

## Pharmacokinetics of lactosaminated recombinant human growth hormone in mice<sup>1</sup>

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**KEY WORDS** somatotropin; pharmacokinetics; recombinant proteins

### ABSTRACT

**AIM:** To study the pharmacokinetic characteristics of lactosaminated recombinant human growth hormone (hGH-L) in mice. **METHODS:** The biodistribution was studied with *in vivo* radioactive tracer technique. The pharmacokinetics was investigated by radioimmunoassay (RIA) method of hGH-L. The results were compared with that of recombinant human growth hormone (hGH). **RESULTS:** <sup>125</sup>I-hGH-L has remarkable liveraxis. The area under drug concentration-time curve ( $32686.9 \mu\text{g} \cdot \text{min} \cdot \text{L}^{-1}$ ) in blood and serum mean residence time (21.4 min) of hGH-L are less than that of hGH ( $36913.1 \mu\text{g} \cdot \text{min} \cdot \text{L}^{-1}$  and 24.9 min) ( $P < 0.05$ ). In target organ liver, hGH-L distribution half life (1.8 min) and elimination half life (11.1 min) are shorter than that of hGH (2.1 min and 27.7 min) ( $P < 0.05$ ). The area under drug concentration-time curve ( $17621.9 \mu\text{g} \cdot \text{min} \cdot \text{L}^{-1}$ ) of hGH-L is bigger than that of hGH ( $12148.2 \mu\text{g} \cdot \text{min} \cdot \text{L}^{-1}$ ) ( $P < 0.05$ ) in liver. **CONCLUSION:** The pharmacokinetic parameters of hGH-L has obvious advantage over that of hGH.

### INTRODUCTION

Recombinant human growth hormone (hGH) enhances bone growth through somatomedin (SOM) that

liver tissue produced by relying on capacity of hGH in liver. So it is widely used in clinic for treating hGH deficient children<sup>[1]</sup>. But hGH is a kind of hormone without any target gland. This decreases its clinical effect. The over-dosage and long-time usage of hGH can cause anti-drug effect and disturb fluid homeostasis<sup>[2,3]</sup>. Therefore, increasing hGH concentration in liver and decreasing its concentration in other tissues can improve its clinical effect and eliminate its side effects. We prepared lactosaminated recombinant human growth hormone (hGH-L) through condensation reaction of hGH with aldehydic glucose fragment of lactose. Here in this study, pharmacokinetics of hGH-L was studied in mice.

### MATERIALS AND METHODS

#### Distribution *in vivo*

<sup>125</sup>I labeling hGH-L and hGH The compounds were labeled according to the method reported<sup>[4]</sup> previously. The labeled compounds were purified through Sephadex G25 Column chromatography and filtered through 220-nm membrane filter. The radiochemical purity (> 98%) was tested through trichloroacetic acid deposit method.

**Biodistribution in mice** BALB/c mice,  $n = 24$ , 13-20 g, were injected with 0.2 mL of <sup>125</sup>I-hGH-L or <sup>125</sup>I-hGH (containing 0.3  $\mu\text{g}$  of chemical content and 31.28 kBq of radioactivity and NaI 0.01 mol/L) through tail vein, then were randomly divided into 8 groups. The mice were sacrificed at different times after injection. Viscera was gained from all mice and radioactivity was measured. The results were expressed as viscera radioactivity percentage of injected radioactivity. The data were presented as  $\bar{x} \pm s$ . The *t* test was carried out between biodistribution of the compounds and level of significance was calculated.

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## Establishment of radioimmunoassay (RIA) method for measuring drug concentration in liver homogenate

