Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 148–153

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Pharmacokinetics, tissue distribution and excretion of porcine fibrinogen after intraperitoneal injection of a porcine-derived fibrin glue to rats

Ying Xie[a,b], Gaoren Zhong[c], Hongbing He[d], Guorong Fan[a,b,*], Yutian Wu[a,b]

[a] Department of Pharmaceutical Analysis, School of Pharmacy, Second Military Medical University, No. 325 Guohe Road, Shanghai 200433, PR China
[b] Shanghai Key Laboratory for Pharmaceutical Metabolite Research, No. 325 Guohe Road, Shanghai 200433, PR China
[c] Institute of Radiation Medicine, Fudan University, No. 2094 Xietu Road, Shanghai 200032, PR China
[d] Shanghai Pine & Power Biotech Co., Ltd, No. 4299 Jindu Road, Shanghai 201108, PR China

ARTICLE INFO

Article history:
Received 16 March 2010
Received in revised form 26 July 2010
Accepted 31 July 2010
Available online 13 August 2010

Keywords:
Porcine-derived fibrin glue
^{125}I-labeled porcine fibrinogen
Pharmacokinetics
Tissue distribution
Excretion

ABSTRACT

The aim of the present study was to characterize the preclinical pharmacokinetics, tissue distribution and excretion profiles of porcine fibrinogen in rats after intraperitoneal injection of a porcine-derived fibrin glue. A sensitive and rapid isotope-labeled assay method was developed and validated for quantitative analysis in biological analysis. Porcine fibrinogen, the major composition of the fibrin glue, was radioiodinated with Na^{125}I using the Iodo-Gen method. Following the purification and identification of ^{125}I-porcine fibrinogen, the fibrin glue containing ^{125}I-porcine fibrinogen was intraperitoneally administered to rats at three single dosages (100, 200, 400 mg/kg of porcine fibrinogen). The results showed that the ^{125}I-labeled assay method was suitable for the quantification of porcine fibrinogen in plasma samples, tissue samples and excreta samples with satisfactory linear ($r^2 > 0.998$), precision (<13%), accuracy (95.9–104.2%) and recovery (>85%). After three single administrations, plasma concentration profiles showed a slow absorption phase with the mean t_{max} of 1.83–5.67 h and a slow elimination proceeding with the terminal elimination half-life ($T_{1/2}$) of 84.5–96.3 h. Porcine fibrinogen was widely distributed to most of the tissues examined after a single intraperitoneal administration at 200 mg/kg to rats. The radioactive porcine fibrinogen showed substantial disposition in liver, kidneys, stomach and intestine. Approximately 79.3% and 17.2% of administered radioactivity were recovered in urine and feces within 528 h post-dosing, which indicated the major elimination route was urinary excretion.

1. Introduction

Fibrin glue (FG), a biological tissue adhesive, has been used for some years to facilitate hemostasis, seal tissues and improve wound healing in a wide variety of clinical settings [1]. FG is frequently applied following surgical procedures such as cardiovascular [2], thoracic [3], vascular [2], neurological [4–7], abdominal [8,9] and oncologic plastic surgery [10]. FG is derived mainly from plasma components, which contains purified and virally inactivated human fibrinogen and thrombin, with different quantities of factor XIII, anti-fibrinolytic agents (such as bovine aprotinin), and calcium chloride [11].

As it is known, human fibrinogen-based glue, which is served as a scarce resource, may contain HIV or HB virus. They may be transmitted to patients while hemostasis is accomplished. Thus, porcine fibrinogen-based glue, a new type of animal-derived FG developed in China since the 2000s, not only prevents transmission of human and porcine blood-borne pathogens but also dramatically reduces production costs of the biomedical materials. It has been verified that this porcine fibrinogen-based glue has the advantages of no virus, good film forming ability and rapid operation by means of innovative biotechnological processing (ISO13485:2003 certified for medical device use). The porcine-derived FG is composed of porcine fibrinogen, thrombin, and small quantity of factor XIII, fibronectin and calcium chloride. When porcine fibrinogen and thrombin are mixed, the fibrinogen component is hydrolyzed into fibrin monomers. Then the stable fibrin polymer (fibrin glue) is formed under the action of factor XIII, fibronectin and calcium chloride.

