

## Biodistribution and Pharmacokinetics of Transgenic Pig-produced Recombinant Human Factor IX (rhFIX) in Rats

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**Abstract.** *Background:* Recombinant human factor IX (rhFIX) is a 56 kDa glycoprotein with full biological activity providing a guarantee of freedom from blood-borne viral contamination in the therapy of hemophilia B, but no data are available on the distribution of transgenic pig-produced rhFIX post injection (p.i.). Therefore, an <sup>131</sup>I-radiolabeled rhFIX was developed to evaluate the distribution of rhFIX in rats. *Materials and Methods:* rhFIX was labeled with the Iodogen method. <sup>131</sup>I-rhFIX (25 µCi/25 µg/200 µl/rat) was intravenously injected through the tail vein in normal Sprague-Dawley (SD) rats and the biodistribution was examined from 5 min to 72 h p.i.. The pharmacokinetics were also evaluated from 5 min to 96 h p.i. *Results:* The radiolabeled efficiency and radiochemical purity of <sup>131</sup>I-rhFIX was over 96% and 98%, respectively. The biodistribution study showed that the rhFIX chiefly accumulated in the liver. The distribution and elimination half-life ( $t_{1/2\alpha}$  and  $t_{1/2\beta}$ ) of <sup>131</sup>I-rhFIX were 0.82 and 9.34 h, respectively. The maximum concentration in the plasma ( $C_{max}$ ) and the area under the concentration versus time curve ( $AUC_{INF}$ ) of <sup>131</sup>I-rhFIX in rats were 3.09% injected dose (ID)/g and 15.3 h×% ID/g. *Conclusion:* The transgenic pig-produced rhFIX is mostly retained in the liver and the preclinical biodistribution and pharmacokinetic studies of <sup>131</sup>I radiolabeled rhFIX are helpful for researching its biological effect in vivo.

Hemophilia B is an X-linked bleeding disorder caused by mutations in the human coagulation factor IX genes, resulting in deficiencies of the procoagulant protein, plasma coagulation factor IX. The disease is usually managed by

direct replenishment with human factor IX. High-purity human plasma-derived factor IX (pdFIX) concentrates have been developed to treat hemophilia B, but the risk of viral infection cannot be totally eliminated (1, 2). Currently, non-human- or animal-derived commercial recombinant human FIX (rhFIX, Benefix<sup>®</sup>) is produced from transgenic Chinese hamster ovary (CHO) cells (3). This is structurally and functionally similar to pdFIX, but with minor differences in posttranslational sulfation and phosphorylation (4-6). The rhFIX is thus free from the risk of transmitting plasma-derived human viruses and has become a unique replenishment product for hemophilia B therapy (7-9).

The production of transgenic livestock has been demonstrated to be feasible for over two decades (10) and this kind of animal has been used in producing human proteins for pharmaceutical use (11). Transgenic pigs and pig biomedical models have also served important roles in researching human biology and commercial applications (12). Although recombinant human clotting factor IX has been produced in the milk of transgenic sheep for clinical applications (13), efforts in developing transgenic techniques to promote the yield of rhFIX during the lactating period and to improve the health of pigs after hFIX and other genes have been co-transferred are still ongoing. A technique has been invented for expressing multiple recombinant proteins in the milk of double-transgenic pigs, which carry both the human clotting factor IX gene and an exogenous porcine lactoferrin gene. The rhFIX and porcine lactoferrin are continuously and stably expressed in the transgenic mammal during its lactation (14). For evaluating its bioactivity, it is valuable to study the distribution and pharmacokinetics (PK) of the transgenic pig-produced rhFIX *in vivo*.

PK studies in preclinical animal and clinical trials are essential for rhFIX approval by the US Food and Drug Administration (FDA) for clinical use. Clotting factor concentrates (CFCs) determined by clotting assay (2) and immunological assay (15) are the conventional techniques to measure the activity and concentration of human clotting factor IX for the PK evaluation. Radio-iodine (iodine-131/125) has been commonly used for PK evaluation of biological products

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**Key Words:** Recombinant human factor IX (rhFIX), radiolabeled, biodistribution, pharmacokinetics, pig.

