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Cytotoxicity of Absorption Enhancers in Caco-2 Cell Monolayers

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Abstract

This study was performed to evaluate the utility of absorption enhancers with reference to mucosal cell cytotoxicity. Overall assessment of the damage to plasma, lysosomal and nuclear membranes by three absorption enhancers, sodium deoxycholate, sodium caprate and dipotassium glycyrrhizinate, was performed on Caco-2 cell monolayers.

The cytotoxicities of sodium deoxycholate (0·02–0·1% w/v), sodium caprate (0·1–0·5% w/v) and dipotassium glycyrrhizinate (0·5–2% w/v) were evaluated by the trypan blue-exclusion test, the protein-release test, the neutral-red assay, the DNA–propidium iodide staining test and the test for recovery of transepithelial electrical resistance (TEER) up to 24 h after treatment with each enhancer. Sodium dodecyl sulphate (SDS; 0·1% w/v), a potent surfactant, was used as positive control. SDS at this level was significantly cytotoxic whereas dipotassium glycyrrhizinate was not cytotoxic in any tests. Results from the trypan blue-exclusion and protein-release tests showed that high concentrations of sodium caprate (0·5% w/v) and sodium deoxycholate (0·1% w/v) were significantly cytotoxic to the plasma membrane. The neutral-red assay, an indicator of damage to lysosomal membranes, revealed that 0·5% (w/v) sodium caprate had no effect whereas the uptake of neutral red was slightly increased by treatment with 0·1% (w/v) sodium deoxycholate, implying that the compound had cell-growth-enhancing activity. Nuclear-membrane damage, as evaluated by the DNA–propidium iodide staining test, was severe in cell monolayers treated with 0·5% (w/v) sodium caprate compared with that induced by 0·1% (w/v) sodium deoxycholate. In the TEER recovery test, TEER failed to recover 24 h after treatment with 0·5% (w/v) sodium caprate and 0·1% (w/v) SDS, but recovered after treatment with 0·1% (w/v) sodium deoxycholate. The recovery of TEER might be related to nuclear membrane damage and cell-growth-enhancing activity.

These results indicate that of the three classes of enhancer, dipotassium glycyrrhizinate was not cytotoxic and that high concentrations of sodium caprate and sodium deoxycholate could damage plasma and nuclear membranes.

Caco-2 cell monolayers, a colon carcinoma cell line from man, has been used as an intestinal epithelial cell model for evaluation of drug permeability and in the screening of absorption enhancers (Pinto et al 1983; Hidalgo et al 1989; Artursson & Karlsson 1991). Sodium deoxycholate, sodium caprate and dipotassium glycyrrhizinate were chosen as typical potent absorption enhancers from the bile acids (Guarini & Ferrari 1985; Hersey & Jackson 1987), fatty acids (Anderberg et al 1993; Takahashi et al 1994) and triterpenes (Aliverti et al 1989; Mishima et al 1989; Tanaka et al 1992), respectively, in a previous experiment which used Caco-2 cell monolayers to determine the absorption-enhancing effects of the compounds. We reported that sodium caprate and sodium deoxycholate enhanced the permeability of a hydrophilic fluorescent model compound mainly via the paracellular route and that of a hydrophobic fluorescent model compound mainly via the transcellular route. Dipotassium glycyrrhizinate had no absorption-enhancing activity for any of the model compounds, despite significant enhancing activity having been shown in an in-vivo study (Aliverti et al 1989; Mishima et al 1989; Tanaka et al 1992). We also reported two different types of response of cell monolayers to sodium caprate and sodium deoxycholate in

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opening the tight junction, i.e. an immediate response to sodium caprate and a more potent but slower response to sodium deoxycholate (Sakai et al 1997). There is little information on the possible occurrence of cytotoxicity along with the absorption-enhancing activity.

Despite the development of numerous methods for evaluation of cytotoxicity, the overall cytotoxicity of sodium caprate, sodium deoxycholate and dipotassium glycyrrhizinate has not been assessed by use of several independent methods. In this study we examined the cytotoxicity of these enhancers by use of the trypan blue-exclusion test, the protein-release test, the neutral-red assay and the DNA–propidium iodide staining assay. Both the trypan blue-exclusion test and the protein-release test can be used to evaluate plasma membrane damage. The uptake of neutral red into cells can be used to evaluate lysosomal function and membrane damage because neutral red, a dye for supravital stain, passively permeates plasma membranes and is concentrated in the lysosomes of viable cells (Borenfreund & Puerner 1985; Babich & Borenfreund 1990). The DNA–propidium iodide staining assay is based on the fluorescence induced by intercalation of propidium iodide with DNA, thus enabling evaluation of the extent of damage to the cell nuclear membrane (Krishan 1975; Wrobel et al 1996).

