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Research paper

Subcutaneous delivery of insulin loaded poly(fumaric-co-sebacic anhydride) microspheres to type 1 diabetic rats

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Abstract

The method of phase inversion nanoencapsulation (PIN) and microencapsulation was used to produce biodegradable poly(fumaric-co-sebacic anhydride) (p(FASA)) microspheres that contain insulin. Microspheres were characterized by SEM and a laser light scattering technique to determine particle size distribution. Insulin stability was determined by RP and SEC HPLC. Release rate studies were conducted and microspheres were administered subcutaneously (SQ) to type 1 diabetic rats to determine the bioactivity of insulin at three different dosages. Pharmacokinetic parameters for SQ experiments were measured using the trapezoidal rule by plotting average plasma insulin level (PIL) vs. time and determining peak concentration (C_P), the time of peak concentration (T_P), duration of PIL curve (D), and relative bioavailability (RB). When our insulin containing formulation was analyzed by HPLC, there was no evidence of high molecular weight transformation (HMWT) or deamidated products. In addition, we effectively altered the onset, peak, and duration of insulin action after SQ injection.

Keywords: Subcutaneous delivery; Insulin; Microspheres; Poly(fumaric-co-sebacic anhydride); Diabetic rat

1. Introduction

Treatment of insulin-dependent diabetes mellitus (IDDM) requires multiple daily subcutaneous insulin injections, as insulin is an unstable protein with a short in vivo half-life of approximately 4.5 min. This, coupled with the pain, tenderness, local tissue necrosis, microbial contamination, and nerve damage which may be associated with frequent subcutaneous (SQ) injections [1], has prompted research on alternative therapy treatments. Recently, investigators have focused their attention on the delivery of insulin via subcutaneous or implanted devices. Such devices include sustained release insulin delivery systems. These devices are capable of decreasing the frequency of insulin injections while subsequently increasing patient compliance and decreasing complications associated with poor glucose control.

Much recent research has focused on sustained insulin delivery systems fabricated by utilizing copolymers of glycolide and lactide [2]; polyphosphazene [3] and poly(acryloyl-L-proline methyl ester)-based hydrogels [4]. Subcutaneous administration of polyphosphazene microspheres to diabetic mice has resulted in approximately 80% reduction of blood glucose levels; however, most of the activity was lost in 100 h [3]. Poly(acryloyl-L-proline methyl ester)-based hydrogels were implanted into diabetic mice resulting in a decrease in the blood glucose concentration that lasted up to 700 h [4]. As an alternative to nanoparticles composed of synthetic polymer, Reithmeier et al. [5] have designed a controlled release system utilizing a lipid, glyceryl tripalmitate, to encapsulate insulin. More recently, Desai et al. investigated an implantable capsule, which allows glucose passage into the device via nanopores subsequently triggering the release of insulin

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by encapsulated cells within the device. As a result, insulin passes from the device into the patient's bloodstream, reducing blood glucose levels [6].

The purpose of our work was to determine whether insulin microspheres are active after fabrication by subcutaneously administering these microspheres to type 1 diabetic rats by analysing the onset, peak, and duration of insulin action. Our approach is to utilize a microencapsulation technique known as phase inversion micro- and nanoencapsulation (PIN) [7], to produce biodegradable poly(fumaric-co-sebacic anhydride) (p(FASA)) microspheres that contain insulin. The ultimate goal for this work is to determine whether the sustained release of insulin microspheres could be used for oral delivery of insulin.

2. Materials and methods

2.1. Materials

20:80 p(FASA) (MW = 14.5 K) synthesized by melt polycondensation in our laboratory was used to encapsulate bovine zinc insulin (BZI) obtained from Gibco Life Technologies (Rockville, MD) (HPLC potency of 27.9 IU/mg) [8]. BZI was micronized by a precipitation method resulting in an average particle size of 300 nm. Other reagents were of analytical grade.