**Feasibility of establishing RIA method** ① Measurement of the affinity of hGH antibody to hGH-L and hGH: twenty BALB/c mice, 20 g – 30 g, were sacrificed. Blood was collected to separate serum. The serum was kept at 4 °C. Livers were collected and mixed with 0.25 mol/L sucrose solutions with the ratio of weight ( $\mu\text{g}$ )/volume (mL) 1:10, then homogenized in ice bath and stored at 4 °C. hGH-L 3.27 mg and 3.14 mg of hGH were precisely weighed and dissolved into 500 mL redistilled water and this was used as hGH-L and hGH stock solution. Stock solution 0.1 mL, 0.25 mL, 0.75 mL, 1.5 mL, 4.0 mL of hGH-L and hGH were separately added into 50 mL volume flasks and diluted to 50 mL. The above solution 0.05 mL was added to 0.45 mL normal mouse serum and this was used as hGH and hGH-L serum working solution. Phosphate buffer solution (PBS, pH 7.0) 100  $\mu\text{L}$  and normal mouse serum 100  $\mu\text{L}$  were regarded as non-specific tube. Normal mouse serum 100  $\mu\text{L}$  was regarded as zero standard sample tubes. hGH-L or hGH serum working solution 100  $\mu\text{L}$  with different concentration was regarded as sample tubes. According to routine RIA method, standard curve was prepared. From the standard curve, the  $\text{ED}_{50}$  (middle value) and  $\text{ED}_{25}$  (half middle value) were got. The affinity constant  $K_a$  of hGH and hGH-L to hGH antibody were calculated according to the equation  $K_a = 6B_0\% / [(1-B_0\%)(2-B_0\%)(4-B_0\%)(3\text{ED}_{50}-\text{ED}_{25})]$ . ② Stability of hGH-L and hGH in liver homogenate: 1 mL of hGH-L stock solution was mixed with 5 mL normal mouse liver homogenate, and was kept in water bath at 37 °C. At 0.5 h, 6 h, 12 h, 24 h, and 36 h respectively, the solutions were tested using Perkin-Elmer DSC-2C differential scanning thermal analysis instrument to get differential scanning thermal (DST) spectra between 273 K to 600 K. The stability of hGH-L and hGH in liver homogenate was tested.

**Preparation of standard curve** ① Working solution of liver homogenate hGH-L and hGH: 0.1 mL, 0.5 mL, 1.0 mL, 2.0 mL, and 4.0 mL of hGH-L stock solutions were respectively diluted to 50.0 mL. Each of these diluted solutions 0.5 mL was respectively added to 4.5 mL normal mouse liver homogenate and then mixed, and kept at 37 °C for 30 min, then rotated to separate for 10 min at a speed of  $1200 \times g$  at 4 °C. The upper clear solution was collected. hGH-L liver homogenate work-

ing solution 1.3 g/L, 6.5 g/L, 13.0 g/L, 26.0 g/L, and 52.3 g/L was thus prepared. hGH liver homogenate working solution (concentration 1.26  $\mu\text{g/L}$ , 6.28  $\mu\text{g/L}$ , 12.56  $\mu\text{g/L}$ , 25.12  $\mu\text{g/L}$ , and 50.24  $\mu\text{g/L}$ ) were prepared as the method described above. ② Standard curve: 100  $\mu\text{L}$  of PBS and 100  $\mu\text{L}$  of normal mouse liver homogenate were regarded as the non-specific sample tube. Normal mouse liver homogenate 100  $\mu\text{L}$  was regarded as zero standard tube. hGH liver homogenate working solution 100  $\mu\text{L}$  (with different concentrations) was regarded as sample tube. Following the routine RIA method, the hGH RIA standard curve was obtained. The hGH-L RIA standard curve was also obtained using the same method.

## Pharmacokinetics

**Sample collection** BALB/c mice,  $n = 30$ , 20 g – 30 g, were injected with hGH-L in the ratio of  $1 \text{ mg} \cdot \text{kg}^{-1}$  and randomly divided into 10 groups. At 1 min, 3 min, 5 min, 8 min, 10 min, 15 min, 30 min, 60 min, 120 min, and 180 min after injection, each group was sacrificed. Blood was collected and serum was separated and kept at 2 °C – 4 °C. Liver without bile duct and gallbladder was mixed with sucrose solution 0.25 mol/L (4 °C) as the ratio of weight ( $\mu\text{g}$ )/volume (mL) 1:10 and then homogenized in ice bath and rotated to separate for 10 min at a speed of  $1200 \times g$  at 4 °C. The upper clear solution was collected and kept at 2 °C – 4 °C. Blood and liver samples of mice injected with hGH were collected using the same method.

**Measurement of blood and liver drug concentration** Blood drug concentration of hGH-L and hGH was estimated using the instructions supplied by hGH RIA commercial kits. Liver homogenate drug concentration of hGH-L and hGH was estimated using the standard curve established as above-mentioned.

**Data handling** The curves were imitated using 3P87 program of pharmacokinetic and the pharmacokinetic parameters of hGH-L and hGH were calculated. The  $t$  test was performed between two groups of parameters.

