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The dose-related hypoglycemic effects of insulin emulsions incorporating highly purified EPA and DHA

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Abstract

The dose-related pharmacological effects of insulin emulsion incorporating highly purified eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were investigated. Water-in-oil-in-water multiple emulsions (insulin dose, 0, 10, 25 and 50 IU/kg) incorporating 2% DHA or EPA were administered directly into the colonic and rectal loops in situ. Serum insulin levels rose and serum glucose levels decreased in an insulin dose-related fashion. The relationship of insulin dose and C_{max} or $AUC_{insulin}$ was linear at the rectum, but a non-linear relationship was observed at the colon. The trend was more predominant in DHA. In the in vivo rectal absorption experiment using emulsions incorporating 2% DHA, 5 IU/kg of insulin emulsion produced a rapid, transitory increase in serum insulin levels and strong reduction of serum glucose levels. The pharmacological availability determined from the dose–response curve by s.c. administration of insulin reached $43.2 \pm 26.3\%$ (mean $\pm$ S.D.). Mucosal irritation caused by administration of emulsions incorporating 2% EPA or DHA was evaluated by a lactate dehydrogenase (LDH) release study, and compared with those of the emulsion incorporating 2% oleic or linolenic acid. Only when emulsion incorporating 2% oleic acid was applied in the intestine did significant LDH release into the mesenteric veins occur. Our results indicate that emulsion incorporating highly purified long-chain polyunsaturated fatty acid, especially DHA, has the potential of becoming the formulation for enteral delivery of insulin.

Keywords: Insulin; Docosahexaenoic acid; Eicosapentaenoic acid; Emulsion; Unsaturated fatty acid; Intestinal absorption

1. Introduction

It has been shown that insulin delivery via the hepatic portal vein after regular feeding demonstrated less hyperinsulinaemia than that observed in a peripheral infusion study (Ritschel and Ritschel, 1984). This finding indicates the importance of insulin delivery via the hepatic portal vein in normalising both blood glucose and insulin levels in the postprandial state. Such portal delivery can be achieved by small intestinal, colonic or rectal insulin administration. There-

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fore, the development of an oral or rectal dosage form providing adequate bioavailability of insulin would revolutionise the treatment of diabetes. Moreover, these dosage forms of insulin administration would be convenient and could improve patient compliance. However, the absorption of insulin from the intestine is limited because of enzymatic degradation in the gastrointestinal tract, high molecular weight, and poor lipophilicity. Researchers have attempted to overcome these hurdles by using various carriers and by adding compounds known as absorption enhancers.

Recently, we showed that water-in-oil-in-water (W/O/W) multiple emulsion incorporating unsaturated fatty acids having 18 carbon (C18) alkyl chain (Morishita et al., 1998; Suzuki et al., 1998), *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA, C22:6ω3) or *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA, C20:5ω3) (Suzuki et al., 1998) could enhance intestinal insulin absorption. We found that both DHA and EPA have a higher enhancing effect than unsaturated fatty acid with a C18 alkyl chain without gross tissue damage (Suzuki et al., 1998). DHA and EPA are present in ω3 polyunsaturated fatty acids of fish oil. DHA is an essential polyunsaturated fatty acid highly enriched in the brainlipids and retinal photoreceptor cell phospholipids. In the late 1970s, people who consume fish or shellfish daily were shown to have significantly lower incidences of coronary heart disease (Dyerberg et al., 1978). Because of this studies on the precise pharmacological effects of fish oil with a high content of DHA and EPA were conducted. As a result, DHA and EPA have been found to exhibit various biological actions (Dyerberg et al., 1978; Wainwright et al., 1991; Takahashi et al., 1993). In addition to these pharmacological effects, the influence of DHA on various biological membranes has been proposed. DHA taken from food stuffs is readily taken up by a variety of cells, where it is incorporated into membrane phospholipids (Jenski et al., 1993). DHA is effective at promoting membrane fusion (Ehringer et al., 1990). DHA has also been shown to increase membrane permeability of T27A murine leukemia cells (Stillwell et al., 1993). In addition, it is suggested that DHA altered the structure and composition of membrane microdomains on the cell surface (Williams et al., 1999). These findings and our previous study suggest a high possibility that DHA can be used in a drug delivery system as an membrane permeability modifying agent.

In the present study, we focused on the potent membrane permeability modifying effect of unsaturated fatty acids, in particular EPA and DHA. In order to verify their potential use in non-invasive enteral delivery, the dose-related pharmacological effects of insulin emulsion incorporating highly purified EPA and DHA were evaluated in in situ absorption. The extent of absorption relative to injected insulin is a primary determinant of the possibility of noninvasive insulin dosage forms. Therefore, the hypoglycemic effect of insulin emulsion incorporating DHA was evaluated in in vivo absorption experiments, and pharmacological availability was determined from the dose–response curve by s.c. administration of insulin. A major limiting factor in the application of the permeability enhancing approach is the potential toxicity of the absorption enhancers themselves. Although our previous histological study showed that emulsion incorporating 2% DHA did not induce gross tissue damage (Suzuki et al., 1998), we carried out additional biochemical evaluation of intestinal damage during emulsion administration.

