ELSEVIER

International Journal of Pharmaceutics 183 (1999) 125–132

international journal of pharmaceutics

Enhanced rectal absorption of insulin-loaded Pluronic® F-127 gels containing unsaturated fatty acids

Jose Mario Barichello [a], Mariko Morishita [a,*], Kozo Takayama [a], Yoshiyuki Chiba [b], Shinji Tokiwa [b], Tsuneji Nagai [a]

[a] *Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hoshi University, Ebara-2-4-41, Shinagawa-Ku, Tokyo 142-8501, Japan*

[b] *Central Research Laboratory, Nippon Suisan Kaisya, Kitano-machi 559-6, Hachioji-shi, Tokyo 192-0906, Japan*

Received 26 November 1998; received in revised form 4 February 1999; accepted 19 February 1999

Abstract

The objective of this study was to prepare and to evaluate Pluronic F-127 (PF127) gel containing unsaturated fatty acids such as oleic acid (18:1), eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6) as a potential formulation for rectal delivery of insulin. The hypoglycemic effect of insulin was examined following rectal administration of the various formulations in normal rats. Rectal insulin absorption was markedly enhanced, and marked hypoglycemia was induced by all PF127 gels (insulin dose, 5 U/kg) containing different unsaturated fatty acids. PF127 gels containing unsaturated fatty acids presented low t_{max} mean values indicating that the absorption of insulin occurred very rapidly in the rectum. The relative hypoglycemic efficacy of PF127 gel formulations containing fatty acids such as oleic acid, eicosapentaenoic (EPA) and docosahexaenoic (DHA) were 28.4 ± 8.1, 26.8 ± 14.3 and $23.1 \pm 5.7\%$, respectively. The finding demonstrated that 20% PF127 gels containing unsaturated fatty acids are potential formulations for rectal delivery of insulin.

Keywords: Insulin; Pluronic F-127 gels; Rectal absorption; Unsaturated fatty acids; Hypoglycemic effect; Sustained release

1. Introduction

With the biotechnological development of various peptide and protein molecules as potential therapeutic agents, problems of formulation and delivery have emerged and limited their widespread utility. Low bioavailability due mainly to degradation by protease enzymes in the gastrointestinal tract, high molecular weight and poor lipophilicity are problems that limits their use by the oral route. Reduced polypeptide degra-

* Corresponding author. Tel./fax: +81-3-5498-5783.
E-mail address: morisita@hoshi.ac.jp (M. Morishita)

0378-5173/99/$ - see front matter
PII: S0378-5173(99)00090-3

dation and partial avoidance of hepatic first pass metabolism have been cited as some advantages for rectal administration of peptide and protein drugs. Another factor that may account for delivery of drugs directly to the general circulation involves lymphatic uptake of drugs, particularly those that are water soluble drugs including insulin (Caldwell et al., 1982). However, it was demonstrated that rectal administration of insulin without the use of adjuvants did not produce any appreciable change in the plasma or lymph insulin concentrations (Caldwell et al., 1982).

It is well established that the epithelial cell layer(s) of the mucosal tissues is a major barrier to absorption of peptide drugs. The epithelial permeability of peptide drugs like insulin has been demonstrated to increase by the addition of absorption enhancers such as surfactants (Ichikawa et al., 1980), bile acids (Ichikawa et al., 1980), salicylates (Nishihata et al., 1981; Liversidge et al., 1985), enamine derivatives (Nishihata et al., 1985), poly- and unsaturated fatty acids (Morishita et al., 1998; Suzuki et al., 1998). The class of unsaturated fatty acids absorption enhancers have the advantages of being endogenous compounds present in human skin lipids (Lampe et al., 1983) and biomembranes (Fischer, 1989). Oleic acid and certain others fatty acids have been shown to alter membrane permeability by increasing the motional freedom or fluidity of membrane phospholipids (Gay et al., 1989; Muranishi, 1990; Wang et al., 1994). In a recent work, Suzuki et al. (1998) demonstrated that long-chain polyunsaturated fatty acids such as eicosapentaenoic (EPA, 20:5ω3) and docosahexaenoic acids (DHA, 22:6ω3) were more effective intestinal absorption enhancers of insulin than C18 unsaturated fatty acids such as oleic, linoleic or linolenic acids using a water-in-oil-in-water (W/O/W) emulsion. Moreover, the emulsion containing DHA, which showed strong hypoglycemic effects, caused only minor damage compared to other unsaturated fatty acids (Suzuki et al., 1998).

