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Pharmacokinetics and tissue levels of a sustained-release recombinant porcine somatotropin in pigs

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돼지에서 서방형 성장호르몬의 약물동태 및 조직잔류성

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초 록 : 서방형 돼지성장호르몬(sustained-release formulation of porcine somatotropin, PST-SR)을 1주 간격으로 6차례 피하 및 근육주사하고 혈액과 조직중의 돼지 성장호르몬(PST)과 insulin-like growth factor 1(IGF-1)의 농도를 측정하여 다음과 같은 결과를 얻었다. 대조군의 혈중 PST와 IGF-1의 농도는 각각 2.41과 95.2 ng/ml 이었다.

1. PST-SR을 투여한 후 PST의 혈중농도는 8시간만에 최대에 도달하여(30 ng/ml) 곧 감소하였다. 혈중농도 반감기(decay half life)는 91~227시간이었다. IGF-1의 혈중농도는 투여후 12시간에 최대에 도달하였으며(165 ng/ml), 이후 서서히 감소되었고 반감기는 77~99시간이었다.

2. 혈중 PST 농도-시간의 자료는 제재에서 PST가 유리되는 과정에는 두단계 즉, 투여후 24시간까지의 유리속도가 빠른 단계와 그 이후의 유리속도가 느린 단계가 있음을 보여주었다.

3. 여섯번의 반복투여기간에는 PST의 혈중농도는 투여직후 증가하여 24시간 이후 다음 투여전까지 지속적으로 감소되는 패턴이 반복되었고, 최종투여후 1주일경에는 정상수준으로 회복되었다. 반면에 투여가 반복됨에 따라 매 투여직후의 PST의 혈중 최고치는 다소 증가되는 경향을 보였다(20~40 ng/ml). IGF-1의 혈중농도는 투여가 반복됨에 따라 누적적인 증가현상이 뚜렷하였으며, 이후 2주일후 까지도 정상농도보다 높게 유지되었다(200

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ng/ml). 임상용량 투여군에서 PST 및 IGF-1의 혈중농도는 투여경로에 따른 차이는 나타나지 않았다.

4. 최종(6번째) 투여후 6, 8, 10, 14일에 조사한 간장, 신장, 소장, 근육, 지방 및 주사부위의 조직중의 PST 농도는 6일째에 이미 대조군 수준으로 회복되었다. IGF-1의 경우 최종투여후 6일에는 간장, 신장, 소장, 지방조직에서 정상보다 높은 농도로 잔류하나 이후 14일까지 모두 대조군 수준으로 감소되었다.

5. 이상의 결과는 본 실험에서 사용된 서방형 PST 제제는 최소 1주간 유효성이 유지되며, 동시에 PST는 투여 6일째에, IGF-1은 투여후 14일에 정상수준으로 회복됨을 보여주고 있다.

Key words : recombinant porcine somatotrophin, IGF-1, pharmacokinetics, tissue residues.

Introduction

The growth promoting effect of porcine somatotropin (PST) was known as early as in 1955[1]. Since then, many reports have confirmed the early findings and potential benefit of PST in pig industry[2-7].

With the advent of molecular biological techniques, it has been now possible to produce a large amount of recombinant PST (rPST) and shown that the effect of rPST is not qualitatively different from natural PST[5,8]. It is also known that rPST improves the growth rate and the feed efficiency of treated pigs and reduces carcass fat[9,10], but rPST has little effect on the processes of pregnancy, paturition and other reproductive functions[11]. Although the mechanism of anabolic effect of PST is not clearly elucidated, it seems to be that insulin-like growth factor 1 (IGF-1) is one of closely related mediators of PST action as in other species[5,12,13].

PST in normal injection formulation should be administered at least once a day because of its short biological half-life[14,15]. To overcome such inconvenience, the sustained-release preparations of PST (PST-SR) were recently introduced[16,17] and it was shown that PST-SR was effective in promoting the rate and efficiency of lean growth as long as 42 days after single treatment.

