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A novel sustained-release formulation of insulin with dramatic reduction in initial rapid release

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Abstract

To ensure a strictly controlled release of insulin, a preparation method for insulin-loaded microcapsules was designed. Microcapsules were prepared with an injectable, biodegradable polymer composed of co-poly(D,L-lactic/glycolic) acids (PLGA) (mean molecular weight 6600, LA/GA ratio 50:50). Morphological examination using scanning electron microphotography demonstrated spherical particles with a main diameter of 15–30 μm. When 3% insulin-loaded PLGA microcapsules were administered subcutaneously as a single dose (250 U/kg) to streptozotocin-induced hyperglycemic rats, plasma insulin levels increased and were sustained at levels showing hypoglycemic effects. When glycerin, ethanol, or distilled water was used throughout the preparation procedure, the resultant microcapsules dramatically reduced the initial burst. The formulation in which glycerin was added to an oil phase containing PLGA, insulin, and ZnO increased plasma insulin levels to 86.7, 108.4, and 84.9 μU/ml at 1, 2, and 6 h, respectively. The levels remained at 36.2–140.7 μU/ml from day 1 to day 9. The $AUC_{0-24\ h}/AUC_{0-336\ h}$ ratio was calculated to be 9.7%. The formulation prepared without additives gave such a rapid insulin release that animals receiving it became transiently hypoglycemic. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Controlled release; Diabetes mellitus (DM); Insulin; Co-poly(D,L-lactic/glycolic) acids (PLGA); Diabetic rat

1. Introduction

For the treatment of insulin-dependent diabetes mellitus (IDDM), insulin therapy by injectable formulation is common and necessary. In addition to mealtime treatment, IDDM patients need a relatively constant basal insulin supply to attain a near-normal physiological pattern of insulin secretion and improve their quality of life [1,2]. At present, one or more doses of intermediate- or long-acting insulin per day are injected subcutaneously to satisfy their basal insulin requirements. If there was an insulin formulation which could release it in a strictly controlled fashion without any initial burst, patients could be partly released from multiple treatments. Such a formulation would contribute not only to an improvement in the patient's condition, but also to a reduction of the risk of catching additional diseases associated with DM.

Sustained-release formulations of insulin have

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been reported by some investigators. Stevenson et al. [3] demonstrated that the application of liposome insulin (0.75 U/kg) to diabetic dogs resulted in a reduction in blood glucose, and immunoreactive insulin in plasma could be detected 24 h after the injection. They also showed that the hypoglycemic effect of the same dose of free insulin virtually ceased within 8 h, and plasma insulin levels returned to baseline 3 h after the injection. Recently, Gershonov et al. [4] reported that a single subcutaneous dose of 9-fluorenylmethoxycarbonyl insulin (3 mg/animal) given to diabetic rats normalized their blood glucose levels, and maintained them in an anabolic state for 2–3 days.

On the other hand, since Langer et al. [5] proposed that polymers were suitable carriers for the sustained release of proteins and other micromolecules, polymers have also been used as a carrier for insulin. Davis [6] used polyacrylamide as a carrier, and studied the insulin release from implants. He showed the benefit by measuring the weights of diabetic animals receiving such implants for 2–3 weeks, however blood glucose was not measured. Creque et al. [7] showed that insulin vinyl acetate–ethylene copolymer implants (60 U/animal) in diabetic rats sustained normalized blood glucose levels for 1 month. They also showed that treated animals gained weight normally. However, these polymers are not biodegradable. Removal of the polymer and periodic implantation would be needed. Furthermore, this might induce inflammatory and/or toxic effects in both animals and humans.

Formulations prepared with biodegradable matrix/microcapsules are attractive. It would be advantageous if the degradation products were not toxic, immunogenic or carcinogenic. Goosen et al. [8] used a biodegradable matrix. The form of insulin and cross-linked albumin microbeads implanted in diabetic rats (750 U/animal) resulted in the release of bioactive insulin for up to 3 weeks. They also showed that the fasting blood glucose levels in treated animals were 88 mg/dl compared with 392 mg/dl for diabetic controls. Implanted diabetic animals were also shown to increase their body weight. However, this formulation was not injectable. There would be the potential disadvantage of repeated removal and implantation. Lin et al. [9] used biodegradable hydroxyl propyl methylcellulose phthalate (HP-55) and polylactic acid (PLA, MW 30,000). They showed that HP-55 microcapsules rapidly released insulin under in vitro conditions and led to a non-sustained action of insulin in vivo. PLA microcapsules were demonstrated to prolong the release of insulin and treatment (10 mg/animal) was reported to maintain normoglycemia for 5 days.