Regarding the protein and peptide absorption enhancement effect by unsaturated fatty acids, it seems important to keep a considerable concentration of drug and enhancer at the absorption site at the time the fatty acid starts its action. For this objective, Pluronic F-127 (PF127) gel containing unsaturated fatty acids as potential formulations for rectal insulin delivery were prepared and evaluated. PF127 is a block copolymer comprising of poly(oxyethylene) (POE) and poly(oxypropylene) (POP) segments which aqueous solutions in the 20–35% range has been used as a drug delivery system for ophthalmic (Miller and Donovan, 1982; Desai and Blanchard, 1998; Edsman et al., 1998), rectal (Miyazaki et al., 1986; Choi et al., 1998a,b), parenteral (Morikawa et al., 1987; Johnston et al., 1992; Pec et al., 1992; Wang and Johnston, 1995; Katakam et al., 1997; Paavola et al., 1998) and percutaneous use (Tobiyama et al., 1994; Miyazaki et al., 1995; Lee et al., 1997), due to its peculiar property of reversal thermal gelation. Another important characteristic of PF127 gels is the enhancement of the stability of proteins loaded in the gel matrix with complete recovery of their full activity when the gel is dissolved in excess buffer (Stratton et al., 1997). Additionally, the PF127 depot can be easily rectally administered as a solution forming a rigid semisolid gel network as it warms with the body temperature.

In this study, various insulin-loaded PF127 gel formulations containing unsaturated fatty acids were prepared and their pharmacological availability following rectal administration was compared. In addition, pharmacokinetic parameters of insulin from the various PF127 formulations are compared.

2. Materials and methods

2.1. Materials

Crystalline porcine insulin (Zn-insulin, 27.0 U/mg) was kindly supplied by Shimizu (Shizuoka, Japan). PF127 was purchased from Sigma (St. Louis, MO). Oleic acid (purity: 99.0%) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). DHA acid (purity: 99.0%) and EPA acid (purity: 99.0%) were provided by Nippon Suisan Kaisya (Tokyo, Japan). All references to water imply the use of MilliQ purified water previously filtered through a 0.2 μm cellulose nitrate membrane. All other chemicals were at least reagent grade and were used without further purification.

2.2. Preparation of the PF127 gels

A stock PF127 solution was firstly prepared by the cold method (Schmolka, 1972). A weighed amount of PF127 was slowly added to an aqueous (5–10°C) solution with gentle mixing until complete dissolution of the polymer. The PF127 solution was stored in a refrigerate overnight to allow complete dissolution of the polymer. Insulin was dissolved in 0.1 N hydrochloride acid adjusted with 0.1 N sodium hydroxide solution to approximately pH 4.0, and then added to an appropriate volume of the stock PF127 solution. The final concentration of PF127 was 20% w/w, and it was used as a control formulation.

The unsaturated fatty acids (DHA, EPA or oleic acid) were added later to the insulin–PF127 solution described above, and dispersed with moderate stirring at low temperature (5–10°C) for 20 min. The final concentrations of PF127 and unsaturated fatty acid were 20% w/w and 5% w/w, respectively.

An unsaturated fatty acid (EPA) dispersed in an insulin pH 7.4 phosphate buffer solution (PBS) with moderate stirring at room temperature was used in this study as another control formulation. The final concentration of EPA in the PBS solution was 5% w/w.

