ELSEVIER

International Journal of Pharmaceutics 172 (1998) 189–198

international journal of pharmaceutics

Improving insulin enteral absorption using water-in-oil-in-water emulsion

Mariko Morishita [a,*], Ayako Matsuzawa [b], Kozo Takayama [a], Koichi Isowa [c], Tsuneji Nagai [a]

[a] *Department of Pharmaceutics, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142-8501, Japan*
[b] *Hospital Pharmacy, Tokyo Women's Medical College, Kawadacho 8-1, Shinjuku-ku, Tokyo 162-0054, Japan*
[c] *The Center of Japan Biological Chemistry, Fukue 52, Kaizu-cho, Kaizu-gun, Gifu 503-0628, Japan*

Received 13 January 1998; received in revised form 17 April 1998; accepted 18 June 1998

Abstract

The purpose of this study was to evaluate the hypoglycemic effects of water-in-oil-in-water (W/O/W) insulin emulsions containing lipoidal absorption enhancer after enteral administration to rats. The hypoglycemic effects of insulin were examined using an in situ loop method in rats. The insulin emulsions prepared with soybean oil, triolein or trilinolein slightly but significantly decreased the serum glucose levels compared to the insulin solution. By addition of 3% limonene or 3% menthol to the triolein emulsion, the hypoglycemic effect of insulin was promoted in the ileum but not in the colon. Strong hypoglycemic effects were observed with the triolein emulsion containing 2% fatty acids such as oleic acid, linoleic acid and linolenic acid. The remarkable enhancing effects occurred more predominately in the colon than in the ileum. The effect of degree of unsaturation of fatty acids was not observed. No tissue damage was noted by light microscopic examination of both regions treated with triolein emulsion, triolein emulsion containing menthol or oleic acid. W/O/W emulsion containing unsaturated fatty acids are able to enhance the ileal and colonic absorption of insulin without tissue damage and may, therefore, be useful in dosage form in enteral delivery system for insulin.

Keywords: Insulin; Enteral absorption; Unsaturated fatty acid; W/O/W emulsion; Hypoglycemic effect

1. Introduction

The water-in-oil-in-water (W/O/W) emulsion has been proposed to enhance the enteral bioavailability of drugs, including peptides (Engel et al., 1968; Shichiri et al., 1975; Oba et al., 1992;

* Corresponding author. Tel.: +81 3 54985783; fax: +81 3 54985783.

0378-5173/98/$ - see front matter
PII S0378-5173(98)00210-5

Matsuzawa et al., 1995; Silva Cunha et al., 1997a). The emulsion has advantages for enteral peptides delivery because it protects peptides against proteolysis (Shichiri et al., 1975; Silva Cunha et al., 1997b) and enhances the absorption of normally non-absorbed water soluble substances (Engel et al., 1968; Shichiri et al., 1975; Oba et al., 1992; Matsuzawa et al., 1995; Silva Cunha et al., 1997a). Further, multiple emulsions are easy to handle and to drink due to its low viscosity (Matsumoto, 1986). Thus, there is a possibility for developing an oral dosage form of insulin by using the emulsion. The most convenient administration route of insulin for patients would be a peroral ingestion of a suitable dosage form. In addition, if insulin enclosed in W/O/W emulsion could be absorbed orally and delivered to the liver via the hepatic portal vein efficiently, it offers a means of improving portal insulin levels and curtails the peripheral hyperinsulinaemia associated with other insulin regiments (Ritschel and Ritschel, 1984; Kennedy, 1991). In a previous study, we have evaluated the usefulness of W/O/W emulsion as an enteral carrier of insulin in segments of the rat intestinal tract (Matsuzawa et al., 1995). By using the W/O/W emulsion, insulin could be absorbed from the ileum and the colon regions while the absorbed amount remained low. Since the multiple emulsion can easily incorporate absorption enhancers in each phase, in accordance with its solubility, it is expected to increase the bioavailability of insulin with formulation improvement.

Several reports in literature have indicated absorption promoting effect of different drugs by lipids such as unsaturated fatty acids (Muranishi, 1990; Wang et al., 1994; Aungst et al., 1996; Baudys et al., 1996) or cyclic monoterpenes (Koyama et al., 1994; Levison et al., 1994; Takayama and Nagai, 1994). Oleic acid and certain fatty acids have been shown to alter membrane permeability by increasing the motional freedom or fluidity of the membrane phospholipids (Gay et al., 1989; Muranishi, 1990; Wang et al., 1994). Cyclic monoterpenes present in essential oils have recently been shown to have pronounced percutaneous absorption enhancement effects (Koyama et al., 1994; Levison et al., 1994; Takayama and Nagai, 1994). If the oily phase of the emulsion is composed of unsaturated fatty acids or cyclic monoterpenes, drug absorption–promoting action would be expected. However, their effects have not been evaluated with W/O/W type of emulsion. Thus, we have focused on W/O/W emulsion containing these lipids as an absorption enhancer.