2.2. Microencapsulation

A phase inversion micro- and nanoencapsulation (PIN) technique was used to fabricate microspheres [7]. An appropriate amount of bovine zinc insulin (Gibco) was weighed, methylene chloride was added, and the suspension was probe sonicated for 15 s at an amplitude of 35, followed by bath sonication for ~20 s until a suspension was formed. The polymer p(FASA) was then added to the solution and vortexed for 1 min. The suspension was then poured rapidly into an unstirred bath of petroleum ether, at a solvent to non-solvent ratio of 1:100 causing microspheres to form spontaneously. Microspheres were filtered, collected, and lyophilized.

2.3. Characterization

A scanning electron microscope (Model S-2700 Hitachi, Ltd, Tokyo, Japan) was used to visualize microsphere structure and morphology. Microspheres were coated using an Emitech K550 sputter coater for approximately 3 min, which resulted in ~25 nm layer of gold covering the sample.

Laser diffraction spectrophotometry (Coulter Corp., Miami, Florida) was also used to determine particle size. This technique allows us to determine the mean diameter of microparticles based on volume statistics. In order to determine size using laser light scattering techniques, microparticles were suspended in 1% hydroxypropylmethyl cellulose:pluronic F127 using a 5 mg/ml ratio. The suspension was then vortexed and bath sonicated to optimize the suspension.

2.4. Insulin integrity using SEC and RP HPLC

Insulin integrity experiments were utilized to determine insulin stability during encapsulation. Insulin can degrade either by hydrolytic reactions (deamidated products) or can transform by the formation of intermolecular covalent bonds with other insulin molecules leading to higher molecular weight transformation (HMWT) or complexed products [11]. The chromatographic system used was a Waters apparatus (Waters, Milford, MA, USA) consisting of a 2690 Separations Module, 996 Photodiode Array Detector, and Millennium32® data acquisition software. All chemicals and reagents used were of HPLC grade. All samples were filtered through 0.2 μm (pore size) filters. The mobile phase (MP) was filtered and degassed.

Insulin analysis was determined by a SEC HPLC method [12]. In this way, the percent loading of insulin in the microsphere formulation was determined. 10 ± 5 mg of microspheres was placed in 1 ml of SEC MP and centrifuged (Marathon 13K/M, Fisher Scientific, Pittsburgh, PA) for 10 min at 12,000 rpm. The supernatant was removed, and the procedure was repeated three more times. All samples were filtered using Acrodisk 0.2 μm PVDF filters (VWR) and were analyzed in duplicate. One hundred microliter of supernatant was injected into an Insulin HMWP column, .3 m long and 7.8 mm ID packed with hydrophilic silica-based gel (Waters, Milford, MA, USA) and carried through by a continuous flow of the mobile phase pumped at a flow rate of 0.5 ml/min at room temperature. Detection was performed at 276 nm.

Insulin analysis was also determined by an RP method [13]. 10 ± 5 mg of microspheres was weighed out and then 1 ml of RP MP was added. Microspheres were centrifuged (Marathon 13K/M, Fisher Scientific, Pittsburgh, PA) for 10 min at 10,000 rpm and then the supernatant was removed, filtered through an Acrodisk 0.2 μm PVDF filter (VWR), and collected for HPLC analysis. 20 μL of supernatant was injected into a Symmetry® C18 column maintained at 40 °C, .15 m long and 3.9 mm ID packed with dimethylactadecylsilyl bonded amorphous silica (Waters, Milford, MA, USA), and carried through by a continuous flow of the MP pumped at a flow rate of 0.8 ml/min. Detection was performed at 220 nm.

2.5. In vitro insulin release rate

The in vitro release rate profile of insulin from microspheres was determined as follows. In triplicate, 10 mg of microspheres were suspended in 1 ml of phosphate buffer solution (PBS) (pH 7.4), and incubated horizontally at 37 °C. At predetermined timepoints, the microspheres was removed from the incubator and centrifuged (Fisher Scientific Marathon 13K/M Centrifuge) at 12,000 rpm for 5 min. Approximately 800 μl of the supernatant was

removed for analysis and then replaced with 800 μl of fresh PBS. All samples were analyzed using a bicinchoninic acid (BCA) assay (Pierce, Rockford, IL) [14,15].

