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Absorption of insulin from Pluronic F-127 gels following subcutaneous administration in rats

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Abstract

The main objective of this work was to evaluate the use of Pluronic (PF127) gels, polylactic-co-glycolic acid (PLGA) nanoparticles and their combination for parenteral delivery of peptides and proteins having short half-lives using insulin as a model drug. The in vitro insulin release profiles of various PF127 formulations were evaluated at 37°C using a membraneless in vitro model. In vivo evaluation of the serum glucose and insulin levels was performed following subcutaneous administration of various insulin formulations in normal rats. The in vitro results demonstrated that the higher the concentration of PF127 in the gel, the slower the release of insulin from the matrices, independent of the vehicle used. The acute hypoglycemic peak resulting from administration of an insulin solution between 0.5 and 2.0 h after administration (peak at 1 h) is replaced after administration of insulin–PLGA nanoparticles by an almost constant hypoglycemic effect with a slower recovery of the serum glucose levels at about 2 h after administration. By loading insulin into PF127 gels, a slower and more prolonged hypoglycemic effect of insulin was obtained in inverse proportion to the polymer concentration. PF127 gel formulations containing insulin–PLGA nanoparticles had the most long-lasting hypoglycemic effects of all formulations. From the current in vitro and in vivo study, we concluded that PF127 gel formulations containing either drug or drug–nanoparticles could be useful for the preparation of a controlled delivery system for peptides and proteins having short half-lives.

Keywords: Insulin; Pluronic F-127 gels; Subcutaneous absorption; Polylactic-co-glycolic acid nanoparticles; Hypoglycemic effect; Sustained release

1. Introduction

Efficient delivery of bioactive agents and peptides or proteins to the systemic circulation and then to target cells or organs has received considerable attention in medicine due to recent advances in biotechnology. Most of the protein and peptide drugs used in therapeutics are administered by parenteral routes, such as the intravenous, intramuscular and subcutaneous (s.c.) routes. This form of delivery has traditionally

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been poorly accepted by patients, except those suffering from life-threatening diseases. This is the case with insulin, a polypeptide used to treat patients with insulin-dependent (type 1) diabetes mellitus, which uses parenteral administration mainly via the s.c. route. Such insulin therapy may also benefit non-insulin-dependent (type 2) diabetes mellitus. The provision of an exogenous supply of insulin that imitates the physiological pattern found in non-diabetic populations with currently available therapeutic regimens has been a real challenge for many years. Recently, pharmacokinetic consideration of new insulin formulations and routes of administration has been reviewed (Hoffman and Ziv, 1997). While much developmental activity has been undertaken on fast-acting insulin analogues, less progress has been made with basal (ultralente) insulin (Hoffman and Ziv, 1997).

Some approaches have been used to improve the existing parenteral dosage forms to increase patient compliance. One of these approaches is the utilization of Pluronic F-127 (PF127) gels. PF127 is a block copolymer comprising poly(-oxyethylene) and poly(oxypropylene) segments with a molecular weight of approximately 12 500 (Schmolka, 1972). The property of reversal thermal gelation exhibited by PF127 aqueous solutions in the 20–35% concentration range has been used as a drug delivery system for ophthalmic (Miller and Donovan, 1982; Bochot et al., 1998; Desai and Blanchard, 1998; Edsman et al., 1998), rectal (Miyazaki et al., 1986), parenteral (Morikawa et al., 1987; Guzmán et al., 1992; Johnston et al., 1992; Pec et al., 1992; Wang and Johnston, 1995; Paavola et al., 1995, 1998; Katakam et al., 1997a,b) and percutaneous use (Tobiyama et al., 1994; Miyazaki et al., 1995; Lee et al., 1997; Suh et al., 1997). Another important characteristic of PF127 gels is the enhancement of the stability of proteins loaded into the gel matrix, with completely recovery of their full activity when the gel is dissolved in excess buffer (Stratton et al., 1997). Additionally, PF127 can easily be administered as a solution, which forms a rigid semisolid gel network upon an increase in temperature, thus avoiding surgical techniques. This kind of 'depot-like' sustained release gel has been applied to many drugs, but no report of its use for the delivery of insulin has been published.