Table 1
Formulations of W/O/W emulsion system

Formulation		*a*	*b*	*c*	*d*	*e*	*f*
Inner aqueous phase (pH7.4 PBS)	(g)	2	2	2	2	2	2
Insulin	(mg)	14.7	14.7	14.7	14.7	14.7	14.7
Gelatin	(%)	5	5	5	5	5	5
Oily phase	(g)	8	8	8	8	8	8
Phosphatidylcholine	(g)	0.28	0.28	0.28	0.28	0.28	0.28
Phosphatidylethanolamine	(g)	0.12	0.12	0.12	0.12	0.12	0.12
Triglyceride							
Soybean oil	(g)	6.0	—	—	—	—	—
Triolein	(g)	—	6.0	—	4.8	5.6	5.2
Trilinolein	(g)	—	—	6.0	—	—	—
Span 80	(g)	1.6	1.6	1.6	1.6	1.6	1.6
Absorption enhancer							
Terpene	(g)	—	—	—	1.2	—	—
Fatty acid	(g)	—	—	—	—	0.4	0.8
Outer aqueous phase (purified water)	(g)	30	30	30	30	30	30
Tween 80	(%)	3	3	3	3	3	3

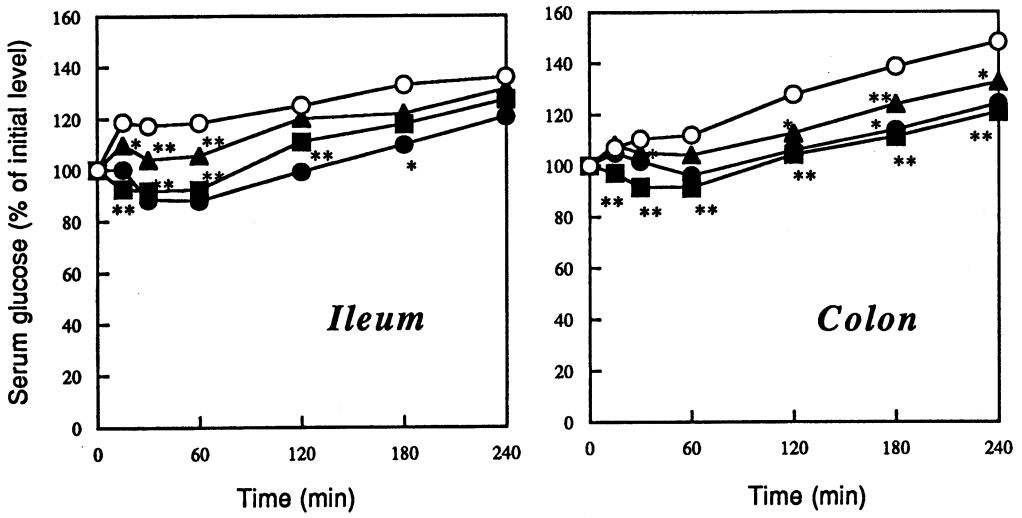

Fig. 1. Effect of intra-ileal and -colonic administration of emulsions prepared with various triglycerides for the oily phase on serum glucose levels. Control (○); emulsion prepared with soybean oil (●); triolein (■); trilinolein (▲). Comparisons calculated at each period against controls: $*p < 0.05$, $**p < 0.01$.

In this study, W/O/W insulin emulsions containing a lipoidal absorption enhancer were prepared using gelatin as a stabilising agent in the inner aqueous phase. The biological effects of the emulsions were compared after administration to the ileum and the colon in the rat intestine. In addition, morphological changes of the intestinal mucosa, after application of these emulsions, were evaluated microscopically.

2. Materials and methods

2.1. Materials

Crystalline porcine insulin (27.3 U/mg) was kindly supplied by Simizu Pharmaceutical (Shizuoka, Japan). Gelatin, a glucose B-Test kit, triolein and sorbitan monooleate (Span 80) were purchased from Wako (Osaka, Japan). Polyoxyethylene sorbitan monooleate (Tween 80), trilinolein, D-(+)-limonene, L-(−)-menthol, oleic acid (99%) and linoleic acid (>98%) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Egg yolk phospholipids (phosphatidylcholine and phosphatidylethanolamine) were purchased from Nippon Oil and Fats (Tokyo, Japan). Linolenic acid (99%) was obtained from Sigma (St. Louis, MO). All other chemicals were of analytical grade and commercially available.

2.2. Preparation of W/O/W emulsion

W/O/W emulsions were prepared by a two-step emulsification procedure using a homogeniser (Ace Homogeniser, Nihonseiki Kaisha, Tokyo, Japan) according to the method reported in the previous paper (Matsuzawa et al., 1995). The formulations of W/O/W emulsion system are shown in Table 1. Briefly, weighed amounts of insulin were dissolved in 200 μl of 0.1 M HCl and then phosphate buffered saline (PBS) containing gelatin (5% of the inner aqueous phase) was added to the solution. The pH value of the solutions was adjusted to pH 7.4 by the addition of 0.1 M NaOH, as required. The oily phase was composed of 5% egg yolk phospholipids (phosphatidylcholine:phosphatidylethanolamine, 7:3), 20% Span 80 and 60–75% triglyceride. Each absorption enhancer was added to the oily phase. The purified water containing 3% Tween 80 was used for the outer aqueous phase. The weight ratio of each phase was as follows: inner aqueous phase:oily phase:outer aqueous phase, 1:4:15.

