

Short Communication

Pharmacokinetics of Recombinant Human Insulin-Like Growth Factor-I in Diabetic Rats

ABSTRACT:

Pharmacokinetics of recombinant human insulin-like growth factor-I (rhIGF-I) was investigated after iv administration (0.32, 1.0, and 3.2 mg/kg) to normal and streptozotocin-induced diabetic rats. rhIGF-I was eliminated from plasma biexponentially in both normal and diabetic rats. Plasma concentrations of rhIGF-I were lower at almost all the time points examined in diabetic rats than in normal rats. The pharmacokinetic parameters of total body clearance (CL_{total}), mean residence time (MRT), and elimination rate constant (k_{el}) indicated that rhIGF-I disappeared more rapidly in diabetic rats than in normal rats at any dosage. The amounts of IGF binding proteins (IGFBPs) in plasma were assessed by determining the endogenous IGF-I and. Levels of the 150 kDa complex, a ternary

complex of IGF-I with IGFBP-3 and an acid-labile subunit, the 50 kDa complex, a complex of IGF-I with IGFBP-2, were found to be lower in diabetic rats than in normal rats. Fractions of rhIGF-I free and bound to the binding proteins were estimated by gel chromatographic separation of rhIGF-I in plasma after iv administration, and the pharmacokinetics of free and bound rhIGF-I was analyzed independently. Plasma concentrations of free and bound rhIGF-I were lower in diabetic rats than in normal rats, especially the concentrations of the 150 kDa complex were much lower. The reduced IGFBP-3 would be responsible for the faster elimination of rhIGF-I in diabetic rats.

IGF-I¹ is a single-chain polypeptide of about 7.6 kDa with proinsulin-like structure (1). rhIGF-I is being used clinically for the therapy of Laron dwarfism, in which the function of growth hormone receptor is deficient (2–4), and of insulin-resistant diabetes (4–6), as rhIGF-I has been available by means of recombinant DNA technology. Although IGF-I is produced by a wide variety of tissues (7), circulating plasma is the largest pool in the body (1). The plasma concentrations of IGF-I are much higher than those of insulin as it is bound to a series of IGFBPs, of which a total of six have been described to date (7). Especially, more than 95% of IGFs in plasma are reported to be bound to IGFBP-2, forming the 50 kDa complex, and IGFBP-3 associated with acid-labile subunit, forming the 150 kDa complex with IGF-I (1). We have already investigated the pharmacokinetics of rhIGF-I after iv administration to normal and hypophysectomized rats. From the aspect of pharmacokinetics, the lack of IGFBP-3 was strongly suggested to be responsible for much more rapid elimination of rhIGF-I in the hypophysectomized rats. Furthermore, we have indicated that the kidney should play an important role in the systemic elimination of rhIGF-I (8).

In the present study, the pharmacokinetics of rhIGF-I was investigated in the streptozotocin-induced diabetic rats, and the relationship between the pharmacokinetics of rhIGF-I and the amount of IGFBPs in plasma was discussed.

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¹ Abbreviations used are: IGF-I, insulin-like growth factor-I; rhIGF-I, recombinant human insulin-like growth factor-I; IGFBP, insulin-like growth factor binding protein; CL_{total} , total body clearance; MRT, mean residence time; AUMC, area under the moment vs. time curve.

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Materials and Methods

Materials. rhIGF-I, a monoclonal antibody (McAb) for rhIGF-I, polyclonal antibody (PcAb) for IGF-I, and ¹²⁵I-rhIGF-I were generous gifts from Fujisawa Pharmaceutical Co. (Osaka, Japan). Streptozotocin was obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade.

Animals. Male Sprague-Dawley rats (Charles River Japan, Inc., Yokohama) at 6 weeks of age were used.

Preparation of Diabetic Rats. Diabetes was induced by a bolus injection of streptozotocin (60 mg/kg) into the tail vein of 6-week-old rats. Streptozotocin was dissolved in isotonic citrate buffer (pH 4.2) in the ice-cold water bath just prior to its injection. Thirteen days later, rats whose plasma glucose levels exceeded 400 mg/dl were used for all the experiments on the next day. Control rats against diabetic rats were treated only with isotonic citrate buffer at 6 weeks and used for studies at 8 weeks of age. In the diabetic rats, urine glucose levels were higher than 2.0 g/dl and the mean plasma glucose level was 615.7 ± 13.3 mg/dl.