2.6. In vivo studies: experimental protocol

Diabetes prone, BBDP, male rats were purchased from Biomedical Research Models, Inc. (Worcester, MA). These rats are genetically predisposed to spontaneously develop type I diabetes via a cell-mediated autoimmune destruction of the pancreatic β cells [9]. For experimentation, type 1 diabetic rats provide the best in vivo model available for our studies as these rats produce no endogenous insulin; therefore, we can be assured that observed plasma insulin concentrations are purely a result of the insulin delivery system [10]. Moreover, the lack of endogenous insulin results in high pre-experimental plasma glucose levels, so pharmacological effects of experimental insulin administration are easily observed from the decrease in plasma glucose levels.

On non-experimental days, rats were housed in individual metabolic cages and were given food and water ad libitum. Testing for glycosuria and ketonuria was done daily using a Keto-diastix test strip (Baxter). Diabetes was diagnosed after a test for glycosuria as well as a blood glucose concentration greater than 250 mg/dl was established. Protamine zinc insulin (PZI) U-40 (Blue Ridge Pharmaceuticals, Inc.), which is a combination of beef/pork insulin, was used for daily insulin injections. PZI has a duration of action of 12–24 h. At noon everyday, animals were injected with the appropriate dose of PZI so that the peak of insulin action (which occurs approximately 8 h after injection) corresponded to the animals' eating cycle. Animals were monitored daily for weight gain, presence/absence of glycosuria and ketonuria, as well as dehydration. On experimental days, rats were not injected with their normal maintenance dose of PZI. Because PZI had a duration of action of 12–24 h, this ensured that all insulin had been metabolized by the rats by the time the experiment had begun approximately 30 h after PZI injection the previous day.

The insulin utilized on experimental days was bovine zinc insulin (BZI). Because p(FASA) is a relatively fast degrading polymer, it was important to choose an insulin whose peak (approximately 2 h post-injection) would coincide with the rate of polymer degradation. There were two types of experiments performed the first being the SQ injection of unencapsulated BZI. The second type of experiment was the SQ injection of BZI encapsulated in microspheres. Prior to SQ administration of either the unencapsulated or encapsulated insulin, the rats were anesthetized using an isoflurane gas chamber and a pre-dose blood sample was taken via tail bleed. It is important to note that, in these experiments, the rats were only under anesthesia for several minutes while the initial incision was made on the tip of the tail. In the several minutes that the rats were anesthetized, blood glucose levels did not decrease. After the predose blood sample was obtained, each of the nine diabetic rats was injected at approximately 6:30 PM. As BZI peaks 2 h after administration, the injection schedule was designed to coincide with the time at which the rats would begin eating.

For control experiments, BZI was suspended in saline at 40 U/ml and injected at a dosage between 5 and 5.7 U/kg. The dosage varied among rats as administering more insulin than the daily maintenance injection could result in hypoglycemia and death. In the insulin microsphere experiments, microspheres were suspended in saline at 6 mg/ml. Dosages were grouped into three categories for average comparisons: six rats at a dosage of 42–46 U/kg (Group 1), three rats at a dosage of 51–56 U/kg insulin (Group 2), and two rats at a dosage of 63–70 U/kg (Group 3).

Blood sampling after the initial injections was performed at predetermined timepoints using rat restraint tubes while the rats were conscious. Blood was collected in heparinized tubes, spun down and the plasma recovered for glucose and insulin analysis.

2.7. Blood analysis

A glucose trinder assay was used to determine plasma glucose levels, (Diagnostic Chemicals Limited, Oxford, CT). For the detection of exogenous bovine zinc insulin in plasma, an ELISA was used (Diagnostic Systems Laboratories, Webster, TX). Samples prepared for the ELISA were treated with a 25% PEG solution in order to remove any insulin that was bound to antibodies present in rat plasma according to the method described by Hwang et al. [16]. The detection limit for bovine zinc insulin was 1 ng/ml.