Despite the extensive use of human-derived FG, published pharmacokinetic studies are scarce. Only some tissue distribution studies of a human-derived fibrin adhesive agent applied to the incision of stomach, lung, and pericardial space of rats were published [12–14]. A good understanding of the pharmacokinetic properties in proper animal models is crucial to any novel drugs including biotech products such as porcine-derived FG. Moreover,

* Corresponding author at: Second Military Medical University, School of Pharmacy, No. 325 Guohe Road, Shanghai 200433, PR China.
Tel.: +86 21 8187 1260; fax: +86 21 8187 1260.
E-mail address: guorfan@yahoo.com.cn (G. Fan).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.07.048

proper knowledge on the distribution and excretion is vital to investigate the major target sites and interpret the in vivo disposition. Therefore, the complete research and evaluation of the preclinical pharmacokinetics, tissue distribution and excretion of this porcine-derived FG is required to make for the efficacy and safety consideration in clinical applications.

Up to now, the commonly used analytical methods by which protein drugs and biomaterials are studied in vivo are immunoassay, isotope trace assay, bioassay and physicochemical analytical techniques [15]. Among them, immunoassay and bioassay techniques including the immunonephelemetric method and the Clauss method have been used to investigate the pharmacokinetic properties of human fibrinogen concentrates in patients with congenital afibrinogenemia or hypofibrinogenemia [16–19]. However, in our experiment, a kind of heterogeneous fibrinogen is served as the research object and the fibrinogen has been converted to the fibrin polymer before entering the body, thus, is not suitable to be determined by using immunoassay and bioassay methods. ^{125}I, a radioisotope with high specific radioactivity, optimum half-life and simple labeled preparation, has been demonstrated to be feasible for labeling of human fibrinogen in pharmacokinetic studies [12–14,20,21], thus, could be used as a selective method for the study of porcine fibrinogen in vivo as well. Besides, the isotope-labeled assay method, with high sensitivity and rapid detection, can intuitively provide all data of plasma level, distribution, metabolism and excretion.

In the present study, a sensitive and rapid isotope-labeled assay method was developed to investigate the pharmacokinetic profiles of porcine fibrinogen in rats following intraperitoneal injections of a porcine-derived FG with three single doses. Furthermore, the tissue distribution and excretion patterns after a single intraperitoneal injection in rats were also studied.

2. Materials and methods

2.1. Materials

Porcine fibrinogen (purity: >98.0%, MW = 340 kDa) used for labeling was purchased from Shanghai Bluegene Biotech Co., Ltd (Shanghai, China). Iodogen (1,3,4,6-tetrchloro-3α,6α-diphenyl glycoluril) and Na^{125}I solutions were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Perkin Elmer Life Sciences Inc. (Boston, MA, USA), respectively. All porcine fibrinogen solutions were formulated in 10 mM phosphate-buffered saline (PBS, pH 7.4), which was produced by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Trichloroacetic acid (TCA) was obtained from Shanghai Qianjin Chemical Reagent Factory (Shanghai, China).

2.2. Preparation of porcine-derived FG containing ^{125}I-porcine fibrinogen

2.2.1. Radioiodination and purification of ^{125}I-porcine fibrinogen

Fibrinogen contains several tyrosine residues enabling it to be ^{125}I-labeled [23,24]. Porcine fibrinogen was radiolabeled with Na^{125}I using the Iodo-Gen method as previously described [22] with some modification. Briefly, 100 μl of fibrinogen (1 μg/μl) was incubated with 1 μl of Na^{125}I (500 μCi) in a reaction tube coated with 5 μg of iodogen at room temperature with gentle stirring for 10 min. Then the incubation was stopped by addition of PBS (10 mM, pH 7.4). After incubation, in order to separate free ^{125}I from fibrinogen-bound ^{125}I, the iodinated porcine fibrinogen was purified on a Sephadex G-25 column (1 cm × 30 cm, Pharmacia Biotech) by eluting with PBS (10 mM, pH 7.4) at a flow rate of 1 ml/min. The effluents were collected at 1 min intervals. Through radioactivity determination, the fractions containing ^{125}I-labeled porcine fibrinogen were collected and the remaining fractions were discarded. Determined by two chromatographic methods, Instant Thin-Layer Chromatography on silica gel (ITLC-SG) and HPLC, the radiochemical purity of ^{125}I-labeled porcine fibrinogen was proved to be more than 95%.