Another approach used for improving the delivery of peptides and proteins has been the use of biodegradable micro- and nanospheres. The most widely used and studied class of biodegradable polymers is the polyesters, including polylactic acid and polylactic-co-glycolic acid (PLGA). Micro- and nanoparticulate systems formulated with these polymers have shown wide applicability to oral (Ammoury et al., 1993; Chacón et al., 1996) and s.c. delivery (Soriano et al., 1996; Uchida et al., 1997) of some drugs. In a previous report, we demonstrated that insulin–PLGA nanoparticles (INP) were effective in reducing the blood glucose levels of normal rats after intra-ileum loop administration (in situ absorption study) (Barichello et al., 1999). Since the investigation of the parenteral mode of delivery is also important for peptide drugs, we also studied the possibility of using this formulation for parenteral delivery of insulin.

The main objective of this work was to evaluate the use of PF127 gels, PLGA nanoparticles, and also the combination of the gel and nanoparticles, for parenteral delivery of peptides and proteins having short half-lives using insulin as a model drug.

2. Materials and methods

2.1. Materials

Crystalline porcine insulin (Zn-insulin, 27.0 U/mg) was kindly supplied by Shimizu Pharmaceutical (Shizuoka, Japan). Pluronic F-127, pluronic F-68 and bovine insulin (Zn-insulin, 28.6 U/mg) were purchased from Sigma Chemical (St. Louis, MO, USA). PLGA with a molecular weight of 10 kD and a lactide:glycolide ratio of 75:25 was purchased from Wako Pure Chemicals (Osaka, 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 PF127 solutions

The PF127 solutions were prepared by the cold method (Schmolka, 1972). A weighed amount of PF127 was slowly added to a cold (5–10°C) solution with gentle mixing until complete dissolution of the polymer. The solutions used included an aqueous solution (PF127-4.0), which exhibited a pH of 4.0 after complete dissolution of the polymer, a phosphate buffered solution of the polymer at pH 5.5 (PF127-5.5) and a phosphate buffered solution of the polymer at pH 7.0 (PF127-7.0). An appropriate amount of 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 with gentle stirring after an overnight refrigeration of the PF127 solution. The final concentration of the PF127 was 20 or 30%.

2.3. Preparation of INP

PLGA nanoparticles were prepared as reported previously (Barichello et al., 1999). Briefly, 75 mg of PLGA was dissolved in 5 ml of acetone. Insulin was dissolved in 150 μl of 0.1 N hydrochloride acid (HCl) adjusted with 0.1 N sodium hydroxide solution to pH 4.0, and added to the polymer phase. This organic phase was poured into 15 ml of pH 5.5 phosphate buffer solution containing 75 mg of Pluronic F-68 with moderate stirring at room temperature. Acetone was removed from the colloidal suspension by using a rotary evaporator. The nanoparticles were purified from the free insulin by gel filtration through Sephadex (Sephadex G-50; Pharmacia Biotech, Sweden) and concentrated to a final volume of 2 ml (approximately 39 mg of INP/ml of suspension). An appropriated volume of the final nanoparticle suspension was centrifuged at 20 000 rpm for 30 min. The sediment (nanoparticles) was dissolved in dichloromethane previously saturated with water and immediately assayed by using a reverse-phase high-performance liquid chromatography (HPLC) system previously reported (Barichello et al., 1999). The insulin content in nanoparticles was $59.8 \pm 3.2\%$. The mean particle size of the nanoparticles was around 128 ± 29 nm with a relatively narrow particle size distribution.

2.4. Preparation of INP-loaded PF127 solutions

The nanoparticles and the PF127 solutions were prepared as already described using a pH 5.5 phosphate buffer solution. An appropriated volume of the INP suspension was added to the PF127 solution with gentle stirring until complete mixing of both phases gave a final PF127 concentration of 20 or 30%.

2.5. Preparation of the insulin control solution

An appropriate amount of porcine insulin was dissolved in 0.1 N hydrochloride acid adjusted with 0.1 N sodium hydroxide solution to approximately pH 4.0. This solution was added to the necessary volume of phosphate buffer solution (pH 7.4) to give the insulin control solutions.