2. Materials and methods

2.1. Materials

Crystalline porcine insulin (26.9 U/mg) was kindly supplied by Simizu Pharmaceutical (Shizuoka, Japan). Gelatin, a glucose B-test kit, a lactate dehydrogenase (LDH) assay kit (LDH-UV), triolein, DL-α-tocopherol and sorbitan monooleate (Span 80) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Polyoxyethylene sorbitan monooleate (Tween 80) and oleic acid (purity, 99.0%) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Egg yolk phospholipids (phosphatidylcholine and phosphatidylethanolamine) were purchased from Nip-

pon Oil & Fats (Tokyo, Japan). Linolenic acid (purity: 98.0%) was obtained from Sigma Chemical (St Louis, MO). Docosahexaenoic acid (purity: 99.0%) and eicosapentaenoic acid (purity: 99.0%) were provided by Nippon Suisan Kaisya (Tokyo, Japan). 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 homogenizer (Ace Homogenizer, Nihonseiki Kaisha, Tokyo, Japan) according to the method reported in a previous paper (Morishita et al., 1998). Briefly, weighed amounts of insulin were dissolved in 200 ml of 0.1 N 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 N NaOH as required. The oily phase was composed of 0.06% DL-α-tocopherol, 5% egg yolk phospholipids (phosphatidylcholine:phosphatidylethanolamine, 7:3), 10% unsaturated fatty acid, 20% Span 80 and 64.94% triolein. Purified water containing 3% Tween 80 was used for the outer aqueous phase. Thus, each emulsion contains 2% of unsaturated fatty acid. The weight ratio of each phase was as follows, inner aqueous phase:oily phase:outer aqueous phase = 1:4:15. Each emulsion was freshly prepared just before the absorption experiments.

2.3. Insulin absorption experiments

In the in situ absorption experiments, male Wistar rats weighing 180–220 g fasted for 48 h prior to the experiments and were anesthetized by an i.p. injection of 50 mg/kg sodium pentobarbital. The rats were restrained in a supine position on a board that was kept at a surface temperature of 37°C. A small midline incision was made in the abdomen. A 5 cm loop of the colon or the rectum was identified and ligated at both ends. The colon loop was made at the ascending colon. The rats were fixed for 1 hr after the operation. The insulin doses were 0, 10, 25 and 50 IU/kg and administered directly into the loops (1 g as the emulsion).

Approximately 5 min before administration, a 0.2 ml aliquot blood sample was taken from the jugular vein. Subsequent blood samples were taken at 15, 30, 60, 120, 180 and 240 min after dosing.

In the in vivo absorption experiments, male Wistar rats weighing 180–220 g fasted for 48 h before the experiments, and were allowed water ad libitum. Rats were restrained in a supine position during administration and at each blood sampling. Approximately 5 min before administration, a 0.2 ml aliquot blood sample was taken from the jugular vein. A 5 IU/kg insulin dose (0.1 g as the emulsion) was administered rectally through a stomach sonde needle for rats (KN-348, Natsume Seisakusyo, Tokyo). The same dose of insulin PBS solution was used as a control. The anus was nipped during a study period by a clip in order to prevent the dosing solution from leaking. Subsequent blood samples were taken at 0.5, 1, 2, 4 and 6 h after dosing.

Serum was separated by centrifugation at 13 000 rpm for 1 min and kept frozen until analysis. The serum glucose levels were determined 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 baseline levels using the trapezoidal method from the percentage of change versus time curves during the experiment ($AUC_{glucose}$).

The serum insulin levels were determined using an enzyme immunoassay (IMx System, DAINABOT, Tokyo, Japan). The basal endogenous insulin concentration was subtracted from all insulin concentrations measured following insulin administration. The serum peak concentration (C_{max}) and the time taken to reach the serum peak level (T_{max}) were determined from the serum insulin concentration–time curves. The areas under the insulin concentration–time curves during the experiment ($AUC_{insulin}$) were determined using a trapezoidal rule. Mean residence time (MRT) was calculated by dividing AUMC by $AUC_{insulin}$, where AUMC is the area under the first moment curve for insulin from 0 to a 4-h point.

The pharmacological availabilities of enterally administered insulin were calculated relative to that administered by the s.c. route. The dose–response curve in the in vivo absorption study was obtained by methods described previously (Morishita et al., 1992). Briefly, insulin solutions were prepared by dissolving an appropriate amount of crystalline porcine insulin in PBS. The s.c. insulin doses were 0.5, 1.0 and 3.0 IU/kg body weight, each in a 0.1 ml volume. Blood samples were collected from the jugular vein before and at 0.25, 0.5, 1, 2, 4 and 6 h after dosing.

2.4. Biochemical evaluation of intestinal damage

The in situ absorption experiments were carried out as described above. After ligation at both ends of the colon or the rectum, a butterfly needle was set at the mesenteric vein. In this experiment insulin-free emulsions incorporating 2% oleic acid, linoleic acid, EPA or DHA were prepared, and administered directly into the loops at a weight of 1 g. Unsaturated fatty acid-free emulsion was used as a control. Before and at 2 and 4 h after administration, a 0.2 ml aliquot blood sample was taken from the mesenteric vein. Plasma LDH concentration was determined using a LDH-UV kit. Post-dose levels were expressed as percentages of the pre-dose level.