(for example, proteins, antibodies) due to its high detection sensitivity (16-18). Proteins labeled with  $^{131}\text{I}$  have been applied in clinical practice to assess the site(s) of tumors, pre-surgical staging, immunotherapy and monitoring for recurrence or response to various treatments (19).

Although PK studies of rhFIX derived by conventional clotting assay have been reported (2, 20, 21), little information is available on the PK evaluation of rhFIX by radiolabeling with  $^{131}\text{I}$ . Additionally, to the best of our knowledge, no information concerning the *in vivo* distribution of transgenic pig-produced  $^{131}\text{I}$ -rhFIX in rats has been reported. To examine the absorption and fate of rhFIX, the biodistribution and PK of  $^{131}\text{I}$ -labeled transgenic pig-produced rhFIX in rats was evaluated.

## Materials and Methods

**Animals and housing.** Eight-week-old male Sprague-Dawley (SD) rats were obtained from the National Animal Center of Taiwan (Taipei). The rats were housed at a temperature of 20-23°C and offered a standard diet (Lab diet, PMI Feeds, St. Louis, MO, USA) with tap water *ad libitum* in the animal house of the Institute of Nuclear Energy Research (INER), Taoyuan (Taiwan). All the animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the INER.

**Preparation of rhFIX.** The rhFIX was produced from double-transgenic pig milk provided by the Animal Technology Institute, Taiwan (14, 22). Different expression plasmids containing the human clotting factor IX gene and the porcine lactoferrin gene consisting of the 5' regulatory sequence (bovine alpha-lactalbumin promoter) were constructed, respectively. Both genes were located behind the regulatory sequence and subject to its control in expression. After transferring the expressing plasmids by gene injection and embryonic implantation in pigs, their products were specifically expressed in the mammary glands and continuously and stably expressed in the transgenic mammal during its lactation. The characteristics and function of the rhFIX have been proved by Western blotting and clotting activity assay (14).

After collecting the pig milk, it was centrifuged at 3000×g for 30 min at 4°C to remove the fat and stored at -80°C until use. A fraction containing the rhFIX was prepared from the skimmed milk by precipitation with phosphate buffer solution and centrifugation at 22,860×g for 10 min at 4°C. The whey fraction was concentrated by ultrafiltration using a polysulfone membrane with a hole diameter of 30 kDa (Tami Industries, France). The rhFIX was subsequently captured and purified by Q Sepharose fast flow chromatography (Amersham Pharmacia Biotech, Sweden) and a heparin-sepharose column (Amersham Pharmacia Biotech). Nanofiltration was used as the viral removal step. The protein had a specific activity of 230±53 IU/mg and purity exceeded 98% (22).

**Radiolabeling of rhFIX.** The rhFIX peptide was radiolabeled with  $\text{Na}^{131}\text{I}$  using the Iodogen (1,3,4,6-tetrachloro-3 $\alpha$ ,6 $\alpha$ -diphenylglycoluril) method as reported previously (23, 24). Briefly, borasilicate test tubes pre-coated with 50  $\mu\text{g}$  of Iodogen (Pierce, Rockford, IL, USA) were prewetted with 1 ml of Tris iodination buffer (25 mM Tris-HCl, pH 7.5, and 0.4 M NaCl) which was

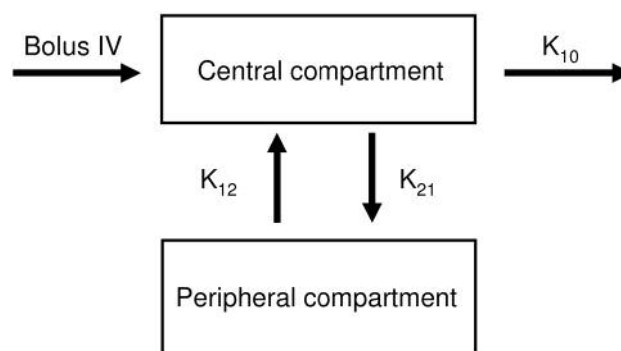


Figure 1. Graphical presentation of two-compartment model applied to plasma data (% ID/g) for  $^{131}\text{I}$ -rhFIX. IV: intravenous, ID: injected dose,  $K_{10}$ ,  $K_{12}$ ,  $K_{21}$ : micro rate constants.

decanted. Subsequently, 10-30 MBq of  $\text{Na}^{131}\text{I}$  in 0.1 M NaOH (Perkin Elmer, Boston, MA, USA) in 100  $\mu\text{l}$  of Tris iodination buffer were added to each tube. The reaction was allowed to proceed for 6 min at room temperature (RT) with a swirling of the tube every 30 seconds. One hundred  $\mu\text{g}$  of rhFIX in 20  $\mu\text{l}$  of Tris iodination buffer were added to react for 6-9 min at RT with mixing by gently flicking the tube every 30 seconds. The sample was transferred to another tube to stop the reaction and stored at -20°C before use.