2.3. In vivo absorption experiments

Male Wistar rats weighing 220 g that had fasted for 48 h were divided into groups of five animals each. A volume of 0.2 ml of a formulation was rectally administered to each rat of the respective group. The dose of insulin was 5 U/kg body weight. Approximately 5 min before administration, a 0.2-ml sample of blood was taken from the jugular vein. Subsequent blood samples (0.2 ml) were taken at 0.5, 1, 2, 4 and 6 after dosing. Serum was separated by centrifugation at 13 000 rpm for 1 min and kept frozen until analysis. The serum glucose level was determined by using a Glucose B-test kit (Wako Pure Chemical Industries, Osaka, Japan). Postdose levels of the serum glucose were expressed as a percentage of the predose level. The percentage change in the serum glucose levels was taken as the percentage of the predose level subtracted from 100. The cumulative percentage change in the serum glucose level was calculated by summing, using the trapezoidal method, the areas below baseline levels in the percentage change versus times curves for 0–6 h ($AUC_{glucose}$).

The serum insulin levels were measured by using an insulin enzyme immunoassay kit (IMx System, DAINABOT, Tokyo, Japan). The basal endogenous insulin level was subtracted from all insulin levels measured following insulin administration. The serum peak level (C_{max}) and the time taken to reach the serum peak level (t_{max}) were determined from the serum insulin level-time curves. The area under the insulin level-time curves for 0–6 h ($AUC_{insulin}$) was determined using the 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 0–6 h point. The hypoglycemic efficacy of the rectally administered insulin was calculated relative to that administered by the subcutaneous route using the methods described by Morishita et al. (1998). The pharmacological availability (PA) was calculated by using the dose-response curve. The bioavailability of insulin (B) was calculated according to Eq. (1) in which $AUC_{s.c.}$ was determined after s.c. administration of 2.0 U/kg of insulin. The values of $AUC_{s.c.}$ described in a previous report (Barichello et al., 1998) was used for the calculation.

$$B = (AUC/dose)/(AUC_{s.c.}/dose_{s.c.}) \quad (1)$$

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 in Hoshi University.

2.4. Statistical analysis

Each value was expressed as the mean ± S.D. For group comparisons, the one-way layout analysis of variance (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 serum insulin and glucose levels following rectal administration of insulin-loaded PF127 gel formulation

PF127 gel shows a very desirable feature that is its tendency to adhere to surfaces, such as the skin (Schmolka, 1972; Lee et al., 1997) and the rectum (Miyazaki et al., 1986; Choi et al., 1998a,b). Juhász et al. (1991) has demonstrated that the strong adhesion property of PF127 gels to the mucous membranes was resulted from its reversal thermal gelation ability, since at low temperature it behaves as a liquid followed by an increasing in viscosity upon warming. The PF127 gel matrix might potentially protects insulin from significant denaturation or degradation by proteases contained in interstitial fluids (Johnston et al., 1992). The intimate contact between the rectal mucous membrane and the PF127 gel matrix might particularly favor rectal absorption of insulin.

Fig. 1 shows the changes in serum insulin and glucose levels following rectal administration of insulin-loaded PF127 gel. Since, the property of reversal thermal gelation of PF127 is just observed in concentrated solutions, it was expected the high concentration used could have some effect on rectal insulin absorption. However, an

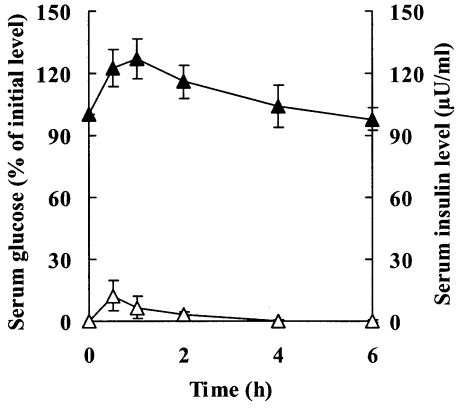

Fig. 1. Serum glucose and insulin levels following rectal administration of an insulin-loaded Pluronic F-127 (PF127) gel formulation in normal rats. Glucose (▲) and insulin (△). Values represent the means of five rats ± S.D.