2.3. In situ absorption experiments

Male Wistar rats weighing 180–220 g were fasted for 24 h prior to the experiments and were anaesthetised by an intraperitoneal (i.p.) injection of 50 mg/kg sodium pentobarbital. The rats were restrained in a supine position on a board which was kept at a surface temperature of 37°C. A small midline incision was made in the abdomen. A 6–7 cm loop of the ileum or the colon was identified and ligated at both ends. The ileum loop was made at the end of the small intestine, just proximal to the ileo-cecal junction. The colon loop was made at the ascending colon. The rats were fixed for 1 h after the operation. The emulsion (1.0 g) was administered directly into the loops. Insulin PBS solution was used as a control. The dose of insulin was fixed at 50 U/kg body weight. A 0.2 ml aliquot of blood sample was taken from the jugular vein ≈ 5 min before administration. Subsequent blood samples were taken at 15, 30, 60, 120, 180 and 240 min after dosing.

The efficacy of the enterally administered insulin was calculated relative to the subcutaneous (s.c.) route, using methods described by Matsuzawa et al., (1995). Briefly, insulin solutions were prepared by dissolving an appropriate amount of crystalline porcine insulin in PBS. The insulin s.c. doses were 0.25, 0.5, 1.0 and 3.0 U/kg body weight. Blood samples were collected from jugular vein before and at 5, 15, 30, 60, 120, 180 and 240 min after dosing. In the intestinal administration experiment, the intrinsic blood glucose levels generally rise due to surgical stress. Thus, the same operation as in the intestinal administration experiment was performed on the rats in the s.c. experiment.

Serum was separated by centrifugation at 3000 rpm for 2 min and kept frozen until analysis. The serum glucose level was determined by using a glucose B-Test kit. Post-dose levels were expressed as a percentage of the pre-dose level. The percentage of change in serum glucose level was taken as the percentage of the pre-dose level subtracted from 100. The cumulative percentage of change in serum glucose level was calculated by summing the areas below base-line levels using the trapezoidal method from the percentage of change versus time curves for 0–4 h.

Experimental procedures above described were performed according to the roles set by the Committee on Ethics in the Care and Use of Laboratory Animals, in Hoshi University.

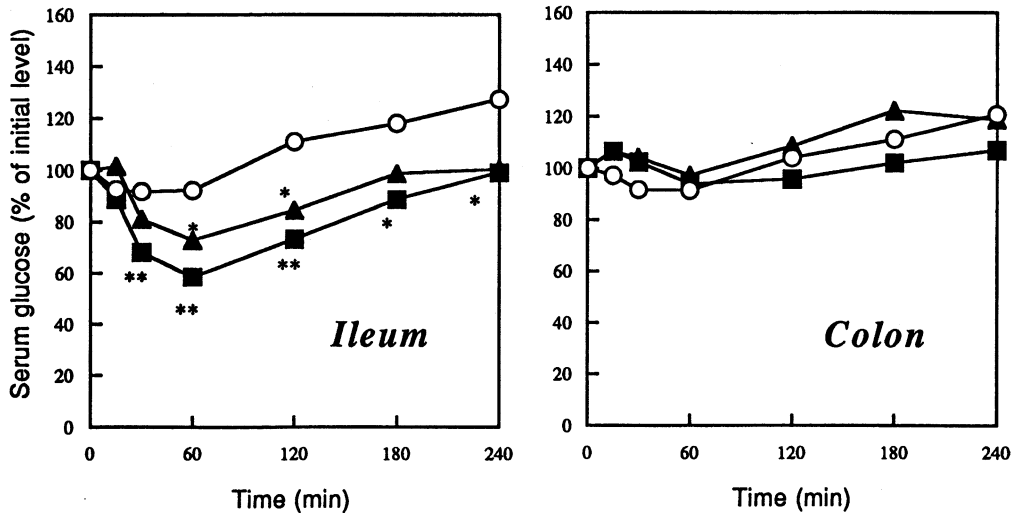

Fig. 2. Effect of intra-ileal and -colonic administration of triolein emulsions containing a terpene on serum glucose levels. Emulsion (○); Emulsion containing 3% limonene (▲) and 3% menthol (■) in the oily phase. Comparisons calculated at each period against controls: $*p < 0.05$, $**p < 0.01$.