In this work, the above methods and the 24-h test for recovery of transepithelial electrical resistance (TEER), which is indicative of the integrity of cell monolayers, were used for overall assessment of the cytotoxicity of the absorption enhancers, sodium deoxycholate, sodium caprate and dipotassium glycyrrhizinate in Caco-2 cells.

Materials and Methods

Materials

Caco-2 cells were purchased from the American Type-Culture Collection (Rockville, MD). Dulbecco's modified Eagle's medium (DMEM), non-essential amino acid, benzylpenicillin G, streptomycin and sodium deoxycholate were obtained from Sigma (St Louis, MO). Foetal bovine serum was purchased from Cytosystems (Castic Hill, Australia). Sodium caprate and dipotassium glycyrrhizinate were purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and Alps (Tokyo, Japan), respectively. Trypan blue was purchased from Life Technologies (Rockville, MD). Propidium iodide, neutral red and sodium dodecylsulphate (SDS) were purchased from Wako (Osaka, Japan).

Cell culture

Caco-2 cells between passages 75 and 90 are usually used for evaluation of absorption enhancers (Artursson & Karlsson 1991; Tomita et al 1995). These were routinely cultured in DMEM supplemented with 1% (w/v) non-essential amino acid solution, 10% (v/v) heat-inactivated foetal bovine serum, benzylpenicillin G (100 units mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37°C in an atmosphere of 95% air–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. They were then resuspended in DMEM and seeded at a density of approximately 1×10^4 cells cm^{-2} on a Transwell insert filter (Costar, Cambridge, MA) with a surface area of 1 cm^2 and a pore size of 3 μm. The culture medium was changed at 48-h intervals. The cells were left for three weeks to reach confluence and to differentiate. The TEER of the monolayers reached a value of at least 600 Ω cm^2 before use.

Treatment of Caco-2 cell monolayers with absorption enhancers

Caco-2 cell monolayers were equilibrated for 1 h in calcium- and magnesium-free Hanks' balanced salt solution (HBSS/CMF) and DMEM respectively on the apical and basal sides. The apical chamber of the cell monolayers was then treated with HBSS/CMF containing various absorption enhancers for 20 min.

Trypan blue-exclusion test

After treatment of a cell monolayer with absorption enhancers the apical and basal sides were washed twice with HBSS/CMF and DMEM, respectively, to wash out the absorption enhancers which had been applied. The cell monolayers were further incubated at 37°C for 160 min with fresh HBSS/CMF and DMEM on the apical and basal sides, respectively. After the medium on the apical and basal chambers had been blotted, 100 μL of 0·4% (w/v) trypan blue aqueous solution was added to the apical chamber. The cell monolayers were then incubated for 1–2 min and gently washed twice with phosphate-buffered saline. The monolayers with the filter were carefully removed from the Transwell insert and placed into a new test-tube. The trypan blue was then extracted from the cell monolayer by adding acetone–0·5% (w/v) Na_2SO_4 (7:3, 1 mL) and leaving overnight at room temperature. After 5-min sonication the material was centrifuged at 3000 rev min^{-1} for 15 min. The supernatant (900 μL) was dried in a stream of N_2 and redissolved in acetone–0·5% (w/v) Na_2SO_4 (7:3, 200 μL). The absorption of a 150-μL portion

of the redissolved solution was measured at 560 nm with a microplate reader (Thermo max, Molecular Devices Corporation, Sunnyvale, USA).