The experimental procedures described above were performed according to the rules set by the Committee on Ethics in the Care and Use of laboratory Animals at Hoshi University.

2.5. Statistical analysis

Each value was expressed as the mean $\pm$ S.D. For group comparisons, one-way layout ANOVA with duplication was applied. Significant differences in mean values were evaluated by the Student's unpaired t-test. A P value of less than 0.05 was considered significant.

3. Results

Fig. 1 shows the changes in serum glucose and insulin levels following the administration of various doses of insulin emulsion incorporating 2% DHA into the colon and the rectum. Clearly, insulin levels rose and the serum glucose levels decreased in an insulin dose-related manner. Similar results were obtained from the administration of insulin emulsion incorporating 2% EPA (Fig. 2). In both emulsions, the 25 and 50 IU/kg dose of insulin administration to the colon resulted in significant reduction in serum glucose levels. In the rectum, both emulsions induced strong hypoglycemic effects at insulin doses as low as 10 IU/kg. Figs. 1 and 2 represent fast insulin absorption with peak systemic concentration attained approximately 1–2 h after colonic administration and 0.54 h after rectal administration. Pharmacokinetic parameters of insulin following intracolonic and rectal administration of emulsions are shown in Table 1. In both emulsions, T_{max} values following rectal administration were less than 1 h and shorter than those following colonic administration. In contrast, the peak insulin concentration was attained at different times in the colon, depending on the dose.

The relationship between C_{max} or $AUC_{insulin}$ following administration of both emulsions and insulin dose is shown in Figs. 3 and 4, respectively. The results show that there was a linear relationship between both parameters and insulin dose in the rectum. Linear regression analysis for dose linearity of $AUC_{insulin}$ yielded correlation coefficients of r, 0.931 (●) for DHA and 0.927 (○) for EPA, and linear regression analysis for dose linearity of C_{max} yielded r, 0.898 (■) for DHA and 0.874 (□) for EPA. In contrast, a non-linear relationship between insulin dose of the emulsion incorporating 2% DHA and C_{max} or $AUC_{insulin}$ was observed in the colon. The same trend was seen in the case of EPA, however, the non-linearity was more predominant in the emulsion incorporating DHA.

Fig. 5 shows the release of LDH, an intracellular enzyme, into the mesenteric vein during the 4-h absorption study with the emulsion incorporating various fatty acids. The emulsions incorporating 2% linolenic acid, EPA or DHA did not release LDH from either administration site, and LDH levels were similar to that of control over the time period of the experiment. On the other

Table 1
Comparison of pharmacokinetic parameters of insulin following enteral administration of insulin emulsions incorporating unsaturated fatty acid[a]

Unsaturated fatty acid	Insulin dose (IU/kg)	$AUC_{insulin}$ (μU · h · ml^{-1})		c_{max} (μU/ml)		T_{max} (h)		MRT (h)	
		Colon	Rectum	Colon	Rectum	Colon	Rectum	Colon	Rectum
	0	NC	NC	NC	NC	NC	NC	NC	NC
DHA									
	10	56.2 ± 35.9	471.9 ± 386.8	59.5 ± 11.7	466.6 ± 375.8	0.4 ± 0.1	0.6 ± 0.4	0.9 ± 0.4	0.9 ± 0.4
	25	1460.2 ± 1071.8	1338.9 ± 794.3	769.6 ± 439.7	1477.6 ± 706.2	1.5 ± 0.6	0.7 ± 0.3	1.6 ± 0.5	0.7 ± 0.2*
	50	6640.7 ± 2092.7**	4088.9 ± 872.6	2703.3 ± 760.2	2417.1 ± 411.9	2.3 ± 1.0	0.7 ± 0.3*	2.2 ± 0.4	1.4 ± 0.1*
	0	NC	NC	NC	NC	NC	NC	NC	NC
EPA									
	10	22.0 ± 22.7	138.2 ± 145.9	34.3 ± 32. 0	213.6 ± 176.1	0.4 ± 0.1	0.5 ± 0.3	0.8 ± 0.5	1.1 ± 0.6
	25	125.3 ± 62.0	1124.0 ± 575.9*	132.4 ± 48.3	1333.2 ± 755.3*	0.6 ± 0.2	0.6 ± 0.3	1.1 ± 0.5	0.9 ± 0.1
	50	2884.3 ± 946.7	3081.4 ± 838.5	2155.1 ± 1166.1	2634.0 ± 930.9	1.3 ± 0.6	0.7 ± 0.3	1.5 ± 0.4	1.0 ± 0.3

[a] Each value represents the mean ± S.D. (n, 3–5). NC denotes not calculated.
* Statistically significant difference between the two administration sites ($P<0.05$).
** Statistically significant difference between the two formulations ($P<0.05$).

hand, of all the tested insulin emulsions, only the emulsion incorporating 2% oleic acid caused significant release of LDH, especially when the emulsion was administered to the rectum. Oleic acid caused the release of LDH approximately twice and three times higher than the baseline levels obtained from the colonic administration and the rectal administration, respectively.