In this work, we present the pharmacokinetic profiles and the residual PST and IGF-1 levels in 6 edible tissues after single or repeated treatment with a sustained-release formulation of rPST (LB00006), which was recently developed by LG Chemical Ltd.

Material and Methods

LB00006, a sustained-release formulation of rPST designed to be effective for 1 week, was provided by LG Chemical Ltd (Taejeon, Korea). Each dose of LB0006 contains 100~125mg of rPST. Clinically heathy gilts and barrows of crossbreeds originating from Landrace-Yorkshire raised in Teaching Farm of Yonam College of Animal Husbandry & Horticulture were used (body weight : 62 ± 4.2kg, mean$\pm$ SD). Eight pigs were housed in each hogpen ($3 \times 5m^2$) and allowed to freely access to feeds (Daehan Jedang) and water under natural day-night shift.

Eighteen pigs, for pharmacokinetic experiments, were randomly assigned to three treatment groups ; SC group treated subcutaneously with a clinical dose (100~125 mg/head), SC2 group treated subcutaneously with twice of a clinical dose and IM group treated intramuscularly with a clinical dose. The clinical dose of rPST was determined based on the optimun daily dose (100 μg/kg/day), and the degradation rate during 7 days of a dosing interval[18]. For the evaluation of residual PST in tissues, 64 pigs were randomly assigned to 4

groups : control, SC, SC2 and IM. Four pigs in each group were sacrificed at the day 6, 8, 10, and 14 after the last treatment. PST-SR was implanted into the neck area subcutaneously in SC and SC2 groups and intramuscularly (Cleidocephalicus muscle) in IM group. The pigs were treated once a week with 100 mg per head for the initial 3 weeks and 125 mg per head for the later 3 weeks.

Blood samples for pharmacokinetic study were taken at the times of 48, 24, and 1 hour before, and 0.25, 0.5, 1, 2, 3, 4, 6 and 7 days after administration of PST-SR. For the evaluation of tissue residues, PST-SR was administered once a week for 6 weeks. Blood were sampled at 48, 24 and 1 hour before the first administration, on 2, 4 and 7 days after following each administration, and on 2, 4, 6, 8, 10 and 14 days after the final administration.

Blood samples (5 ml) were collected via venifuncture of the jugular vein into heparinized syringes with 24G needle. Plasma was separated within 30 minutes by centrifugation for 10 min at 3,000 rpm. Plasma from each animal was transferred into two small tubes (1 ml). Animal name and sampling time were labelled on each tube. Those samples were stored at -20℃ until the assay. Tissues (~50 g) of muscle (hind leg), injection site, liver, kidney, small intestine and fat (abdominal) were also sampled at 6, 8. 10, and 14 days after the final treatment, labelled and stored at -20℃ until the assay.

PST and IGF-1 levels were determined by the radioimmunoassay of Choi *et al*[19]. The slope factor that indicates the sensitivity of procedures was 1.167±0.268 ng/ml (n=12) for PST and 0.930±0.141 ng/ml (n=15) for IGF-1 determined by the "four parameter logistic equation" of De Lean *et al*[20]. To evaluate the detection limit of method, the tissue samples containing known amount of PST or IGF-1 were serially diluted by 7, 14, 28, 56, 224, 448, 896, 1792 and 3584 times and PST or IGF-1 levels in diluted samples were assayed. Then, we took the lowest levels that keep the correlation (or linearity) between the measured and the expected concentrations. The detection limit for PST was in the range of 0.17~0.25 ng/gm (average, 0.204±0.028) and that for IGF-1 was in the range of 0.23~0.51 ng/gm in different tissues (average, 0.295±0.096 ng/gm). The coefficient of variance that reflects reproducibility of the assay was 5.80±2.54% (n=20) for PST and 7.1±2.85% (n=21) for IGF-1. The recovery rate of PST or IGF-1 from tissues ranged 71~79%.