We therefore attempted to prepare an injectable formulation which could maintain insulin release in a zero-order kinetic fashion following a single subcutaneous administration to diabetic rats. In order to achieve this, human recombinant insulin was encapsulated in an injectable, biodegradable polymer composed of co-poly(D,L-lactic/glycolic) acids (PLGA).

2. Materials and methods

2.1. Reagents

Human recombinant insulin was purchased from Wako Pure Chemical (Osaka, Japan). PLGA (LA/GA ratio 50:50, average molecular weights 4100, 6600, and 7900) was synthesized and kindly provided by Wako Pure Chemical.

2.2. Insulin-containing PLGA formulations

Human insulin usually contains 0.27–0.35 w/w% zinc. The zinc content of the insulin was reduced by dialysis and then lyophilized to produce a size of about 2–3 μm. The zinc content of the resulting lyophilized insulin (Zn-free insulin) was ≤0.0001%, as determined by a polarized Zeeman atomic absorption spectrometer (Hitachi, Ibaraki, Japan).

Insulin-loaded PLGA microcapsules were prepared substantially as previously reported [10] by the solvent evaporation method. In brief, in the case of microcapsules containing 3% insulin, 240 mg Zn-free insulin, 3.99 mg ZnO, and 7.84 g PLGA dissolved in 8 ml CH_2Cl_2 were mixed and agitated vigorously to form a S/O suspension (oil phase). The appropriate amount of glycerin, ethanol or distilled water was then added and agitated vigorously to reduce the optical density of this suspension from 3.8 to <1.5 at 400 nm. The resultant suspension/solution was poured into 1.6 l 0.1% (w/v) polyvinyl

alcohol (PVA, GOHSENOL: EG-25, average molecular weight 45,000, Nippon Gosei Kagaku, Tokyo, Japan) solution containing 0.7% $(CH_3COO)_2Zn$ under stirring. This (S)/O/W emulsion was stirred at 1200 rpm using a propeller-type agitator with three blades (Heidon 600G, Shinto Scientific, Japan) for 3 h to evaporate the organic solvent to obtain the microcapsules. The microcapsules were washed three times with distilled water by centrifugation and then sieved using a 125 μm screen to remove larger particles. Mannitol was added to the resulting microcapsules to prevent aggregation, and they were then lyophilized. The insulin contents of the prepared microcapsules were determined after extraction with CH_2Cl_2 and 0.01 N HCl according to the method of Lowry et al. [11], using Zn-free insulin as a standard. The encapsulation efficiency was >70%.

2.3. Electron microscopic findings

The insulin-loaded microcapsules were examined under a SEM 4300 scanning electron microscope (Hitachi, Ibaraki, Japan) at an acceleration voltage of 1.0 kV.

2.4. Animals

Seven-week-old male Wistar rats (200–240 g) were purchased from SLC Experimental Animals (Shizuoka, Japan). Animals were housed at a constant temperature (23±1°C) and humidity (50–60%) with free access to a standard diet and water. The animal room had a 12-h light/dark cycle (lights on 06:30 to 18:30).

The study protocol was approved by the animal experimentation committee of St. Marianna University.

2.5. Animal experiments

Animal experiments were conducted using streptozotocin (STZ)-induced hyperglycemic rats. Three days after rats received 60 mg/kg of STZ dissolved in 10 mM citrate–citrate Na buffer (pH 4.5) intravenously, they were given a single subcutaneous injection of insulin-loaded PLGA microcapsules (250 U/kg). Just before administration, the insulin formulations were dispersed (20% w/v) in 5% mannitol solution, pH 6.5, containing 5% carboxymethylcellulose and 0.1% Tween 80. Blood samples were taken from the inferior ophthalmic vein before and after treatment to determine plasma insulin and blood glucose levels. These samples were all taken in the morning (09:00–11:30), except for that taken 6 h after dosing, until the 14th day.

Plasma insulin was determined using a RIA kit (Shionogi Seiyaku, Osaka, Japan). The area under the plasma insulin concentration vs. time curve (AUC) was calculated according to the trapezoidal method. Blood glucose levels were also determined by the glucose oxidase method using a glucose analyzer (Glucoster-M, Sankyo, Tokyo, Japan) immediately after blood collection.