2.8. Calculations for PIL and relative bioavailability

Pharmacokinetic parameters for SQ experiments were measured using the trapezoidal rule by plotting average plasma insulin level (PIL) vs. time and determining peak concentration (C_P), the time of peak concentration (T_P), duration of PIL curve (D), and relative bioavailability (RB). For relative bioavailability (RB) calculations, each rat was used as its own control. This was done in order to take into account that each rat has a different resistance to insulin. The RB was calculated comparing the SQ insulin microspheres to the SQ injection of unencapsulated insulin for the same rat according to the following equation:

$$\text{relative bioavailability} = \frac{[\text{AUC}]_A/\text{dose A}}{[\text{AUC}]_B/\text{dose B}} \times 100, \qquad (1)$$

where the AUC is the area under the curve measured using the trapezoidal rule by plotting average plasma insulin level (PIL) vs. time. A represents the insulin microspheres and B represents the unencapsulated insulin.

3. Results and discussion

3.1. Microencapsulation

During the phase inversion micro- and nanoencapsulation (PIN) technique, microspheres are fabricated by the spontaneous phase inversion of dilute polymer solutions, which are then quickly dispersed into an excess of non-solvent for the polymer. As solvent leaves the polymer solution and enters the bulk non-solvent phase, polymer instantaneously precipitates into the form of microspheres in what appears to be a self-assembling system [17].

3.2. Characterization

A SEM of a representative sample of the microspheres used in the SQ experiments can be seen in Fig. 1. Using laser light scattering techniques, 90% of the microspheres were found to have a diameter of 5.9 μm or less by volume.

3.3. Insulin integrity using SEC

Polymeric insulin complexes or the covalent insulin–protamine complex elute first in the retention time interval time of approximately 13–17 min. Covalent insulin dimer elutes at a retention time of approximately 17.5 min, followed by elution of the insulin monomer at a retention time of approximately 20 min. Salts are eluted after the passage of one column volume of MP, i.e., at a retention time of approximately 22 min [12]. When our insulin containing formulation was analyzed, there was no evidence of HMWT products, only dimeric insulin which eluted at 18.1 min. These results indicated that the chemical integrity of the insulin molecule was retained after our microencapsulation process. In addition, when a sample of blank p(FASA) microspheres was run by SEC, a sharp peak was present at a retention time of approximately 22.5 min, the p(FASA) degradation peak.

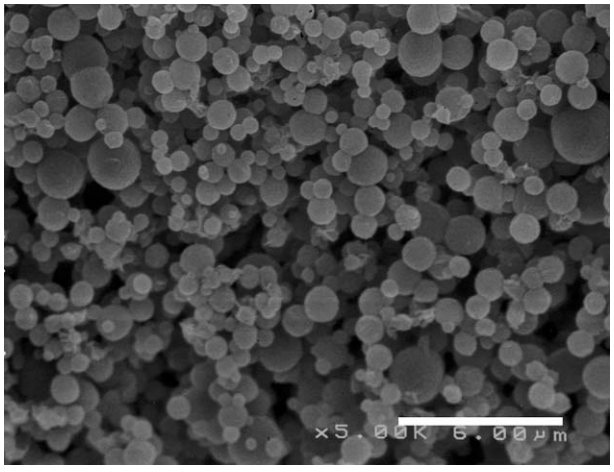

Fig. 1. A representative electron micrograph of FA:SA spheres loaded with insulin. The image was taken at 5,000×; the scale bar (in white) measures 6.0 micrometers.

The insulin percent loading was calculated by dividing the calculated experimental loading by the theoretical load and multiplying × 100 and was determined to be approximately 100%.

3.4. Insulin integrity using RP

It has been established that insulin deteriorates rapidly under acidic conditions at residue Asn A21. In neutral solutions the chemical stability of insulin is improved, but during prolonged storage significant transformation of insulin still occurs and is found to take place at residue Asn B3 [11,18].