## RESULTS

### Biodistribution in mice

Tab 1 lists the biodistribution results of  $^{125}\text{I}$ -hGH-L and  $^{125}\text{I}$ -hGH in mice.  $^{125}\text{I}$ -hGH-L reaches the highest value in liver, which is 69 % of injected dose at 5 min after injection. However, liver uptake of  $^{125}\text{I}$ -hGH

peaked at 10 min after injection. The uptake being only 35 % of the injected dose which is about half of  $^{125}\text{I}$ -hGH-L in liver. As the time increased, the drug distribution rate in liver decreased gradually. During the observation time period, the uptake rate of  $^{125}\text{I}$ -hGH-L in liver is about 2-fold of  $^{125}\text{I}$ -hGH. The distribution of  $^{125}\text{I}$ -hGH-L in blood is much less than that of  $^{125}\text{I}$ -hGH and has the characteristic of re-distribution in blood that is only possessed by liver target drug. The distribution rate of  $^{125}\text{I}$ -hGH-L is lower than that of  $^{125}\text{I}$ -hGH in kidney.  $^{125}\text{I}$ -hGH-L and  $^{125}\text{I}$ -hGH are mainly cleared from liver, gall and intestine. The *t* test results show that drug distribution of the two drugs in mice blood, liver, and kidney was markedly different, (blood, *t*: 2.65,  $P < 0.05$ . liver, *t*: 4.06,  $P < 0.01$ . kidney, *t*: 4.01,  $P < 0.01$ ).

### RIA reliability test

#### Feasibility of RIA method of liver homogenate

The affinity constant  $K_a$  of hGH antibody to hGH-L is  $2.02 \times 10^9 \text{ L/mol}$ ,  $K_a'$  of hGH to its antibody equals  $1.90 \times 10^9 \text{ L/mol}$ .  $K_a \approx K_a'$ , this means that the two drugs have almost equal affinity for hGH antibody. The DST spectra of hGH-L and hGH in liver homogenate are the same during the period of 0.5 h – 36 h. This means that the two drugs in liver homogenate undergo no structural change during the time period (0.5 h – 36 h) and are quite stable. It is thus feasible to establish an RIA method of hGH-L and hGH in liver homogenate.

**Standard curve** In blood, the standard curve range of hGH-L and hGH are 1.26 – 52.30  $\mu\text{g/L}$  and 1.00 – 50.00  $\mu\text{g/L}$  respectively, the *r* values are 0.999 and 1.000 respectively, the minimal limits (sensitivity) are 1.10  $\mu\text{g/L}$  and 0.70  $\mu\text{g/L}$  respectively. In liver homogenate, the standard curve ranges of hGH-L and hGH are 1.30 – 52.30  $\mu\text{g/L}$  and 1.26 – 50.25  $\mu\text{g/L}$  respectively, and the *r* values are 0.998 and 1.000 respectively, the minimal limits are 0.70  $\mu\text{g/L}$  and 0.80  $\mu\text{g/L}$  respectively.

**Dilution linear test** After sample dilution, good linear relationship existed between the tested concentration and folds of dilution ( $r \approx 1$ ). Based on the results, when the sample concentration exceeded the standard curve range, the sample was tested after dilution and the original concentration was calculated according to its fold of dilution.

**Recovery experiment** The recovery rates (%) of hGH-L and hGH in liver homogenate are  $94 \pm 7$  and

$93.8 \pm 2.3$  respectively; The recovery rates (%) of hGH-L and hGH in blood are  $99.5 \pm 2.2$  and  $98 \pm 6$  respectively.

**Accuracy** The tested blood drug concentration accuracy of hGH-L and hGH is 0.40 % – 2.86 % and 0.99 % – 3.18 % respectively. The accuracy of liver drug concentration is 0.62 % – 3.68 % and 0.44 % – 4.32 % respectively.

**Repetition experiment** Difference in one group is 1.64 % – 3.25 %. Difference among groups is 2.92 % – 6.36 %

### Pharmacokinetics of hGH-L and hGH

The blood drug concentration-time curves of hGH-L and hGH are shown in Fig 1. The drug concentration-time curves of hGH-L and hGH in liver are shown in Fig 2. Tab 2 lists the pharmacokinetic parameters of hGH-L and hGH. The distribution half life ( $T_{1/2\alpha} = 1.8 \text{ min}$ ) and the elimination half life ( $T_{1/2\beta} = 11.1 \text{ min}$ ) of hGH-L in liver are less than that of hGH in liver ( $T_{1/2\alpha} = 2.1 \text{ min}$ ,  $T_{1/2\beta} = 27.7 \text{ min}$ ) ( $P < 0.05$ ). The area under curve (AUC) of drug concentration-time of hGH-L in liver ( $17621.9 \mu\text{g} \cdot \text{min} \cdot \text{L}^{-1}$ ) is 1.5 folds than that of hGH ( $12148.2 \mu\text{g} \cdot \text{min} \cdot \text{L}^{-1}$ ) ( $P < 0.05$ ). This means that the hGH-L absorbed by liver is 150 % of that of hGH and its distribution and elimination by liver are faster than that of hGH. As the blood pharmacokinetic parameters of hGH-L can not be calculated with 3P87 program, the concentration changes of hGH-L and hGH in blood were estimated. The results show that AUC of blood drug concentration-time curve of hGH-L and hGH are  $32686.9 \mu\text{g} \cdot \text{min} \cdot \text{L}^{-1}$  and  $36913.1 \mu\text{g} \cdot \text{min} \cdot \text{L}^{-1}$  respectively ( $P < 0.05$ ), the mean residence time (MRT) of hGH-L and hGH in blood are 21.4 min and 24.9 min respectively ( $P < 0.05$ ). This means that the distribution of hGH-L in blood is less than that of hGH and hGH-L is eliminated faster than hGH.