2.6. In vitro insulin and INP release from PF127 gel formulations

A membraneless dissolution model was used for the in vitro studies. The cold PF127 solutions (3 g) containing insulin (1mg/g of gel) or INP (26 mg/g of gel) were transferred into test tubes and placed in a 37°C water bath. The PF127 solutions gelled upon equilibration for 20 min at the 37°C. One milliliter of the release medium (a pH 4.0 aqueous solution), pre-equilibrated at the experimental temperature, was layered over the surface of the PF127 gel containing insulin or INP. At sampling times, the supernatant was completely removed and replaced with fresh solution, in order to maintain sink conditions. The amount of insulin in the released medium was determined as already described. For INP-loaded PF127 gel, the released medium was separated from the nanoparticles by centrifugation at 20 000 rpm for 30 min. The amount of insulin present in the release medium and the nanoparticles was determined as described earlier. Bovine insulin was used for the in vitro experiments.

2.7. In vivo study in rats

Male Wistar rats weighing 220 g that had fasted for 24 h was divided into groups of five animals each. A volume of 0.2 ml of a formulation was subcutaneously administered to each rat of the respective group. For the in vivo experiments, porcine insulin in a dose 2 U/kg body weight was used. 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, 6 and 12 h after dosing. Serum was separated by centrifugation at 13 000 rpm for 1 min and kept frozen until analysis. The serum insulin levels were measured by using an insulin enzyme immunoassay kit (Dainabot, Tokyo, Japan). The serum glucose level was determined by the glucose oxidase method using the Glucose B-test kit (Wako Pure Chemical Industries, Osaka, Japan).

2.8. Statistical analysis

Each value was expressed as the mean $\pm$ SD. 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. In vitro insulin release from PF127 gel formulations

The in vitro release of insulin from the PF127 gel formulations at 37°C is shown in Fig. 1. The slope of the regression line of insulin released from the PF127 gels versus time is shown in Table 1. The results of the in vitro study indicated that 20% gels released insulin approximately twice as fast as 30% gels. The cumulative percentage of insulin released at 12 h from 20% PF127-7.0, 20% PF127-4.0, 20% PF127-5.5, 30% PF127-7.0, 30% PF127-5.5 and 30% PF127-4.0 gel formulations were 14.2 ± 0.5, 11.7 ± 1.5, 9.9 ± 1.3, 8.6 ± 2.1, 5.8 ± 0.6 and 5.6 ± 0.7%, respectively. In general,

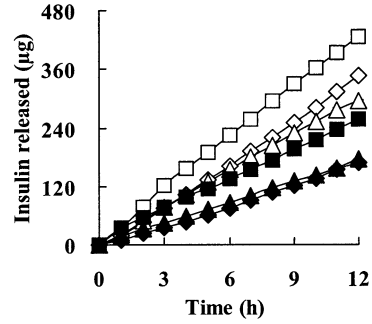

Fig. 1. In vitro release profiles of insulin from 20 and 30% PF127 gel formulations prepared in different pH solutions at 37°C: 20% PF127-4.0 (○); 20% PF127-5.5 (△); 20% PF127-7.0 (□); 30% PF127-4.0 (●); 30% PF127-5.5 (▲) and 30% PF127-7.0 (■). Values represent the mean of three experiments. To improve clarity, the error bars for each data point were not plotted unless they were larger than the symbols used.

the PF127 gel formation occurs due to the progressive dehydration of the polymer micelles as temperature increases, leading to increased chain entanglement. This entanglement is more marked at higher concentrations of PF127, yielding an increase of gel strength and consequently, a decrease of the release rate (Guzmán et al., 1992). In any case, release of insulin from the PF127 gels was observed to follow zero-order release kinetics. Although we have no direct evidence about the mechanism of insulin release from the PF127 formulations, the zero-order profiles suggest gel erosion and possibly some diffusion as the predominant mechanisms, as previously suggested by others (Guzmán et al., 1992; Johnston et al., 1992; Wang and Johnston, 1995).