Protein-release test

When the cell monolayers from the apical chamber were treated with HBSS/CMF containing absorption enhancers for 20 min, foetal bovine serum-free DMEM was used in the basal chamber. Because protein could be released from the cell monolayers during treatment with absorption enhancers for 20 min, 50 μL of the medium was sampled from the apical chamber before washing out the absorption enhancers. Another sample (50 μL) of the medium from the apical chamber was taken after incubation for 160 min in 0·5 mL fresh HBSS/CMF and 1·0 mL fresh foetal bovine serum-free DMEM from the apical and basal chambers, respectively. The amount of released protein in the two samples was determined by the modified Lowry method (Peterson 1979). The solution was aspirated from the apical and basal chambers and the total amount of protein determined by dissolving the cells in Triton-X (0·1% v/v; 1 mL). The amount of released protein was calculated as a percentage of the total amount of protein.

Neutral-red assay

Cell monolayers treated with absorption enhancers were incubated for 160 min with fresh HBSS/CMF and DMEM on the apical and basal sides, respectively. The medium in the apical and basal chambers was replaced with HBSS (0·5 mL) containing neutral red (50 μg mL^{-1}) and the monolayers were further incubated overnight at 37°C. The solution in the apical and basal chambers was blotted and formaldehyde solution (1% v/v; 0·5 mL) containing $CaCl_2$ (1% w/v) was added to both chambers. After 2–3 min fixation of the cells, the monolayer with filter was gently removed from the Transwell insert and placed in a new test-tube. An aqueous ethanol (50% v/v) solution of acetic acid (1% v/v; 1 mL) was added, and the neutral red was extracted from the cell overnight at room temperature. After centrifugation at 3000 rev min^{-1} for 15 min, 100 μL of the supernatant was measured with a microplate reader at a wavelength of 540 nm.

DNA–propidium iodide-staining test

Because phenol red affects the measurement of propidium iodide, phenol red-free HBSS/CMF and phenol red-free DMEM were used for the apical and basal chambers, respectively. The monolayers were treated with absorption enhancers for 20 min and a sample (0·5 mL) of medium was immediately taken from the apical side. After 160-min incubation another sample (0·5 mL) of apical medium was sampled. The sample (0·5 mL) was added to propidium iodide solution (50 μg mL^{-1}, 300 μL) and the fluorescence was measured at 490 nm (excitation) and 630 nm (emission) (F-2000 spectro-fluorimeter; Hitachi, Tokyo, Japan). The amount of DNA released by treatment with the absorption enhancers was calculated as the ratio of fluorescent intensity to that of the control.

Measurement of recovery of transepithelial electrical resistance (TEER)

After preincubation of cell monolayers for 1 h, TEER of cell monolayers at $t = 0$ was measured with the Millicell electrical resistance system (Millipore Corporation, Bedford, MA). This reading was regarded as the initial value. HBSS/CMF in the apical chamber was replaced with 0·5 mL HBSS/CMF containing the enhancer or enhancer-free HBSS/CMF. After 20-min treatment with absorption enhancer the apical and basal chambers were gently washed twice with fresh HBSS/CMF and DMEM, respectively. Fresh HBSS/CMF (0·5 mL) and DMEM (1 mL) were added to the apical and basal sides, respectively. To study the recovery of TEER for 24 h, HBSS/CMF in the apical chamber was replaced with DMEM at 3 h. The resistance of the cell monolayers was measured at onset ($t = 0$) and 20, 40 and 60 min and 2, 3, 6 and 24 h later. The resistance was expressed as the percentage of the initial value (i.e. that at $t = 0$).

Statistical analysis

Tukey's multiple rank test was used to analyse the data. P values < 0.05 were considered to be indicative of statistical significance. Results are expressed as means ± standard deviation (s.d.).

Results

The concentrations of absorption enhancers

It has been reported that the activity of absorption enhancers correlates well with TEER in Caco-2 cell monolayers (Tomita et al 1995). On the basis of the TEER value reported elsewhere (Sakai et al 1997), three concentrations of sodium caprate and sodium deoxycholate were selected for determination of cytotoxicity to Caco-2 cell monolayers: 0·1% (w/v) sodium caprate and 0·02% (w/v) sodium deoxycholate, at which TEER is slightly reduced; 0·2% (w/v) sodium caprate and 0·05% (w/v) sodium deoxycholate, at which TEER is reduced by more than 50%; and 0·5% (w/v) sodium caprate and 0·1% (w/v) sodium deoxycholate at which TEER is reduced by approximately 80%. Because dipotassium glycyrrhizinate had no TEER-lowering

effect, concentrations higher than those used for sodium caprate and sodium deoxycholate, i.e. 0·5, 1 and 2% (w/v) were selected. Sodium dodecyl sulphate (SDS), a potent surfactant with cytotoxic activity (Augustin & Damour 1995; Nishi et al 1995), was used as positive control at a concentration of 0·1% (w/v) at which TEER was reduced by approximately 80% in a preliminary dose-finding study.