2.6. In vitro release of insulin from microcapsules

The in vitro release of insulin from various formulations was evaluated using phosphate-buffered saline (pH 7.4). The insulin-loaded PLGA microcapsules (80 mg) were suspended in 0.4 ml of the buffer in a test tube and kept at 37°C. At appropriate times, supernatants were collected after centrifugation, and 0.4 ml of the buffer was added and incubated. The insulin content of the supernatant was determined by the method of Lowry using Zn-free insulin as a standard.

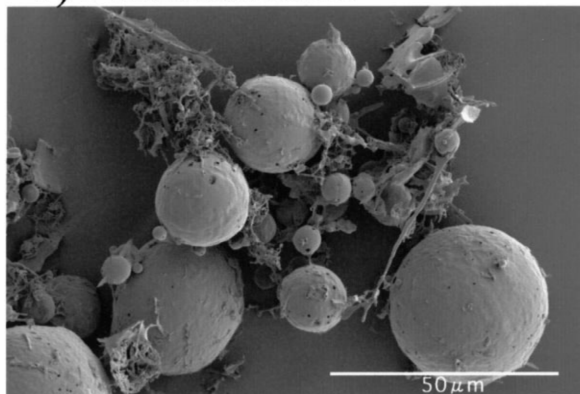
2.7. Statistical analysis

Results are presented as the mean (±S.D.). Determination of the significance of differences was carried out using the Mann–Whitney *U*-test, and $P<0.05$ was taken as the criterion of significance.

3. Results

3.1. Electron microscopic findings

Morphological examination using scanning electron microphotography showed spherical PLGA particles with a main diameter of 15–30 μm (Fig. 1). There were no significant differences between the lots. Particle size was also determined using a particle analyzer (Multisizer II_E, Beckman Coulter,



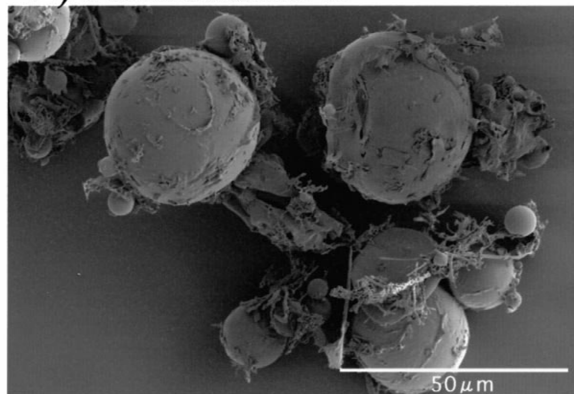


Fig. 1. Insulin-loaded PLGA microcapsules. The photograph was taken using a scanning electron microscope with ×1000 magnification. (1) Formulation A, (2) formulation C.

Tokyo, Japan), with no significant differences (data not shown).

3.2. Insulin release from PLGA microcapsules in streptozotocin (STZ)-induced hyperglycemic rats

3.2.1. Effect of additives

Blood glucose concentrations of the STZ-treated rats tested in this study were all >290 mg/dl. Their plasma insulin levels were 3.0–5.6 μU/ml throughout the experiment. The insulin levels of normal animals were 20.4–51.4 μU/ml, and their blood glucose levels were 98–150 mg/dl when measured in the morning. All animals were housed with free access to a standard diet and water.

Several kinds of PLGA microcapsules containing 3% insulin were prepared (Table 1). When 250 U/kg of insulin-loaded PLGA microcapsules prepared without any additives (formulation A) was given subcutaneously to diabetic rats, plasma insulin levels increased rapidly (Fig. 2). The mean plasma insulin level was 131.0 μU/ml at 1 h. Levels at 2 and 6 h were 993.1 and 2440.5 μU/ml, respectively, 7.6 and 18.6 times higher than that at 1 h. Diabetic animals receiving this formulation became transiently hypoglycemic (64.7 mg/dl). Plasma insulin levels then decreased with time, reaching 56.5 μU/ml at 24 h. After remaining relatively low, the levels increased again to 174.7–196.5 μU/ml on days 6–8. The burst induced by this formulation is clearly indicated by the mean $AUC_{0-24\ h}/AUC_{0-336\ h}$ ratio, which was calculated to be 57.6%.