Table 1
Slope of the regression line of insulin released versus time from PF127 gels and INP-loaded 20% PF127 gel

pH	Slope (µg/h)	
	20% PF127	30% PF127
4.0	29.8 ± 0.91	14.6 ± 0.65*
5.5	25.1 ± 0.41	14.3 ± 1.12*
7.0	35.1 ± 0.82	20.0 ± 0.92*
INP	23.2 ± 0.67	–

* Significant ($P < 0.01$) difference in the mean values compared with 20% PF127 gels using the Students' t-test.

One important characteristic of PF127 gels is their loss of gelation ability in the presence of inorganic salts (Pandit and Kisaka, 1996) and some additives (Gilbert et al., 1987). This is very important, since inorganic salts are commonly used to control pH in such gels and some other additives may be required to stabilize the formulation. As shown in Fig. 1 and Table 1, PF127-7.0 produced the fastest release of insulin among the formulations with the two concentrations of PF127 studied. The release profiles of insulin from the PF127-5.5 and PF127-4.0 gels were similar at the concentration of 30%, but slightly different at the concentration of 20%. Pandit and Kisaka (1996) have demonstrated that salts with multivalent anions have a very dramatic effect on the sol–gel transition temperature of PF127 gel, leading to a loss of PF127 gelation ability at certain concentrations. Moreover, the alteration of transition temperatures may alter the diffusion of drugs in the gels, hence influencing release rates as observed for the vehicles used here.

The physicochemical properties of model drugs are also known to affect the drug release characteristics from formulations (Paavola et al., 1998). In the PF127-5.5 formulations, the pH of the vehicle is closer to the isoelectric point of insulin than it is in the PF127-4.0 and PF127-7.0 formulations, which may affect the physicochemical properties of the insulin, at least at the lower PF127 concentration (Paavola et al., 1998). At the higher concentration of PF127, the intermicellar distance and the degree of micellar swelling necessary for polymers to contact one another will be reduced, decreasing the gelation temperature and, therefore, altering the viscosity and the consistency characteristics of the formulation. This is probably the most important factor influencing the release characteristics of the drug (Schmolka, 1972; Chen-Chow and Frank, 1981; Wanka et al., 1990; Lu and Jun, 1998). The PF127-5.5 formulation was chosen for the in vivo study based on the results obtained from the in vitro study.

The in vitro release of insulin from INP-loaded PF127 gel at 37°C is shown in Fig. 2. A slower release of insulin was observed for INP- than for insulin-loaded 20% PF127 gels. As observed in Fig. 2, just 65% of the total insulin were directly released from the INP-loaded PF127 gel system. Almost 35% of the total insulin remained in the INP after its release from the PF127 gel system. It is expected the association of INP and PF127 gels could provide different insulin absorption profiles to those obtained of a simple PF127 gel formulation.

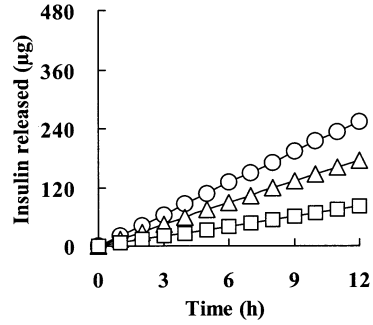

Fig. 2. In vitro insulin release profile from a INP-loaded 20% PF127 gel formulation: INP-loaded 20% PF127 (○); insulin in the PF127 gel phase (△) and insulin in the INP phase (□). Values represent the mean of three experiments ± SD. To improve clarity, the error bars for each data point were not plotted unless they were larger than the symbols used.

3.2. Changes in serum insulin and glucose levels following subcutaneous administration of an insulin solution

Management of diabetes by the patient involves a daily inconvenience, since insulin requires parenteral administration mainly via the s.c. route. The inconvenience of multiple administration due to the relatively short time of action of the insulin seems to be one of the biggest problems to overcome. As shown in Fig. 3, the s.c. administration of an insulin solution is characterized by an acute and relatively short hypoglycemic effect. The s.c. region is well supplied by capillary and lymphatic vessels. Although it has not been determined whether the absorption route is via capillaries, lymphatic, or both, many drugs in solution injected into s.c. sites behave as if their absorption were taking place by passive diffusion (Banerjee et al., 1991). The major barrier to absorption from the s.c. site is believed to

be the capillary endothelial membrane or cell wall, which is formed by a single layer of flat cells of approximately 0.0025-mm width (Banerjee et al., 1991). A prime factor in controlling the absorption of insulin is presumed to be its molecular weight, which is affected by its aggregation behavior in solution (monomer, dimer, hexamer, etc.).

