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Pharmacokinetics of Porcine Somatotropin Liposomes

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ABSTRACT

This paper focuses on the effects of pharmacokinetics in fattening pigs as well as changes in serum resulting from the use of Porcine Somatotropin (PST) and its liposomes. A slow-release model was determined to be relevant following an examination of all dynamic parameters. The results indicated that the slow-release effect of PST liposomes was significant, with an extended release time of over seven days.

Key Words: Porcine somatotropin (PST) liposomes; Pharmacokinetics; Slow-release

INTRODUCTION

PST belongs to a biologically active peptide hormone. Porcine somatotropin (PST) increases the protein syntheses and decreases the grease deposition mainly through adjusting nutrient substance's distribution in muscle and fatty tissue. It is a new and ideal growth promoter and carcass quality improver.

It is difficult to obtain a good result when PST is used because its half-life *in vivo* is short. To increase the dose and the frequency of PST will bring about some inconvenience. Liposomes are regarded as a multi-function medical carrier, one of the great characteristics of liposomes is sustained-release and long-run effects, and their half-life of medicine in vivo can be efficiently extended (Qineng Ping, 1998).

Research from molecular biology has shown that porcine somatotropin (PST) can increase IGF-I level (insulin growth factor-I) in tissues. The main cause is that PST can promote liver and muscle action to produce IGF-I. PST helps enforce the transcriptional regulation of IGF-IDNA to IGF-ImRNA, producing more IGF-I to accelerate cytodifferentiation, protein synthesis, and muscle growth. In addition to liver cells, many other tissues (skeletal muscle, adipose tissue, & primitive fatty cells) are able to synthesize IGF-I via self-secretion or side-secretion mechanisms under stimulation from PST. Local IGF-I may play a major part in the growth of bone and gristles, with PST having a direct effect on targeted tissues in IGF-I-dependent circumstances. Some studies have indicated that PST inhibited fatty-acid synthetase via the transcription of IGF-ImRNA in fibrous hepar and adipose tissues (Mildner & Clarke, 1991; Clarke *et al.,* 1990, 1990a; Clarke, 1993; Harris *et al.,* 1993).

The purpose of this study was to examine the metabolic rules of PST liposomes in piglets and offer a theoretical basic for the application of PST liposomes according to animal patterns.

MATERIALS AND METHODS

PST liposomes preparation. PST liposomes were prepared with reverse evaporating Lecithin (East China Normal University factory provided) cholesterol (Biological Agent Factory .Shanghai) and octadecane (Fluka) at 7 mol: 2 mol: 1 mol then transferred to a round flask. PST was added to 25 mL PBS (phosphate buffer 0.01 mol/L) then transferred to the flask. The mixture was subjected to ultrasonic waves for 5min (intensity 40%, at intervals 1s), then decompressed evaporating (38) to gum or membrane state. 25 mL PBS (0.01 mol) was used to wash down the membrane completely. Decompress evaporation (38) was done to remove all ether at 3000 rpm, with 5 min centrifuge to eliminate dissociating drug, then stored after filling with nitrogen.

Animals and PST. Eighteen Taihu two-way cross boars each weighing 20 kg were chosen for testing. They were allowed free access to food and water for 7 days prior to use in the experiments. The pigs were randomly divided into three groups: A) a controlled group (n=6), B) a trial group I (n=6), C) a trial group (n=6). PST solids were brought into 1 mg/mL solution with asepic super pure water. The PST liposomes were taken for radiant sterilization using Co^{60} for 24 h to make sure the liposomes were germ-free, with a final PST concentration of 7.03 mg/mL.

Experimental procedures. For testing, all pigs from Group B were injected with 4 mL PST liquid in neck muscles at 8:00 every day. Pigs in Group C were injected with 28 mg PST liposomes every week throughout the experiment. Following the first injection, the blood was sampled using approximately 2 mL from auricle veins in all pigs. The sampling schedule for the first two days after injection occurred at 1, 2, 3.5, 5.5, 7.5, 10, 16, and 20 h. On the 3rd day, blood was sampled from group C four times, with blood sampled twice each day in the following 4 days. On the 14th day, blood was sampled from all groups at 8, 12, 16, and 20 o'clock. The blood samples were centrifuged at 3000

rpm for 5 min to separate the blood plasma.