To analyze plasma-concentration time profile, we used the following equation considering absorption and elimination processes,

$$y = a*\alpha / (\alpha-\beta) * (e^{-\beta*t} - e^{-\alpha*t}) + y_o \text{ ---------- } (1)$$

Where 'a' is a constant representing F*Dose/Vd, and F, Dose and Vd are bioavailability, amount of drug administered and volume of distribution of the drug, respectively[21]. Parameter 'α' is an initial absorption rate constant and 'β', elimination rate constant. Parameters 'y and y_o' are measured and background plasma levels of PST or IGF-1, respectively. Decay half life was obtained from the relation $t_{1/2} = 0.693/\beta$. Clearance and volume of distribution were not attempted to obtain because of the nature of slow-release formulation. Area under the curve (AUC) was obtained by linear trapezoidal extrapolation method of Rowland and Tozer[21]. The normal levels of PST and IGF-1 were not included in this calculation. Area under the first moment of the concentration-time curve (AUMC) was obtained by integrating the curve obtained by multiplying concentration-time data and time. Mean residence time (MRT) was obtained by dividing AUMC with AUC. Actual non-linear curve fitting and integrations were done by using a graphic software, Origin (Ver 4.1, Microcal Software, Inc. Northampton, MA 01060 USA). Statistical significance was determined by *t*-test at p values less than 0.05.

Results

Pharmacokinetic properties of PST-SR : Normal plasma levels of PST and IGF-1 in pigs (60 kg BW) were 2.41±1.50 ng/ml (mean±SD, n=29) and 95.2±4.1 ng/ml (n = 36). Subcutaneous implantation of PST-SR increased plasma PST and IGF-1 levels above their control levels (Fig 1). Fig 1A illustrates that plasma PST levels rapidly increased and reached to the peak at 8 hour (30 ng/ml) after the treatment and then slowly decreased afterwards. Decay half-lives ob-

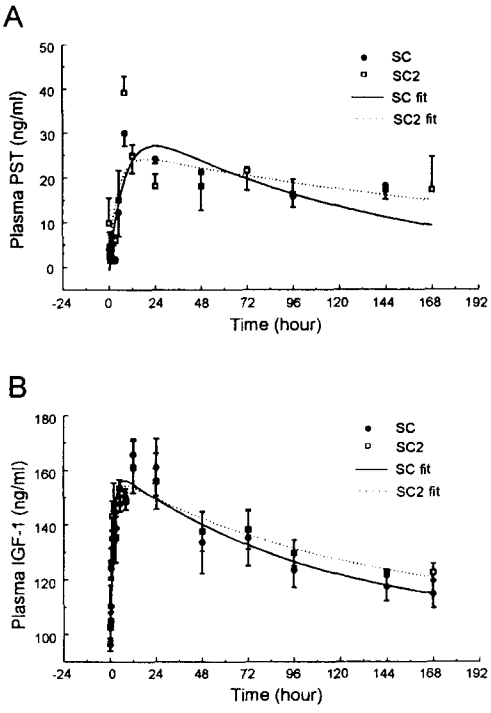

Fig 1. Plasma concentration-time profile of PST (A) and IGF-1(B). Sustained-release preparation of PST (LB00006) was injected subcutaneously at two doses, 100 (SC) and 200mg/pig (SC2). Lines are drawn by the best-fit parameters of equation (1) described in Materials and Methods for SC (solid line) and SC2 (dotted line). Symbols and bars are averages of 4-6 measurement and standard error of mean, respectively.

tained by nonlinear regression as described in Materials and Methods were 91 and 182 hours for the groups of SC and SC2, respec-tively (Table 1). In one week after the treatment, the plasma PST levels in SC group returned to the baseline level, while those in SC group remained higher than the normal level. The solid and dotted lines drawn by the equation (1) with the best-fit parameters obtained by a curve fitting routine were reasonably well simulating the measured data points except the peak values. We also tried to fit the data shown in Fig 1A and 1B with an equation containing two exponentials, but the results were not better than the model described in the equation (1). Plasma concentration-time profiles of IGF-1 shown in Fig 1B was similar to those of PST. However, the time to peak (165 ng/ml) of plasma IGF-1 occurred at 12 hours after the treatment which was slower than PST by 4 hours. Decay half-life of plasma IGF-1 was 75 and 90 hours in SC and SC2 groups, respectively (Table 1).

