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Promoting effect of Azone on intestinal absorption of poorly absorbable drugs in rats

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Summary

The effect of Azone (1-dodecylazacycloheptan-2-one) on the intestinal absorption of two water-soluble compounds, 6-carboxylfluorescein (CF) and sodium cefazolin (CEZ), was investigated using the in situ closed loop method in rats. It was found that Azone solubilized with HCO-60 (polyoxyethylated (60 M) hydrogenated caster oil) markedly enhanced the absorption of both compounds, predominantly in the large intestine. The potentiated absorption of CF gave rise to an apparent plateau in the concentrations of Azone above 20 mM. The inclusively complexed solution of Azone with α-cyclodextrin (α-CD) could also enhance the absorption of CF but not with β-cyclodextrin (β-CD). However, HCO-60 exerted much more potential adjuvant effect on the intestinal absorption of CF as compared to α-CD. The presence of either the high concentration of divalent cations (i.e. calcium and magnesium) or a strong chelating agent (i.e. phytate) has no influence on the adjuvant effect of Azone. These observations may exclude the possible involvement of the divalent cations at least in the luminal side on the mechanism of the absorption promoting effect of Azone. On the other hand, the histological studies demonstrated that Azone did not induce gross morphological changes on the structure of the intestinal mucosa.

Introduction

Recently, many attempts have been made so as to improve the bioavailability of the drugs, which are normally poorly absorbed from the gastrointestinal tract, by means of some absorption promoters (Kakemi et al., 1970a; Hori et al., 1977; Nishihata et al., 1980; Murakami et al., 1981). We have also proposed that the fusogenic lipids (Ahkong et al., 1973), such as linoleic acid, oleic acid and glyceryl monooleate, have a strong absorption promoting effect in the micellar state (Muranishi et al., 1977, 1979; Taniguchi et al., 1980). We have also investigated the mechanism by which these fusogenic lipids used as pharmaceutical adjuvant could enhance the absorption of poorly absorbable drugs via the enteral route (Muranushi et al., 1980, 1981; Murakami et al., 1985).

Azone, which was developed as a transdermal penetration promoter (Stoughton, 1982), has some physicochemical resemblance to these fusogenic lipids, e.g. low melting point, high hydrophobicity, unbranched and long hydrocarbon chain and

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carbonyl group in its molecular structure. Therefore, we investigated the effect of Azone on the permeability of the intestinal mucosa for poorly absorbable drugs and found out that the solubilized Azone could markedly enhance the intestinal absorption of them. In this paper, we will discuss the characteristics of the adjuvant effect of Azone in comparison with that of the fusogenic lipids.

Materials and Methods

Materials

Azone (1-dodecylazacycloheptan-2-one) and HCO-60 (a derivertive of polyoxyethylene hydrogenated caster oil) were kindly supplied from Teikoku Pharmaceutical Co. (Kagawa, Japan) and Nikko Chemical Co. (Tokyo, Japan), respectively. Sodium cefazolin (CEZ) was obtained from the Fujisawa Pharmaceutical Industry Co. (Osaka, Japan) and α- and β-cyclodextrin (α-CD and β-CD) were also from Sanraku Ocean Co. (Tokyo, Japan). 6-Carboxyfluorescein (CF) was purchased from Eastman Kodak Co. (Rochester, NY). All other chemicals were of reagent grade quality.

Preparation of test solution

The 0.01 w/v % CF solution and the 0.6 w/v % CEZ solution were prepared by dissolving the compounds in 100 mM Tris-HCl buffered solution of pH 7.4. Micellar solution was prepared by mixing Azone and HCO-60 into the above drug solutions.

Preparation of inclusion complexes of Azone with α- and β-CD

Complexation of Azone with α- and β-CD was performed according to the co-precipitation method. Azone dissolved in acetone was poured into the hypersaturated solution of α- or β-CD, followed by agitating with a magnetic stirrer overnight. The resulting precipitate was collected and washed three times with a small amount of the solvent, and then lyophilized to remove the residual solvent and water. The complexation of Azone with α- and β-CD was ascertained by their IR spectra.