Using RP, the formation of degraded insulin by hydrolytic reactions (deamidated products) was determined. The A21 monodesamido insulin standard eluted at 15.0 min and the B3 monodesamido insulin standard eluted at a retention time of 17.4 min. When blank p(FASA) microspheres were run, two peaks were evident. One is a large peak at a retention time of 1.5 min while the other is a small peak at a retention time of approximately 14.5 min. Neither peak interferes with the insulin peaks, and is attributed to p(FASA) degradation products. It can thus be concluded that the formation of deamidated products did not occur during the microencapsulation process.

3.5. In vitro release rate

Figs. 2a and b show the in vitro release profile for insulin from 0 to 24 h and from 0 to 12 days, respectively. After

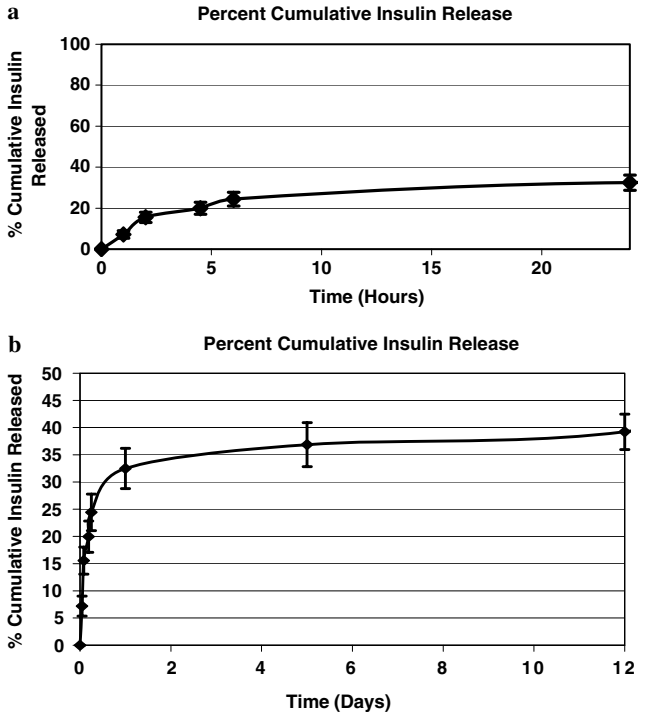

Fig. 2. (a and b) Release rate curve of the insulin containing formulation used in the experiments for 24 h and 12 days, respectively.

24 h of release, it can be seen that approximately 35% of the insulin has been released from the microspheres. After 12 days, approximately 40% of the insulin has been released. Only 40% of the insulin could be accounted for during the in vitro release; however, when the insulin was extracted from the microspheres immediately after fabrication, we were able to account for almost all the protein. It is possible that the remainder of insulin was unable to be detected by the BCA assay as the time between timepoints was too long such that the insulin had degraded in solution.

3.6. Plasma glucose levels (PGL)

Fig. 3 shows the average plasma glucose levels (PGL) over time for both the SQ control as well as the three different dosage SQ insulin microsphere experiments. It is evident that PGLs decrease similarly in all experiments regardless of dose. We obtain a linear dose–response between dose administered and PGL reduction only up to a SQ dose of 5 U/kg. The dosage administered in our experiments is larger than 5 U/kg. This may explain the nonlinear behavior we observe.

Since the insulin microspheres when administered SQ initiated a pharmacological response by decreasing the amount of glucose in the blood, we concluded that the insulin microspheres retained biological activity.

3.7. Plasma insulin levels (PIL)

Fig. 4 shows the average plasma insulin levels (PIL) over time for the control as well as the three different dosages of insulin microspheres injected SQ. In all experiments, exogenous insulin was detected in plasma samples. The AUC for the insulin curves were calculated using the trapezoidal rule and values are listed in Table 1. In the unencapsulated insulin control, a bolus of insulin was administered at time 0 resulting in an AUC of 16.06 μg h/L ± 3.03. By encapsulating the insulin, we were able to increase the AUC considerably as well as