Fig 2 shows the plasma concentration-time profiles of PST and IGF-1 of IM group. The overall trend was similar to those of group SC and SC2 although the average decay half-life of PST in IM group appeared larger than those of other two groups.

A common feature of Figures 1A and 2A was a biphasic decay pattern of plasma PST levels showing initial large increase and later small increase. Fig 3 illustrates three typical

Table 1. Pharmacokinetic parameters for sustained-release formulation of PST

	Plasma PST			Plasma IGF-1		
	SC	SC2	IM	SC	SC2	IM
α (h^{-1})	0.13	0.22	0.16	0.60	0.56	0.98
t_{max} (h)	8	8	8	12	12	12
β (h^{-1})	0.076	0.0038	0.0026	0.0093	0.0077	0.0070
$t_{1/2}$ (h)	91	182	227	75	90	99
y_o (ng/ml)	-0.69	2.39	0.89	103	107	98
x^2	31	49	19.4	61	63	52
AUC (ng/ml/h)	3,084	3,093	2,437	5,041	4,725	2,437
AUMC (ng/ml/h^2)	231,445	250,624	206,311	308,863	307,307	206,311
MRT (h)	75.0	81.0	84.7	61.3	65.0	84.7

α, initial absorption rate constant; t_{max} to peak plasma concentration; y_o, background level of PST (porcine somatotropin) or IGF-1 (insulin-like growth factor 1); β, elimination rate constant; AUC, area under the concentration-time curve; AUMC, area under the first moment of the concentration-time curve; MRT, mean residence time; x^2, parameter for evaluation of goodness of the fit.

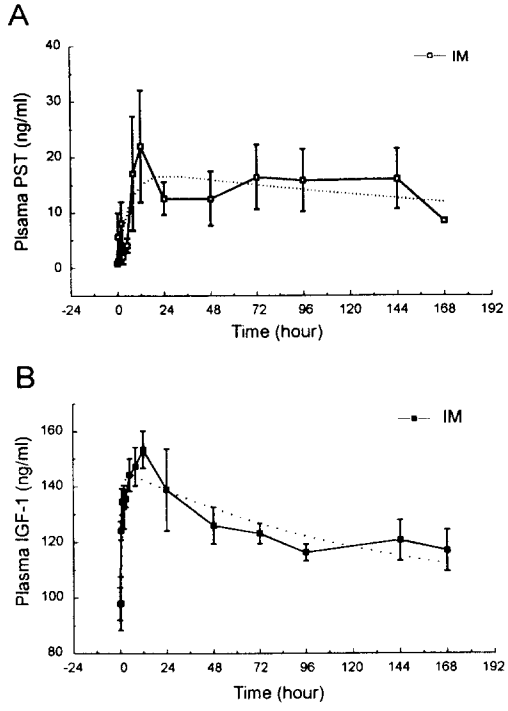

Fig 2. Plasma concentration-time profiles of PST(A) and IGF-1(B). LB00006 was injected intramuscularly at t = 0. The dotted lines in A and B were drawn by the best fit parameters to equation (1). Other conditions are similar to those in Fig 1. Note that PST levels is temporarily lower during 24~48 hours after the administration.

data sets showing such a trend, a rapid initial increase and decrease during the initial 24~48 hours and a slight increase and sustaining at a steady level during 48~144 hours after the treatment. This pattern of plasma concentration-time profiles of PST indicate that there are two phases in the release of PST from the formulation, initial rapid and later slow phases.

Table 1 is a summary of various pharmacokinetic parameters. The decay half-lives of PST (91~227 hours) after the implantation of PST-SR are much larger than those of daily injection formulation which is less than 1 hour[14,15], indicating the sustained nature of the preparation used in our study.