Trypan blue-exclusion test

Plasma membrane damage was evaluated by uptake of trypan blue into the cell. As shown in Table 1, the result for the positive control, 0·1% (w/v) SDS treatment, was approximately nine times higher than that for the control monolayers. Results from treatment with 0·1% (w/v) sodium deoxycholate and 0·5% (w/v) sodium caprate were approximately three and four times higher, respectively, than that from the control, indicating significant damage to the plasma membrane. The absorbance after treatment with 0·5–2% (w/v) dipotassium glycyrrhizinate was similar to that of the control, suggesting no damage to the plasma membrane.

Table 1. Cytotoxicity of sodium deoxycholate, sodium caprate, dipotassium glycyrrhizinate and sodium dodecyl sulphate as evaluated by the trypan blue-exclusion test.

Enhancer	Concn (% w/v)	Absorbance (560 nm)
Control	–	0·018 ± 0·004
Sodium deoxycholate	0·02	0·016 ± 0·002
	0·05	0·021 ± 0·002
	0·1	0·063 ± 0·023*
Sodium caprate	0·1	0·018 ± 0·001
	0·2	0·019 ± 0·005
	0·5	0·079 ± 0·002*
Dipotassium glycyrrhizinate	0·5	0·020 ± 0·011
	1·0	0·021 ± 0·003
	2·0	0·011 ± 0·007
Sodium dodecyl sulphate	0·1	0·153 ± 0·005*

Data are means ± s.d. (n = 3). *$P < 0·05$, significantly different from control result (Tukey's multiple rank test).

Protein-release test

Table 2 shows the amount of protein released from monolayers immediately after 20-min treatment with absorption enhancers and after further incubation for 160 min in fresh medium. During the 20-min treatment 0·1% (w/v) sodium deoxycholate, 0·5% (w/v) sodium caprate and 0·1% (w/v) SDS induced significant protein release (40–60% of protein) in comparison with the control. There was no significant difference between the amount of protein released after treatment with 0·1% (w/v) sodium deoxycholate and 0·5% (w/v) sodium caprate. The release of protein after 160-min incubation was affected only by 0·1% (w/v) SDS, the positive control, despite approx-imately 50% of the protein having been released at 20 min. Protein release by 0·5–2% (w/v) dipotassium glycyrrhizinate was similar to that in the control during treatment, although these values decreased after subsequent incubation, in comparison with the control.

Neutral-red assay

The uptake of neutral red into lysosomes is listed in Table 3. Neutral red is a weakly cationic dye which accumulates in the lysosomes of living cells after passive diffusion through the plasma cell membrane (Borenfreund & Puerner 1985), thus the uptake of neutral red indicates cell viability and stability of lysosomal membrane. After treatment with sodium deoxycholate, sodium caprate and

Table 2. Cytotoxicity of sodium deoxycholate, sodium caprate, dipotassium glycyrrhizinate and sodium dodecylsulphate as evaluated by protein release from cell monolayers.

Enhancer	Concn (% w/v)	Protein released†	
		20 min	3 h
Control	–	3·9 ± 0·9	4·0 ± 0·6
Sodium deoxycholate	0·02	4·8 ± 0·7	5·5 ± 1·9
	0·05	7·1 ± 0·7	5·0 ± 0·8
	0·1	42·2 ± 1·8*	4·8 ± 0·9
Sodium caprate	0·1	4·0 ± 0·9	2·3 ± 0·6
	0·2	7·8 ± 0·4	4·9 ± 0·6
	0·5	35·4 ± 3·0*	5·5 ± 0·7
Dipotassium glycyrrhizinate	0·5	5·5 ± 1·4	2·2 ± 0·3
	1·0	9·5 ± 0·5	1·8 ± 0·1*
	2·0	4·8 ± 0·6	1·5 ± 0·3*
Sodium dodecylsulphate	0·1	52·9 ± 6·7*	9·7 ± 1·0*

†Expressed as a percentage of total protein released at the given times after treatment with enhancer. Data are means ± s.d. (n = 4). *$P < 0·05$, significantly different from control result (Tukey's multiple rank test).