**Quality control of radiolabeled  $^{131}\text{I}$ -rhFIX.** The labeling efficiency of rhFIX with  $^{131}\text{I}$  was determined using radio-thin-layer chromatography (Radio-TLC). Radio-TLC was performed by spotting samples on instant thin-layer chromatography silica gel strips (ITLC-SG; Gelman, Ann Arbor, MI, USA), which were then developed in methanol and water (85/15, v/v) as the mobile phase. The developed strips were scanned by a Bioscan Imaging Scanner (Bioscan, Inc., Washington, DC, USA).

**Biodistribution of  $^{131}\text{I}$ -rhFIX.** Forty-five normal male SD rats (five rats at each time-point) were used and each rat was intravenously injected with 25  $\mu\text{Ci}$   $^{131}\text{I}$ -rhFIX per 25  $\mu\text{g}$  total protein per 0.2 ml normal saline. The rats were sacrificed by  $\text{CO}_2$  asphyxiation at 5, 30, 45 min, 1, 3, 5, 8, 24 and 72 h post injection (*p.i.*) of  $^{131}\text{I}$ -rhFIX. Whole blood, plasma, and the organs of interest including stomach, large intestine, small intestine, bone, skin, muscle, lung, heart, liver, kidney, spleen, brain, testis and bladder, were removed and weighed and the radioactivity was measured with a gamma counter (1470 WIZARD Gamma Counter; Wallac, Turku, Finland). The radioactivity of the carcass was measured by a CRC-15R dose calibrator (Capintec, Ramsay, NJ, USA) and the decay correction of all radioactivity data was calculated from the injection time. The percentage of injected dose (% ID) and the % ID/g were calculated by comparison with standards representing the injected dose per animal. The data were expressed as mean±standard error of mean.

**Pharmacokinetics of  $^{131}\text{I}$ -rhFIX.** Five normal male SD rats were used and each rat was intravenously injected with 25  $\mu\text{Ci}$   $^{131}\text{I}$ -rhFIX per 25  $\mu\text{g}$  total protein per 0.2 ml normal saline. Blood samples (0.2 ml) were collected by heart puncture under 2% isofluorine anesthesia at 5 and 30 min, 1, 3, 8, 24, 30, 48, 72 and 96 h *p.i.* of the  $^{131}\text{I}$ -rhFIX protein. The samples were centrifuged at 3000 rpm to acquire the plasma. The concentrations of radioactivity in the blood

Table I. Radioactivity in major organs after intravenous injection of  $^{131}\text{I}$ -rhFIX into normal rats. Values are presented as the percent age injected dose per organ (% ID/organ, mean $\pm$ SEM, n=5 at each time-point).