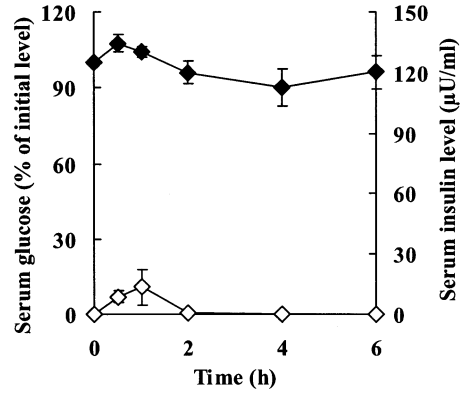

Fig. 2. Serum glucose and insulin levels following rectal administration of an eicosapentaenoic (EPA)-dispersed insulin phosphate buffer solution in normal rats. Glucose (◆) and insulin (◇). Values represent the means of five rats ± S.D.

obvious hypoglycemic effect was not observed. Others previously (Ichikawa et al., 1980) reported the effects of a variety of surfactants on the rectal absorption of insulin. Polyoxyethylene(9)lauryl ether, a nonionic surfactant, was the most effective surfactant in promoting rectal absorption of insulin from suppositories (Ichikawa et al., 1980). However, this and other studies demonstrated the permeation of the rectal mucosa appears to be rate limiting and, an increase in the insulin dose does not always result in a proportional greater decrease in serum glucose levels, resulting in an overall low bioavailability of insulin (Ichikawa et al., 1980; Yamasaki et al., 1981).

3.2. Changes in serum insulin and glucose levels following rectal administration of various Insulin-loaded PF127 gel formulations containing unsaturated fatty acids

Fig. 2 shows the changes in serum insulin and glucose levels following rectal administration of an EPA-dispersed insulin PBS solution. As discussed before, the intestinal absorption of molecules having a mass larger than 2 kDa is generally recognized to be extremely limited. However, the simple dispersion of EPA into a insulin PBS solution demonstrated to enhance rectal insulin absorption (Fig. 2), but no expressively change of the serum glucose levels has been

observed. Muranishi (1985) has proposed that long chain fatty acids could promote rectal absorption whenever the fatty acid were taken up into the epithelial cells. It was also demonstrated that fatty acids perturb the membrane structural integrity by being incorporated into the plasma membrane (Muranishi et al., 1981). It seems this perturbation by fatty acids occurs in both the lipid fraction and protein fraction of the membrane (Nishihata and Rytting, 1997). In the situation where the enhanced drug permeability is in part related to the lipid membrane perturbation by free fatty acids, it is required to maintain as high a concentration as possible of both drug and enhancer at the site of absorption to guarantee an efficiently high drug bioavailability. Also, if the drug could be in solution in the same medium where the enhancer will be dispersed or dissolved, it will make the drug readily available for absorption when the enhancer starts its action.

In order to provide these conditions, PF127 gels of insulin containing unsaturated fatty acids have been prepared. The reversible sol–gel property of a PF127 solution allows the cool solution to flow into the rectum changing to a gel as it warms with the body temperature. Since rectal mucous lining consists of oligosaccharide chains with siacil acid, the hydrophilic groups (POE comprise about 70% of the total molecular weight of PF127) such as hydroxyl groups can strongly bind to the oligosaccharide chains, resulting in a considerable bioadhesive force (Choi et al., 1998b).

The changes in serum insulin and glucose levels following rectal administration of PF127 gels containing various unsaturated fatty acids in normal rats are shown in Fig. 3. Clearly, rectal insulin absorption was markedly enhanced, and marked hypoglycemia was induced by all PF127 gel formulations. Insulin and fatty acids being dispersed in a polymer matrix only contact with the interstitial fluid and mucous membrane surface after their diffusion through the polymer network. In addition, the concentration of PF127 might be enough for aiding solubilization of the insulin, thus reducing the propensity for insulin unfolding (Wang and Hanson, 1988). Nonionic surfactants, such as PF127, tend to be less structure perturbing to proteins causing fewer directional interactions than ionic surfactant due to its generally lower critical micelle concentration and because electrostatic binding is absent (Wang and Hanson, 1988).