Table 1
Insulin-loaded PLGA(6k) microcapsule formulations tested in this study

Formulation	Insulin content (%)	Mean MW	Additive	Oil phase[a] (mg/ml)	Zinc compounds		$AUC_{0-24\ h}$/ $AUC_{0-336\ h}$ ratio (%)
					Oil phase	Water phase	
A	2.1	6600	–		ZnO	$(CH_3COO)_2Zn$	57.6#
B	2.5	6600	Distilled water	3.65	ZnO	$(CH_3COO)_2Zn$	13.2*#
C	2.4	6600	Ethanol	6.08	ZnO	$(CH_3COO)_2Zn$	7.2*#
D	2.3	6600	Glycerin	3.65	ZnO	$(CH_3COO)_2Zn$	9.7*#
E	2.3	6600	–		–	–	78.0

*$P<0.01$ vs. formulation A; #$P<0.01$ vs. formulation E. The $AUC_{0-24\ h}/AUC_{0-336\ h}$ ratio was calculated from plasma insulin levels after treatment.

[a] Values represent the amount of the respective additive (mg) in 1 ml of oil phase containing 1 g of PLGA, insulin, and ZnO.

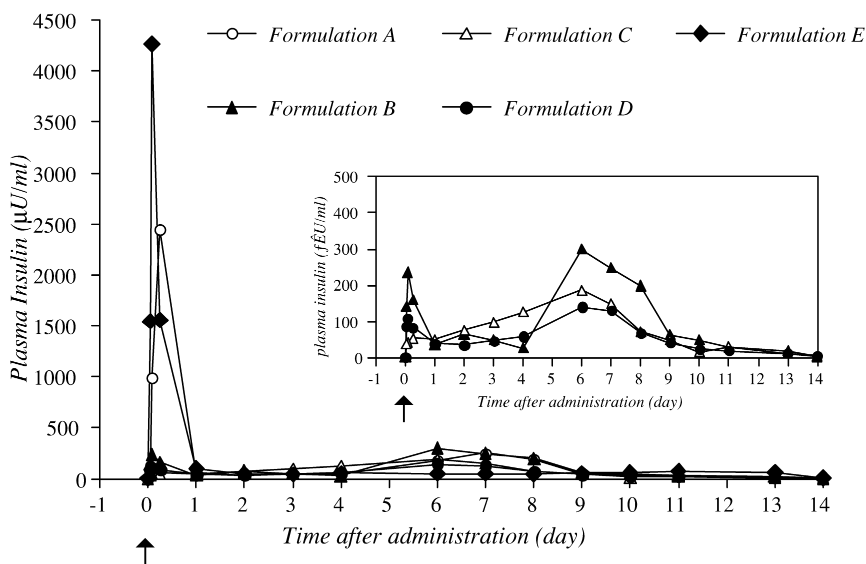


Fig. 2. Plasma insulin levels following a single subcutaneous administration of insulin-loaded PLGA microcapsules. Three insulin-loaded PLGA microcapsules (formulations A, B, C, D, and E) were administered subcutaneously (250 U/kg) as a single dose to STZ-induced hyperglycemic rats. Plasma insulin levels were monitored. Details of the formulations are shown in Table 1. $N = 3–7$. Data represent the mean.

In the preparation procedure for formulation B, 3.65 mg of distilled water was added to 1 ml of oil phase containing 1 g of PLGA, insulin, and ZnO. As a result of this addition, the optical density of the oil phase decreased from 3.8 to 1.5 at 400 nm (Fig. 3). The same dose of formulation B dramatically reduced the initial burst. Although the level at 1 h was comparable to that of formulation A, the respective levels at 2 and 6 h were 236.6 and 159.8 μU/ml. Plasma insulin levels remained around 50 μU/ml, and again increased to 300.1 μU/ml on day 6, levels high enough to exert a hypoglycemic effect. Reduction of the burst was evident from the $AUC_{0-24\ h}/AUC_{0-336\ h}$ ratio, which was calculated to be 13.2% ($P=0.002$ vs. formulation A).

In the preparation procedure of formulation C, 6.08 mg of ethanol was added to 1 ml of the oil phase to improve the turbidity. Plasma levels at 2 and 6 h after administration were 43.0 and 54.7 μU/ml, respectively. A similar level (52.6 μU/ml) still remained at 24 h. As observed with formulation B, the peak was seen on day 7 (187.0 μU/ml). The $AUC_{0-24\ h}/AUC_{0-336\ h}$ ratio for this formulation was calculated to be 7.2% ($P=0.021$ vs. formulation A), showing a dramatic reduction in the burst.