Table 3. Cytotoxicity of sodium deoxycholate, sodium caprate, dipotassium glycyrrhizinate and sodium dodecyl sulphate as evaluated by the neutral-red assay.

Enhancer	Concn (% w/v)	Absorbance (540 nm)
Control	–	0·250 ± 0·043
Sodium deoxycholate	0·02	0·251 ± 0·027
	0·05	0·317 ± 0·065
	0·1	0·303 ± 0·003
Sodium caprate	0·1	0·234 ± 0·012
	0·2	0·246 ± 0·016
	0·5	0·292 ± 0·017
Dipotassium glycyrrhizinate	0·5	0·216 ± 0·035
	1·0	0·208 ± 0·026
	2·0	0·222 ± 0·042
Sodium dodecylsulphate	0·1	0·018 ± 0·004*

Data are means ± s.d. (n = 3). *$P < 0·05$, significantly different from control result (Tukey's multiple rank test).

dipotassium glycyrrhizinate the uptake of neutral red was not significantly different from the control uptake. Treatment with 0·1% (w/v) SDS resulted in a significant decrease in uptake compared with the control.

DNA–propidium iodide staining test

The DNA released from cells to the apical medium was detected by propidium iodide staining. The amount of DNA released by treatment with absorption enhancers is shown in Table 4 as a ratio to the amount of DNA released from control monolayers. Treatment for 20 min with 0·5% (w/v) sodium caprate induced DNA release that was significantly greater than that from control cell monolayers. The release of DNA induced by 0·1% (w/v) sodium deoxycholate was greater that that from the control but the difference was not significant. Subsequent DNA release during 160-min incubation in fresh medium was found to be dose-dependent for both sodium caprate and sodium deoxycholate. Treatment with 1% (w/v) dipotassium glycyrrhizinate tended to increase DNA release compared with that from the treated control, but the values were not significant.

Although treatment with 0·1% (w/v) SDS significantly increased the release of DNA, the amount released could not be measured because of interference from severe cell damage; the result is, therefore, not listed in Table 4.

Recovery of transepithelial electrical resistance (TEER)

The effect on TEER of 20-min treatment with absorption enhancers was examined after 24 h; the results are shown in Figure 1. A range of 100–120% was measured for the control cell monolayers compared with the initial value. Treatment with 0·02% (w/v) sodium deoxycholate resulted in TEER values identical with those of the control, except for a slight decrease in TEER immediately after treatment. Treatment with 0·05% (w/v) sodium deoxycholate led to a continuous decrease in TEER up to 2 h after wash-out, and the TEER was maintained for up to 3 h. However, TEER recovered after changing from HBSS/CMF to DMEM in the apical side and returned to the control level after 24 h. Although 0·1% (w/v) sodium deoxycholate had a stronger reducing effect on TEER (80–90% decrease) than 0·05% (w/v) sodium deoxycholate from 20 min to 3 h after the treatment, TEER returned to the control level after 24 h. In addition, 0·1 and 0·2% (w/v) sodium caprate led to a transient decrease in TEER after 20 min treatment, but the decrease in TEER induced by 0·1% (w/v) sodium caprate recovered before the change from HBSS/CMF to DMEM,

Table 4. Cytotoxicity of sodium deoxycholate, sodium caprate and dipotassium glycyrrhizinate as evaluated by the DNA–propidium iodide staining assay.

Enhancer	Concn (% w/v)	Ratio to control monolayer†	
		20 min	3 h
Control	–	1·0 ± 0·8	1·0 ± 0·5
Sodium deoxycholate	0·02	0·6 ± 0·3	1·7 ± 0·1
	0·05	0·8 ± 0·5	1·9 ± 0·3
	0·1	67·2 ± 51·4	5·0 ± 1·0*
Sodium caprate	0·1	1·1 ± 0·4	1·3 ± 0·0
	0·2	0·9 ± 0·5	1·4 ± 0·2
	0·5	366·0 ± 69·5*	2·9 ± 1·1*
Dipotassium glycyrrhizinate	0·5	1·2 ± 0·2	1·4 ± 0·2
	1·0	18·4 ± 15·1	2·4 ± 0·6
	2·0	7·2 ± 0·8	1·3 ± 0·2

Data are means ± s.d. (n = 3 or 4). *$P < 0·05$, significantly different from control result (Tukey's multiple rank test). †At the given times after treatment with enhancer.