PST and IGF-1 concentrations during repeated administration : Fig 4 illustrates the plasma levels of PST and

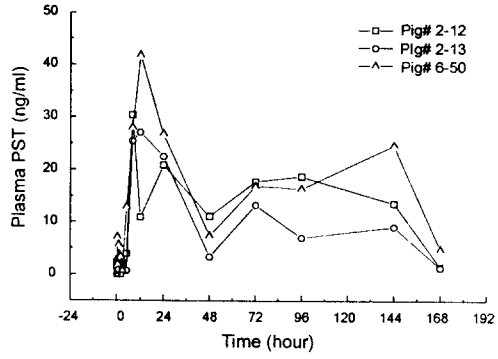

Fig 3. Typical plasma concentration-time profiles of PST showing two phases of increase in plasma PST. PST (100 mg/pig) was administered subcutaneously at time zero.

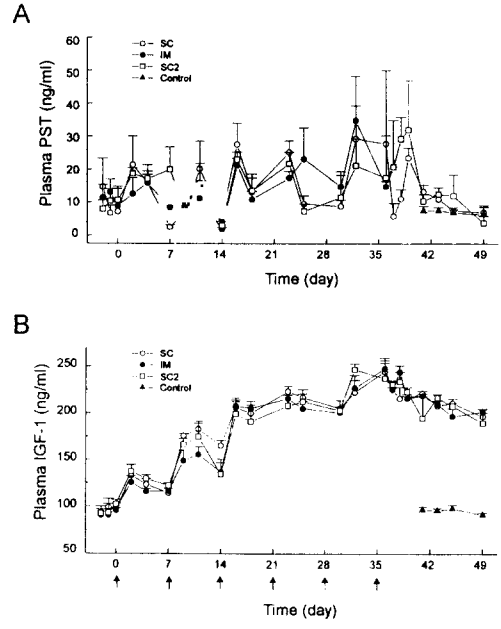

Fig 4. Plasma concentration-time profiles of PST(A) and IGF-1(B) after repeated administration of PST. PST was administered once a week on day 0, 7, 14, 21, 28, and 35 for 6 weeks (marked by arrows). SC: the group treated subcutaneously (100 mg/head), SC2: the group treated subcutaneously with (200 mg/head), IM: the group treated intramusculary (100 mg/head).

IGF-1 in response to consecutive administrations of PST-SR at 1 week intervals. PST levels increased after each ad-