Formulation D, in which 3.65 mg of glycerin was used to reduce the optical density in 1 ml of the oil phase, also showed a reduced burst. Plasma insulin levels of 86.7, 108.4, and 84.9 μU/ml were detected at 1, 2, and 6 h, respectively. Levels remained steady (36.2–140.7 μU/ml) up to the ninth day. The $AUC_{0-24\ h}/AUC_{0-336\ h}$ ratio was calculated to be 9.7% ($P=0.003$ vs. formulation A). There were no significant differences in these ratios between formulations B, C, and D.

In addition to no additive, formulation E was prepared without Zn compounds (<0.3 mg per 100 mg microcapsules). This formulation increased plasma insulin levels to 4264.1 μU/ml (2 h). The formulation yielded an $AUC_{0-24\ h}/AUC_{0-336\ h}$ ratio of 78.0%.

Several formulations in which the amount of glycerin added to the oil phase was changed were also evaluated. The results are shown in Fig. 4. Formulation D-1, where 1.68 mg of glycerin was added to 1 ml of oil phase, reduced the burst. Levels in animals administered this formulation were 20.2–59.6 μU/ml. Although the levels were a little higher (128.4 μU/ml) on day 6, they were within a similar range to those of formulation D. Similar results were

Fig. 3. The appropriate amount of additives improved the turbidity of the oil phase containing PLGA, insulin, and ZnO. Insulin was present in the oil phase containing PLGA as a dispersed suspension (right). The appropriate amount of distilled water improved the turbidity (left). The optical density of this suspension was reduced from 3.8 to 1.5 at 400 nm. The appropriate amount of ethanol and glycerin had the same effect.

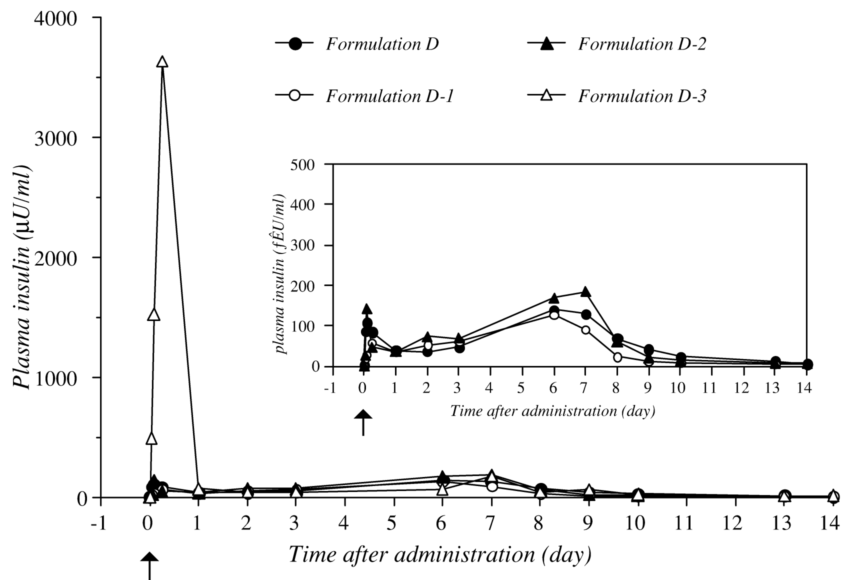


Fig. 4. Plasma insulin levels following a single subcutaneous administration of insulin-loaded PLGA microcapsules. Plasma insulin levels were monitored following a single subcutaneous administration of 3% insulin-loaded PLGA microcapsules (250 U/kg) to STZ-induced hyperglycemic rats. In the preparation procedure, 3.65 (formulation D), 1.68 (formulation D-1), 1.17 (formulation D-2), or 0.655 mg (formulation D-3) of glycerin was added to 1 ml of oil phase containing PLGA, insulin, and ZnO. The mean molecular weight of PLGA was 6600. $N = 2$–5. Data represent the mean.

also obtained with formulation D-2. A small amount of glycerin did not improve the optical density of the oil phase containing PLGA, insulin, and ZnO. Formulation D-3 could not prevent the burst, with a peak of 3637.5 μU/ml at 6 h. There was no significant difference between formulation D-3 and formulation A.

There were hardly any microcapsules remaining in the injected area by the 14th day. Insulin contents were approximately 0.013% of the original dose when determined by RIA ($n=5$).

The changes in body weight of the animals in the experiment are shown in Fig. 5. Weight gain was depressed as a result of the administration of STZ. Animals administered STZ only gained little weight throughout the experiment. Animals administered insulin-loaded PLGA, including formulation A, gained weight at a rate of approximately 40 g per week up to the eighth day. Formulations D-3 and E showed similar changes as observed for formulation A. Weights decreased with reduced plasma insulin levels.