Procedure for animal experiment

Male Wistar albino rats weighing between 220 and 280 g were fasted for 16 h prior to experiments and anesthetized with sodium pentobarbital during the experimental period. Absorption experiments were carried out according to the in situ closed loop method as previously described in detail (Murakami et al., 1986). Closed loop was made using the jejunum (15 cm) or the entire large intestine (colon and rectum) of rats. About 0.2 ml of blood samples were periodically collected via a catheter placed into the carotid artery, following the introduction of the warmed test solution at 37°C into the loop. The dose of CF and CEZ were 1 mg and 60 mg/kg of rat body weight, respectively. Bile was excluded from the body through the bile fistula during the experiments.

Analytical methods

CF in the plasma samples was extracted as previously reported (Hashida et al., 1984), followed by measuring its fluorescence with a spectrofluorometer (Hitachi model 650-10S) at 520 nm using an excitation wavelength of 490 nm. The detected limit for CF in plasma was around 5 ng/ml in this assay method.

For the assay of CEZ, the paper disc method using *Bacillus subtilis* ATCC 6633 as a test microorganism was employed. The minimal sensitivity of the method was 0.7 μg/ml. Any other constituent of the micellar solution did not affect the antimicrobial activity of CEZ.

Histology

Histological examinations were performed on jejunal and colorectal segments from the loops which were surgically excised at 30 min after dosing with Azone in micellar solution. These segments for light microscopy were fixed in 10% w/v buffered formalin, paraffin-sectioned and stained with hematoxylin and eosin.

Results

Effect of the concentration ratio of HCO-60 to Azone on the absorption of CF

Our preceding work on the fusogenic fatty acids

showed that the mixed micellar solution formed with HCO-60 was the best absorption promoting system among all the lipid dispersion systems such as vesicles and micellar solutions employed (Murakami et al., 1985). Therefore, at first we used HCO-60 as a non-ionic surfactant to solubilize Azone. Exploratory experiments were conducted toward obtaining the optimal preparation of Azone in micellar solution to elucidate maximal promoting effect of Azone on the intestinal absorption of CF. At first, the relationship between the degree of the solubilization of Azone with HCO-60 and the amount of CF absorbed from the large intestinal tract was studied. The ratio of HCO-60 to Azone was changed, while the Azone concentration was held constant at 20 mM. Azone markedly enhanced the absorption of CF from the large intestine in any case (Fig. 1), though CF was hardly absorbed in the absence of this adjuvant. The maximal plasma concentrations of CF were obtained during the first 30 min after dosing, and no significant difference was detected among them. The faster elimination of CF was observed in the case of the molar ratio of 20 : 1 (Azone:HCO-60) as compared to other cases. The area under the concentration–time curve of CF during first 240 min ($AUC_{0-240\ min}$) was maximum when HCO-60 was used at 2 mM (i.e. Azone : HCO-60 = 10 : 1). Though little difference was detected between the molar ratio of 10 : 1 and 5 : 1, the AUC value was reduced in increasing the molar ratio to 2 : 1 (Fig. 1). A similar result was obtained when the Azone concentration in micellar solution was 5 mM. Therefore, the optimal molar ratio between Azone and HCO-60 was thought to be around 10 : 1 to 5 : 1.

Effect of the Azone concentration on the absorption of CF

The effect of the various concentrations of Azone on the absorption of CF was investigated in the large intestine. The concentration ratio of Azone-to-HCO-60 was fixed at 10 : 1 so as to obtain the maximum adjuvant effect of Azone and minimize the possible side-effect of the surfactant on the intestinal mucosa. It was found that Azone could enhance the intestinal absorption of CF, though being dependent on its concentrations (Fig. 2). In the presence of Azone at the concentrations above 10 mM, no difference was observed in the maximal plasma concentration (C_{max}) of CF. The AUC in the presence of 20 mM Azone was signifi-