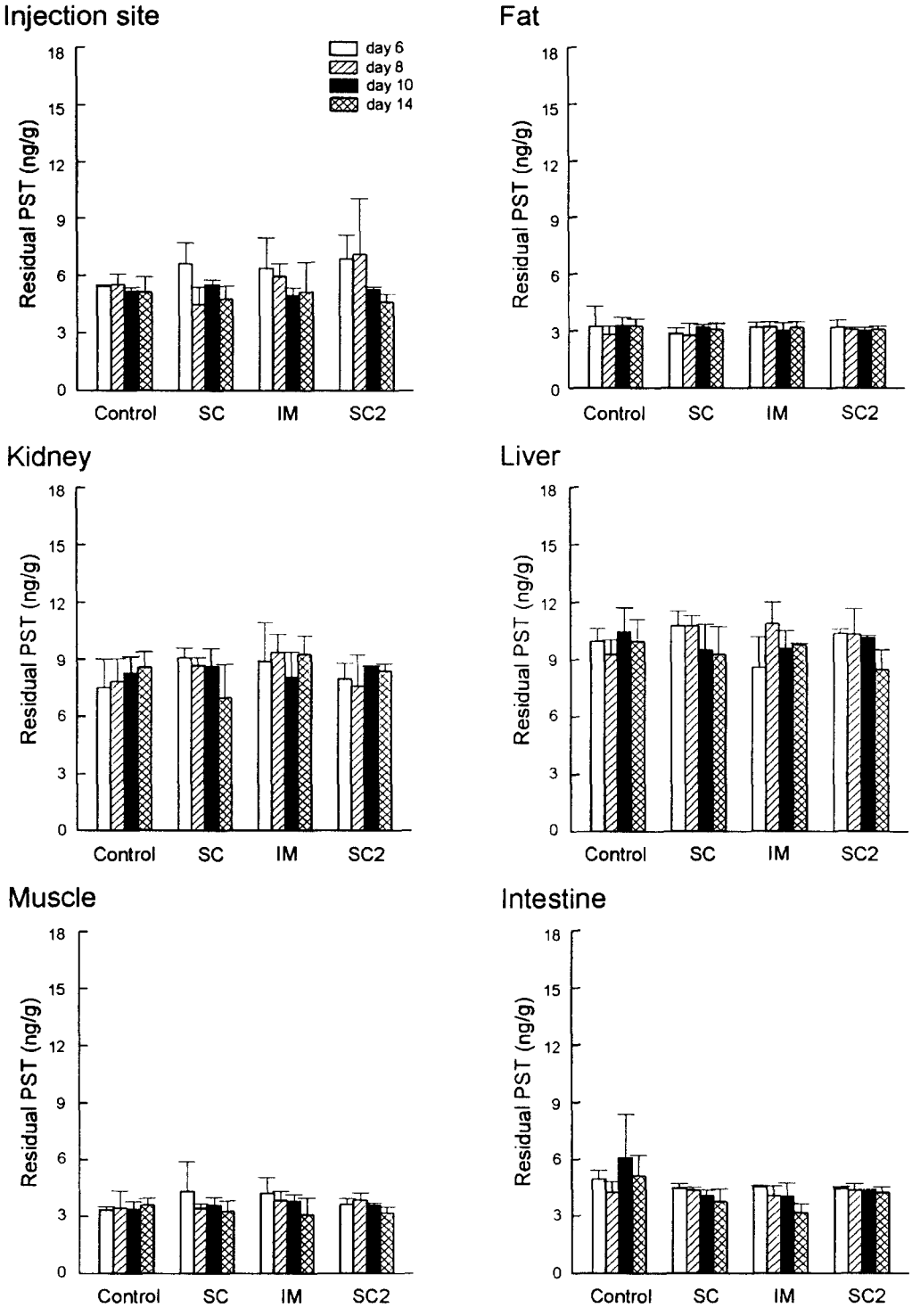

Fig 5. Tissue PST levels on day 6, 8, 10 and 14 after last (6th) administration of PST. Symbols are mean values obtained from 9~10 animals (day 6 and 8), 5~9 animals (day 10) and 3~4 animals (day 14). Error bars indicate standard error of means.