3.3. Changes in serum insulin and glucose levels following subcutaneous administration of an INP formulation

The insulin monomers self-association in aqueous solutions and in the presence of certain salts, solvents and hydrophobic surfaces has been studied for many years (Chawla et al., 1985; Sluzky et al., 1991; Tokihiro et al., 1997). These known insulin characteristics were considered in preparing INP, in which just 20% of the insulin was encapsulated into the nanoparticles. Surprisingly, an in situ absorption study of this INP formulation demonstrated a considerable hypoglycemic effect of insulin absorbed from the ileum loop of rats (Barichello et al., 1999). In a previously reported study, insulin adsorbed to hydrolyzable nanoparticles was effective in reducing the blood glucose levels of diabetic rats after s.c. administration, but no change of the glucose level was observed after oral administration of the same hydrolyzable nanoparticles (Couvreur et al., 1980). Since the investigation of the parenteral mode of delivery is also important for peptide drugs, the hypoglycemic effect of the insulin from an INP formulation following s.c. administration in normal rats was evaluated (Fig. 4). The time-course of the hypoglycemic effect of insulin in the INP formulation is different from the time-course of the insulin solution in at least one aspect. The acute hypoglycemic peak characteristics of an insulin solution between 0.5 and 2.0 h after administration (peak at 1 h) are replaced by an almost constant hypoglycemic effect with a slower recovery of the serum glucose levels during the 2 h after administration of the INP formulation. In a diffusion-controlled process, large molecules would be expected to have lower penetration rates than smaller ones. It appears that molecules of low molecular weight (< 20.000) are absorbed primarily via the capillaries, while molecules having high molecular weights are absorbed primarily via lymph vessel (Banerjee et al., 1991). In addition, the absorption from the s.c. site also depends on the pH of the vehicle, volume of injection, concentration of solute, osmolarity of the solution, polymorphic form and particle size (Banerjee et al., 1991), which makes it difficult to compare the hypoglycemic profiles of the insulin solution and INP.

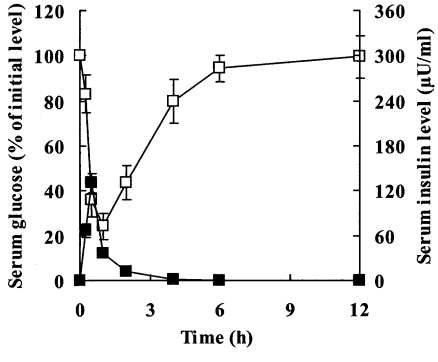

Fig. 3. Serum glucose and insulin levels following subcutaneous administration of an insulin solution in normal rats: glucose (□) and insulin (■). Values represent the means of five rats ± SD.

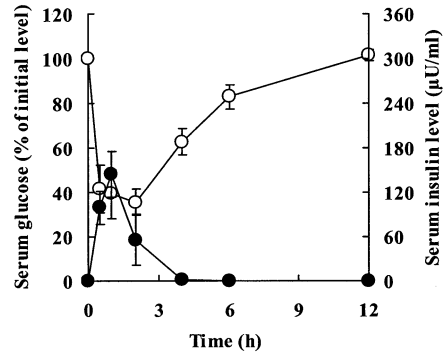

Fig. 4. Serum glucose and insulin levels following subcutaneous administration of an INP suspension in normal rats: glucose (○) and insulin (●). Values represent the means of five rats ± SD.