### DISCUSSION

SOM is produced by hGH in liver tissue and liberated into blood to promote bone growth. The amount of SOM in blood is controlled by the amount of hGH in liver tissue<sup>[1]</sup>. But hGH is a gland hormone without target gland. Injected hGH only induces low concentrations of SOM in the blood and its drug efficiency is thus inhibited. Therefore, increasing hGH concentration in liver is the key point to improve its curing efficiency. The *in vivo* distribution results show that hGH-L has obvious

Tab 1. Biodistribution results of  $^{125}\text{I}$ -hGH-L and  $^{125}\text{I}$ -hGH in mice (ID %).  $n=3$ .  $\bar{x} \pm s$ .

Viscera	Drug	Time/min							
		1	3	5	10	15	30	60	120
Blood	A	24 ± 3	8 ± 1	5 ± 0	5 ± 1	4 ± 0	19 ± 0	5 ± 1	6 ± 1
	B	43 ± 2	23 ± 2	18 ± 1	10 ± 2	10 ± 2	7 ± 1	7 ± 2	6 ± 1
Liver	A	53 ± 5	64 ± 4	69 ± 5	65 ± 1	60 ± 4	22 ± 1	10 ± 0	5 ± 0
	B	19 ± 2	24 ± 2	28 ± 1	35 ± 4	34 ± 3	21 ± 1	6 ± 1	4 ± 0
Kidney	A	8 ± 2	9 ± 2	14 ± 2	11 ± 2	8 ± 2	5 ± 0	3 ± 1	2 ± 1
	B	11 ± 2	20 ± 2	21 ± 2	18 ± 5	16 ± 3	5 ± 0	2 ± 0	1 ± 1
Intestine	A	3 ± 0	2 ± 0	2 ± 0	3 ± 1	3 ± 1	10 ± 1	8 ± 1	7 ± 1
	B	5 ± 1	5 ± 0	7 ± 1	6 ± 1	7 ± 2	8 ± 2	7 ± 1	6 ± 1

Taking 7 % of body weight as blood weight. A:  $^{125}\text{I}$ -hGH-L. B:  $^{125}\text{I}$ -hGH.

Tab 2. Pharmacokinetic parameters of hGH-L and hGH 0.3  $\mu\text{g}$  iv in mice.

		Pharmacokinetic parameters										CL ( $\text{mL} \cdot \text{min}^{-1}$ )	AUC ( $\mu\text{g} \cdot \text{min} \cdot \text{L}^{-1}$ )
		a ( $\text{min}^{-1}$ )	b ( $\text{min}^{-1}$ )	$\alpha$ ( $\text{min}^{-1}$ )	$\beta$ ( $\text{min}^{-1}$ )	V/F ( $\text{mL}$ )	$T_{1/2\alpha}$ ( $\text{min}$ )	$T_{1/2\beta}$ ( $\text{min}$ )	$K_{21}$ ( $\text{min}^{-1}$ )	$K_{10}$ ( $\text{min}^{-1}$ )	$K_{12}$ ( $\text{min}^{-1}$ )		
Liver	C	285.2	9.3	0.3	0.01	0.2	2.1	27.7	0.04	0.1	0.2	0.02	12148.2
	D	5.0	106.2	0.4	0.1	0.02	1.8	11.1	0.4	0.1	-0.002	0.001	17621.9
Blood	C	3139.6	50.6	0.1	0.01	0.01	4.7	28.4	0.01	0.1	0.3	0.001	36913.1

D Because of double peaks of blood drug concentration-time curve, pharmacokinetic parameters could not be obtained with the current computer program.