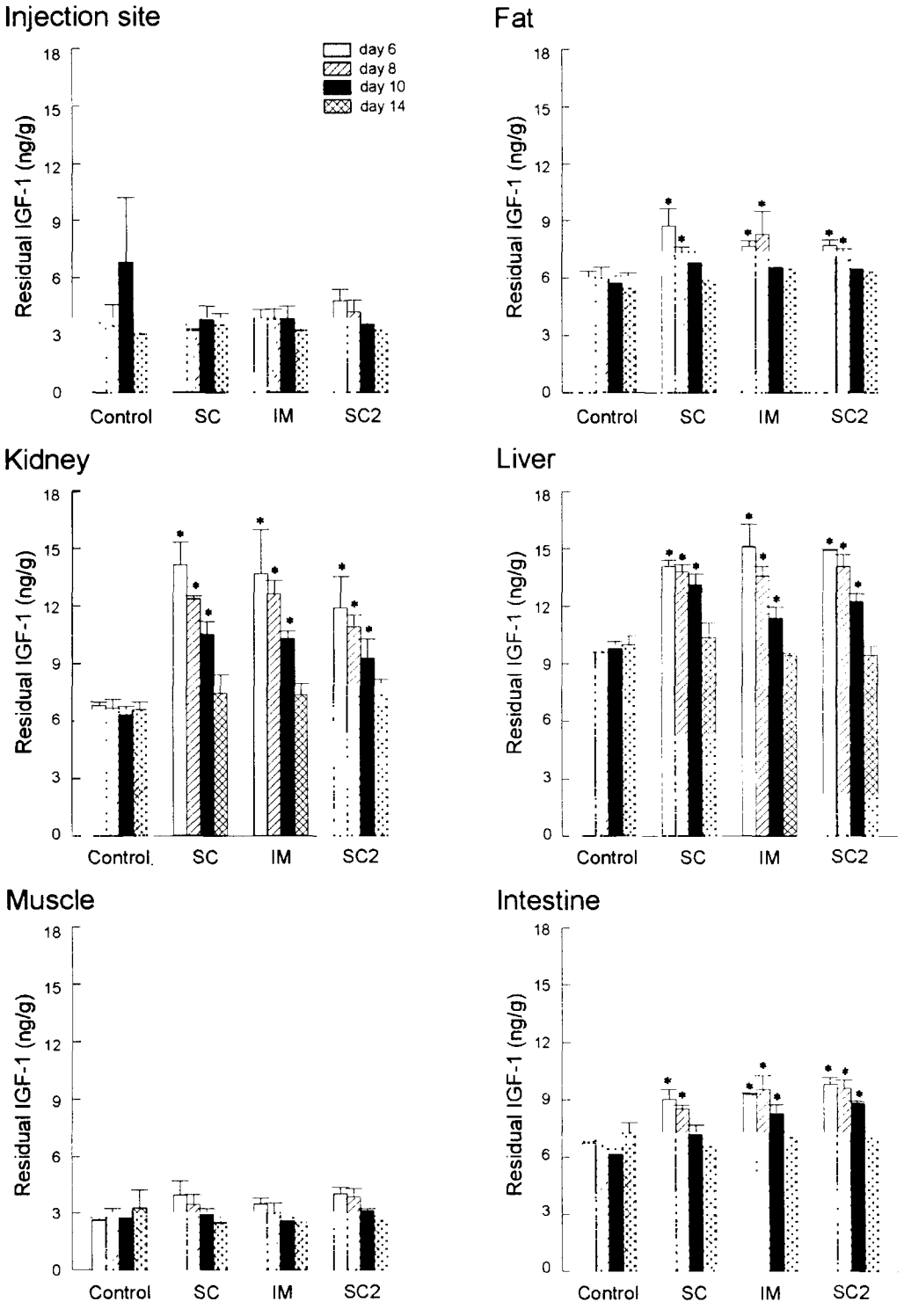

Fig 6. Tissue IGF-1 levels on day 6, 8, 10 and 14 after last (6th) administration of IGF-1. Symbols are mean values obtained from 9~10 animals (day 6 and 8), 5~9 animals (day 10 and 3~4 animals (day 14). Error bars indicate standard error of means(*$p < 0.05$).

ministration and decreased with time, showing a cyclic fluctuation of increase and decrease. After the last administration, plasma PST levels were slightly higher than those after the first administration, although the differences were not statistically significant. During the 6 week treatment period, peak plasma PST levels were in the range of 20~40 ng/ml which was 10~20 times higher than the control level. However, PST levels were gradually decreased after the last treatment and returned to the control level in 8 days. In contrast, plasma IGF-1 levels did not fluctuate much and gradually increased with repeated administration in all treatment groups (Fig 4B). After the final administration, the IGF-1 level reached the peak which was about two and half times of the control and remained at significantly higher levels up to 14 days after the last dose.

In this work, we also compared two routes of admini-stration for PST-SR, subcutaneous and intramuscular routes. As shown in Fig 4, the overall plasma concentration-time profiles of PST and IGF-1 are not different between the two routes.

Tissue residue levels of PST and IGF-1 after the last treatment : Figures 5 and 6 show the concentration of PST and IGF-1 in muscle, liver, kidney, fat, small intestine and injection site at days of 6, 8, 10 and 14 after the last administration. At day 6 after the last administration, tissue PST concentrations were already near the control levels. The residual PST levels were highest in the liver (8.5~11 ng/g), then followed by the kidney (7.4~10.7 ng/g), small intestine and muscle (3.1~4.8 ng/g) and fat (2.8~3.2 ng/g). In addition, the PST levels in injection sites were not significantly different from that of the similar sites in untreated pigs. The tissue levels of IGF-1 also tend to decrease with time as shown in Fig 6, but the IGF-1 levels in the liver, kidney, fat and intestine were significantly higher than those of respective controls at day 6 after the last administration ($p<0.01$). However, on day 14 after the last administration, none of these tissues showed the higher PST concentrations than the control. The tissue IGF-1 levels were highest in the liver and the kidney (7.4~15ng/g), then followed by small intestine and fat (6.2~9.6 ng/g) and muscle (2.6~4.1 ng/g).