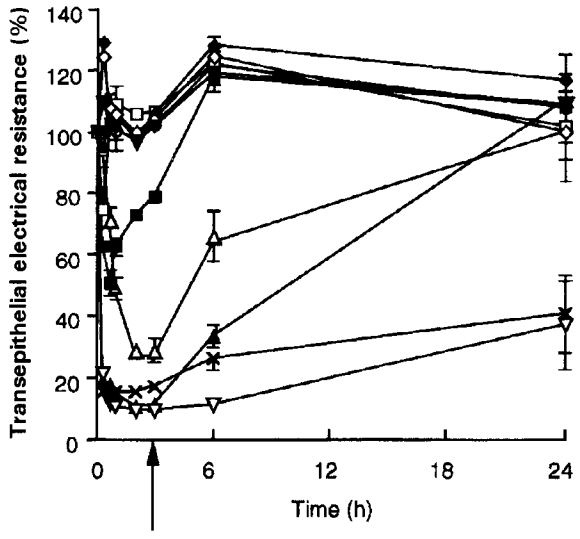

Figure 1. Recovery of transepithelial electrical resistance after treatment with sodium deoxycholate, sodium caprate, dipotassium glycyrrhizinate or sodium dodecyl sulphate. The treatment period for absorption enhancers was 0 to 20 min at concentrations of 0% (○) control, 0·02% (●), 0·05% (△) and 0·1% (▲) sodium deoxycholate, 0·1% (□), 0·2% (■) and 0·5% (▽) sodium caprate, 0·5% (▼), 1% (◇) and 2·0% (◆) dipotassium glycyrrhizinate and 0·1% (×) sodium dodecyl-sulphate. The arrow at 3 h indicates when apical calcium- and magnesium-free Hanks' balanced salt solution was replaced with Dulbecco's modified Eagle's medium. Data are means ± s.d. (n = 4).

and that induced by 0·2% (w/v) sodium caprate recovered to the control level after 6 h. Treatment with 0·5% (w/v) sodium caprate led to a decrease of approximately 80–90% immediately after the 20-min treatment, and this effect persisted for up to 6 h. No recovery of TEER was observed after 24 h. The TEER time-course after treatment with 0·5% (w/v) sodium caprate was similar to that for 0·1% (w/v) SDS. The TEER time-course after treatment with 0·5% (w/v) dipotassium glycyrrhizinate was nearly identical with that of the control. Treatment with 1 and 2% (w/v) dipotassium glycyrrhizinate resulted in a transient increase of 20–30% immediately after the 20-min treatment and a TEER time-course almost identical with that of the control.

Discussion

An overall assessment of the utility of three absorption enhancers was performed using four methods to determine the cytotoxicity of the compounds. For 0·1% (w/v) SDS, significant cytotoxicity was shown by all the testing methods whereas dipotassium glycyrrhizinate was not cytotoxic at the concentrations used in the tests. High concentrations of sodium deoxycholate (0·1% w/v) and sodium caprate (0·5% w/v) resulted in severe damage to plasma and nuclear membranes. Although further study is required the different cytotoxicities of the absorption enhancers and SDS to lysosomal membranes seemed to be reflected by the compounds' different membrane-solubilizing strengths. The critical micelle concentration (CMC) for sodium deoxycholate, sodium caprate and dipotassium glycyrrhizinate in HBSS/CMF was approximately 1% (w/v) (data not shown). The cytotoxicities of these three absorption enhancers were examined at concentrations below their CMCs whereas SDS was used at its CMC (0·1% w/v) at which its solubilizing activity is substantial.