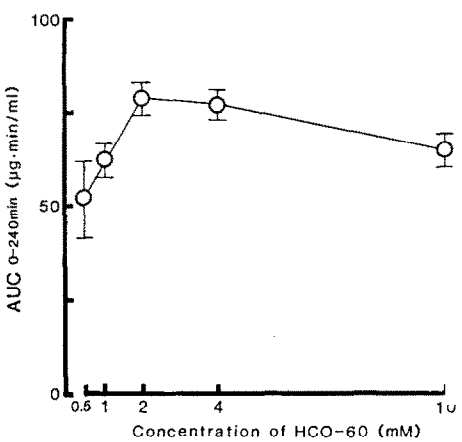

Fig. 1. Effect of different HCO-60 concentrations on the absorption promoting efficiency of Azone. The concentration of Azone is constant at 20 mM. Azone micellar solution was infused into the large intestinal loop. Each value is the mean with standard error for 3–4 experiments.

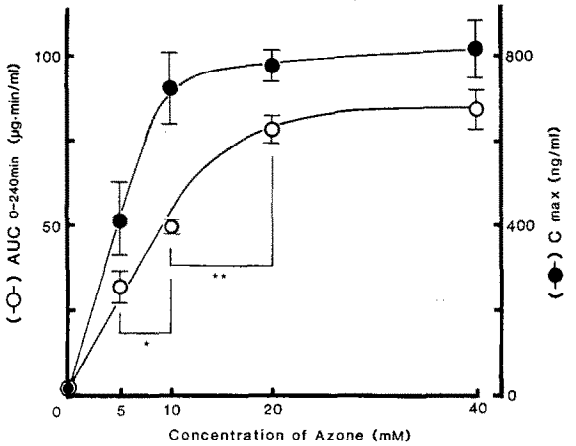

Fig. 2. Relationship between the concentration of Azone and the absorption efficiencies of CF. The molar ratio of HCO-60-to-Azone is 1 : 10. Each value is the mean with standard error for 3–4 experiments. Statistical comparisons were done by Student's *t*-test: $*P < 0.05$; $**P < 0.01$.

TABLE 1

COMPARISON BETWEEN THE SMALL INTESTINAL AND THE LARGE INTESTINAL ABSORPTION OF CF IN THE PRESENCE OF AZONE

Site of administration	Adjuvant	C_{max} (ng/ml)	$AUC_{0\text{-}240\,min}$ ($\mu g \cdot min/ml$)
Small intestine	None	17 ± 5 [b]	3.43 ± 0.89 [d]
	Azone (20 mM) [a]	386 ± 34 [c]	54.13 ± 3.85 [e]
Large intestine	None	12 ± 4 [b]	1.77 ± 0.31 [d]
	Azone (20 mM) [a]	778 ± 37 [c]	78.58 ± 3.84 [e]

Each value is the mean with standard error for 3–4 experiments.
[a] Azone was solubilized with 2 mM HCO-60.
[b] NS: not significant (Student's *t*-test).
[c] $P < 0.01$.
[d] NS.
[e] $P < 0.05$.

cantly greater than that in the presence of 10 mM Azone ($P < 0.01$). However, there was no significant difference in AUC value between the presence of 20 mM and 40 mM Azone. Consequently, the absorption promoting effect of Azone was thought to reach a plateau at a concentration of about 20 mM.

Adjuvant effect of Azone on the small intestinal absorption of CF

The effect of Azone on the absorption of CF from the upper small intestine (jejunum), of which different susceptibility from the large intestine were reported for some other absorption promoters (Muranishi et al., 1977; Murakami et al., 1981), was investigated. As shown in Table 1, the absorption of CF in the small intestine was significantly enhanced due to the presence of Azone in the micellar state, though less effective than in the large intestine. These observations were consistent with the results of the fusogenic lipids previously reported from our laboratory (Muranishi et al., 1977; Taniguchi et al., 1978; Murakami et al., 1985, 1986).