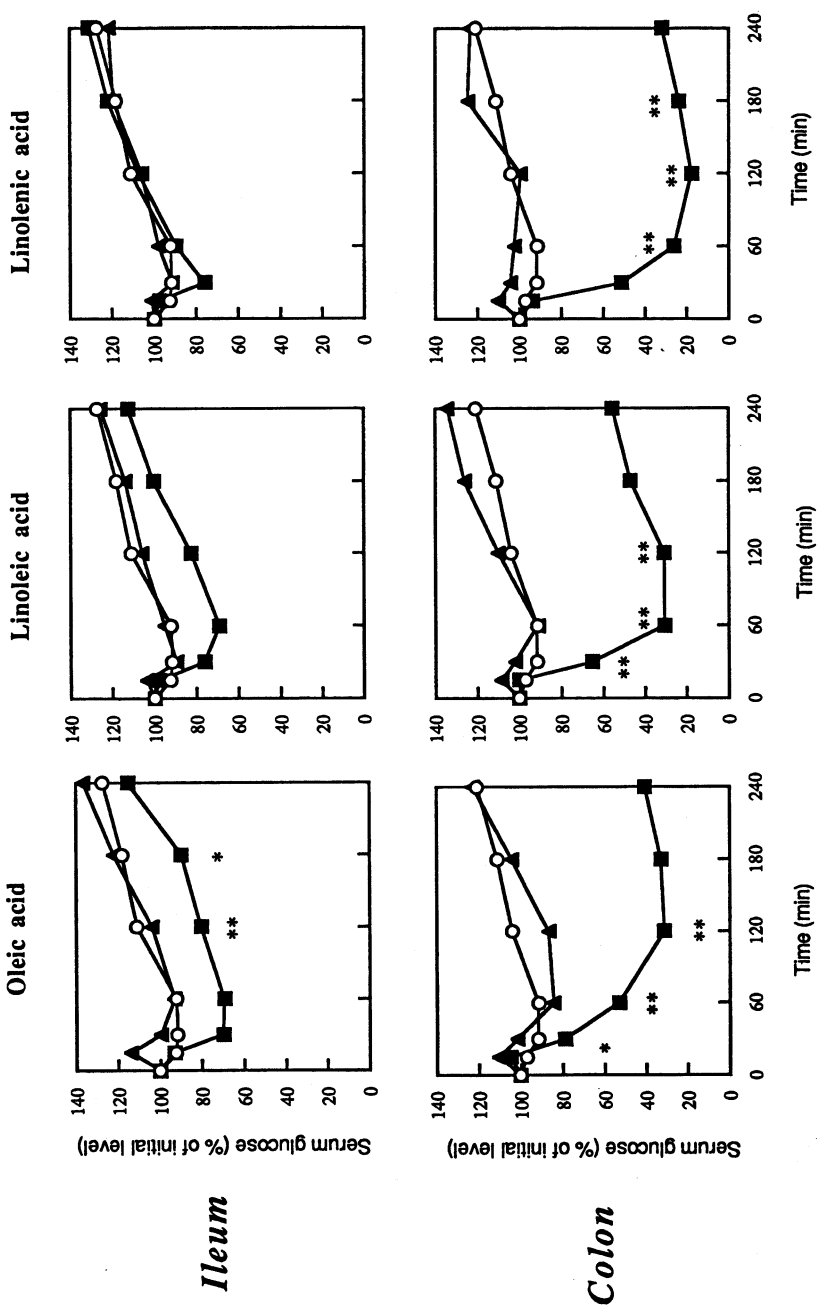

Fig. 3. Effect of intra-ileal and -colonic administration of triolein emulsions containing a fatty acid on serum glucose levels. Emulsion (○); Emulsion containing 1% (▲) and 2% (■) fatty acid in the oily phase. Comparisons calculated at each period against controls: $^{*}p < 0.05$, $^{**}p < 0.01$.