Pharmacokinetic studies. rhIGF-I dissolved in saline (1.0 mg/ml) was injected to the femoral vein at the doses of 0.32, 1.0, and 3.2 mg/kg. The blood samples were periodically collected from the jugular vein. Immediately, the blood was centrifuged at 9000 rpm for 5 min to obtain the plasma. For the diabetic and the control rats, urine was collected for 18 hr after the dosing of 1.0 mg/kg.

Gel chromatography of IGF-I. Two milliliters of a plasma sample were applied to a Sephacryl S200HR column (16 mm i.d. × 600 mm) (Pharmacia LKB Biotechnology, Tokyo, Japan) previously equilibrated with 10 mM phosphate buffer (pH 7.4) containing 0.02% Na₃N and 25 mM EDTA. The elution was carried out at a flow rate of 28.8 ml/hr and 2.4 ml of each fraction was collected. The column was calibrated with several standard materials (8).

Analytical Procedure. IGF was determined by radioimmunoassay (RIA) using McAb for rhIGF-I and/or PcAb for IGF-I following the method reported in the previous studies (8, 9). The former can detect only rhIGF-I, while the latter can detect total IGF-I, including the endogenous IGF-I. Plasma glucose was determined following the glucose oxidase method using Iatro-Chrome GLU-Lq (Iatron Laboratories Co., Tokyo, Japan). Urinary glucose was assayed by reagent strips for urine analysis (Diastix, Miles-Sankyo Co., Tokyo, Japan).

Data Analysis. Plasma concentration (C_p) vs. time curves of total rhIGF-I

after iv administration were fitted to a biexponential equation (eq. 1) and the kinetic parameters were calculated by using the nonlinear least-square regression program MULTI (10).

$$C_p = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \quad (1)$$

AUC and AUMC were calculated following eqs. 2 and 3, respectively.

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} \quad (2)$$

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2} \quad (3)$$

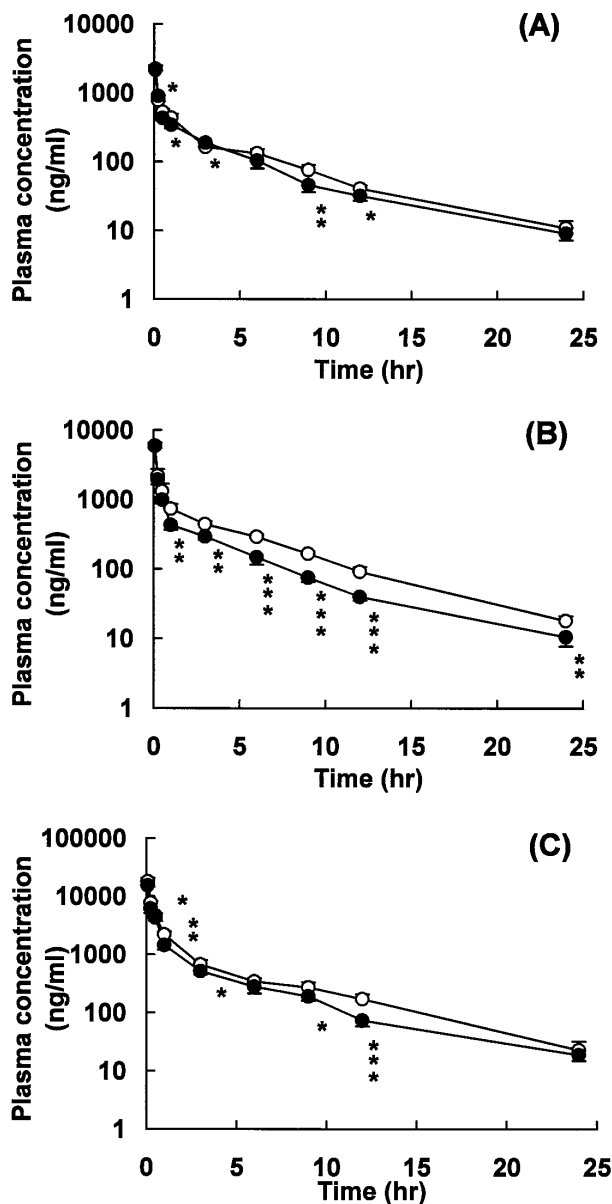


FIG. 1. Plasma concentrations of rhIGF-I after iv administration to normal and diabetic rats.