Effect of Azone on the intestinal absorption of CEZ

Based on the above results, we attempted to ascertain the adjuvant effect of Azone in the large intestine using CEZ, a poorly absorbable antibiotic, as a real therapeutic drug. The 20 mM : 2 mM (Azone : HCO-60) micellar solution was used as the most effective preparation. It was demonstrated that Azone in micellar state could remarkably enhance the intestinal absorption of CEZ as well as CF, though CEZ was hardly absorbed from the gut as a free from (Fig. 3). Significantly high plasma levels were obtained over at least 4 h following the administration of CEZ with Azone as compared to CEZ alone. The plasma CEZ concentration attained the maximum about 0.5 h after dosing with Azone. No significant difference was detected on the extent of bioavailability of CEZ between in this case and the intramuscular administration (Fig. 3).

Inclusion complexes of Azone with α- and β-CD

It is previously reported that the CDs protect unsaturated fatty acids against autoxidation (Schlenke et al., 1955). Simultaneously, they markedly increased the water solubilities of these guest molecules. Judging from the structural similarity of Azone molecules to these fatty acids, it is supposed that Azone may be included within the CD molecules without diminishing its absorption promoting ability.

Therefore, the effect of Azone included within α- or β-CD on the intestinal absorption of CF was investigated in the large intestine of rats. When Azone–β-CD complex was administered at 20 mM as a suspension, only slight increase was detected

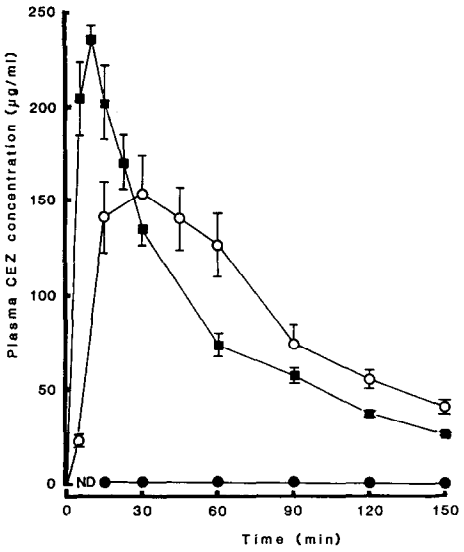

Fig. 3. Plasma CEZ concentration following administration of Azone micellar solution into the large intestine (l.i.). ●, l.i. administration of CEZ; ○, l.i. administration of CEZ with 20 mM Azone+2 mM HCO-60; ■, intramuscular administration of CEZ. Each value is the mean with standard error for 6 animals. ND, none detected.

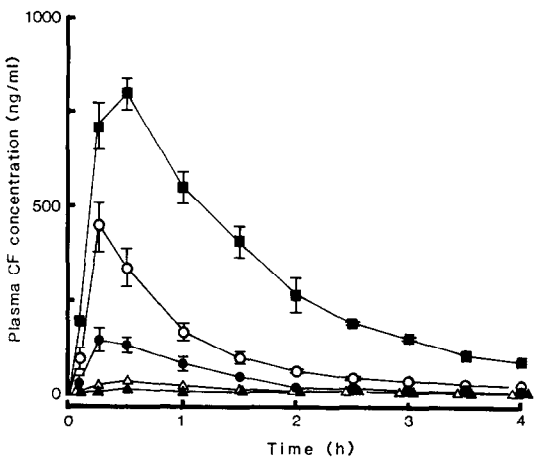

Fig. 4. Plasma CF concentration following administration of Azone–CD complex into the large intestine. ■, 20 mM Azone +2 mM HCO-60; ○, 20 mM Azone–α-CD; ●, 5 mM Azone–α-CD; △, 20 mM Azone–β-CD; ▲, none (free CF). Each value is the mean with standard error for 3 animals.

in the plasma CF level (Fig. 4). On the contrary, Azone–α-CD complex caused remarkable elevation of plasma CF levels depending on their concentrations (Fig. 4). In the presence of 20 mM Azone–α-CD complex, about a 3-fold higher plasma CF level of CF was observed in comparison with that in the case of 5 mM. However, the absorption promoting effect of Azone was more pronounced in the micellar state than in the inclusively complexed form (Fig. 4). CDs failed to enhance the absorption of CF without Azone within the range of these concentrations (data not shown). These results suggested that the accelerated absorption of CF from the gut could be ascribed to Azone, an essential promoting component of these systems.