The serum insulin levels were sustained and reached baseline levels after 6-h administration. Insulin and fatty acids might achieve the mucous membrane by diffusion through extramicellar aqueous channels of the gel matrix and by decomposition of the PF127 gel matrix by itself (Chen-Chow and Frank, 1981; Miyazaki et al., 1984; Morikawa et al., 1987; Paavola et al., 1998). In

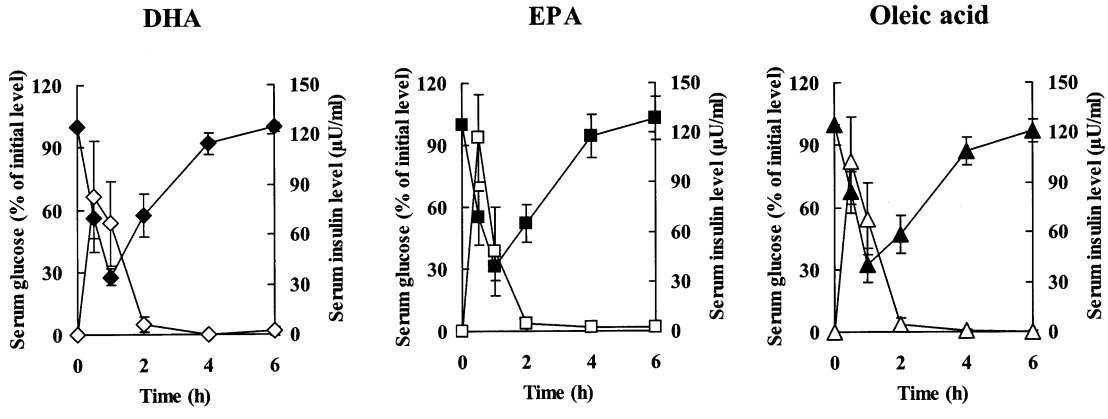

Fig. 3. Serum glucose and insulin levels following rectal administration of various Pluronic F-127 (PF127) gel formulations containing different unsaturated fatty acids in normal rats. Glucose (closed symbols) and insulin (open symbols). Values represent the means of five rats ± S.D.

Table 1
Comparison of the insulin pharmacokinetic parameters following rectal administration of various formulations containing insulin in normal rats[a]

Formulation	C_{max} (μU/ml)	t_{max} (h)	MRT (h)
EPA–insulin solution	15.3 ± 7.7	0.9 ± 0.2	1.1 ± 0.3
Insulin–PF127 gel	14.8 ± 7.8	0.7 ± 0.2	1.3 ± 0.4
EPA–insulin –PF127 gel	126.7 ± 52.5*	0.6 ± 0.2	1.0 ± 0.2
DHA–insulin –PF127 gel	112.8 ± 43.2*	0.7 ± 0.2	1.0 ± 0.2
Oleic acid–insulin–PF127 gel	115.4 ± 31.4*	0.7 ± 0.2	0.9 ± 0.1

[a] Each value represents the mean of five rats ± S.D.
* Significant ($P<0.01$) difference in the mean values compared to the control groups (eicosapentaenoic (EPA)-dispersed insulin phosphate buffer solution and insulin–Pluronic F-127 (PF127) gel) using the Students' t-test.

this regard, the microviscosity and the size of the extramicellar channels and the partition of drug and fatty acid between the micellar phase and the extramicellar aqueous channels during the sol to gel transition phenomenon might play the roles of diffusion and release of these two compounds from the PF127 gel matrix (Chen-Chow and Frank, 1981; Miyazaki et al., 1984; Tung, 1994; Lu and Jun, 1998; Paavola et al., 1998). Further investigation is needed to clarify the insulin and fatty acid release mechanism(s) as well as the effective concentration of fatty acid involved in the rectal insulin absorption.