Fig. 6 shows the changes in serum insulin and glucose levels following rectal administration of the emulsion incorporating 2% DHA It is obvious that 5 IU/kg of insulin emulsion was highly effective in attaining a rapid, transitory increase in serum insulin levels and considerable reduction in serum glucose levels. In order to obtain accurate pharmacological availability of insulin emulsion, in the present study, we determined the dose–response curve by s.c. administration of insulin and then the following equation was obtained.

Cummulative % change, $219.29 \times \log \text{dose} + 145.96$; r, 0.856; $P < 0.01$. Table 2 clearly shows the very high pharmacological availability of the insulin emulsion incorporating DHA, calculated from the equation. The low bioavailability of insulin PBS solution was greatly improved by using the emulsion incorporating 2% DHA The results indicate that only nearly twice the s. c. injection dose is required in order to obtain the same hypoglycemic effect.

4. Discussion

The results from the present study clearly demonstrate that emulsions incorporating long-chain polyunsaturated fatty acids, such as DHA and EPA, significantly potentiates the biological effect of enterally administered insulin in a dose-

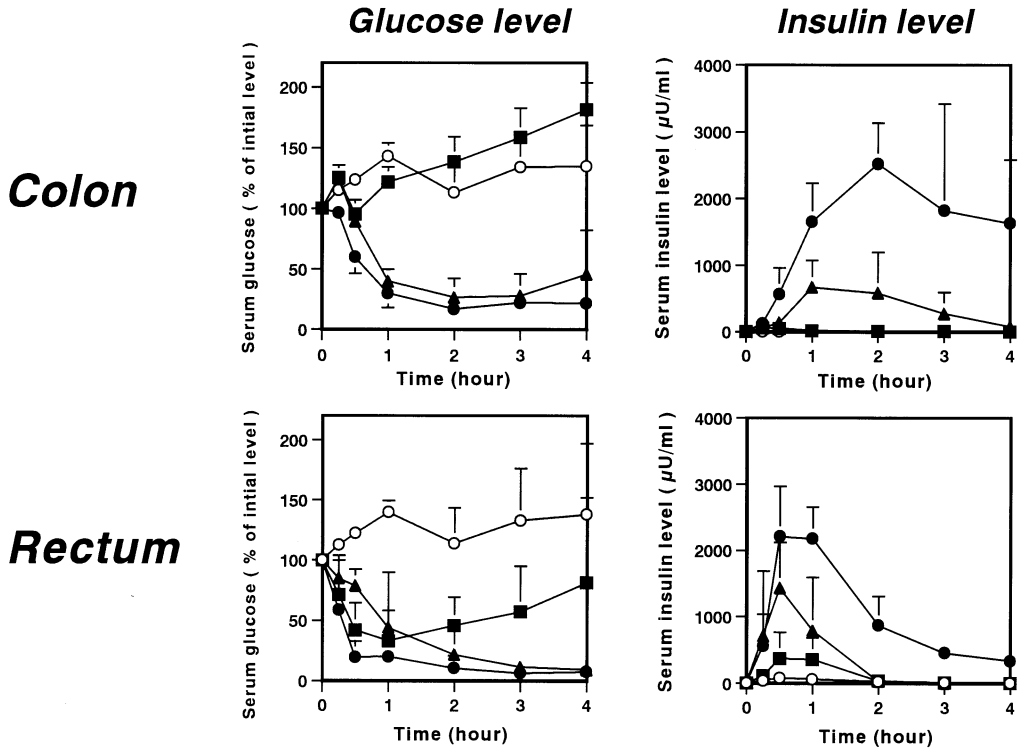

Fig. 1. Effect of intra-colonic and -rectal administration of four different doses of insulin emulsions incorporating 2% DHA on serum glucose and insulin levels in situ. Serum glucose levels were normalised to a percentage of the initial concentration. Insulin doses were 0 IU/kg (○), 10 IU/kg (■), 25 IU/kg (▲) and 50 IUikg (●). DHA dose was 100 mg/kg. Symbols represent the mean ± S.D. from three to five rats.