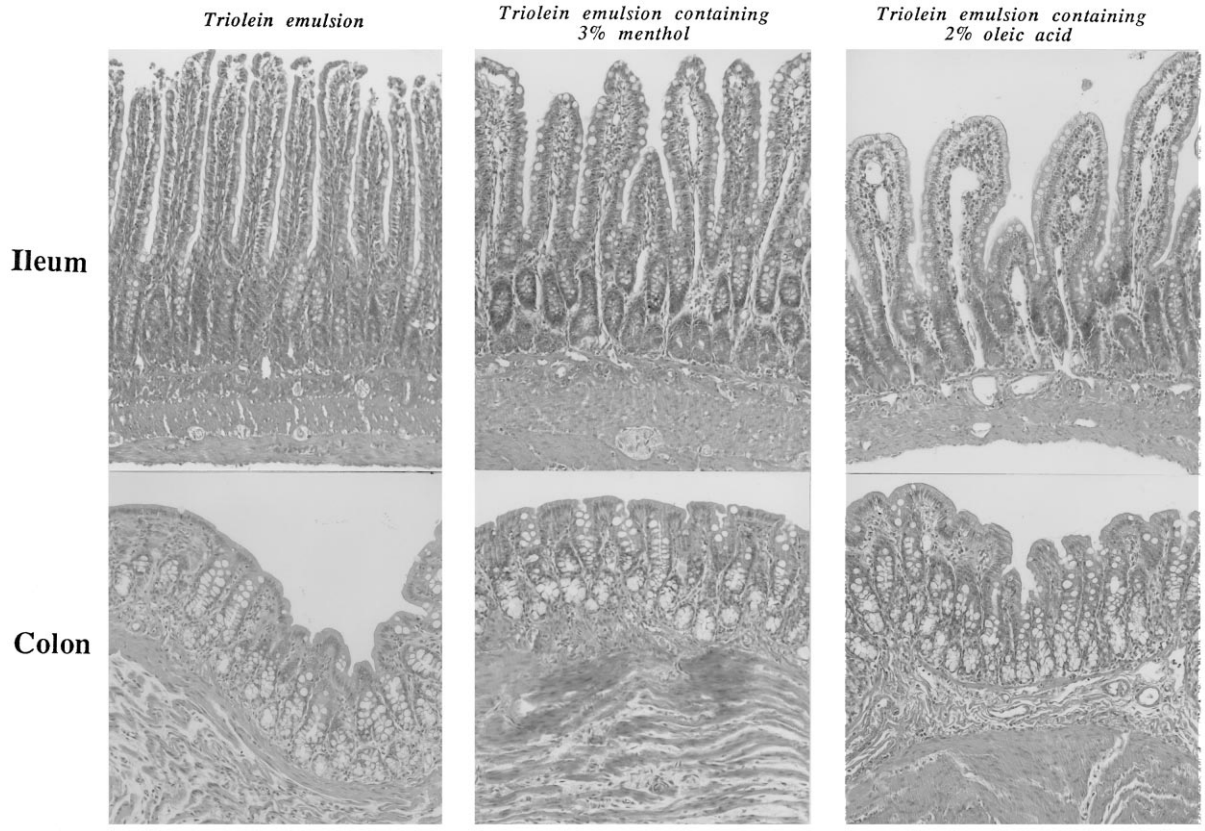

Fig. 4. Light micrographs (H & E stain, × 100) of rat ileal and colonic mucosa after 4 h application of various emulsions.

2.4. Microscopic study

The ileum and the colon were excised just after 4 h application of various emulsions. The separated intestine was fixed in 10% neutral carbonate-buffered formalin, paraffin-sectioned and stained with hematoxylin and eosin. All tissues were examined by light microscopy.

2.5. Statistical analysis

Each value was expressed as the mean or the mean ± S.D. of the mean. For group comparisons, the one-way layout ANOVA with duplication was applied. Significant differences of the mean values were evaluated by student's unpaired t-test. A p value of < 0.05 was considered significant.

3. Results and discussion

3.1. Changes in blood glucose level after administration of W/O/W emulsion prepared with various triglycerides

The biological efficacy of the emulsion is largely dependent on the lipid that constitutes the oily phase. It has been reported that the triglycerides were shown to be almost inactive in modifications of the intestinal barrier function (Muranishi, 1990). However, medium-chain triglycerides have been used in emulsion formulations as absorption enhancers and reported to promote the oral absorption of drugs (Palin et al., 1986). On the other hand, mixed micelles of monoolein increased the in situ intestinal absorption of various compounds in rats, whereas diolein and triolein had no effects (Muranushi, et al., 1980). Insulin absorption en-

hancement effects could be expected to differ among triglycerides used for the oily phase, therefore, the emulsions were prepared with soybean oil, triolein or trilinolein. Fig. 1 shows the mean serum glucose concentrations after administration of three emulsions to the ileum and the colon regions. Although clear hypoglycemic effects could not be observed, all emulsions slightly, but significantly, decreased the serum glucose levels compared to the control. Since pancreatic lipase is present in the small intestine, lipase-generated fatty acids or monoglycerides may be responsible for the permeability increase (Swenson and Curatolo, 1992). However, the site differences of the hypoglycemic effects of these emulsions were not clearly seen, indicating that such a small amount of fatty acids could not induce strong modification of the intestinal barrier. The hypoglycemic effects obtained by administration of triolein emulsion were almost the same in the ileum, or slightly higher in the colon, than those obtained by soybean oil and trilinolein emulsion. The effects of triolein emulsion were highly reproducible with very small S.D. Therefore, we used triolein for the lipid that constitutes mainly the oily phase.

3.2. Effect of monoterpene on the hypoglycemic effect of insulin emulsion

Cyclic monoterpenes have been found to be effective as percutaneous absorption promoters (Koyama, et al., 1994; Levison et al., 1994; Takayama and Nagai, 1994). Among the terpenes, D-limonene possesses a single-ring structure which might cause a disruption of normal lipid packing and increase diffusivity of the lipid bilayer (Koyama, et al., 1994). Fig. 2 shows the changes in serum glucose level following the administration of insulin emulsion containing 3% limonene or 3% menthol into the ileum and the colon. Both menthol and limonene significantly promoted the hypoglycemic effect of insulin at the ileum region. However, the W/O/W emulsion containing a monoterpene had failed to promote the colonic insulin absorption.