The dosages of rhIGF-I are (A) 0.1 mg/kg, (B) 0.32 mg/kg and (C) 3.2 mg/kg. ○, control rats; ●, diabetic rats. Results are expressed as the mean with the vertical bar showing the SD of 4 to 5 experiments. Statistically significant differences between normal and diabetic rats are as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

AUC and AUMC values of free rhIGF-I and rhIGF-I bound to the binding proteins were calculated following the linear trapezoidal rule with a monoexponential extrapolation to infinity. The mean residence time (MRT) was calculated by the following equation:

$$MRT = \frac{AUMC}{AUC} \quad (4)$$

Statistical Analysis. Statistically significant difference was determined by Student's t test with or without Welch's correction after ANOVA was performed by F -test.

Results and Discussion

Pharmacokinetics of rhIGF-I in Diabetic Rats. rhIGF-I was eliminated from plasma by biexponential mode in both groups of rats, and the plasma concentrations in normal rats were higher than those in diabetic rats at every dose (fig. 1). Table 1 represents the pharmacokinetic parameters of rhIGF-I. In both diabetic and normal rats, CL_{total} increased and MRT decreased as the dosage increased from 0.32 to 3.2 mg/kg, suggesting the dose-dependent and nonlinear elimination of rhIGF-I. This would be caused by the gradual increase in free fraction of rhIGF-I according to the increment of dosage since the amount of IGFBP-3 needed for the residence in the circulation is constant. Diabetic rats showed the significantly larger value of CL_{total} than normal rats, and the value of MRT in diabetic rats tended to be shorter than that in normal rats in every dose. As for the apparent volume of distribution at steady state ($V_{d_{ss}}$) and the elimination rate constant (k_{el}), those for diabetic rats were larger than or almost equal to those for normal rats, although there was no significant difference between two groups in $t_{1/2}$ at β phase ($t_{1/2\beta}$). These results suggest the faster distribution and/or elimination of rhIGF-I at early time periods after dosing in diabetic rats. Urinary recovery of unchanged rhIGF-I was examined in the case of 1 mg/kg dosing. rhIGF-I was hardly excreted in urine as the unchanged form in both normal ($0.002 \pm 0.001\%$) and diabetic ($0.005 \pm 0.003\%$) rats.

Estimation of IGFBPs Levels in Plasma of Diabetic Rats. The amounts of IGFBP-2 and -3 in the plasma were assessed by determining the endogenous IGF-I bound to them by RIA using PcAb for IGF-I. Fig. 2 shows typical gel chromatograms of IGF-I in normal rat plasma and diabetic rat plasma. The peaks around the 22nd and 28th fractions correspond to IGF-I bound to IGFBP-3 and an acid-labile subunit (150 kDa complex) and one to IGFBP-2 (50 kDa complex), respectively. The addition of rhIGF-I did not change the height and the pattern of these peaks, but it generated another peak around 33rd fraction corresponding to free rhIGF-I and/or IGF-I. These phenomena indicate that almost all of IGFBP-2 and IGFBP-3 were bound to

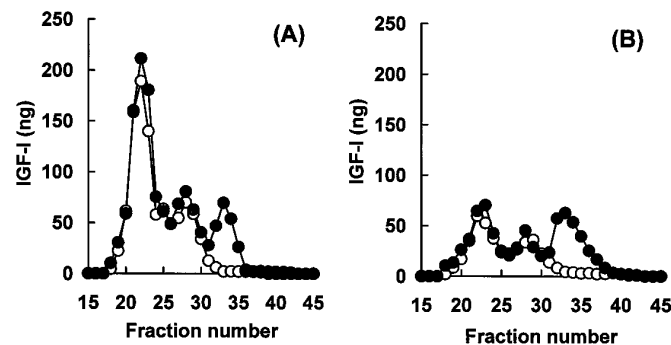


FIG. 2. Typical gel chromatograms of IGF-I in plasma of (A) normal and (B) diabetic rats.

○, endogenous IGF-I; ●, plasma with the addition of rhIGF-I (300 ng/ml).