C: hGH, D: hGH-L

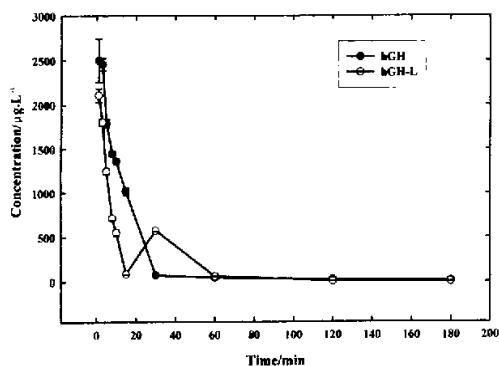


Fig 1. The blood drug concentration-time curve of hGH-L and hGH.  $n=3$ .  $\bar{x} \pm s$ .

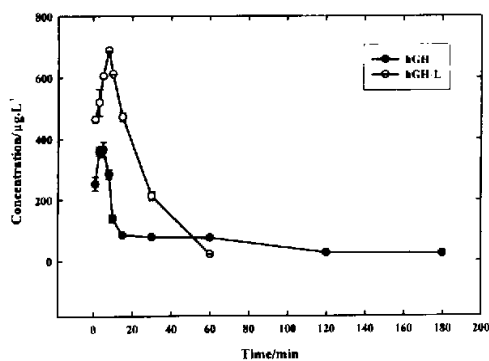


Fig 2. The drug concentration-time curve of hGH-L and hGH in liver.  $n=3$ .  $\bar{x} \pm s$ .

liver and increases hGH concentration in liver and hGH-L circulating in blood and present in kidney is far less than hGH. The reason may be that asialoglycoproteins receptors presenting only on hepatic cell membranes of mammal specifically bind to hGH-L<sup>[5-7]</sup>. This is one of the most important advantage for using hGH-L for treating children who are deficient in hGH. The lower concentration of hGH-L in blood soften

the effect of injected hGH to non-target tissue and therefore side effect of hGH is relieved<sup>[2,8]</sup>. The lower amount of hGH-L in kidney decrease the hGH effect to hydro-sodium catabolism and hydro-sodium retention<sup>[3]</sup>.

Pharmacokinetic research results show that hGH-L has a greater potential clinical value, as the literature reports that hGH concentration change frequency has more effect than the hGH retention time *in vivo* for the growth

promoting effect<sup>[2,8]</sup>. High concentrations of hGH-L in liver in a short time is beneficial not only for the liver to be induced to produce more SOM, but also to increase the effectiveness of hGH and to avoid the patient long time exposures to injected hGH. This reduces the possibility of production of the hGH antibody and decrease the possibility of anti-drug effects of hGH. In the meantime, hGH-L has a shorter distribution and retention time than hGH, which decreases the hGH interaction with non-target tissue and decreases the side effects of hGH<sup>[2]</sup>. Our results show that  $T_{1/2\beta}$  of hGH in serum is 24.9 min - 28.4 min which coincides with the reported value, 19 min - 29 min<sup>[9]</sup>. In addition,  $^{125}\text{I}$ -hGH-L and hGH-L exists as two peaks in the blood drug concentration-time curve, further demonstrating that hGH-L is a liver target drug which possesses this feature<sup>[4]</sup>.

From the chemical point of view, hGH-L is the lactose derivative of hGH. It can exist stably in mice blood and liver. From this report and our former results<sup>[7]</sup>, the conclusion can be drawn that hGH-L and hGH have different biological characteristics and hGH-L may be treated as a new compound. And our experiments prove that hGH-L and hGH possesses identical bioactivity. So, hGH-L may be a potential drug to replace hGH for the treatment of hGH deficiency.

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## 乳糖化重组人生长激素在小鼠体内的药物动力学<sup>1</sup>

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**关键词** 生长激素; 药物动力学; 重组蛋白质类

**目的:** 研究乳糖化基因重组人生长激素(hGH-L)在小鼠体内的药代动力学特征. **方法:** 用放射性核素体内示踪技术研究体内分布. 建立 hGH-L 放射免疫分析(RIA)方法, 研究其药代动力学特征, 并对比研究基因重组人生长激素(hGH). **结果:**  $^{125}\text{I}$ -hGH-L 具有明显的趋势肝性. hGH-L 的血药时曲线下面积和在血清的平均驻留时间均小于 hGH,  $P < 0.01$ ; 而靶器官肝脏的 hGH-L 分布半衰期、消除半衰期小于 hGH,  $P < 0.05$ , 其药时曲线下面积大于 hGH,  $P < 0.05$ . **结论:** hGH-L 的药代动力学特征明显优于 hGH.

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