When insulin was protected from proteolysis by using protease inhibitors (Morishita et al., 1993) or the emulsion form (Matsuzawa et al., 1995), insulin could be absorbed from the ileum without any absorption enhancers. On the other hand, insulin could be absorbed only when given with

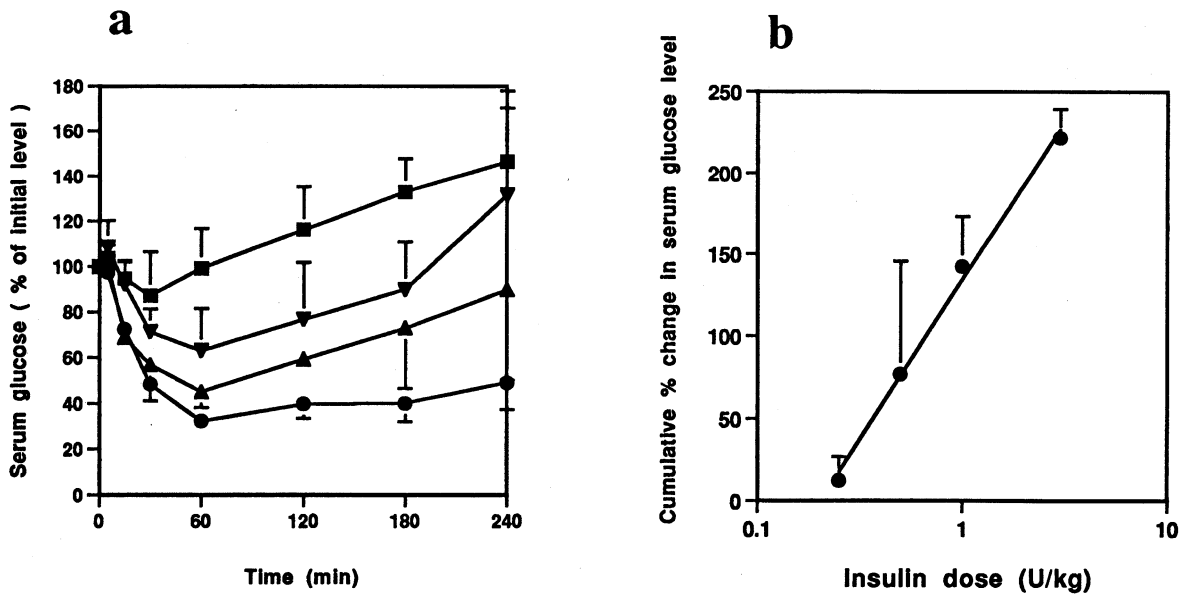

Fig. 5. (a) Serum glucose levels normalised to percentage of the initial level after s.c. administration of insulin in doses of 0.25 U/kg (■), 0.5 U/kg (▼), 1.0 U/kg (▲), 3.0 U/kg (●). (b) Relationship between s.c. insulin dose and efficacy, expressed as the cumulative percentage of change in serum glucose level.

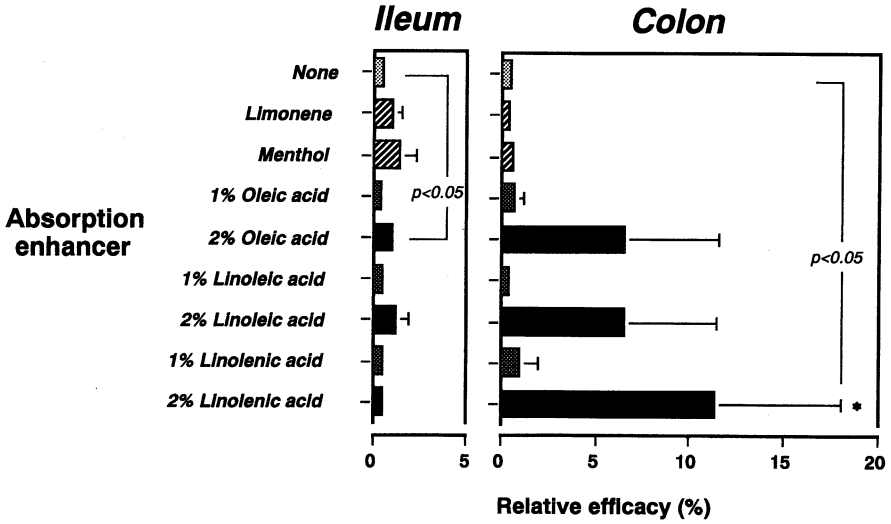

Fig. 6. Comparison of relative hypoglycemic efficacy of various emulsions. An asterisk denote significant difference from intra-ileal administration: $*p < 0.05$.

absorption enhancers in the colon (Morishita et al., 1993). Although the detailed mechanisms of its action have not yet been clarified, it seems likely that monoterpenes can not be interacted with the colonic lipid layer and therefore the intestinal membrane is not greatly altered by these compounds.