2.2.2. Final formulation preparation

The tested porcine-derived FG, supplied by Pine & Power Biotech Co., Ltd (Shanghai, China), consists of solution A (46.67 mg/ml porcine fibrinogen, small quantity of factor XIII and fibronectin dissolved in 10 mM PBS) and solution B (754 IU/ml thrombin dissolved in 10 mM calcium chloride). To achieve the final mixture solution for dosing in rat experiments, the above ^{125}I-labeled porcine fibrinogen was added to 3 ml of solution A, which was prepared based on the concentration of porcine fibrinogen to be administered. In this way, each animal in the three single dosage groups received equivalent to 100 mg/kg, 200 mg/kg and 400 mg/kg of porcine fibrinogen containing ^{125}I-labeled and unlabeled porcine fibrinogen.

2.3. Animals

Healthy Sprague–Dawley rats (male and female) weighing 220–250 g were obtained from Department of Laboratory Animal Science, Fudan University (Shanghai, China). The animals were housed with free access to food and water, and maintained on a 12 h light-dark cycle (light on from 8:00 to 20:00) at ambient temperature (18–20 °C) and roughly 50% relative humidity. The rats were fasted for 12 h before all studies. Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals and the related ethical regulations of Fudan University.

2.4. Validation for radioactivity determination in plasma, tissues and excreta by TCA precipitation assay

The contents of porcine fibrinogen in biological samples were defined by the radioactivity of samples, which was determined by using a SN-695 gamma counter (Rihuan Instrument Factory of Shanghai Atomic Nucleus Institute, China). The radioactivity was denoted by counts per minute (cpm). Precipitation of the iodinated porcine fibrinogen in plasma and tissues by 10% TCA solution (diluted by distilled water) was used to remove free ^{125}I or ^{125}I associated with the fragmented peptide. TCA precipitable radioactivity rather than total radioactivity was used to calculate the iodinated protein concentration in plasma and tissue homogenate samples, while total radioactivity instead of TCA precipitated radioactivity recovered from the feces and urine was used in the excretion studies [25] to obtain mass balance information.

Each of the radioactive plasma and tissue homogenate samples (100 μl) was well mixed with 1.5 ml of 10% TCA solution. After centrifugation for 10 min at 3000 × *g*, the supernatant was removed and then the radioactivity of precipitate was determined. Each spiked radioactive excreta sample was directly determined after appropriate vortex. A series of calibration standards were prepared by adding seven concentrations of radiolabeled porcine fibrinogen (1.0–187.5 μg/ml) into the blank rat plasma, tissue homogenate and excreta samples. After suitable treatments, the relationship between the added concentrations and counted radioactivity of the standards was evaluated. Considering the measuring time and statistics requirements of radionuclide, the lower limit of detection of the ^{125}I-labeling was calculated based on the variability in the background cpm levels. Quality control (QC) samples in plasma, liver, urine and feces at four different concentration levels (1.0, 5.0, 50.0 and 150.0 μg/ml) were prepared to determine the accuracy (deviation form nominal values, %) and precision (relative standard deviation, R.S.D.) of this method. Intra-day precision was evaluated

by analyzing QC samples in six replicates over 1 day, while inter-day precision was evaluated from the analysis of each control once on each of 6 days. The accuracy was estimated for each QC sample by comparing the measured concentration to the actual concentration. The absolute recovery was determined by calculating the ratio of the responses of three QC biological samples (low, medium and high) finally obtained against those originally untreated.