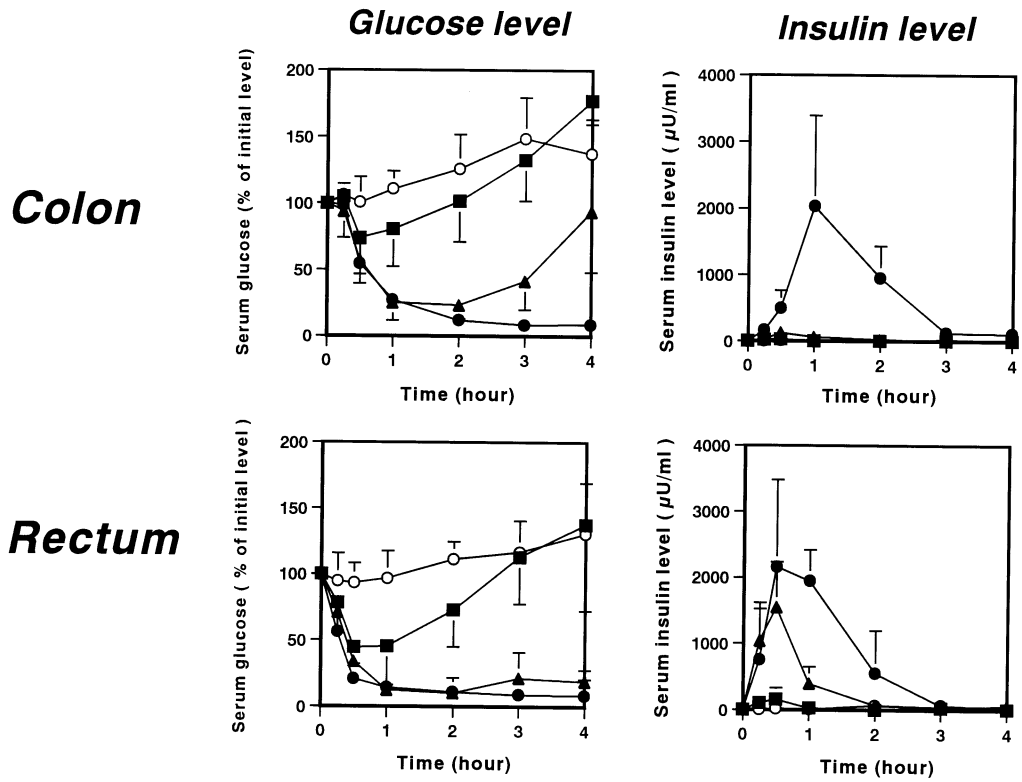

Fig. 2. Effect of intra-colonic and rectal administration of four different doses of insulin emulsions incorporating 2% EPA on serum glucose and insulin levels in situ. Serum glucose levels were normalised to a percentage of the initial concentration. Insulin doses were 0 IU/kg (○), 10 IU/kg (■), 25 IU/kg (▲) and 50 IU/kg (●). EPA dose was 100 mg/kg. Symbols represent the mean ± S.D. from three to five rats.

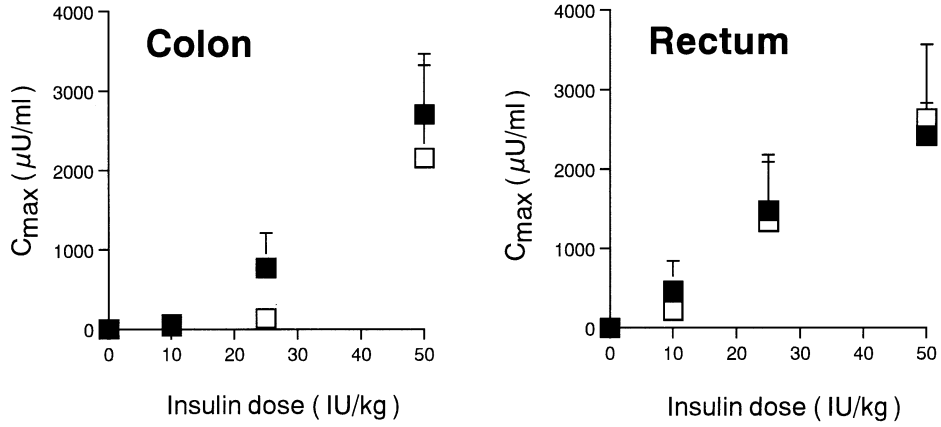

Fig. 3. Relationship between insulin dose and C_{max} values of insulin following the administration of emulsion incorporating 2% DHA (●) and EPA(○). C_{max} represents the peak concentration determined from the insulin concentration–time curves. Each value represents the mean ± S.D. from three to five rats.

related fashion. The time course of the glucose reduction provided by the insulin emulsion incorporating 2% polyunsaturated fatty acids was very rapid (Figs. 1 and 2) (Table 1), and the profiles are similar to that of the only insulin monomeric analogue for which a clinical use has been found, insulin lispro (Howey et al., 1994). It was reported that the pharmacokinetic and pharmacodynamic profiles of insulin lispro mimic more closely those of physiological mealtime insulin (Hoffman and Ziv, 1997). Therefore, there may be a high possibility of achieving postprandial hyperglycemia control by rectal administration of emulsion incorporating polyunsaturated fatty acid as a way that is non-invasive and provides a relatively rapid insulin peak concentration.