3.3. Effect of fatty acids on the hypoglycemic effect of insulin emulsion

Free fatty acids and their monoglycerides which have polar groups attached were demonstrated in the form of mixed micelles to act on the intestinal barrier as enhancers (Taniguchi et al., 1980; Muranishi, 1990). The lipoidal absorption enhancers may enter the lipid bilayer structure in intestinal brush border membrane and disrupt the configuration of the lipid region (Muranishi, 1990). However, the potential usefulness of fatty acids as an absorption enhancer of the insulin emulsion has not been evaluated. Fig. 3 shows the changes in serum glucose level following the administration of insulin emulsion containing a long-chain unsaturated fatty acid into the ileum and the colon. Strong hypoglycemic effects were observed with the emulsion containing 2% fatty acid. The effects were sustained and did not reach to the base-line levels after 4 h administration. The remarkable enhancing effects occurred predominantly in the colon rather than in the ileum. This observation substantially agrees with previous findings, which demonstrated that the absorption enhancement effects were more predominant in the large intestine than in the small intestine (Muranishi, 1990, Morishita et al., 1993; Aungst et al., 1996). The apical microvillus membrane provides the major physical barrier to drug absorption from the small intestine. The apical membrane is rich in glycolipids, which would serve to rigidify this membrane at body temperature due to the high T_m of these lipids (Muranushi et al., 1980). Scanning calorimetry and fluorescence polarisation studies have demonstrated that the microvillus membrane is less fluid than most membranes (Schachter and Shinitzky, 1977). The composition of the brush border membrane is finely balanced to permit proper functioning of transmembrane pumps while assuring structural stability against the onslaught of the osmotically variable and detergent-laded intestinal lumen (Muranushi et al., 1980). Therefore, the upper portion of intestine is thought to be resistant to enhancer action. In contrast, the microvilli in the colon are much less closely packed and the fuzzy coat is less compact (Kararli, 1989).

The effect of degree of unsaturation of fatty acids was not observed. All three fatty acids exhibited similar and comparable insulin absorption enhancement effects. Similar results were reported by Wang et al. (1994), who examined the alveolar monolayer permeability using unsaturated fatty acids with a chain length equal to that of stearic acid. The results showed an insignificant role of degree of unsaturation in the monolayer permeability and cellular calcium. Similarly, the mixed micelles consisting of bile acid and unsaturated fatty acids enhanced drug absorption independently of the degree of unsaturation of the lipids (Ishizawa et al., 1987).

In this study, the insulin emulsions containing an unsaturated fatty acid did not reduce the electrical resistance of rat colonic membrane (data not shown). The electrical resistance are thought to express mainly the structural feature of the tight junctional portion of the membrane. Thus, the effect of fatty acid on the paracellular pathway for insulin permeation was thought to be small. The details of the above mechanism will be described in future reports.

3.4. Morphological changes of the mucosal surface

The histology of the ileum and the colon mucosa after a 4 h application of various emulsions is shown in Fig. 4. No tissue damage was noted by light microscopic examination of both regions treated with three type of emulsions. Even in the emulsion containing oleic acid, which showed strong hypoglycemic effect, membrane damage was not detected. Thus, the insulin emulsions containing an unsaturated fatty acid are acceptable for use in oral delivery system.

The central lacterials of villi in the ileum were obviously expanded after administration of the emulsion containing oleic acid, suggesting a enhancement of lymphatic insulin delivery. This is consistent with the fact that the lipid-based formulation could become the lymphotropic carrier (Kararli, 1989; Muranishi, 1990; Oba et al., 1992).

3.5. Hypoglycemic efficacy relative to subcutaneous

In order to calculate the value of percentage efficacy relative to s.c., the relationship between s.c. insulin dose and efficacy, expressed as the cumulative percentage of change in serum glucose levels, was obtained from the results of the s.c. administration study. Fig. 5a shows average serum glucose level versus time profiles after s.c. administration of insulin at several doses to rats. The relationship between the logarithm of s.c. insulin doses and the average values of cumulative percentages of change in serum glucose levels is shown in Fig. 5b. The dose–response curve gave the following equation.

$$\text{Cumulative \% change} = 194.0 \times \log \text{dose} + 134.1;$$

$$r = 0.922;\ p < 0.01$$

As shown in Fig. 6, relative efficacies clearly indicated that unsaturated fatty acids enhanced the colonic insulin absorption. The highest relative efficacy, $\approx 10\%$, was achieved with the insulin emulsion containing 2% linolenic acid.

In addition to the altering membrane permeability by unsaturated fatty acids, the absorption enhancement effect of insulin by W/O/W emulsion system probably results from the increase of the stability of insulin in the intestinal tract as suggested by Silva Cunha et al. (1997a). In conclusion, W/O/W emulsion incorporating unsaturated fatty acid were demonstrated to be useful carriers for enhancing insulin absorption via the intestinal tract.

Acknowledgements

We thank Asuka Ono for technical assistance. The gift of insulin from Simizu Pharmaceutical is gratefully acknowledged. This work was supported by the Ministry of Education, Science, Sports and Culture of Japan.