3.2.2. Effect of PLGA molecular weight

Different kinds of PLGA microcapsules containing 3% insulin (formulations MC-8k and MC-4k) were prepared (Table 2). Glycerin was used as an additive in the preparation procedure.

Table 2
Characteristics of PLGA used in this study

PLGA	M_w	M_n	Terminal –OH ratio	
			LA	GA
4k	4100	3000	36.11	63.89
6k	6600	3480	56.82	43.18
8k	7900	3780	38.36	61.64

M_w, weight-average molecular weight; M_n, number-average molecular weight. M_w and M_n were measured by GPC (gel permeation chromatography). –OH ratio, terminal –OH ratio of LA(lactate) and GA(glycolate). –OH ratio was determined by NMR.

MC-8k induced a rise in plasma insulin concentration (Fig. 6). The levels at 1 h (190.8 μU/ml) and 2 h (265.2 μU/ml) were both higher than those with MC-6k (formulation D). These levels were followed by a lag phase, and a subsequent gradual augmentation was observed. It should be noted that higher levels were detected on later days. Even on day 10, the level was 162.1 μU/ml ($P<0.01$ vs. MC-6k).

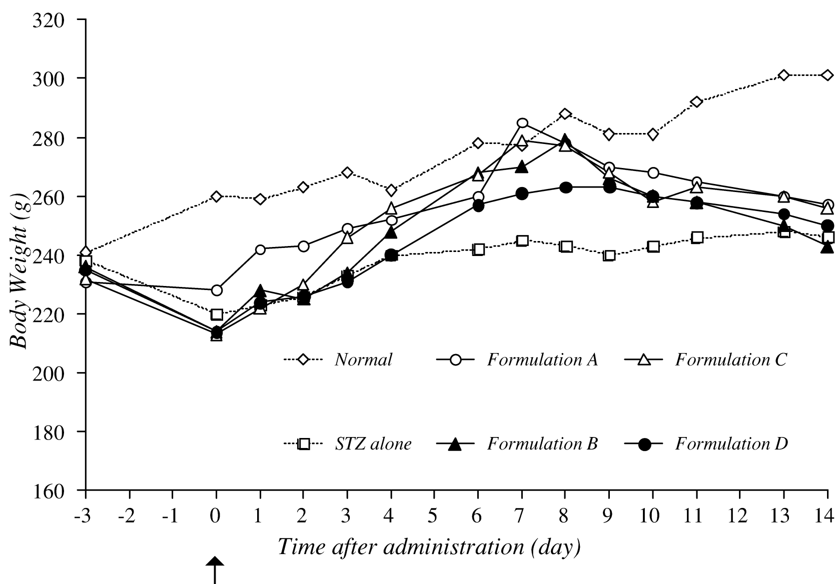


Fig. 5. Body weight changes following a single subcutaneous administration of insulin-loaded PLGA microcapsules. Body weights were monitored following a single subcutaneous administration of 3% insulin-loaded PLGA microcapsules (250 U/kg) (formulations A, B, C, and D). Body weights of normal animals and STZ-treated animals are also shown. $N=3–7$. Data represent the mean.