3.3. Pharmacokinetic parameters of insulin following rectal administration of various PF127 gel formulations containing different unsaturated fatty acids

The pharmacokinetic parameters of insulin following rectal administration of various PF127 formulations containing unsaturated fatty acids are shown in Table 1. Recently, Suzuki et al. (1998) have shown that the three C18 unsaturated fatty acids evaluated exhibited similar and comparable $AUC_{insulin}$ mean values. Otherwise, $AUC_{insulin}$ of the emulsions incorporating DHA and EPA has tended to be higher than those for C18 unsaturated fatty acids.

In general, insulin absorption occurred very rapidly in the rectum. Suzuki et al. (1998) have demonstrated that the t_{max} mean values following rectal administration were less than 1 h and shorter than those following colonic administration. As observed in Table 1, insulin-loaded PF127 gels containing unsaturated fatty acids presented low t_{max} mean values. Although approximately 50% of the insulin absorbed from the intestine is considered to be extracted into the liver, in the case of rectal administration using PF127 gels, most of the absorbed insulin is considered to by pass the liver and enter the systemic circulation directly (Choi et al., 1998a). This might contribute to the shorter t_{max} observed for PF127 gels compared to previous works.

3.4. Hypoglycemic efficacy and insulin bioavailability relative to subcutaneous administration

To calculate the pharmacological availability, a previously reported relationship between the logarithm of subcutaneous doses and $AUC_{glucose}$ was used in this study (Morishita et al., 1998). The bioavailability of insulin was calculated from a single subcutaneous administration of 2.0 U/kg of insulin using the values of $AUC_{s.c.}$ described in a previous report (Barichello et al., 1998). As shown in Table 2, no significant differences could be observed among the relative hypoglycemic efficiencies and the insulin bioavailabilities of the different unsaturated fatty acids. However, it is observed that the bioavailability of insulin was a little higher than the hypoglycemic efficiency of the different unsaturated fatty acids. The pharmacodynamics of insulin is rather complex, and depends on various factors such as route of administration, liver function, glucose concentration, etc (Hoffman and Ziv, 1997). The complexity results from the fact that the apparent hypoglycemic effect are the sum of several biochemical and physiological processes that occur at different sites (Hoffman and Ziv, 1997). These

Table 2
Hypoglycemic efficacy and insulin bioavailability of various insulin formulations rectally administered relative to subcutaneous administration[a]

Formulation	AUC		Bioavailability	
	Glucose (% glu.reduc. ·h)	Insulin (μU h ml^{-1})	PA (%)	B (%)
EPA–insulin solution	29.7 ± 23.4	16.2 ± 6.4	6.1 ± 1.6	5.7 ± 2.2
Insulin–PF127 gel	9.2 ± 12.4	15.5 ± 7.7	3.1 ± 2.8	5.4 ± 2.7
EPA–insulin–PF127 gel	164.8 ± 44.9*	108.6 ± 29.6*	26.8 ± 14.3*	38.0 ± 10.4*
DHA–insulin–PF127 gel	157.4 ± 22.1*	103.8 ± 29.5*	23.1 ± 5.7*	36.3 ± 10.3*
Oleic acid–insulin–PF127 gel	175.9 ± 29.9*	110.7 ± 32.6*	28.4 ± 8.1*	38.7 ± 11.4*

[a] Each value represents the mean ± S.D.
* Significant ($P < 0.01$) difference in the mean value compared to the controls (eicosapentaenoic (EPA)-dispersed insulin phosphate buffer solution (PBS) solution and insulin–Pluronic F-127 (PF127) gel) using the Students' t-test.

partly might explain the discrepancy observed between the hypoglycemic efficiency and insulin bioavailability mean values of the different unsaturated fatty acids.

Even though the effectiveness of insulin administration by rectal suppositories were demonstrated in various animals and in normal and diabetic subjects, the most part of them have not shown encouraging results to proceed with long-term clinical applications (Ichikawa et al., 1980; Yamasaki et al., 1981; Liversidge et al., 1985; Nishihata et al., 1985). Therefore, a more detail study is needed to clarify the effectiveness and toxicity of the PF127 gel formulations containing unsaturated fatty acids in multiple dose study.

