficient of fluorescein isothiocyanate dextran in Caco-2 cell monolayers treated with a combination of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate.

Enhancer	Apparent permeability coefficient (10^{-7} cm s^{-1})
Control*	0·36 ± 0·11
Control with H7	0·39 ± 0·24
Sodium deoxycholate, 0·02% (w/v) + dipotassium glycyrrhizinate, 1% (w/v)	1·72 ± 0·34†
Sodium deoxycholate, 0·02% (w/v) + dipotassium glycyrrhizinate1% (w/v) + H7	0·81 ± 0·04‡

Data are means ± standard deviation (n = 3). *Calcium- and magnesium-free Hanks' balanced salt solution. † $P < 0{\cdot}05$, significantly different from control result (Tukey's multiple rank test); ‡ $P < 0{\cdot}05$, significantly different from result from 0·02% (w/v) sodium deoxycholate + 1% (w/v) dipotassium glycyrrhizinate (Student's *t*-test).

and dipotassium glycyrrhizinate was greater for fluorescein isothiocyanate dextran (MW 4400) than for sodium fluorescein, a small molecule. This is because the cellular permeability of high-molecular weight compounds is highly limited by the tight-junction, in contrast with that of low-molecular weight compounds. The enhanced transport of fluorescein isothiocyanate dextran as a result of combined treatment with sodium deoxycholate and dipotassium glycyrrhizinate prompted us to expect a similar enhancement effect in the transport of peptide and protein drugs.

The effect of the combined enhancers was observed only when they were present together (Figure 2). The TEER-reducing activity of the combination was sufficient even when the treatment period was 10 min, although this was weaker than that observed after 20 min treatment.

In general, activated phospholipase C cleaves inositol diphosphate to form the intracellular mediators inositol trisphosphate and diacylglycerol. Inositol trisphosphate releases calcium ion from intracellular stores, and diacylglycerol activates PKC in the cell. It has been reported that these mediators regulate the paracellular permeability in Caco-2 cell monolayers (Lindmark et al 1998). Tomita et al (1996) demonstrated that sodium caprate acts on cell membranes and induces an increase in the intracellular calcium concentration via inositol trisphosphate which is produced by activation of phospholipase C in the plasma membrane. The calcium-calmodulin activates myosin light chain kinase, which provokes the contraction of actin-myosin filaments resulting in the opening of the paracellular route. This suggests that the increase in intracellular calcium-ion concentration is one of the enhancing mechanisms in drug absorption. In our previous study, however, intracellular calcium concentration was not significantly affected by sodium deoxycholate but was reduced by dipotassium glycyrrhizinate (Sakai et al 1998). We thus proposed that the regulation of PKC activity via diacylglycerol is another factor in the opening of the tight-junction. The decrease in TEER as a result of the combined use of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate was reduced to half by pretreatment with H7, a PKC inhibitor (Figure 4). In addition, the enhanced transport of fluorescein isothiocyanate dextran by combined treatment with sodium deoxycholate and dipotassium glycyrrhizinate was inhibited by H7 (Table 2). The concentration of H7 used, 200 μM, had no effect on TEER in the control and treatment with 0·02% (w/v) sodium deoxycholate or 1% (w/v) dipotassium glycyrrhizinate individually. A similar observation has been reported for EDTA, which opens the tight junction by activation of PKC (Tomita et al 1996). It is possible that the activation of PKC might, at least in part, be involved in the mechanism of the enhancement of absorption resulting from the combination of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate. Huang et al (1992) reported that sodium deoxycholate enhances PKC activity in cells. The reduction of TEER by high concentra-

tions (0·05% w/v) of sodium deoxycholate on TEER was diminished by (approx.) 10–20% by H7 (data not shown). Considering that sodium deoxycholate enhances PKC activity in cells, dipotassium glycyrrhizinate could conceivably enhance the activation of PKC by sodium deoxycholate.

Glycyrrhizinate-salt has been reported as a drug with anti-inflammatory, anti-hepatotoxic, anti-allergic and anti-viral activity (Kuroyanagi et al 1962; Amagaya et al 1984; Kiso et al 1984; Hirabayashi et al 1991). In the pharmaceutical field, dipotassium glycyrrhizinate and its ammonium salt have been reported to have absorption-enhancing activity (Aliverti et al 1989; Mishima et al 1989; Tanaka et al 1992; Reardon et al 1993). The use of dipotassium glycyrrhizinate alone neither reduced the TEER of Caco-2 cell monolayers nor enhanced the cellular permeability of water-soluble model compounds. The reason for such a discrepancy, in terms of the presence or absence of the absorption-enhancing activity of dipotassium glycyrrhizinate, remains unknown and the mechanism by which dipotassium glycyrrhizinate enhances the activation of PKC is unclear; both are subjects for future studies. Sodium deoxycholate has been reported to enhance the absorption of several drugs (Guarini & Ferrari 1985; Lin & Shen 1991) and there have been several reports that sodium deoxycholate causes considerable damage to the epithelium when used at high concentrations (Guarini et al 1986; Hersey & Jackson 1987; Hosoya et al 1994). The trypan blue exclusion test was conducted to verify the cytotoxicity of the enhancers. There was no difference among the toxicities of 0·02% (w/v) sodium deoxycholate used alone, the combination of 0·02% (w/v) sodium deoxycholate and 1% (w/v) dipotassium glycyrrhizinate, and the control (data not shown). This could be because of the very low sodium deoxycholate concentration used in this experiment. These data indicate the potential for the safe use of a combination of sodium deoxycholate and dipotassium glycyrrhizinate.