2.5. Pharmacokinetic studies

Three single doses (equivalent to 100 mg/kg, 200 mg/kg and 400 mg/kg of porcine fibrinogen containing ^{125}I-labeled and unlabeled porcine fibrinogen) of solution A and an equivalent volume of solution B were intraperitoneally injected to rats through the duplex syringes (Pine & Power Biotech Co., Ltd, Shanghai, China). The rats were grouped randomly (three groups, $n = 6$ per group) based on their genders and body weights. Blood samples (approximately 200 μl each) were collected from tail vein into heparinized tubes at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 72, 168 and 240 h, and then centrifuged at 3000 × *g* for 10 min. 100 μl of plasma samples were harvested. The radioactivity in plasma samples was determined by the gamma counter after precipitation with TCA (10%, v/v). The result of ^{125}I-labeled porcine fibrinogen concentration in each time phase was expressed as micrograms per milliliter (μg/ml), calculated by standard curve equation.

2.6. Tissue distribution studies

Seven groups of rats ($n = 6$ per group) were intraperitoneally injected at a single dose of 200 mg/kg by the same way as described above. Tissues including heart, lungs, liver, spleen, kidneys, stomach, intestine, brain, uterus, testicle, muscle and injection site were removed at 2, 8, 24, 72, 168, 240 and 528 h after dosing and dried with filter paper. Following weighed on a Sartorius digital electronic balance, the radioactivity of tissue homogenates was determined by the gamma counter after precipitation with TCA (10%, v/v). Finally, the radioactive distribution of tissues was expressed as the percentage of the injected dose per gram of tissue (%ID/g).

2.7. Elimination studies

Six rats were intraperitoneally injected at a single dose of 200 mg/kg by the same way as described above and then individually placed in a stainless-steel metabolic cage, which allowed separate collection of urine and feces. The urine and feces samples were collected at 0–8, 8–24, 24–48, 48–72, 72–96, 96–144, 144–168, 168–192, 192–216, 216–240, 240–264, 264–312, 312–336, 336–360, 360–384, 384–408, 408–432, 432–504 and 504–528 h after dosing. The volume of urine samples and the weight of feces samples were measured prior to determination of radioactivity. The urinary and fecal excretion rates (%) were calculated as the percentage of dose excreted.

2.8. Data analysis

The concentration versus time profiles was analyzed by a non-compartmental model. Data were expressed as mean ± standard deviation (S.D.). Statistical differences in the pharmacokinetic parameters among different doses were tested by the one-way ANOVA. $P < 0.05$ was considered as statistical significance.

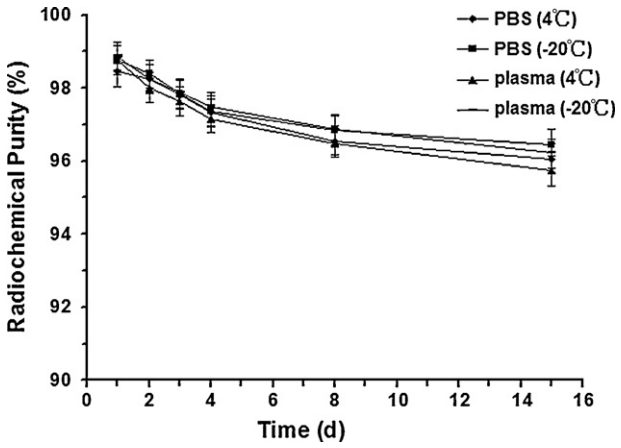

Fig. 1. Stability of ^{125}I-labeled porcine fibrinogen stored at 4 °C and −20 °C in PBS and plasma ($n = 5$).