Acknowledgements

This work was supported by the Ministry of Education, Science, Sports, and Culture of Japan. The gift of insulin from Shimizu is gratefully acknowledged.

References

Barichello, J.M., Morishita, M., Takayama, K., Nagai, T., 1998. Absorption of insulin from PF127 gels following subcutaneous administration in rats. Int. J. Pharm. (submitted).

Caldwell, L., Nishihata, T., Rytting, J.H., Higuchi, T., 1982. Lymphatic uptake of water-soluble drugs after rectal administration. J. Pharm. Pharmacol. 34, 520–522.

Chen-Chow, P., Frank, S.G., 1981. In vitro release of lidocaine from Pluronic F-127 gels. Int. J. Pharm. 8, 89–99.

Choi, H.-G., Oh, Y.-K., Kim, C.-K., 1998a. In situ gelling and mucoadhesive liquid suppository containing acetaminophen: enhanced bioavailability. Int. J. Pharm. 165, 23–32.

Choi, H.-G., Jung, J.-H., Ryu, J.-M., Yoon, S.-J., Oh, Y.-K., Kim, C.-K., 1998b. Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository. Int. J. Pharm. 165, 33–44.

Desai, S.D., Blanchard, J., 1998. In vitro evaluation of Pluronic F127-based controlled-release ocular delivery system for pilocarpine. J. Pharm. Sci. 87, 226–230.

Edsman, K., Carlfors, J., Petersson, R., 1998. Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. Eur. J. Pharm. Sci. 6, 105–112.

Fischer, S., 1989. Dietary polyunsaturated fatty acids and eicosanoid formation in humans. Adv. Lipid Res. 23, 160–198.

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.

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

Ichikawa, K., Ohata, I., Mitomi, M., Kawamura, S., Maeno, H., Kawata, H., 1980. Rectal absorption of insulin suppositories in rabbits. J. Pharm. Pharmacol. 32, 314–318.

Johnston, T.P., Punjabi, M.A., Froelich, C.F., 1992. Sustained delivery of interleukin-2 from a poloxamer 407 gel matrix following intraperitoneal injection in mice. Pharm. Res. 9, 425–434.

Juhász, J., Pimienta, C., Lenaerts, V., 1991. Adhesion of poloxamer 407 formulations on dog ileal segments in vitro. Eur. J. Pharm. Biopharm. 37, 262–265.

Katakam, M., Ravis, W.R., Golden, D.L., Banga, A.K., 1997. Controlled release of human growth hormone following

subcutaneous administration in dogs. Int. J. Pharm. 152, 53–58.

Lampe, M.A., Burlingame, A.L., Whitney, J.A., Williams, M.L., Brown, B.E., Roitman, E., Elias, P.M., 1983. Human stratum corneum lipids: characterization and regional variations. J. Lipid Res. 24, 120–130.

Lee, B.-J., Lee, T.-S., Cha, B-J., Kim, S.-H., Kim, W.-B., 1997. Percutaneous absorption and histopathology of a poloxamer-based formulation of capsaicin analog. Int. J. Pharm. 159, 105–114.

Liversidge, G.G., Nishihata, T., Engle, K.K., Higuchi, T., 1985. Effect of rectal suppository formulation on the rectal release of insulin and on the glucose plasma levels in dogs. Int. J. Pharm. 23, 87–95.

Lu, G., Jun, H.W., 1998. Diffusion studies of methotrexate in carbopol and poloxamer gels. Int. J. Pharm. 160, 1–9.

Miller, S.C., Donovan, M.D., 1982. Effect of poloxamer 407 gels on the miotic activity of pilocarpine nitrate in rabbits. Int. J. Pharm. 12, 147–152.

Miyazaki, S., Takeuchi, S., Yokouchi, T., Takada, M., 1984. Pluronic F-127 gels as a vehicle for topical administration of anticancer agents. Chem. Pharm. Bull. 32, 4205–4208.