Concentration of PST and IGF-I in blood plasma. A radio immunological kit was used to detect PST levels in the blood plasma. For determination of IGF-I**,** 0.1 mL blood plasma was added to 0.9ml alcohol acid (21.6 mL HCl, 103.4 mL twice-distillated H_2O, 875 mL water free ethanol for 1L Solution). The solution was incubated at ambient temperature for 30 min and centrifuged at 3500 rpm at 4^0C for 30 minutes, with the upper 0.2 mL transparent liquid plus 0.1 mL Tris Base fluid extracted. (0.855M Tris Base; 103.54 g Tris resolved into 1L twice distilled H_2O, pH11.0). It was then incubated at ambient temperature for 30min prior to centrifuging (30 min, 3500 rpm, 4^0C). Following this, we again took the upper transparent liquid plus 0.8 mL PBS buffer solution and mixed it up. The extraction of alcohol acid was then taken to the radio immunity box for RIA analysis (double antibody methods): adds PBS, ^{125}I-IGF-I, Ab1, Ab2, into the extraction treated with alcohol acid and sequentially incubated 18-24 h and 16-18 h, respectively. Finally, the concentration was determined with a radio immunological kit and counting recorder to work out the IGF-I level (mg/mL) for each sample.

Statistical analyses. Data obtained from the study were analyzed using 3P87software, and analyzed with the Pharmacokinetic Parameters Estimated procedure.

RESULTS AND DISCUSSION

From Fig I, two sharp peaks can be seen in Group A and Group B during a single day. PST pulse release of group B lapped on the basic of group A, while the PST concentration in blood plasma of group B rose immediately after injection, coinciding with past research. The PST concentration of Group C rose gradually on the first day and reached the peak at 24 o'clock, expressing the fast-release phase of liposome. It decreased gradually until the 3rd day, where it reached a level equal to group B, and remained this level during the next 7 days (demonstrating the slow-release span of PST liposome is at least 7 days).

The concentration-time curve of PST in blood plasma was analyzed with 3p87 software. Comparing the three cellular models (1, 1/ C, 1/C/C), there were no apparent differences ($P>0.05$), with the AIC (Akaike Information Criterion) value of 1/C/C the least. The lower AIC value, however, was close to the actual release pattern (Ling & Qi Hui, 1996; Xiao *et al.*, 1998; Wang & Li, 1999; Chen *et al.*, 2000; Wei *et al.*, 2000), indicating a quick equilibrium was obtained in the body. Thereafter, the concentration of drug in the blood plasma decreased at single-phase.

Table I shows metabolic parameters of PST liposomes where the absorption half-life period of PST liposomes is only 0.3 h and reaches to the peak 0.84 h later, and the declining half-life period is 36.93 h; far longer than 7min previously reported. In drug delivery, the area under the curve can reach 215.2 ng/mL per hour.

Analyses of the mechanism parameters. After injection, PST liposome is released quickly, reaching a peak fairly soon; which is the typical fast-release phase? The declining phase half-life is typical slow-release, so the release of PST liposome in the body experienced fast-release phase and a slow-release phase. Usually, the drug is released at rate "I" (drug-transship or transform by fixed percent in unit time). If drug declines at rate "I," the removal of the drug will show a direct ratio with the amount in the body at that time. Release rate of this test according with rate "I" had fast-release phage.

IGF-I value in blood plasma. Following the first day's injection, the IGF-I of Group C increased remarkably, reaching a peak 6 h later. In comparison with group A, however, there were no differences on the first day. On the second day, it began to rise, reaching a peak at 4:00A.M. On the 3rd day, IGF-I in blood plasma didn't rise for 12 h, since the PST in blood plasma had risen, possibly indicating that the concentration of IGF-I in the blood plasma changes with time and is somehow connected with PST. IGF-I Concentration-time diagram after injection in two days was shown on Table II and Fig. 2.