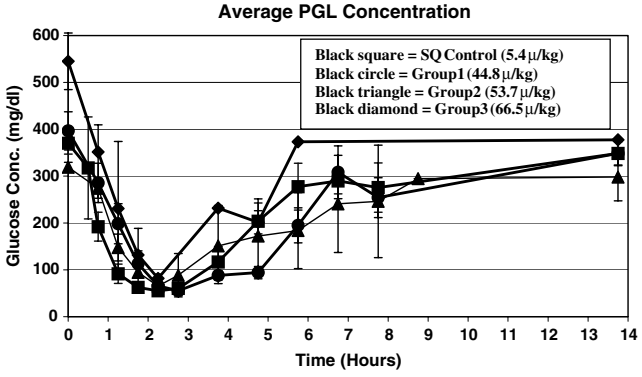

Fig. 3. Average plasma glucose levels over time for a SQ unencapsulated insulin control as well as for the SQ administration of three dosages of insulin encapsulated in p(FASA).

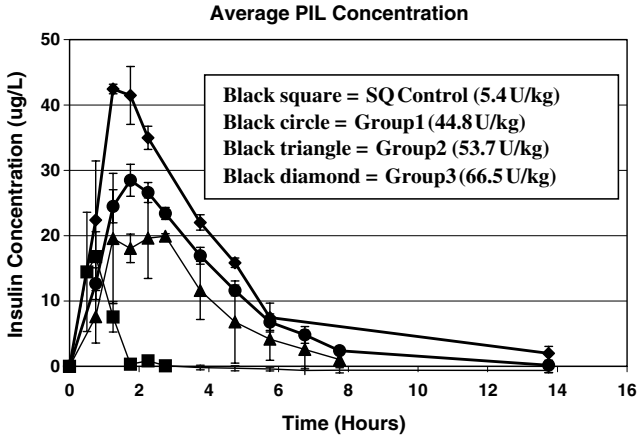

Fig. 4. Average insulin levels over time for a SQ unencapsulated insulin control as well as for the SQ administration of three dosages of insulin encapsulated in p(FASA).

increase the peak concentration, time of peak plasma level and the duration of drug activity all of which will be discussed further. Group 1 (dosage of 44.8 U/kg) resulted in an AUC of 116.35 μg h/L ± 7.43 while Group 2 (dosage of 53.7 U/kg) and Group 3 (dosage of 66.5 U/kg) resulted in an AUC of 82.43 μg h/L ± 8.63 and 179.95 μg h/L ± 8.98, respectively. Increasing the dosage of insulin administered did not result in a linear increase in the AUC.

It is interesting to note that the lowering of plasma glucose levels for the control and encapsulated insulin experiments is very similar (Fig. 3) while the plasma insulin level for the control experiment is substantially smaller than for the encapsulated insulin dosages. As was stated in the section above which discusses PGL, we state that we obtain a linear dose–response between dose administered and reduction of PGL only up to 5 U/kg. This nonlinear behavior may explain the discrepancy we observe between the PGL and PIL data.

3.8. Peak concentration, C_P

Table 1 shows average peak concentration comparisons for the control and for Groups 1, 2, and 3. The average C_P increased in all three SQ insulin microsphere dosage groups compared to the control. C_P for Group 1 averaged approximately 9 μg/L higher than the control while the C_P for Group 2 and Group 3 averaged 14.5 and 26 μg/L higher than the control, respectively. These data indicate that the higher the dose of insulin microspheres administered, the higher the peak concentration.

3.9. Time of peak plasma level, T_P, and duration of drug activity, D

Table 1 shows average time to peak comparisons between Groups 1, 2, and 3 and the control. The average T_P occurred later in all three SQ insulin microsphere

Table 1
Pharmacokinetic values obtained for SQ delivery of unencapsulated insulin and insulin containing microspheres to type 1 diabetic rats