TABLE 1

Pharmacokinetic parameters of rhIGF-I after iv administration to normal and diabetic rats

Dose (mg/kg)	Rat	AUC ($\mu\text{g} \cdot \text{hr}/\text{ml}$)	MRT (hr)	CL _{total} (ml/hr/kg)	Vd _{ss} (ml/kg)	k _{el} (hr ⁻¹)	t _{1/2} β (hr)
0.32	Normal	2.35 ± 0.24	5.79 ± 0.59	137 ± 13	795 ± 128	0.79 ± 0.17	5.00 ± 0.44
	Diabetic	2.05 ± 0.10	4.51 ± 0.64	156 ± 7	754 ± 103	1.55 ± 0.26	4.51 ± 0.64
	<i>p</i> ^a	0.1	0.1	0.05	0.8	0.005	0.5
1.0	Normal	5.89 ± 0.57 ^d	4.73 ± 0.11 ^b	171 ± 16 ^c	810 ± 92	1.36 ± 0.19 ^c	4.28 ± 0.08 ^b
	Diabetic	3.66 ± 0.33 ^d	3.80 ± 0.09 ^b	275 ± 24 ^d	1044 ± 96 ^c	2.09 ± 0.34 ^b	4.32 ± 0.41
	<i>p</i> ^a	0.0005	0.0005	0.0002	0.05	0.01	1.00
3.2	Normal	12.22 ± 1.10 ^{d,g}	3.48 ± 0.51 ^{c,f}	263 ± 23 ^{d,g}	913 ± 113 ^b	1.37 ± 0.08 ^{d,g}	4.37 ± 0.43
	Diabetic	9.27 ± 8.85 ^{d,g}	3.03 ± 0.37 ^{d,f}	348 ± 36 ^{d,e}	1060 ± 212	1.65 ± 0.29	4.30 ± 0.54
	<i>p</i> ^a	0.005	0.2	0.005	0.5	0.1	1.00

Results are expressed as the mean ± SD of more than 4 rats. ^a, Probability level for comparison between normal and diabetic rats. Statistically significant differences from 0.32 mg/kg dosing in each group were indicated as follows: ^b, $p < 0.05$; ^c, $p < 0.01$; ^d, $p < 0.001$. Statistically significant differences between 1.0 and 3.2 mg/kg dosing in each group were indicated as follows: ^e, $p < 0.05$; ^f, $p < 0.01$; ^g, $p < 0.001$.

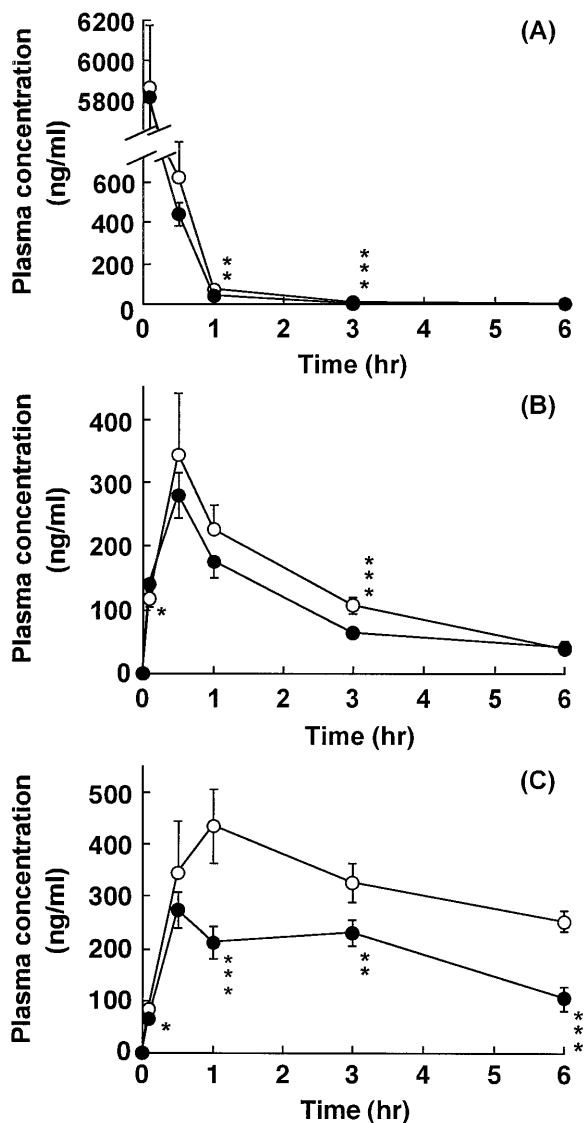


FIG. 3. Plasma concentration of rhIGF-I (A) free form, (B) as 50 kDa complex and (C) as 150 kDa complex after iv administration (1 mg/kg) to normal and diabetic rats.