3.4. Changes in serum insulin and glucose levels following subcutaneous administration of insulin-loaded PF127 gels

Fig. 5 shows the changes in serum glucose and insulin levels following s.c. administration of insulin-loaded PF127 gels in normal rats. The typical profile obtained with an insulin solution was changed to a delayed and more prolonged hypoglycemic profile when insulin was loaded into PF127 gels. As observed in Fig. 5, the effect of the concentration of PF127 on the release of insulin showed an inverse dependence, i.e. the higher the concentration of PF127, the slower and more prolonged was the release of insulin. The decreased absorption rate can be explained by the decreased diffusion of insulin due to obstruction by the micellar entanglement, depending on the PF127 concentration (Chen-Chow and Frank, 1981). Viscosity may also delay or impede diffusion of the drug into body fluids (Lu and Jun, 1998). These results are in good agreement with the in vitro results described earlier. The use of a PF127 gel matrix might also serve to stabilize insulin against its self-aggregation responsible for the loss of its biological potency observed in a variety of delivery environments (Chawla et al., 1985) and processing stresses (Wang and Johnston, 1995; Katakam et al., 1997a; Stratton et al., 1997). It might also protect insulin against local proteolytic enzymes while the peptide is entrapped in the gel matrix.

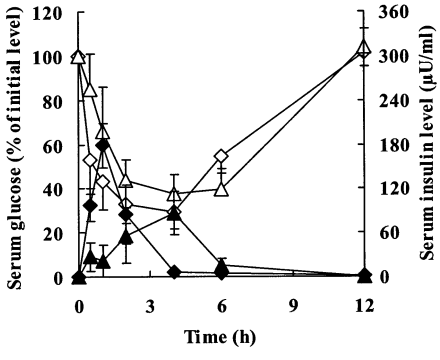

Fig. 5. Serum glucose and insulin levels following subcutaneous administration of PF127 gels containing insulin in normal rats: 20% PF127 gel (◇,◆); 30% PF127 gel (△,▲); glucose (△,◇) and insulin (▲,◆). Values represent the means of five rats ± SD.

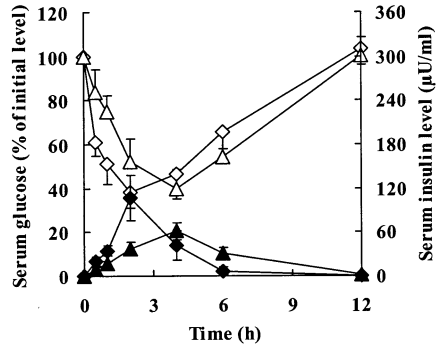

Fig. 6. Serum glucose and insulin levels following subcutaneous administration of INP-loaded PF127 gels in normal rats: INP-loaded 20% PF127 gel (◇,◆); INP-loaded 30% PF127 gel (△,▲); glucose (△,◇) and insulin (▲,◆). Values represent the means of five rats ± SD.

3.5. Changes in serum insulin and glucose levels following subcutaneous administration of INP-loaded PF127 gels

The effect of INP-loaded PF127 gels on serum glucose and insulin levels following s.c. administration in normal rats is seen in Fig. 6. PF127 gel formulations containing insulin–PLGA nanoparticles had the most long-lasting hypoglycemic effects of all formulations. The administration of such a formulation resulted in hypoglycemic effect profiles of an almost trapezoidal shape. The local distribution of a solution injected subcutaneously is of interest because the permeation rate of the drug depends in part on the geometry and the resulting area of the depot exposed to the tissue. As observed, INP-loaded PF127 gels produced a burst of serum insulin more pronounced at the lower concentration of PF127, not observed in the case of insulin-loaded PF127 gels. Usually, the nanoparticles (INP) occupy a larger volume in solution and this might be effective enough at disrupting the stability of the PF127 micellar aggregates, directly affecting the critical gel formation temperature. Such effect might be more pronounced at lower PF127 concentration than at higher PF127 concentrations. Further in

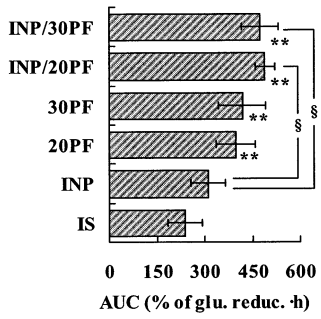

Fig. 7. AUC values of the serum glucose levels following subcutaneous administration of various insulin formulations in normal rats: IS, insulin solution; INP, insulin nanoparticles; 20PF, 20% PF127 gel; 30PF, 30% PF127 gel; INP/20PF, INP-loaded 20% PF127 gel; and INP/30PF, INP-loaded 30% PF127 gel. Values represent the mean of five rats ± SD. Significant (** $P < 0.01$) difference in the AUC mean values compared with the control group using the Student's t-test. Significant (§ $P < 0.01$) difference in the AUC mean values compared with the control group using the Student's t-test.

vestigation is needed to clarify the effect of nanoparticles (INP) on the thermal gelation property of PF127 solutions.