As described above, we have confirmed that the combined use of sodium deoxycholate and dipotassium glycyrrhizinate has a synergistic absorption-enhancing effect. This information will be useful in selecting absorption enhancers for the future pharmaceutical design of poorly absorbed drugs.

References

Aliverti, V., Dorigotti, L., Fonio, T., Pinza, M. (1989) Pharmaceutical Compositions, UK Patent 2212062A.

Amagaya, S., Sugishita, E., Ogihara, Y., Ogawa, S., Okada, K., Aizawa, T. (1984) Comparative studies of the stereoisomers of glycyrrhetinic acid on anti-inflammatory activities. J. Pharmacobiodyn. 7: 923–928

Guarini, S., Ferrari, W. (1985) Sodium deoxycholate promotes the absorption of heparin administered orally, probably by acting on gastrointestinal mucosa, in rats. Experientia 41: 350–352

Guarini, S., Fano, R. A., Rompianesi, E., Martinelli, A. M., Ferrari, W. (1986) The effect of sodium deoxycholate given by gavage with heparin on the histology of the intestinal mucosa of the rat. J. Pharm. Pharmacol. 38: 922–924

Hersey, S. J., Jackson, R. T. (1987) Effect of bile salts on nasal permeability in vitro. J. Pharm. Sci. 76: 876–879

Hirabayashi, K., Iwata, S., Matsumoto, H., Mori, T., Shibata, S., Baba, M., Ito, M., Shigeta, S., Nakashima, H., Yamamoto, N. (1991) Antiviral activities of glycyrrhizin and its modified compounds against human immunodeficiency virus type 1 (HIV-1) and herpes simplex virus type I (HSV-1) in vitro. Chem. Pharm. Bull. 39: 112–115

Hosoya, K., Kubo, H., Natsume, H., Sugibayashi, K., Morimoto, Y. (1994) Evaluation of enhancers to increase nasal absorption using Ussing chamber technique. Biol. Pharm. Bull. 17: 316–322

Huang, X. P., Fan, X. T., Desjeux, J. F., Castagna, M. (1992) Bile acids, non-phorbol-ester-type tumor promoters, stimulate the phosphorylation of protein kinase C substrates in human platelets and colon cell line HT29. Int. J. Cancer 52: 444–450

Kiso, Y., Tohkin, M., Hikino, H., Hattori, M., Sakamoto, T., Namba, T. (1984) Mechanism of antihepatotoxic activity of glycyrrhizin. 1: Effect on free radical generation and lipid peroxidation. Planta Med. 50: 298–302

Kuroyanagi, T., Kurisu, A., Sugiyama, H., Saito, M. (1962) Studies on experimental allergic hepatitis 2. Effect of prednisolone and glycyrrhizin on allergic hepatic lesions. Jpn J. Med. Prog. 49: 458–465

Lin, Y.-J., Shen, W.-C. (1991) Effects of deoxycholate on the transepithelial transport of sucrose and horseradish peroxidase in filter-grown Madin-Darby canine kidney (MDCK) cells. Pharm. Res. 8: 498–501

Lindmark, T., Kimura, Y., Artursson, P. (1998) Absorption enhancement through intracellular regulation of tight junction permeability by medium chain fatty acids in Caco-2 cells. J. Pharmacol. Exp. Ther. 284: 362–369

Mishima, M., Okada, S., Wakita, Y., Nakano, M. (1989) Promotion of nasal absorption of insulin by glycyrrhetinic acid derivatives. 1. J. Pharmacobiodyn. 12: 31–36

Reardon, P. M., Gochoco, C. H., Audus, K. L., Wilson, G., Smith, P. L. (1993) In vitro nasal transport across ovine mucosa: effects of ammonium glycyrrhizinate on electrical properties and permeability of growth hormone releasing peptide, mannitol, and lucifer yellow. Pharm. Res. 10: 553–561

Sakai, M., Imai, T., Ohtake, H., Azuma, H., Otagiri, M. (1997) Effects of absorption enhancers on the transport of model compounds in Caco-2 cell monolayers: assessment by confocal laser scanning microscopy. J. Pharm. Sci. 86: 779–785

Sakai, M., Imai, T., Ohtake, H., Azuma, H., Otagiri, M. (1998) Effects of absorption enhancers on cytoskeletal actin filaments in Caco-2 cell monolayers. Life Sci. 63: 45–54

Tanaka, M., Takahashi, M., Kuwahara, E., Koyama, O., Ohkubo, K., Yotsuyanagi, T. (1992) Effect of glycyrrhizinate on dissolution behavior and rectal absorption of amphotericin B in rabbits. Chem. Pharm. Bull. 40: 1559–1562

Tomita, M., Hayashi, M., Awazu, S. (1996) Absorption-enhancing mechanism of EDTA, caprate, and decanoylcarnitine in Caco-2 cells. J. Pharm. Sci. 85: 608–611