Effects of divalent cations and the natural chelating agent on the adjuvant effect of Azone

It has been proposed that the calcium ion and/or its chelation might have participated in the mechanism by which several kinds of absorption promoters could exert their adjuvant effect on the gastrointestinal absorption of poorly absorbable drugs (Kamada et al., 1981). Then, we investigated whether divalent cations on the luminal side were involved in eliciting the absorption promoting effect of Azone or not. The higher concentration of divalent cations could not influence the absorption of CF in the presence of Azone solubilized with HCO-60 (Fig. 5). On the other hand, phytate, which is a natural chelating agent comparable to EDTA and EGTA, potentiated the adjuvant effects of Azone on the absorption of CF in the large intestine at the earlier stage after dosing (Fig. 6). Phytate alone slightly enhanced the absorption of CF in the slowly effecting fashion. These synergistic effects of phytate indicate that the action site of Azone might be different from that of phytate.

Histological evaluation

The influence of Azone on the structural integrity of the intestinal mucosa was unknown. Hence, we evaluated the histological changes of the intestinal segments using light microscopy. Observation was carried out at 30 min after the intraluminal administration of Azone (10 mM) when its absorption promoting effects attained the

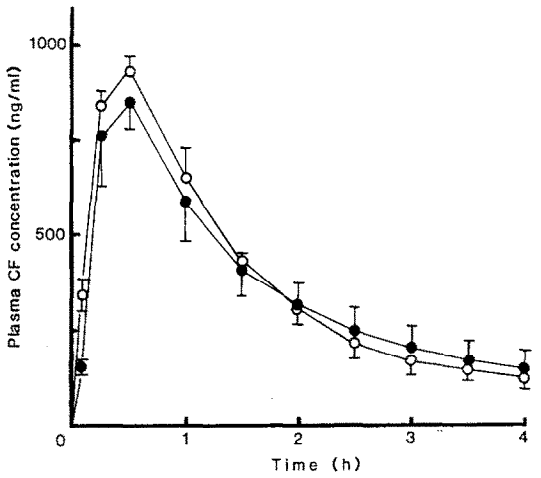

Fig. 5. Effect of co-administration of Azone and divalent cation on the large intestinal absorption of CF. ○, 30 mM Azone+3 mM HCO-60/15 mM $CaCl_2$; ●, 30 mM Azone+3 mM HCO-60/15 mM $MgCl_2$. Each value is the mean with standard error for 3–4 animals.

maximum to both CEZ and CF. No gross morphological changes were detected in both jejunal and colorectal specimens. It therefore appeared that the potential absorption of CEZ and CF from the intestinal tract in the presence of Azone should not be ascribed to the mucosal trauma such as the loss of the epithelial cells nor to the leaky junctions induced by local edema and inflammation.

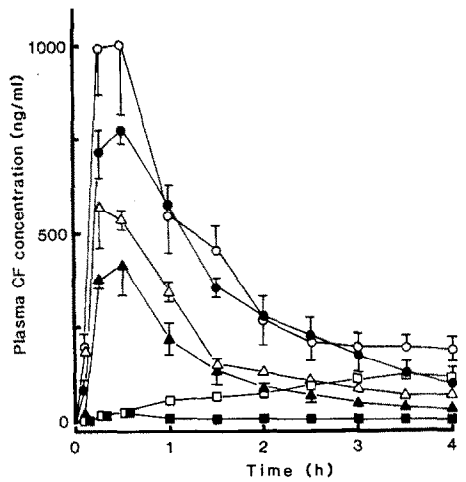

Fig. 6. Effect of co-administration of Azone and phytate on the large intestinal absorption of CF. ○, 20 mM Azone+2 mM HCO-60/5 mM phytate; ●, 20 mM Azone+2 mM HCO-60; △, 5 mM Azone+0.5 mM HCO-60/5 mM phytate; ▲, 5 mM Azone+0.5 mM HCO-60; □, 5 mM phytate; ■, none (free CF). Each value is the mean with standard error for 3 animals.