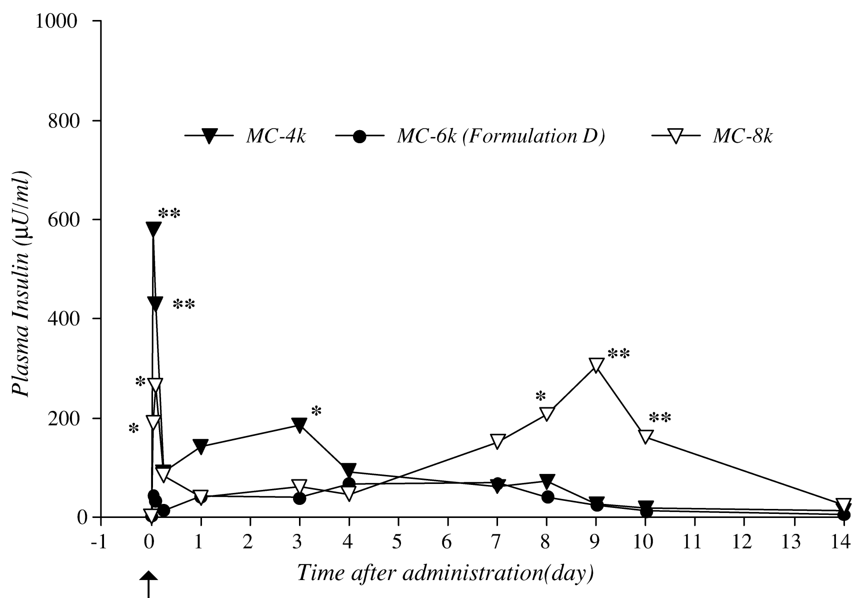


Fig. 6. Effect of PLGA molecular weight of insulin-loaded PLGA microcapsules on plasma insulin levels following a single subcutaneous administration. Three percent insulin-loaded PLGA microcapsules (MC-4k, MC-6k, and MC-8k) were administered subcutaneously (250 U/kg) to hyperglycemic rats, and plasma insulin levels were monitored. In the preparation procedure, glycerin was used as an additive. $N = 3–5$. Data represent the mean. *$P<0.05$, **$P<0.01$ vs. MC-6k (formulation D).

MC-4k also gave an insulin burst, which was more significant than that of MC-8k. One and 2 h after administration to diabetic rats, plasma insulin levels were 579.2 and 430.3 μU/ml, respectively. Unlike MC-8k, MC-4k continued to release a steady level of insulin. Levels from the first to the fourth day were significantly higher than those measured with MC-6k and MC-8k. The level on day 9 was about one-tenth of that with MC-8k ($P<0.01$).

3.2. In vitro release of insulin from microcapsules (MC-4k, MC-6k, and MC-8k)

The in vitro release of insulin from microcapsules (MC-4k, MC-6k, and MC-8k) was examined. Formulation A (PLGA 6k), prepared without additives, released 3.9% of its insulin content after 1 h incubation (Fig. 7). The cumulative insulin release of this formulation was 11.3% (2 h), 15.6% (6 h), and 17.3% (day 1). The percent release of MC-4k, MC-6k (formulation D), and MC-8k after 1 h incubation was only a little lower than that seen with formulation A. Release from MC-8k was the most rapid. More than 16.5% of the insulin was released from MC-8k. However, there was then little insulin release for several days. Acceleration of drug release was again seen after 10 days incubation. A relatively constant release was seen from both MC-6k and MC-4k.

4. Discussion

PLGA is an attractive biodegradable carrier for drugs and has been used to achieve sustained release of not only insulin, but also other drugs [12–21]. LHRH analogue-loaded PLGAs have already been used in the clinical setting, and have made a contribution to improving the condition of patients with prostate cancer, ovarian hyperthecosis, early precocious puberty, endometriosis, etc.

Several investigators [4,6–8] have demonstrated that a sustained-release formulation of insulin constantly augmented the body weight of diabetic animals. Hyperglycemic animals that received insulin-loaded PLGA formulations gained body weight as fast as normal animals for the first 8 days. This result shows that it is important to maintain more

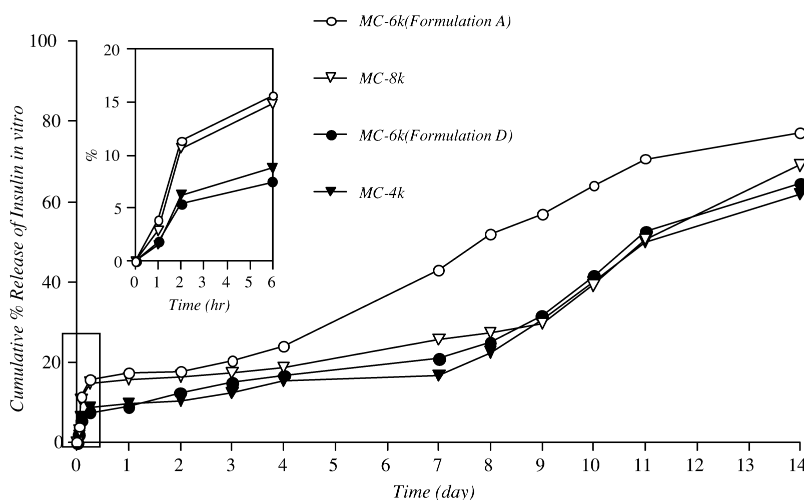


Fig. 7. In vitro insulin release from PLGA microcapsules. In vitro insulin release from MC-4k, MC-6k, MC-8k and formulation A (PLGA 6k) was evaluated. MC-6k is formulation D. Data represent the mean obtained from three to five separate experiments.

than a certain level of insulin in order to improve the condition of diabetic rats. However, these formulations are not in clinical use. One reason for this is that it is difficult to prevent the initial rapid release (burst). Other determining factors are whether or not the formulation is injectable, and whether or not carriers including the polymer/matrix are biodegradable. It should be noted that our formulation is not only injectable, but is also prepared with a biodegradable polymer.