Open and closed symbols represent the control and diabetic rats, respectively. Results are the mean with the vertical bar showing the SD of 4 to 5 experiments. Statistically significant differences between normal and diabetic rats are as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

the endogenous IGF-I, and the determination of the bound complexes made it possible to estimate the amount of the binding proteins in both normal and diabetic rats. Therefore, fig. 2 indicates that endogenous IGF-I, 150 kDa complex, and 50 kDa complex in plasma decrease in diabetes. In the diabetic state, endogenous IGF-I in plasma is reported to decrease in comparison with the normal condition in men (11, 12), monkeys (13), and rats (14, 15–17). Furthermore, plasma levels of IGFBP-3 are also recognized to be lower in diabetic men (18) and rats (15–17). Our results have supported previous reports. IGFBP-1 is reported to increase significantly in serum in the diabetic state (17). However, we could not recognize the substantial amount of IGFBP-1 associated with rhIGF-I and/or endogenous IGF-I as a peak around the 30th fraction compared with those of IGFBP-2 and IGFBP-3 in both groups of rats. The reason may be that the level of IGFBP-1 is under one-hundredth and its binding affinity for IGF-I is about one-third of IGFBP-3 (19, 20).

Pharmacokinetics of rhIGF-I Unbound and Bound to IGFBPs in Diabetic Rats. To examine pharmacokinetics of rhIGF-I unbound and bound to IGFBPs, gel chromatographic analyses of plasma samples were performed after iv administration of rhIGF-I (1 mg/kg). From the estimated rate of each fraction and the data of fig. 1, the concentrations of free rhIGF-I and the high molecular complexes were calculated (fig. 3). In both groups of rats, rhIGF-I in plasma was almost free form just after the bolus injection, and then the free rhIGF-I decreased abruptly. On the other hand, rhIGF-I bound to the binding proteins increased. Especially, the 150 kDa complex maintained the relatively high level in plasma up to 6 hr after dosing. Plasma concentration profile of every fraction was higher in normal rats than that in diabetic rats. AUC values of free rhIGF-I, 50 kDa complex, and 150 kDa complex are 2.20 ± 0.26 , 0.795 ± 0.105 , and 1.92 ± 0.21 in normal rats and 2.08 ± 0.12 , 0.607 ± 0.038 ($p < 0.001$), and $1.14 \pm 0.08 \mu\text{g} \cdot \text{hr}/\text{ml}$ ($p < 0.001$) in diabetic rats, respectively. IGFBP-3 is well known as one of the major determinants for the plasma levels of IGFs (7), and the plasma concentrations of IGF-I (16) or rhIGF-I (8, 15) have been reported to be correlated with those of IGFBP-3. In the present study, the decrease in the concentration of 150 kDa complex was the greatest in the diabetic rats, and this would be mainly responsible for the higher clearance of rhIGF-I in the disease state. In diabetic state, biosynthesis of endogenous IGF-I would be reduced because of the lower level of growth hormone (21), which may be one of the reasons for the lower levels of endogenous IGF-I in diabetes. However, the levels of IGF-I in the kidney, a main organ for the elimination of IGF-I (8), is reported to be much higher in the diabetic rats (21, 22). Phillip *et al.* (21) indicated that the elevation of local production of IGF-I in the kidney should not

be responsible for the higher level of IGF-I in the kidney, suggesting the increase in IGF-I is trapped in the kidney. This can be explained by the higher rate of glomerular filtration resulting from the increase in free IGF-I because of the decrease in IGFBP-3, as is the case of rhIGF-I administered to diabetic rats in the present study.

MRT of only free rhIGF-I was significantly shorter in diabetic rats (0.132 ± 0.007 hr, $p < 0.05$) than that in normal rats (0.179 ± 0.013 hr), indicating that free rhIGF-I was eliminated more rapidly in diabetic rats than in normal rats. On the other hand, there was no difference in MRT of rhIGF-I in the forms of 150 kDa complex and of 50 kDa complex between diabetic (50 kDa, 1.89 ± 0.07 hr; 150 kDa, 2.63 ± 0.09 hr) and normal rats (50 kDa, 1.91 ± 0.11 hr; 150 kDa, 2.79 ± 0.11 hr).

In conclusion, we have investigated the pharmacokinetics of rhIGF-I after iv administration to diabetic rats. rhIGF-I administered was distributed and/or eliminated more rapidly in diabetic rats, and the faster disappearance coincided with the lower plasma levels of 150 kDa complex. Therefore, the decrease in the circulating 150 kDa complex caused by decreased IGFBP-3 would be one of the major factors for the rapid distribution and/or elimination of rhIGF-I in diabetic rats.

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