Discussion

The optimal molar concentration ratio of HCO-60-to-Azone was observed as described above (Fig. 1). Previously, the authors reported that the concentration of a surfactant such as polysolbate 80 (PS-80) affected the intestinal absorption of solubilized vitamin A in the adsorptive step onto the brush border membrane (Kakemi et al., 1970b). In that case, the absorption of vitamin A was dependent on the initial concentration of PS-80, especially for the earlier stage of dosing, and the optimal effective concentration of PS-80 was observed. Furthermore, we suggested in the preceding report that HCO-60 may improve the solubility of the adjuvants and their diffusibility through the mucus gel overlying the epithelial cell surface, though HCO-60 cannot improve the bioavailability of poorly absorbable drugs by itself (Murakami et al., 1985). Presumably, these surface-active agents promote the transfer of the adjuvants from the lumen onto the apical membrane of the absorptive cells across the overlying mucus gel. The results of the adjuvant effect of the Azone–CD inclusion complexes (Fig. 4) may support the above assumption. Although Azone was completely dissolved as an included form within α-CD, its adjuvant effect was not so strong as that observed in the micellar state (Fig. 4). It is assumed that the CD molecules might be less diffusible across the mucus gel and/or less efficiently release the Azone monomers than those with HCO-60 micelles. On the other hand, the optimal concentration concerning Azone, which is often observed in the case of its topical application (Stoughton, 1982), was not detected for the two water-soluble compounds within the concentration range employed in the present work.

It is to be noticed that the absorption promoting effect of Azone was much greater in the large intestine than in the small intestine as reported for some other adjuvants (Table 1). The different sites

of the gastrointestinal mucosa appear to possess essentially different sensitivity to these adjuvants (Muranishi, 1985). However, the reason why the promoting effect of the adjuvants was more pronounced in the lower site of the alimentary canal is under debate, and no convincing explanation can be given.

Maggio and Lucy (1972) suggested that a low melting fusogenic lipid might be incorporated into the lipid bilayer to cause its increased permeability. Recently, the mechanism of the absorption enhancement by these lipids has been investigated involving the physiological modification such as lipid–membrane perturbation as reviewed by Muranishi (1985). The authors reported that there was good correlation between the enhanced intestinal absorption of drugs by the fusogenic lipid and its melting or freezing point which represents the flexibility of the acyl chain of it. It should be noticed that Azone has a low melting point of $-7°C$ and also has a longer and saturated carbon chain in its molecular structure. Saturated medium chain fatty acids can also enhance the intestinal absorption of poorly absorbable drugs less effectively than the *cis*-unsaturated long chain fatty acids, and the other saturated fatty acids having the shorter acyl chain fail to potentiate (Muranushi, 1980).

Yata and co-workers (1983) suggested that capric acid might elicit the adjuvant effect through their chelating ability of calcium ion. However, the results obtained in the present studies (Figs. 5 and 6) seem to indicate that the chelating action probably is not the main mechanism for the absorption promoting effect of Azone, though its effect on the intestine is very similar to that of fusogenic lipids. Azone essentially fails to have chelating ability for calcium ion. The more detailed mode of action of Azone is now under investigation.

In conclusion, it was suggested in this work that Azone could be potential and harmless absorption promoter comparable with the fusogenic lipids. However, the physiological affections should be clarified in order to utilize it for practical pharmaceutical use.

References

Ahkong, Q.F., Fisher, D., Tampion, W. and Lucy, A.C., The fusion of erythrocytes by fatty acids, esters, retinol and α-tocopherol. Biochem. J., 136 (1973) 147–155.

Hori, R., Okumura, K., Inui, K., Nakamura, N., Miyoshi, A. and Suyama, T., Pharmaceutical approach to the oral dosage form of macromolecules: effect of bile salts and oil-in-water emulsions on the intestinal absorption of urogastrone in the rat. Chem. Pharm. Bull., 25 (1977) 1974–1979.

Kakemi, K., Sezaki, H., Konishi, R., Kimura, T. and Murakami, M., Effect of bile salts on gastrointestinal absorption of drugs. I. Chem. Pharm. Bull., 18 (1970a) 275–280.