	Dose (U/kg)	PIL				PGL			RB
		T_P (h)	C_P (μg/L)	D (h)	AUC (μg h/L)	T_L (h)	C_L (%)	D (h)	
Control									
SQ Micronized insulin (5–5.7 U/kg)									
Rat 1	5.2	0.75	10.6	2	9.2	1.75	9.6	7.75	100
Rat 2	5.6	0.75	16.792	1.75	16.8	2.25	8.7	*	100
Rat 3	5.4	0.5	20.91	3	23.8	2.75	10.6	5	100
Rat 4	5	1.25	6.429	2.75	6.4	2.75	16.6	*	100
Rat 5	5	0.75	18.199	1.25	10.8	2.75	9.8	*	100
Rat 6	5.3	0.75	9.532	3.75	12.2	2.75	12.8	12	100
Rat 7	5.7	1.25	21.858	3	22.3	1.75	14.6	6	100
Rat 8	5.7	0.75	41.603	3.75	32.6	1.75	10.8	*	100
Rat 9	5.4	0.75	10.915	2.75	10.4	2.75	18.9	14	100
Average	5.37	0.83	17.43	2.67	16.06				100
Standard Error	0.10	0.09	3.73	0.30	3.03				
Group 1									
SQ insulin containing microspheres (44–46 U/kg)									
Rat 1	42.9	1.75	39.935	12.75	142.7	2.75	8.128	11	
Rat 3	44	2.25	29.563	13	102.8	2.25	13.9	*	
Rat 4	45	2.25	30.279	13.75	98.4	2.75	22.3	8	
Rat 6	46	1.25	33.737	13.75	131	2.25	14.7	12	
Rat 7	46	2.25	23.056	13.75	101.7	2.75	15.9	*	
Rat 9	45	1.75	28.858	14	121.5	2.75	8.5	*	
Average	44.82	1.64	26.49	11.57	116.35				86.78
Standard Error	0.54	0.18	2.51	0.22	7.43				
Group 2									
SQ insulin containing microspheres (51.7–56 U/kg)									
Rat 2	53.5	1.25	46.9	13.75	93.9	2.25	28.4	5.75	
Rat 5	56	1.25	26.616	9	69.6	2.75	17.3	*	
Rat 8	51.7	2.75	20.178	13.75	83.8	2.75	14.4	6.5	
Average	53.85	2.00	23.40	11.38	82.43				51.17
Standard Error	3.04	1.06	4.55	3.36	8.63				
Group 3									
SQ insulin containing microspheres (63–70 U/kg)									
Rat 7	63	1.25	41.929	14	173.6	2.25	12.8	*	
Rat 8	70	1.75	44.604	13.75	186.3	2.25	17.5	*	
Average	66.5	1.5	43.267	13.88	179.95				90.45
Standard Error	4.9	0.4	1.9	0.2	8.98				

* Denotes duration exceeded experimental time-frame.

dosage groups as compared to SQ unencapsulated insulin control. T_P for all Groups averaged just less than 1 h later than the control group. These data demonstrate that the maximum PIL concentration occurred substantially later in SQ insulin microsphere experiments than in SQ unencapsulated insulin experiments. There was no difference between the T_P values between the three SQ experimental groups. This is an important point since the time to peak for all formulations were the same, indicating the reproducibility of the delivery system to release insulin in vivo.

Table 1 shows average duration of drug activity (D) comparisons between Groups 1–3 and the control group. The average duration was longer in all three SQ insulin containing dosage groups compared to the SQ unencapsulated insulin control. The duration for Group 1 averaged almost 9 h longer than the control group, the duration for Group 2 averaged 6.5 h longer and the duration for Group 3 averaged more than 11 h longer. Interestingly, in every microsphere experiment (in all dosage groups) the PIL was still slightly above zero at the final time point of the night (14 h after administration). This indicates that all but one microsphere experiment had a plasma insulin curve that lasted 7.75 h or longer. On the other hand, not a single unencapsulated insulin experiment had a plasma insulin curve that lasted longer than 3.75 h.

The increased duration of drug activity in the insulin microsphere dosage groups in comparison to the control demonstrates that the controlled release of insulin was achieved in that the insulin was released more slowly in the encapsulated insulin experiments. As the plasma half-life of insulin is approximately 4.5 min, 2 h after the unencapsulated insulin is administered, there is no evidence of it in the plasma. For the encapsulated insulin experiments, insulin is detected in the plasma up to almost 14 h in the plasma in several of the rats. This finding is of utmost importance and demonstrates the ability of the micro-

spheres to be present and to release insulin for long periods of time while in the body.