On the 7th day, the IGF-I concentration in the blood plasma of Groups B and C were quite a few times greater than that in Group A. However, IGF-I concentration in blood plasma of group C on 14th day had declined significantly.

Metabolic mechanism pattern. The PST liposome used in this study has both fast- and slow-release phases controlled by release rate "I." The pattern of metabolic mechanism is described in the following equation:

Table I. Metabolic parameters of PST liposomes

Parameter	Unit	Value	S.E.
A(drug concentration)	ng/ml	4.07655	0.00000
Ke(elimination constant)	1/hr	0.01877	0.00000
Ka(absorption constant)	1/hr	2.27577	0.00000
Lag time	hr	0.84087	0.00000
$T1/2_{(Ka)}$(absorption half life)	hr	0.30458	
$T1/2_{(Ke)}$(elimination half life)	hr	36.92899	
T(peak)	hr	2.96662	
C(max)	ng/ml	3.88479	
AUC(area under curve)	(ng/ml)*hr	215.39610	
CL/F(s)(clearance)	mg/hr/(ng/ml)	0.12999	
V/F(c)(apparent volume distribution)	of (mg)/(ng/ml)	6.92568	

Table II. IGF-I content in different time between groups (ng/ml)

	The 7th day				The 14th day			
	8:00	12:00	16:00	20:00	8:00	12:00	16:00	20:00
Group A	36	61.5	50.5	59.5	40	45	55	58
Group B	199	275	184.5	147	121	284	193.5	192
Group C	143	486	146	173	27	81	30	60

Fig. 1 Changes of PST in blood plasma with time; A——group A, B——group B, C——group C

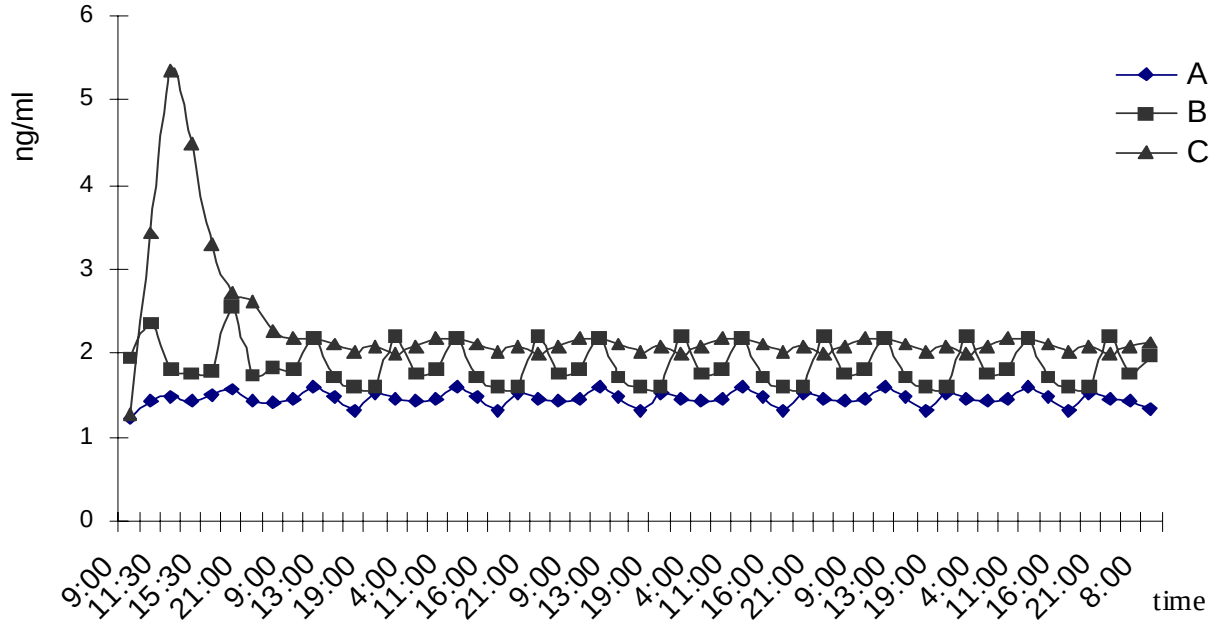
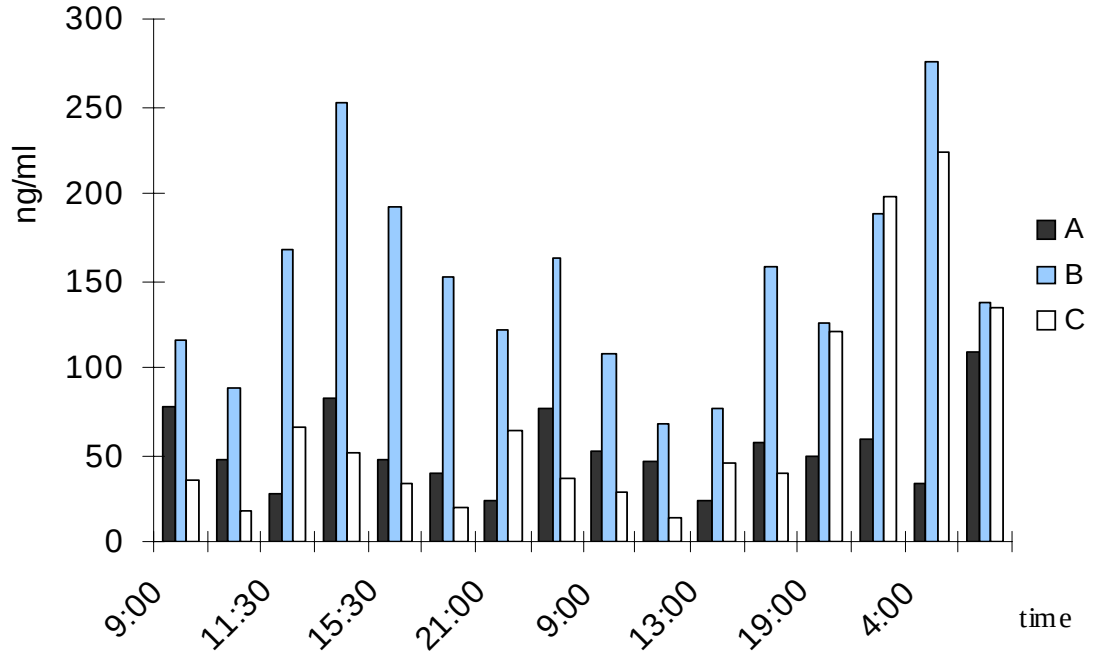