Recovery in TEER up to 24 h after treatment was examined. The results obtained up to 3 h after treatment showed good reproducibility when compared with those reported previously (Sakai et al 1997). We have recently observed histomorphological changes to actin filaments and to intracellular calcium ion levels as a result of 20-min treatment of Caco-2 cell monolayers with sodium caprate, sodium deoxycholate and dipotassium glycyrrhizinate (Sakai et al 1998). The structure of actin filaments, in microvilli (near apical side) was slightly modified by 0·5% (w/v) dipotassium glycyrrhizinate and was significantly changed by 0·2% (w/v) sodium caprate and 0·05% (w/v) sodium deoxycholate. All the enhancers except dipotassium glycyrrhizinate induced significant histomorphological changes in the actin filaments on the middle depth and basal side of the cells. The altered structure of the actin filaments in the monolayers was restored 160 min after removal of sodium caprate and dipotassium glycyrrhizinate, but not sodium deoxycholate. These time-courses are identical with that of TEER derived from treatment with 0·2% (w/v) sodium caprate or 0·05% (w/v) sodium deoxycholate (Figure 1). In the current study TEER failed to recover 24 h after treatment with 0·5% (w/v) sodium caprate and 0·1% (w/v) SDS. The effect of 0·1% (w/v) sodium deoxycholate or 0·5% (w/v) sodium caprate on actin filaments might be more severe than that of low concentrations of the compounds. The structure of actin filaments was more severely damaged by 0·5% (w/v) sodium caprate than by 0·1% sodium deoxycholate.

Similar results were obtained from the DNA–propidium iodide staining assay—0·5% (w/v) sodium caprate led to substantial and rapid release of DNA compared with the effect of 0·1% (w/v) sodium deoxycholate (Table 4). The severe effect of 0·5% (w/v) sodium caprate on the nuclear membrane might explain the non-recovery of TEER, because the damage to plasma and lysosomal membranes was almost the same after treatment

with 0·1% (w/v) sodium deoxycholate and 0·5% (w/v) sodium caprate, as shown by the trypan blue-exclusion test, protein-release test and neutral-red assay. It was clear from the previous report that the level of intracellular calcium ion was dose-dependently increased by sodium caprate and not affected by sodium deoxycholate. It is possible that increasing intracellular calcium ion levels is responsible for the cytotoxicity of sodium caprate.

It has been reported that 0·1% (w/v) sodium deoxycholate enhances cell growth (Bartram et al 1994). The relatively high uptake of neutral red 3 h after treatment with 0·05 and 0·1% (w/v) sodium deoxycholate (Table 3) indicates the possible cell-growth-enhancing effect of sodium deoxycholate. The cell-growth-enhancing activity of 0·1% (w/v) sodium deoxycholate was examined by means of the neutral-red assay 24 h after the onset of treatment with sodium deoxycholate. The results clearly showed that 0·1% (w/v) sodium deoxycholate had significant cell-growth-enhancing activity compared with the control (data not shown).

On the basis of the results from all the cytotoxicity tests, therefore, the following are believed to have occurred. Treatment with 0·1% (w/v) sodium deoxycholate provoked transient cell damage except for lysosomal function. It caused less damage to nuclear membranes than did 0·5% (w/v) sodium caprate and had little effect on intracellular calcium ion levels. It enhanced the growth of the remaining cells via reconstruction of cell monolayers, as indicated by the recovery of TEER 24 h after treatment. Even though dipotassium glycyrrhizinate had little effect on the transepithelial transport of hydrophilic and hydrophobic model compounds (Sakai et al 1997), it led to a transient increase in TEER (Figure 1) and significantly inhibited protein release 180 min after the onset of treatment (Table 2). We have no explanation of the action of dipotassium glycyrrhizinate. Glycyrrhiza with anti-inflammatory and anti-allergic properties, which contain dipotassium glycyrrhizinate, have been reported to stabilize the membranes of the inflamed cells; dipotassium glycyrrhizinate also significantly reduces intracellular calcium ion levels (Sakai et al 1998). This membrane-stabilizing action, together with the reduction of calcium ion levels, might be correlated with the increase in TEER and the inhibition of protein release observed in this experiment.

We have previously reported that 0·05% (w/v) sodium deoxycholate and 0·2% (w/v) sodium caprate had significant absorption-enhancing activity whereas dipotassium glycyrrhizinate did not (Sakai et al 1997). The results obtained in the current study imply that the absorption-enhancing activity of 0·05% (w/v) sodium deoxycholate and 0·2% (w/v) sodium caprate occur at concentrations which are not cytotoxic, thus indicating the usefulness of these compounds. However, increases in the concentration of the enhancers would inevitably provoke cytotoxicity. In particular, nuclear membrane damage was more severe after treatment with sodium caprate than with sodium deoxycholate. These findings indicate the need for caution in the use of these absorption enhancers.

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