Organ	5 min	30 min	45 min	1 h	3 h	5 h	8 h	24 h	72 h
Stomach	0.21 $\pm$ 0.03	1.38 $\pm$ 0.33	2.35 $\pm$ 0.19	2.52 $\pm$ 0.27	2.04 $\pm$ 0.46	1.20 $\pm$ 0.11	0.73 $\pm$ 0.07	0.10 $\pm$ 0.01	0.03 $\pm$ 0.00
Lung	0.92 $\pm$ 0.15	1.24 $\pm$ 0.28	1.40 $\pm$ 0.20	2.16 $\pm$ 0.95	0.59 $\pm$ 0.05	0.48 $\pm$ 0.07	0.34 $\pm$ 0.03	0.35 $\pm$ 0.20	0.04 $\pm$ 0.01
Heart	0.43 $\pm$ 0.07	0.40 $\pm$ 0.07	0.38 $\pm$ 0.04	0.38 $\pm$ 0.02	0.21 $\pm$ 0.01	0.16 $\pm$ 0.00	0.10 $\pm$ 0.01	0.03 $\pm$ 0.00	0.01 $\pm$ 0.00
Liver	89.78 $\pm$ 13.6	33.75 $\pm$ 2.85	21.95 $\pm$ 3.10	20.26 $\pm$ 0.35	10.01 $\pm$ 0.09	8.41 $\pm$ 0.43	5.83 $\pm$ 0.12	2.89 $\pm$ 0.27	1.40 $\pm$ 0.08
Kidney	5.49 $\pm$ 0.83	6.39 $\pm$ 0.78	5.67 $\pm$ 0.54	4.76 $\pm$ 0.49	3.06 $\pm$ 0.19	2.05 $\pm$ 0.26	1.45 $\pm$ 0.11	0.83 $\pm$ 0.33	0.33 $\pm$ 0.05
Spleen	1.10 $\pm$ 0.25	0.83 $\pm$ 0.18	0.53 $\pm$ 0.09	0.69 $\pm$ 0.11	0.34 $\pm$ 0.07	0.23 $\pm$ 0.03	0.17 $\pm$ 0.01	0.07 $\pm$ 0.01	0.03 $\pm$ 0.00
Brain	0.10 $\pm$ 0.02	0.11 $\pm$ 0.02	0.11 $\pm$ 0.01	0.10 $\pm$ 0.00	0.05 $\pm$ 0.00	0.05 $\pm$ 0.01	0.03 $\pm$ 0.00	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00

Table II. Radioactivity in organs after intravenous injection of  $^{131}\text{I}$ -rhFIX into normal rats. Values are presented as the percent age injected dose per gram (% ID/g, mean $\pm$ SEM, n=5 at each time-point).

Organ	5 min	30 min	45 min	1 h	3 h	5 h	8 h	24 h	72 h
Blood	1.64 $\pm$ 0.23	1.29 $\pm$ 0.22	1.28 $\pm$ 0.14	1.22 $\pm$ 0.03	0.56 $\pm$ 0.02	0.46 $\pm$ 0.03	0.29 $\pm$ 0.01	0.10 $\pm$ 0.01	0.04 $\pm$ 0.00
Plasma	3.08 $\pm$ 0.45	1.78 $\pm$ 0.29	1.69 $\pm$ 0.18	1.53 $\pm$ 0.02	0.69 $\pm$ 0.01	0.59 $\pm$ 0.06	0.41 $\pm$ 0.02	0.25 $\pm$ 0.03	0.12 $\pm$ 0.02
Stomach	0.13 $\pm$ 0.02	0.83 $\pm$ 0.18	1.50 $\pm$ 0.18	1.63 $\pm$ 0.26	1.20 $\pm$ 0.25	0.73 $\pm$ 0.10	0.48 $\pm$ 0.05	0.06 $\pm$ 0.01	0.02 $\pm$ 0.00
L.I	0.10 $\pm$ 0.02	0.24 $\pm$ 0.05	0.25 $\pm$ 0.04	0.39 $\pm$ 0.08	0.18 $\pm$ 0.04	0.16 $\pm$ 0.02	0.16 $\pm$ 0.03	0.04 $\pm$ 0.00	0.02 $\pm$ 0.00
S.I	0.30 $\pm$ 0.05	0.66 $\pm$ 0.19	0.81 $\pm$ 0.19	0.77 $\pm$ 0.25	0.28 $\pm$ 0.05	0.22 $\pm$ 0.06	0.21 $\pm$ 0.03	0.05 $\pm$ 0.01	0.02 $\pm$ 0.00
Bone	0.23 $\pm$ 0.03	0.38 $\pm$ 0.07	0.38 $\pm$ 0.05	0.43 $\pm$ 0.01	0.24 $\pm$ 0.01	0.23 $\pm$ 0.02	0.19 $\pm$ 0.02	0.08 $\pm$ 0.01	0.05 $\pm$ 0.00
Muscle	0.05 $\pm$ 0.01	0.15 $\pm$ 0.03	0.10 $\pm$ 0.03	0.10 $\pm$ 0.02	0.07 $\pm$ 0.01	0.05 $\pm$ 0.01	0.03 $\pm$ 0.01	0.01 $\pm$ 0.00	0.00 $\pm$ 0.00
Lung	0.54 $\pm$ 0.06	0.72 $\pm$ 0.14	0.81 $\pm$ 0.13	1.46 $\pm$ 0.64	0.40 $\pm$ 0.03	0.31 $\pm$ 0.06	0.22 $\pm$ 0.02	0.20 $\pm$ 0.01	0.02 $\pm$ 0.00
Heart	0.34 $\pm$ 0.05	0.33 $\pm$ 0.05	0.33 $\pm$ 0.04	0.33 $\pm$ 0.02	0.16 $\pm$ 0.01	0.14 $\pm$ 0.01	0.08 $\pm$ 0.00	0.02 $\pm$ 0.00	0.01 $\pm$ 0.00
Liver	4.78 $\pm$ 0.87	1.52 $\pm$ 0.34	1.22 $\pm$ 0.18	1.15 $\pm$ 0.07	0.44 $\pm$ 0.02	0.44 $\pm$ 0.04	0.29 $\pm$ 0.01	0.15 $\pm$ 0.02	0.08 $\pm$ 0.01
Kidney	1.40 $\pm$ 0.23	1.64 $\pm$ 0.23	1.54 $\pm$ 0.15	1.33 $\pm$ 0.13	0.75 $\pm$ 0.07	0.57 $\pm$ 0.10	0.34 $\pm$ 0.03	0.21 $\pm$ 0.08	0.08 $\pm$ 0.01
Spleen	1.51 $\pm$ 0.27	1.19 $\pm$ 0.26	0.90 $\pm$ 0.16	0.87 $\pm$ 0.15	0.39 $\pm$ 0.07	0.31 $\pm$ 0.03	0.22 $\pm$ 0.01	0.10 $\pm$ 0.02	0.04 $\pm$ 0.00
Brain	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.06 $\pm$ 0.00	0.05 $\pm$ 0.00	0.03 $\pm$ 0.00	0.03 $\pm$ 0.01	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00
Testis	0.06 $\pm$ 0.01	0.16 $\pm$ 0.03	0.18 $\pm$ 0.02	0.27 $\pm$ 0.01	0.18 $\pm$ 0.01	0.18 $\pm$ 0.02	0.12 $\pm$ 0.01	0.02 $\pm$ 0.00	0.01 $\pm$ 0.00