Miyazaki, S., Yokouchi, C., Nakamura, T., Hashiguchi, N., Hou, W.-M., Takada, M., 1986. Pluronic F-127 gels as a novel vehicle for rectal administration on indomethacin. Chem. Pharm. Bull. 34, 1801–1808.

Miyazaki, S., Tobiyama, T., Takada, M., Attwood, D., 1995. Percutaneous absorption of indomethacin from Pluronic F-127 gels in rats. J. Pharm. Pharmacol. 47, 455–457.

Morikawa, K., Okada, F., Hosokawa, M., Kobayashi, H., 1987. Enhancement of therapeutic effects of recombinant interleukin-2 on a transplantable rat fibrosarcoma by the use of a sustained release vehicle, Pluronic gel. Cancer Res. 47, 37–41.

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

Muranishi, S., 1985. Modification of intestinal absorption of drugs by lipoidal adjuvants. Pharm. Res. 2, 108–118.

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

Muranishi, S., Takagi, N., Sezaki, H., 1981. Effect of fatty acids and monoglycerides on permeability of lipid bilayer. Chem. Phys. Lipids 28, 269–279.

Nishihata, T., Rytting, J.H., 1997. Absorption promoter adjuvants: enhancing action on rectal absorption. Adv. Drug Deliv. Rev. 28, 229–237.

Nishihata, T., Rytting, J.H., Kamada, A., Higuchi, T., 1981. Enhanced intestinal absorption of insulin in rats in the presence of sodium 5-methoxysalicylate. Diabetes 30, 1065–1067.

Nishihata, T., Okamura, Y., Kamada, A., Higuchi, T., Yagi, T., Kawamori, R., Shichiri, M., 1985. Enhanced bioavailability of insulin after rectal administration with enamine as adjuvant in depancreatized dogs. J. Pharm. Pharmacol. 37, 22–26.

Paavola, A., Yliruusi, J., Rosemberg, P., 1998. Controlled release and dura matter permeability of lidocaine and ibuprofen from injectable poloxamer-based gels. J. Control. Rel. 52, 169–178.

Pec, E.A., Wout, Z.G., Johnston, T.P., 1992. Biological activity of urease formulated in poloxamer 407 after intraperitonial injection in the rat. J. Pharm. Sci. 81, 626–630.

Schmolka, I.R., 1972. Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns. J. Biomed. Mater. Res. 6, 571–582.

Stratton, L.P., Dong, A., Manning, M.C., Carpenter, J.F., 1997. Drug delivery matrix containing native protein precipitates suspended in a poloxamer gel. J. Pharm. Sci. 86, 1006–1010.

Suzuki, A., Morishita, M., Kajita, M., Takayama, K., Isowa, K., Chiba, Y., Tokiwa, S., Nagai, T., 1998. Enhanced colonic and rectal absorption of insulin using a multiple emulsion containing eicosapentaenoic acid and docosahexaenoic acid. J. Pharm. Sci. 87, 1196–1202.

Tobiyama, T., Miyazaki, S., Takada, M., 1994. Use of Pluronic F-127 gels as a vehicle for percutaneous absorption. Yakuzaigaku 54, 205–213.

Tung, I.C., 1994. Rheological behavior of poloxamer 407 aqueous solutions during sol–gel and dehydration process. Int. J. Pharm. 107, 85–90.

Wang, Y.J., Hanson, M., 1988. Parenteral formulations of proteins and peptides: stability and stabilizers. J. Parent. Sci. Technol., Tech. Rep. 42 (10), S1–S23.

Wang, P.-L., Johnston, T.P., 1995. Sustained-release interleukin-2 following intramuscular injection in rats. Int. J. Pharm. 113, 73–81.

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

Yamasaki, Y., Shichiri, M., Kawamori, R., Kikuchi, M., Yagi, T., Arai, S., Tohdo, R., Hakui, N., Oji, N., Abe, H., 1981. The effectiveness of rectal administration of insulin suppository on normal and diabetic subjects. Diabetes Care 4, 454–458.