Kakemi, K., Sezaki, H., Muranishi, S., and Yano, A., Mechanism of drug absorption from micellar solution. I. Absorption of solubilized vitamin A from the rat intestine. Chem. Pharm. Bull., 18 (1970b) 1563–1568.

Kamada, A., Nishihata, T., Kim, S., Yamamoto, M. and Yata, N., Study of enamine derivatives of phenylglycine as adjuvants for the rectal absorption of insulin. Chem. Pharm. Bull., 29 (1981) 2012–2019.

Maggio, B. and Lucy, J.A., Polar-group behaviour in mixed monolayers of phospholipids and fusogenic lipids. Biochem. J., 155 (1976) 353–364.

Murakami, M., Masuda, Y., Fukui, H., Yoshikawa, H., Kanji, T. and Muranishi, S., Role of the dispersion systems containing fusogenic lipids on enhanced absorption of poorly absorbable drugs from the gastrointestinal tract. J. Pharmacobio-Dyn., 8 (1985) S131.

Murakami, M., Yoshikawa, H., Kanji, T. and Muranishi, S., Impact of oleic acid vesicles on intestinal absorption of carboxyfluorescein in rats. Pharm. Res., in press.

Murakami, T., Tamachi, H., Yamazaki, M., Kubo, K., Kamada, A. and Yata, N., Biopharmaceutical study on the oral and rectal administrations of enamine prodrugs of amino acid-like β-lactam antibiotics in rabbits. Chem. Pharm. Bull., 29 (1981) 1986–1997.

Muranishi, S., Tokunaga, Y., Taniguchi, K. and Sezaki, H., Potential absorption of heparin from the small and the large intestine in the presence of monoolein mixed micelles. Chem. Pharm. Bull., 25 (1977) 1159–1161.

Muranishi, S., Muranishi, N. and Sezaki, H., Improvement of absolute bioavailability of normally poorly absorbed drugs: inducement of the intestinal absorption of streptomycin and gentamycin by lipid–bile salt mixed micelles in rat and rabbit. Int. J. Pharm., 2 (1979) 101–111.

Muranishi, S., Modification of intestinal absorption of drugs by lipoidal adjuvants. Pharm. Res., 1 (1985) 97–150.

Muranushi, N., Kinugawa, M., Nakajima, Y., Muranishi, S. and Sezaki, H., Mechanism for the inducement of the intestinal absorption of poorly absorbed drugs by mixed micelles I. Effects of various lipid–bile salt mixed micelles on the intestinal absorption of streptomycin. Int. J. Pharm., 4 (1980) 271–1279.

Muranushi, N., Nakajima, Y., Kinugawa, M., Muranishi, S. and Sezaki, H., Mechanism for the inducement of the intestinal absorption of poorly absorbed drugs by mixed

micelles. II. Effect of the incorporation of various lipids on the permeability of liposomal membranes. Int. J. Pharm., 4 (1980) 281–290.

Nishihata, T., Rytting, J.H. and Higuchi, T., Enhanced rectal absorption of theophylline, lidocaine, cefmetazole, and levodopa by several adjuvants. J. Pharm. Sci., 69 (1980) 744–745.

Schlenke, H., Sand, D.M. and Tillotson, J.A., Stabilization of autoxidizable materials by means of inclusion. J. Am. Chem. Soc., 77 (1955) 3587–3590.

Stoughton, R.B., Enhanced percutaneous penetration with 1-dodecylazacycloheptan-2-one. Arch. Dermatol., 118 (1982) 474–477.

Stoughton, R.B., Azone: a new non-toxic enhancer of cutaneous penetration. Drug Dev. Ind. Pharm., 9 (1983) 725–744.

Taniguchi, K., Muranishi, S. and Sezaki, H., Enhanced intestinal permeability to macromolecules II. Improvement of the large intestinal absorption of heparin by lipid–surfactant mixed micelles in rat. Int. J. Pharm., 4 (1980) 219–228.

Yata, N., Higashi, Y., Murakami, T., Yamajo, R., Wu, W.M., Taku, K., Sasaki, Y. and Hideshima, Y., A possible mechanism of absorption promoters. J. Pharmacobio-Dyn., 6 (1983) s-78.