Fig 2. IGF-I Concentration-time diagram after injection in two days; A——group A, B——group B, C——groupC



$$C = FK_a D_f \frac{e^{-kt} - e^{-kat}}{K_a - K} V + FK_{r1} D_s \frac{e_{-kt} - e^{kr1t}}{K_{r1} - K} V$$

Where F: Bioavailability, K_a: absorption rate constant, K: Removing rate constant, K_{r1}: Constant of rate I release, V: Apparent Scattered Space, $D_{F:}$ fast-release dosage, Ds: Slow-release dosage

And $K_{r1} = Ke^{-K}$ t_{max}^{s}; $D_s = KD_b/K_{r1}$; t_{max}^{f}=2.303LgK_a/K/(K_a-K); $D_f=D_b-D_s \cdot K_{r1} \cdot t_{max}^{f}$; $D_0=D_f+D_s$; T_{max}^{s} : moment of slow-release peak; Db: depending on materials or studies before design; T_{max}^{f}: moment of fast-release peak; Do: whole dosage in body

($V=F \cdot D_0/K \cdot AUC$)

AUC: Area under the curve responsing absorption degree of drugs.

CONCLUSIONS

We carried out a one-time drug injection in muscle, and built a pattern of release rate "I" in pigs using PST liposome. The drug release process was divided into two phases: fast-release and slow-release. Compared with earlier research, the declining half-life-period of PST liposome in animals had a prolonged time span of 37 h, while the existing time of PST was 7 min with a slow-release span beyond 7 days. As such, increasing levels of PST in pig bodies raises the level of IGF-I. The analysis indicates that PST liposomes (slow-release doses) are effect with a slow-release span over 7 days.

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