Under fasting conditions, the pancreas secretes about 1 unit of insulin per hour into the portal vein to achieve a concentration of insulin in portal blood of 50–100 μU/ml and in the peripheral

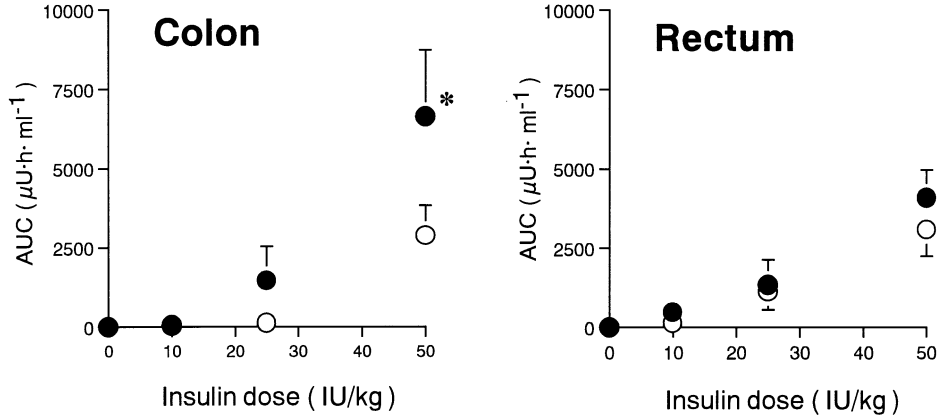

Fig. 4. Relationship between insulin dose and AUC values of insulin following the administration of emulsions incorporating 2% DHA (●) and EPA (○). AUC represents the area under the serum insulin concentration–time curve during the 4-h absorption experiment determined with the use of the trapezoidal rule. Each value represents the mean ± S.D. from three to five rats. *, $P < 0.05$ when comparing data obtained from the two formulations at each insulin dose.

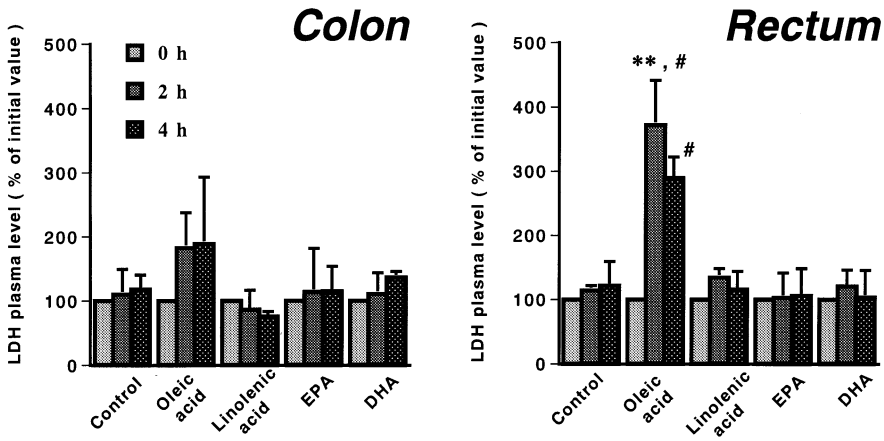

Fig. 5. Changes in LDH plasma concentration after application of an emulsion without unsaturated fatty acid (control) and incorporating 2% unsaturated fatty acid. Each value represents the mean ± S.D. from three to five rats. **, $P < 0.01$ versus control at each time in the same administration site. # $P < 0.01$ when comparing data obtained from the two administration sites at each time point.

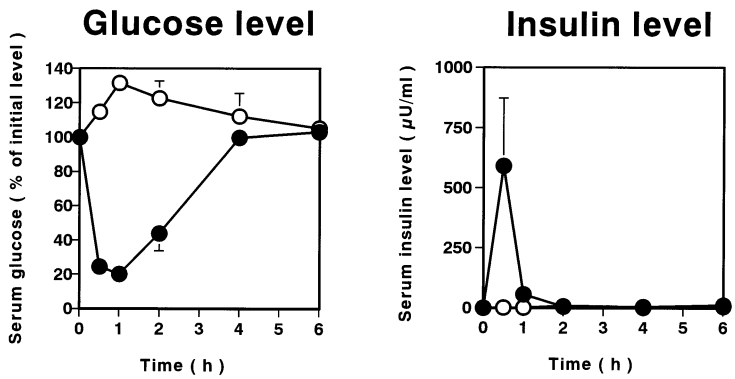

Fig. 6. Serum glucose and insulin levels after rectal administration of insulin solution (○) and insulin emulsion incorporating 2% DHA (●) in vivo. Insulin and DHA doses were 5 IU/kg and 10 mg/kg, respectively. Each value represents the mean ± S.D. from four to five rats.

circulation of 12 μU/ml (Kahn and Shechter, 1990). Degradation of insulin occurs primarily in the liver, kidney and muscles. About 50% of the insulin that reaches the liver via the portal vein is destroyed, however, hepatic degradation of insulin operates near its maximal capacity (Duckworth, 1988). Therefore, it is reasonable to suppose that non-physiological high insulin concentration provided by emulsions incorporating 2% DHA or EPA, as shown in Figs. 1 and 2, may induce saturation of insulin degradation in the liver. On the other hand, rectally administered insulin is thought to bypass the liver and enter into the systemic circulation directly (de Boer et al., 1980). Thus, it is thought that a linear dose-C_{max} or $AUC_{insulin}$ relationship can be obtained.