The increased T_P and D in insulin containing microsphere experiments are important characteristics of our drug delivery system. In control experiments, a bolus of insulin is injected into the body where it enters into circulation and bombards the tissues at a rate faster than these tissues can process the insulin. Consequently, tissue receptors quickly become saturated and the remaining unbound insulin is rapidly degraded by virtue of its short half-life and degradative enzymes in the body [19]. This results in an average T_P that occurs a mere 50 min following the SQ injection, and an average D that only lasts 2.5 h (see Table 1). These T_P and D values imply that unencapsulated insulin has an extremely high rate of absorption for a very brief period of time at the start of the experiment after which, the insulin has been taken up or degraded and is no longer present in the body for the remainder of the experiment. The inherent disadvantage with this type of unencapsulated insulin is that the short insulin duration fails to provide insulin for controlled glucose regulation throughout the entire experiment and is therefore unable to maintain basal plasma glucose levels past the initial hours of the experiment. In this respect, SQ delivery of unencapsulated insulin fails to mimic the normal physiological insulin secretion. Additionally, excessive exposure of tissues and muscle to injected insulin is associated with a plethora of diabetic complications.

In contrast, the insulin containing microspheres degrade slowly over a period of several hours, thereby providing closely controlled insulin diffusion throughout the majority of the experiment. The implications of such a system can be seen in Groups 1, 2, and 3. These experiments have average T_P values ranging from 1.5 to 1.8 h and the D in all but one trial was greater than 7.75 h (see Table 1). These data show that insulin is absorbed at a slower rate for a longer duration in microsphere experiments. The major benefit of this system is insulin is presented to tissue receptors at a constant employable rate, as opposed to pure insulin injections where an overload of insulin results in a high degree of insulin degradation. This prevents excessive tissue and muscle exposure to insulin and makes insulin available to regulate glucose levels in the body for a substantially longer time than SQ unencapsulated insulin administrations. Therefore, this type of release more closely resembles the body's normal physiological processes whereby insulin is constantly secreted to keep plasma glucose at a basal level.

3.10. Relative bioavailability (RB)

Table 1 and Fig. 5 show average RB comparisons between Groups 1–3 and the control group. RB is a measure of bioavailability as compared to our recognized standard of unencapsulated insulin administered subcutaneously (RB for the control group always equals 100%). RB was calculated after determining the area under the plasma insulin curves (AUC) using the trapezoidal rule.

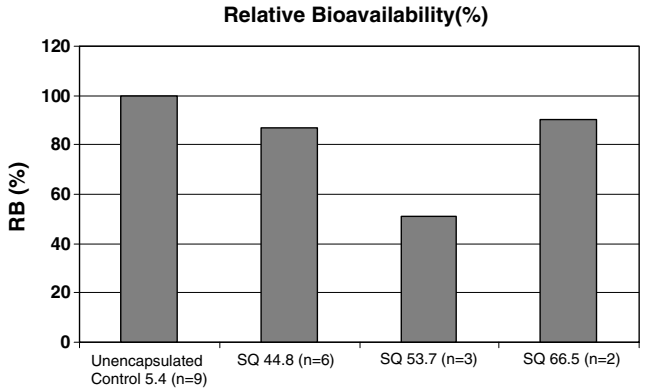

Fig. 5. Average relative bioavailability data a SQ unencapsulated insulin control as well as for the SQ administration of three dosages of insulin encapsulated in p(FASA).

Fig. 5 shows that average RB was close to 100% for Groups 1 and 3 and 51% for Group 2. Yet, one must keep in mind that in humans, relative bioavailability (SQ) is approximately 70% that of intravenous administration (IV); thus, our estimated absolute bioavailability for Groups 1 and 3 would be about 61–63%, while our estimated absolute bioavailability for Group 2 would be about 36%.

4. Conclusions

After insulin encapsulation by p(FASA), we effectively altered the onset, peak, and duration of insulin action after SQ injection to type 1 diabetic rats. This work has importance in that we demonstrated that insulin remains bioactive after the encapsulation process.

Acknowledgement

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