Discussion

In this work we determined the pharmacokinetic properties and residue levels of PST and IGF-1 after single or repeated treatment of a sustained-release formulation of PST (PST-SR) developed by LG Chemical Ltd. The results indicate that PST-SR used in this work is effective for at least 1 week while the residual levels of PST and IGF-1 in edible tissues returned to the resting levels within 2 weeks after the 6 consecutive treatments at 1 week intervals.

The elimination half-life of plasma growth hormone in porcine was reported as 7~12 min[22], 14~21 min[14] and 40 min[15]. Therefore, PST of daily injection formulation must be administered at least once a day and such a frequent administration is not desirable because of the cost and stress to the pigs. Our results demonstrate that the sustained-release formulation used in the present work can the decay half life of PST from less than 1 hour to 91~227 hours. Previously Klindt *et al*[10] and Buonomo *et al*[16] also reported on sustained-release implants for porcine somatotropin. Their implants were designed to steadily release at a rate of 2 mg PST/day/implant and the peak plasma PST was about 35 and 60 ng/ml at the release rates of 2 and 4 mg per day, respectively. The terminal half-life of PST was approximately 16 days judged from the Fig 1 of Klindt *et al*[10], while that of PST-SR used in this work(3.8~9.5 days) was shorter by 0.6~4 times than that of Klindt *et al*[10], indicating a slower or little release of PST from the present preparation at later period of a dosing interval. It may be difficult to obtain the correct time-to-peak value from the works of Klindt *et al*[10] and Buonomo *et al*[16] because their PST levels already reached the peak at their first measurement at day 7 after the treatment. This value of time-to-peak is much larger than that in the present formulation (8 hours, Table 1). Nevertheless, the peak values of two PST-SR preparations were comparable and in the range 30~60 ng/ml. In another report of the same group[17], daily injection of rPST in sodium bicarbonate induced a rapid increase of IGF-1 within two days and remained at a steady level around 300 ng/ml during the treatment. Therefore, these

comparisons suggest that the release rate during the initial phase (Fig 3) from the PST-SR used in this study should be much faster than the those (4 mg/day/implant) from the formulation of Klindt *et al* [10] and Buonomo *et al* [16]. In contrast, the release rate from LB00006 at later periods for example at 24~72 hours after the treatment should be much less than the release rate of 2 mg/day/implant from the formulations used by Klindt *et al* [10] and Buonomo *et al* [16]. Such difference between two formulations was not unexpected because the PST formulation used by Buonomo *et al* [16] and Klindt *et al* [10] released PST at a steady rate, but the PST-SR formulation used in this work released PST in a time dependent manner ; a rapid release during first 24 hours and following slow release during next 5 days.

Termination half-life of IGF-1 after single treatment of PST was shorter than that of PST (Figures 1 and 2), but plasma IGF-1 levels showed a gradual accumulation during the repeated treatment (Fig 4). Such accumulation was not evident in plasma PST levels. However, this apparently contradictory observations may not be surprising if one realizes the fact that the formation of IGF-1 in response to PST occurred more slowly than the effects of plasma PST levels and the synthetic reaction of IGF-1, once triggered by PST, lasts longer than the effect of PST. This possibility was also supported by the results that the time to peak of plasma IGF-1 was slower by several hours than that of PST (Fig 1 of this work) and that IGF-1 levels remained elevated after plasma PST levels returned to its normal level[16] (Fig 4 of this work). So, the sustained-release formulation of PST seems to maintain plasma IGF-1 concentration at significantly higher levels over 7 days after single or 6 consecutive treatments, respectively. The residue levels in six different edible tissues were returned to the control levels at 14 days after the 6 consecutive treatments. Taken together, our results indicate that LB00006 could be an effective as well as safe dosage form of PST for the repeated treatment at 1 week intervals in swine industry.

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