Since the presence of even one double bond greatly effects the steric structure and function of fatty acid in membranes, it seems reasonable that the large number and location of the fatty acyl unsaturations associated with DHA and EPA could confer a unique structure on and hence alter the permeability of membranes. Our previous findings (Suzuki et al., 1998) and the present study also verified that these polyunsaturated fatty acids render the intestinal membrane more permeable to insulin. These data indicated that DHA is equivalent to or even more efficient than EPA, and more effective than C18 unsaturated fatty acid in enhancing insulin absorption from the intestine. It was reported that unsaturated fatty acids, including linoleic, alph-linolenic, arachidonic, and eicosapentaenoic acids, are less effective than DHA at influencing membrane properties and membrane-associated cellular events (Williams et al., 1999). The insulin absorption enhancement effects provided by DHA and EPA, therefore, might be consistent with these differences in the membrane-associated functions of unsaturated fatty acids.

Intact insulin can be absorbed from the intestine (Bendayan et al., 1990; Hoffman and Ziv, 1997). Bendayan et al. showed that insulin was absorbed into ileal enterocytes and suggested that its absorption was through a transcytotic pathway (Bendayan et al., 1990). In addition, the presence of insulin receptors on intestinal enterocytes has been demonstrated (Gingerich et al., 1987; MacDonald et al., 1993). In using rabbit intestinal brush-border membrane vesicles, the colon was shown to have a higher density of insulin receptors than the small intestine (Pillion et al., 1985). On the other hand, a recent report suggested the existence and abundance of insulin degradation enzymes in the epithelial cells, including the colon and the rectum (Chang et al., 1997). It has also been reported that free fatty acids impair insulin degradation in isolated rat hepatocytes (Wiesenthal et al., 1999). Based on these recent findings there seems to be some possibility that DHA might predominantly act not only on membrane fluidity, but also on the inhibition of insulin degradation in the cytosol or hepatocytes, or on the facilitation of receptor-mediated trans-

port of insulin. A more detailed study is needed to clarify the predominant enhancement effect of DHA on the insulin absorption.

In our previous work, the administration of insulin emulsion (50 IU/kg) induced a rapid decrease in serum glucose level, but the exact pharmacological availabilities were not calculated because of continued hypoglycemic effects during the study period (Suzuki et al., 1998). Furthermore, the intrinsic blood glucose levels generally rose due to surgical stress in in situ administration experiments. This also makes it difficult to estimate the efficacy from response measurements. Thus, an in vivo absorption study with a lower insulin dose (5 IU/kg) was performed in order to accurately determine pharmacological availability. Accurate assessment of insulin efficacy by alternative routes of administration requires defining the dose–response profile by a standard administration, such as s.c. The relationship was determined in the present study and the pharmacological availability of the emulsion incorporating 2% DHA was calculated. As a result, it became clear that such emulsions had very high pharmacological availability (Table 2). DHA, therefore, could be a very promising absorption promoting agent for insulin.

The release of LDH into the plasma suggests that some intestinal wall damage occurs in the presence of oleic acid (Fig. 5). These observations are in agreement with our results previously reported in a histological study (Suzuki et al., 1998). The study showed that the emulsion incorporating 2% oleic acid caused a small amount of damage to the rectum, and no tissue damage was noted in the colon. In addition, the emulsion incorporating 2% DHA caused only minor membrane damage. *N*-3 polyunsaturated fatty acid was reported to decrease the production of cytokaine and eicosanoid that originated from *n*-6 PUFA (Hamazaki, 1992), and to reduce inflammation (Horie et al., 1998). Furthermore, a recent study shows that DHA exhibits potent protection of the small intestine from methotrexate-induced damage in mice (Tashiro et al., 1998). These findings indicate that DHA might act to protect the membrane from damage, while exhibiting a more potent mucosal absorption enhancement effect for insulin than that of oleic acid.

Table 2
Efficacy of rectal administration of insulin[a]

Preparation	$AUC_{glucose}$ (% glucose reduction · h)	Pharmacological availability (%)
Insulin solution	1.7 ± 2.9	1.5 ± 2.6
Insulin emulsion incorporating DHA	202.6 ± 62.4	42.7 ± 26.5

[a] Each value represents the mean ± S.D. (*n*, 4–5).

In conclusion, our results clearly indicate that emulsions incorporating 2% DHA or EPA may strongly enhance the intestinal absorption of insulin without causing LDH release. Very high pharmacological availability was obtained by rectal administration of the emulsion incorporating 2% DHA. This value is at least 43% of that resulting from an injection of insulin. Emulsions incorporating highly purified long-chain polyunsaturated fatty acids could be, therefore, very promising for non-invasive enteral delivery of insulin.

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References

Bendayan, M., Ziv, E., Ben-Sasson, R., Bar-On, H., Kidron, M., 1990. Morphocytochemical and biochemical evidence for insulin absorption by rat ileal epithelium. Diabetologia 33, 197–204.

deBoer, A.G., Breimer, D.D., Pronk, J., Gubbens-Stibbe, M., 1980. Rectal bioavailability of lidocaine in rats: absence of significant first-pass elimination. J. Pharm. Sci. 69, 804–807.