L.I: Large intestine; S.I: small intestine.

were expressed as % ID/ml (25). The data were fitted to a two-compartment model (Figure 1) and the PK parameters were derived by the computer software WinNonlin 5.0 (Pharsight Corporation, Mountain View, CA, USA). The macro rate constants (A, B,  $\alpha$ ,  $\beta$ ) were derived. The following biexponential equations were used:

$$C(t) = A \exp(-\alpha t) + B \exp(-\beta t) \text{ and} \\ A = (D/V_1) \times (\alpha - K_{21}) / (\alpha - \beta); B = (D/V_1) \times (K_{21} - \beta) / (\alpha - \beta).$$

$C(t)$  is the concentration in the plasma at time  $t$ ,  $A$  and  $B$  are the ordinate intercepts,  $\alpha$  is the first-order rate constant associated with the distribution phase and  $\beta$  is the first-order rate constant associated with the elimination phase;  $D$  is the injected dose. The micro rate constants ( $K_{10}$ ,  $K_{12}$  and  $K_{21}$ ) and the apparent volume of the central compartment ( $V_1$ ) were also calculated.  $K_{10}$  is the elimination rate constant of the drug leaving the system from the central compartment.  $K_{12}$  and  $K_{21}$  are the transfer rate constants of the drug from the central compartment to the peripheral compartment and vice versa, respectively (Figure 1). The distribution half-life ( $t_{1/2\alpha}$ ), elimination half-life ( $t_{1/2\beta}$ ), maximum area under the  $^{131}\text{I}$ -rhFIX concentration *versus* time curve (AUC), maximum plasma concentration reached ( $C_{\max}$ ) and the mean residence time (MRT) were calculated.

## Results

**Radiolabeling of  $^{131}\text{I}$ -rhFIX.** The radiolabeling efficiencies and radiochemical purity of  $^{131}\text{I}$ -rhFIX were 96.00 $\pm$ 1.39% and >95%, respectively. Because the labeling efficiencies exceeded 95%, no further purification was performed for the animal studies.