3.6. Statistical analysis of the areas under the curve of serum glucose obtained from various insulin formulations

The area under the curve (AUC_{glu}) of the serum glucose levels following s.c. administration of various insulin formulations in normal rats is shown in Fig. 7. A highly significant ($P < 0.01$) increase in the AUC_{glu} occurred when insulin was subcutaneously administered as a PF127 gel or as a INP-loaded PF127 gel formulation compared with the corresponding AUC_{glu} when insulin was subcutaneously administered as an aqueous solution. A highly significant ($P < 0.01$) increase in the AUC_{glu} was also observed when insulin was subcutaneously administered as an INP-loaded PF127 gel formulation compared with the corresponding AUC_{glu} when insulin was subcutaneously administered as an INP formulation.

3.7. Pharmacokinetic parameters of insulin following subcutaneous administration of various insulin formulations

The pharmacokinetics of insulin after extravascular administration have been extensively studied (Banerjee et al., 1991; Hoffman and Ziv, 1997). The process by which insulin is absorbed is extremely complex, involving spatial and temporal factors. A precise determination of the amount of insulin cleared from the injection site per unit time requires measurement of the levels of blood and lymph throughout the tissue and of the arteriovenous concentration difference. This is impracticable in humans and difficult in animal models.

The comparison of the pharmacokinetic parameters of insulin following s.c. administration with various formulations containing insulin in

Table 2
Comparison of the pharmacokinetic parameters of insulin following subcutaneous administration of various formulations containing insulin in normal rats[a]

Formulation	$AUC_{insulin}$ (μU·h·ml^{-1})	C_{max} (μU/ml)	t_{max} (h)	MRT (h)
Insulin solution	114.4 ± 14.3	130.9 ± 11.6	0.5 ± 0.1	0.9 ± 0.2
INP	248.4 ± 26.5**	150.2 ± 20.6	1.2 ± 0.4*	1.3 ± 0.3*
20% PF127	294.4 ± 34.0**	102.7 ± 16.0*	2.4 ± 0.8**	2.8 ± 0.5**
30% PF127	315.7 ± 17.6**	61.7 ± 11.3**	4.0 ± 0.9**	4.6 ± 0.3**
INP-loaded 20% PF127	355.9 ± 36.7**,†	189.8 ± 49.2	1.4 ± 0.5*	2.1 ± 0.4**,†
INP-loaded 30% PF127	355.8 ± 30.8**,†	97.2 ± 13.4**,†	3.6 ± 0.8**,†	3.8 ± 0.5**,†

[a] Each value represents the mean of five rats ± SD.
* Significant difference ($P < 0.05$) in the mean value compared with insulin solution using the Students' t-test.
** Significant difference ($P < 0.01$) in the mean value compared with insulin solution using the Students' t-test.
† Significant difference ($P < 0.01$) in the mean value compared with INP using the Students' t-test.

normal rats is presented in Table 2. The blood sampling time point (t_{max}) required to achieve the maximum plasma insulin concentration (C_{max}) and the MRT values obtained after s.c. administration of the various formulations suggest that 30% PF127 gels containing either insulin or INP were the best formulations for controlling the release of insulin among the formulations studied. However, the serum insulin levels obtained from the various formulations, at least at the doses studied, seem to be much higher than the normally required basal insulin levels (i.e. 10–40 μU/ml) (Hoffman and Ziv, 1997).

From the current in vitro and in vivo study, we concluded that PF127 gel formulations containing either insulin or insulin nanoparticles could be useful for the preparation of a controlled insulin delivery system for s.c. administration. A controlled release formulation of insulin can improve patient compliance and therapy. Moreover, this method can be applied to another peptides and proteins having short half-lives.

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