Chang, L.L., Stout, L.E., Wong, W.D., et al., 1997. Immunohistochemical localization of insulin-degrading enzyme along the rat intestine, in the human colon adenocarcinoma cell line (Caco-2), and in human ileum. J. Pharm. Sci. 86, 116–119.

Duckworth, W.C., 1988. Insulin degradation: mechanisms, products and significance. Endocr. Rev. 9, 319–345.

Dyerberg, J., Bang, H.O., Stoffersen, E., Moncada, S., Vane, J.R., 1978. Eicosapentaenoic acid and prevention of thrombosis and atheroscleosis? Lancet 15, 117–119.

Ehringer, W., Belcher, D., Wassall, S.R., Stillwell, W., 1990. A comparison of the effects of linolenic (18:3 omega3) and docosahexaenoic (22:6 omega3) acids on phospholipid bilayers. Chem. Phys. Lipids 54, 79–88.

Gingerich, R.L., Gilbert, W.R., Comens, P.G., Gavin, J.R., 1987. Identification and characterization of insulin receptors in basolateral membranes of dog intestinal mucosa. Diabetes 36, 1124–1129.

Hamazaki, T., 1992. Intravenous infusion of *n*-3 polyunsaturated fatty acids. Proc. Soc. Exp. Biol. Med. 200, 171–173.

Hoffman, A., Ziv, E., 1997. Pharmacokinetic considerations of new insulin formulations and routes of administration. Clin. Pharmacokinet. 33, 285–301.

Horie, T., Nakamaru, M., Masubuchi, Y., 1998. Docosahexaenoic acid exhibits a potent protection of small intestine from methotrexate-induced damage in mice. Life Sci. 62, 1333–1338.

Howey, D.C., Bowsher, R.R., Brunelle, R.L., Woodworth, J.R., 1994. [Lys(B28), Pro(B29)]1-human insulin: a rapidly absorbed analogue of human insulin. Diabetes 43, 396–402.

Jenski, L.J., Sturdevant, L.K., Ehringer, W.D., Stillwell, W., 1993. Omega-3 fatty acid modification of membrane structure and function: I: dietary manipulation of tumor cell susceptibility to cell- and complement-mediated lysis. Nutr. Cancer 19, 135–146.

Kahn, C.R., Shechter, Y., 1990. Insulin, oral hypoglycemic agent, and the pharmacology of the endocrine pancreas. In: Gilman, A.G., Nies, T.W., Taylor, P. (Eds.), The Pharmacological Basis of Therapeutics. Pergamon, New York, pp. 1463–1495.

MacDonald, R.S., Thornton, W.H., Bean, T.L., 1993. Insulin and WFA receptors in a human intestinal adenocarcinoma cell line (Caco-2): regulation of Na^+ glucose transport across the brush-border. J. Recept. Res. 13, 1093–1113.

Morishita, I., Morishita, M., Takayama, K., Machida, Y., Nagai, T., 1992. Hypoglycemice effect of novel oral microspheres of insulin with protease inhibitor in normal and diabetic rats. Int. J. Pharm. 78, 9–16.

Morishita, M., Matsuzawa, A., Takayama, K., lsowa, K., Nagai, T., 1998. Improving insulin enteral absorption using water-in-oil-in-water emulsion. Int. J. Pharm. 172, 189–198.

Pillion, D.J., Ganapathy, V., Leibach, F.H., 1985. Identification of insulin receptors on the mucosal surface of colon epithelial cells. J. Biol. Chem. 260, 5244–5247.

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

Stillwell, W., Ehringer, D.W., Jenski, L.J., 1993. Docosahexaenoic acid increases permeability of lipid vesicles and tumor cells. Lipids 28, 103–108.

Suzuki, A., Morishita, M., Kajita, M., et al., 1998. Enhanced colonic and rectal absorption of insulin using the multiple emulsion containing eicosapentaenoic acid and docosahexaenoic acid. J. Pharm. Sci. 87, 1196–1202.

Takahashi, M., Minamoto, T., Yamashita, N., et al., 1993. Reduction in formation and growth of 1, 2-dimethylhydrazine-induced aberrant crypt foci in rat colon by docosahexaenoic acid. Cancer Res. 53, 2786–2789.

Tashiro, T., Yamamori, H., Morishima, Y., et al., 1998. *N*-3 polyunsaturated fatty acids in surgical nutrition. Nippon Geka Gakkai Zasshi 99, 256–263.

Wainwright, P.E., Huang, Y.S., Bubnan-Fleming, B., et al., 1991. The role of *n*-3 essential fatty acids in brain and behavioral development: a cross-fostering study in the mouse. Lipids 26, 37–45.

Wiesenthal, S.R., Sandhu, H., McCall, R.H., et al., 1999. Free fatty acids impair hepatic insulin extraction in vivo. Diabetes 48, 766–774.

Williams, E.E., May, B.D., Stillwell, W., Jenski, L.J., 1999. Docosahexaenoic acid (DHA) alters the phospholipid molecular species composition of membranous vesicles exfoliated from the surface of a murine leukemia cell line. Biochimica Biophysica Acta 1418, 185–196.