**Biodistribution of  $^{131}\text{I}$ -rhFIX.** The total radioactivity of the intravenously injected  $^{131}\text{I}$ -rhFIX (% ID) in the major organs is shown in Table I. The radioactivity was found predominantly in the liver at each time-point. It reached maximum levels of 89.8 $\pm$ 13.6 % ID at 5 min, then declined to 33.8 $\pm$ 2.85% ID at 30 min and 1.4 $\pm$ 0.08 % ID at 72 h. The radioactivity also accumulated in the kidneys. The biodistribution data of the  $^{131}\text{I}$ -rhFIX (% ID/g) in selected organs from the normal SD rats at 5, 30, 45 min, 1, 3, 5, 8, 24, 48 and 72 h *p.i.* are presented in Table II. The uptake in the liver reached the highest value (4.78 $\pm$ 0.87% ID/g) at 5 min, then declined to 0.44 $\pm$ 0.03 % ID/g at 3 h and 0.08 $\pm$ 0.01 % ID/g at 72 h. The

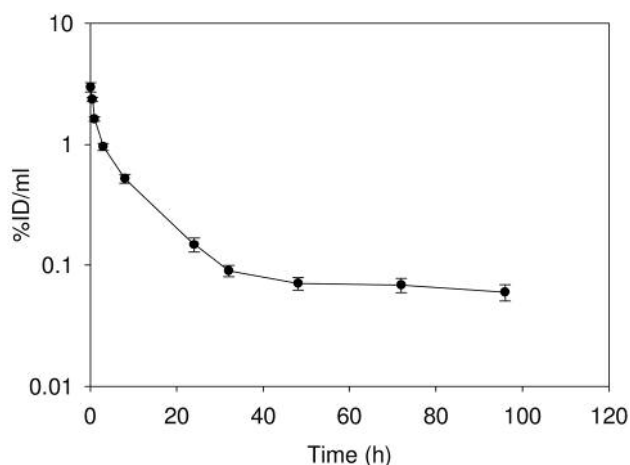


Figure 2. Radioactivity in plasma after intravenous administration of 25  $\mu\text{Ci}/25 \mu\text{g}$   $^{131}\text{I}$ -rhFIX into normal SD rats. Data are presented as mean  $\pm$  standard error of mean ( $n=5$ ). ID: injected dose.

radioactivity accumulating in the kidneys peaked at 30 min *p.i.* Very low radioactivity accumulation was found in the brain and testis.

**Pharmacokinetics of  $^{131}\text{I}$ -rhFIX.** The mean radioactivity (% ID/ml) in the plasma versus time curve is shown in Figure 2. The clearance curve of  $^{131}\text{I}$ -rhFIX was biphasic, with an initial rapid distribution phase followed by a slow elimination phase of longer duration. The calculated PK parameters of  $^{131}\text{I}$ -rhFIX derived from the two-compartment model are shown in Table III.

## Discussion

The double-transgenic swine not only express a high yield of rhFIX in the secreted milk, but also express exogenous porcine lactoferrin, which can help boost the immunity and resistance of the nursing offspring, reducing diarrhea and fighting inflammation (14). This advantage makes these double-transgenic pigs highly valuable in the swine industry because they produce high quality rhFIX for clinical use.

Iodine-131 with its physical properties (mainly gamma emission at 363 keV, half-life 8.02 days) has been widely applied in many fields of research (16-18), including one therapeutic product approved by the FDA (19). The rhFIX contains tyrosine residues in position 5 for radioiodination. The radiolabeled factor IX in the present study displayed identical activity to the unlabeled factor IX reported by Fuchs and colleagues (23). Their studies demonstrated that most of the radioactivity (% ID) was contained within the liver at 2 min (86% RD, recovered dose) after injection of  $^{125}\text{I}$ -rhFIX, compared with the present results at 5 min

Table III. Pharmacokinetic parameter estimates from radioactivity in plasma after intravenous injection of  $^{131}\text{I}$ -rhFIX (25  $\mu\text{g}/\text{rat}$ ) in rats (Each value represents mean  $\pm$  SEM of 5 rats).