References

Aungst, B.J., Saitoh, H., Burcham, D.L., Huang, S.M., Mousa, S.A., Hussain, M.A., 1996. Enhancement of the intestinal absorption of peptides and non-peptides. J. Control. Release 41, 19–31.

Baudys, M., Mix, D., Kim, S.W., 1996. Stabilization and intestinal absorption of human calcitonin. J. Control. Release 39, 145–151.

Engel, R.H., Riggi, S.J., Fahrenbach, M.J., 1968. Insulin: intestinal absorption as water-in-oil-in-water emulsions. Nature 219, 856–857.

Gay, C.L., Murphy, T.M., Hadgraft, J., Kellaway, I.W., Evans, J.C., Rowlands, C.C., 1989. An electron spin resonance study of skin penetration enhancers. Int. J. Pharm. 49, 39–45.

Ishizawa, T., Hayashi, M., Awazu, S., 1987. Enhancement of jejunal and colonic absorption of fosfomycin by promoters in the rat. J. Pharm. Pharmacol. 39, 892–895.

Kararli, T.T., 1989. Gastrointestinal absorption of drugs. Crit. Rev. Ther. Drug Carr. Syst. 6, 39–86.

Kennedy, F.P., 1991. Recent developments in insulin delivery techniques: current status and future potential. Drugs 42, 213–227.

Koyama, Y., Bando, H., Yamashita, F., Takakura, Y., Sezaki, H., Hashida, M., 1994. Comparative analysis of percutaneous absorption enhancement by *d*-limonene and oleic acid based on a skin diffusion model. Pharm. Res. 11, 377–383.

Levison, K.K., Takayama, K., Isowa, K., Okabe, K., Nagai, T., 1994. Formulation optimisation of indomethacin gels containing a combination of three kinds of cyclic monoterpenes as percutaneous penetration enhancers. J. Pharm. Sci. 83, 1367–1372.

Matsumoto, S., 1986. W/O/W-type multiple emulsions with a view to possible food applications. J. Texture Stud. 17, 141–159.

Matsuzawa, A., Morishita, M., Takayama, K., Nagai, T., 1995. Absorption of insulin using water-in-oil-in-water emulsion from enteral loop in rats. Biol. Pharm. Bull. 18, 1718–1723.

Morishita, M., Morishita, I., Takayama, K., Machida, Y., Nagai, T., 1993. Site-dependent effect of aprotinin, sodium caprate, Na_2EDTA and sodium glycocholate on intestinal absorption of insulin. Biol. Pharm. Bull. 16, 68–72.

Muranishi, S., 1990. Absorption enhancers. Crit. Rev. Ther. Drug Carr. Syst. 7, 1–33.

Muranushi, N., Nakajima, Y., Kinugawa, M., Muranishi, S., Sezaki, H., 1980. 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, 281–290.

Oba, N., Sugimura, H., Umehara, Y., Yoshida, M., Kimura, T., Yamaguchi, T., 1992. Evaluation of an oleic acid water-in-oil-in-water-type multiple emulsion as potential drug carrier via the enteral route. Lipids 27, 701–705.

Palin, K.J., Phillips, A.J., Ning, A., 1986. The oral absorption of cefoxitin from oil and emulsion vesicles in rats. Int. J. Pharm. 33, 99–104.

Ritschel, W.A., Ritschel, G.E., 1984. Rectal administration of insulin. Methods Find. Exp. Clin. Pharmacol. 6, 513–529.

Schachter, D., Shinitzky, M., 1977. Fluorescence polarisation studies of rat intestinal microvillus membranes. J. Clin. Invest. 59, 536–548.

Shichiri, M., Kawamori, R., Yoshida, M., et al., 1975. Short-term treatment of alloxan-diabetic rats with intrajejunal administration of water-in-oil-in-water insulin emulsions. Diabetes 24, 971–976.

Silva Cunha, A., Grossiord, J.L., Puisieux, F., Seiller, M., 1997a. Insulin in W/O/W multiple emulsions: preparation, characterisation and determination of stability towards proteases in vitro. J. Microencapsul. 14, 311–319.

Silva Cunha, A., Grossiord, J.L., Puisieux, F., Seiller, M., 1997b. Insulin in W/O/W multiple emulsions: biological activity after oral administration in normal and diabetic rats. J. Microencapsul. 14, 321–333.

Swenson, E.S., Curatolo, W.J., 1992. Intestinal permeability enhancement for proteins, peptides and other polar drugs: mechanisms and potential toxicity. Adv. Drug Deliv. Rev. 8, 39–92.

Takayama, K., Nagai, T., 1994. Limonene and related compounds as potential skin penetration promoters. Drug Dev. Ind. Pharm. 20, 677–684.

Taniguchi, K., Muranishi, S., Sezaki, H., 1980. 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, 219–228.

Wang, L.Y., Ma, J.K.H., Pan, W.F., Toledo-Velasquez, D., Malanga, C.J., Rojanasakul, Y., 1994. Alveolar permeability enhancement by oleic acid and related fatty acids: evidence for a calcium-dependent mechanism. Pharm. Res. 11, 513–517.