The burst, which causes severe hypoglycemia, is a critical factor that must be overcome. As reported by Tracy [22], since it is important to prevent rapid release the size of the insulin particles was reduced to 2–3 μm. It was confirmed that the formulation prepared with human insulin produced a burst (data not shown). Zinc compounds were also necessary. Formulation E prepared without any zinc compound caused a rapid release of insulin. Zinc compounds have been reported to not only stabilize the protein, but also to control the release from the microcapsules [22,23]. We used ZnO in the oil phase and $(CH_3COO)_2Zn$ in the water phase. As a result, the burst was partly suppressed, showing a $AUC_{0-24\ h}/AUC_{0-336\ h}$ ratio from 78.0 to 56.7%.

The addition of L-arginine [16] in the preparation of microcapsules has been reported to contribute to a reduction of the burst. However, there have been no reports that glycerin, ethanol, or distilled water display a similar effect. These additives dramatically changed the turbidity of the oil phase containing insulin and PLGA. When 6k PLGA was used, the insulin itself was insoluble in $PLGA/CH_2Cl_2$ oil phase. Its optical density at 400 nm was >3.8. All the additives used lowered the optical density. A smaller amount of glycerin did not improve the turbidity, and the resultant formulation produced a burst. The evidence leads us to believe that the additives must have minimized the insulin particle unit in the oil phase. This would result partly from strengthening of the interactions between PLGA and insulin. Furthermore, the addition of the additives changed the insulin localization in the resultant formulation. We have recently confirmed that the initial insulin release from PLGA microcapsules is dependent on the insulin localization (manuscript in preparation).

The molecular weight of the PLGAs was also a determining factor for the initial release from microcapsules. MC-8k produced a higher plasma insulin level than MC-6k. Formulations prepared with higher-molecular-weight PLGAs (9k or 10k) increased the plasma insulin level to a greater degree (data not shown). This finding was also reported by Jhonson et al. [24]. The maximum serum rhGH concentration after subcutaneous administration of 10k PLGA formulation to rats was higher than that of the 8k formulation. Higher-molecular-weight

PLGAs may interact more weakly with the insulin than 6k PLGAs, leading to a relatively rapid insulin release. MC-4k caused significantly higher insulin levels than MC-6k and MC-8k. One reason for this is that 4k PLGAs were susceptible to release with the insulin into aqueous fluid because of their greater solubility. This is supported by the fact that plasma insulin levels in the MC-4k-treated group were significantly higher from the first to the fourth day than those measured for MC-6k and MC-8k. It should be noted that there was a coincidence between the in vivo and in vitro release patterns of insulin from MC-8k, MC-6k (formulation A) and MC-6k (formulation D), although the in vitro PLGA degradation profile from microcapsules has been reported to be different from that in vivo [25]. However, the initial in vitro release from MC-4k was different from the in vivo release. This may be partly ascribed to the lower glass transition temperature (T_g) of PLGAs. Since the T_g of MC-4k is around 36°C, microcapsules can easily fuse under physiological conditions. The centrifugation procedure in the in vitro study might have accelerated this phenomenon. This also suggests the possibility that the in vitro drug release profile from microcapsules prepared from lower-molecular-weight PLGAs such as 4k does not necessarily reflect that of in vivo release.

The long duration is attributed to the fact that the insulin was gradually released associated with PLGA degradation. Compared to the desired flat patterns, plasma insulin levels showed a lag phase and then gradual augmentation. The in vitro release study showed similar patterns. In general, it takes longer for degradation to occur, the higher the molecular weight of the polymer [26]. The delay in the release of insulin observed after treatment with formulation MC-8k can be explained by the higher molecular weight of the polymer used in this formulation.

The present study has shown that a single subcutaneous administration of insulin-loaded PLGA microcapsules to diabetic rats can produce a sustained release with little burst. This formulation is injectable and biodegradable. The encapsulation efficiency was >70%. Since higher insulin levels can induce hypoglycemia [27], a lower peak/trough ratio is desirable. Factors controlling drug release include not only the molecular weight of the PLGAs, but also the LA/GA ratio of the PLGAs and the particle size of the microcapsules. In addition, it would be desirable to investigate further the optimum additives in order to determine the best formulation.

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