Parameter	Unit	Value
A	(% ID/ml)	2.10 $\pm$ 0.26
B	(% ID/ml)	0.99 $\pm$ 0.09
$\alpha$	(h <sup>-1</sup> )	0.96 $\pm$ 0.18
$\beta$	(h <sup>-1</sup> )	0.08 $\pm$ 0.01
$t_{1/2\alpha}$	(h)	0.82 $\pm$ 0.13
$t_{1/2\beta}$	(h)	9.34 $\pm$ 0.96
MRT	(h)	9.08 $\pm$ 1.16
$K_{10}$	(h <sup>-1</sup> )	0.20 $\pm$ 0.02
$K_{12}$	(h <sup>-1</sup> )	0.47 $\pm$ 0.11
$K_{21}$	(h <sup>-1</sup> )	0.36 $\pm$ 0.06
$V_1$	(ml)	33.85 $\pm$ 3.42
$C_{\text{max}}$	(% ID/g)	3.09 $\pm$ 0.33
$\text{AUC}_{\text{INF}}$	(h $\times$ % ID/g)	15.30 $\pm$ 1.17

A, B,  $\alpha$ ,  $\beta$ : macro rate constants;  $t_{1/2\alpha}$ ,  $t_{1/2\beta}$ : distribution and elimination half-life, respectively; MRT: mean residence time;  $K_{10}$ ,  $K_{12}$ ,  $K_{21}$ : micro rate constants;  $V_1$ : apparent volume of the central compartment;  $C_{\text{max}}$ : maximum concentration in plasma;  $\text{AUC}_{\text{INF}}$ : area under concentration of  $^{131}\text{I}$ -rhFIX versus time curve.

(89.8% ID, ID was equivalent to RD in these studies). The high uptake of  $^{131}\text{I}$ -rhFIX in the liver suggested that the  $^{131}\text{I}$ -rhFIX retained its bioactivity and bound to the thrombin-binding sites on the endothelial cells in the liver (Table I) (23). The radioactivity accumulated in the kidneys suggested the urinary system would be one of the routes for excretion of  $^{131}\text{I}$ -rhFIX.

Although the radioactivity (% ID/g) of  $^{131}\text{I}$ -rhFIX was clearly noted in the liver, it was very low in the brain (Tables I and II) showing that rhFIX did not cross the blood-brain barrier. The PK of intravenously administered rhFIX was also studied in Sprague-Dawley rats by Keith *et al.* (15). The plasma levels of the CHO cell-produced rhFIX were determined by a conventional monoclonal sandwich ELISA assay in their study and the elimination half-life ( $t_{1/2\beta}$ ) was roughly 5.0 h (15). In the present study, the  $t_{1/2\beta}$  of the transgenic pig-produced rhFIX after a single intravenous administration was 9.34 h (Table III). Although the exact reasons need to be further determined, the analytical methods or differences in posttranslational modification of rhFIX by the Chinese hamster ovary cells and the transgenic mammal may be responsible for these differences.

Some important parameters need to be considered in designing PK studies using radioisotopes, including the physical property of the radioisotopes, the position of the radiolabel in the molecule, the specific activity, radiochemical purity and the analysis protocol. Iodine-131 has the advantages of a well-known chemistry, longer half-life (8 days), easy availability and low cost for medical research and radiotherapy (26). These advantages also make

it useful for monitoring the location and distribution of bio-macromolecules during the biological response. Although the PK of rhFIX have been reported previously (15, 20, 27), the PK of double-transgenic pig-produced  $^{131}\text{I}$ -rhFIX was first reported in this study.

In conclusion, radioactivity of  $^{131}\text{I}$ -rhFIX chiefly accumulates in the liver, and no specific radioactivity accumulation is found in brain and testis. These preclinical biodistribution and pharmacokinetic data of  $^{131}\text{I}$ -rhFIX are helpful for future examination of its biological effect *in vivo*.

## Acknowledgements

The authors would like to thank the Ministry of Economic Affairs of the Republic of China, Taiwan for financially supporting this research and thank L.C. Chen, C.Y. Yu, W.C. Hsu, C.L. Ho, Y.J. Chang, M.H. Chen and W.C. Lee for their technical assistance.

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Received May 16, 2008

Revised July 15, 2008

Accepted August 14, 2008