3. Results and discussion

3.1. Preparation of porcine-derived FG containing ^{125}I-labeled porcine fibrinogen

Considering that the labeling process is based on the atom exchange, chemical reaction and physicochemical treatments, the stability and biological activity of labeled porcine fibrinogen were the main factors to be taken into account. To examine the stability in vitro of ^{125}I-labeled porcine fibrinogen stored at 4 °C and −20 °C in PBS and plasma, the radiochemical purity was determined at 1, 2, 3, 4, 8 and 15 d by ITLC-SG method using 85% ethanol as the mobile phase. The results showed that the radiochemical purities of all radioactive samples were more than 95% in 15 days, thus this kind of radioactive sample was stable in both PBS and plasma (Fig. 1). The short-term temperature stability and post-preparative stability of radioactive plasma samples were determined after keeping the samples at room temperature for 2 h. The results showed that the radiochemical purities were more than 98%, which indicated the acceptable stability in vitro at room temperature. To investigate the influence of labeling on biological activity of porcine fibrinogen, the labeled porcine fibrinogen–thrombin binding experiment was carried out. The results indicated that the labeled fibrinogen–thrombin binding percentage was more than 85%, calculated by the ratio of radioactivity in the labeled porcine fibrinogen-thrombin coagulum to that in the labeled porcine fibrinogen, thus proving that the biological activity was not interfered by labeling.

3.2. Validation for radioactivity determination in plasma, tissues and excreta

The calibration model was selected based on the data obtained by linear regression with $1/x^2$ weighting factor. The calibration curves for all matrices showed good linearity ($r^2 > 0.998$) over the concentration ranges tested. The absolute recoveries of different matrices were more than 85%. The intra- and inter-day precision and accuracy in different matrices are summarized in Table 1. The intra- and inter-day precision were less than 12.2% and the accuracy was from 95.9% to 104.2% for QC samples. The precision of LLOQ was below 12.9% and accuracy was from 97.4% to 103.7%. The results showed acceptable precision, accuracy and recovery. Consequently, the ^{125}I-labeled assay method was demonstrated to be suitable for the quantification of porcine fibrinogen in plasma samples, tissue samples and excreta samples.

Table 1
Intra- and inter-day precision and accuracy of ^{125}I-porcine fibrinogen assay in rat plasma, liver, urine and feces ($n = 6$).

Sample matrix	Spiked concentration (μg/ml)	Intra-day precision (R.S.D., %)	Inter-day precision (R.S.D., %)	Accuracy (mean ± S.D., %)
Plasma	1.0	12.9	12.8	102.2 ± 13.2
	5.0	11.0	12.2	99.2 ± 10.9
	50.0	3.4	11.0	100.7 ± 3.4
	150.0	2.4	4.4	99.7 ± 2.4
Liver	1.0	10.2	11.3	98.7 ± 10.1
	5.0	9.3	5.7	96.4 ± 9.0
	50.0	4.7	7.1	101.5 ± 4.8
	150.0	2.5	3.7	99.6 ± 2.5
Urine	1.0	8.7	7.3	103.7 ± 9.1
	5.0	4.9	6.4	104.2 ± 5.1
	50.0	4.9	4.5	99.0 ± 4.8
	150.0	3.2	1.9	99.3 ± 3.2
Feces	1.0	11.7	12.8	97.4 ± 11.4
	5.0	7.7	8.3	101.9 ± 7.8
	50.0	8.9	6.3	95.9 ± 8.6
	150.0	5.4	3.5	99.7 ± 5.4

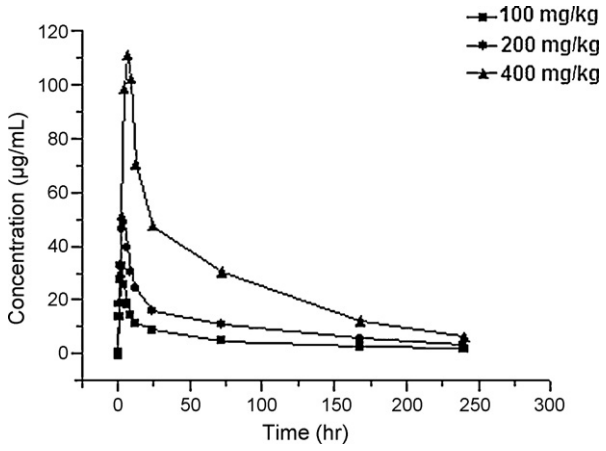

Fig. 2. Plasma concentration–time profiles of ^{125}I-porcine fibrinogen after intraperitoneal injection of porcine-derived FG to rats ($n = 6$). Three investigated dosages are 100, 200, 400 mg/kg of porcine fibrinogen containing ^{125}I-labeled and unlabeled porcine fibrinogen.

3.3. Plasma pharmacokinetics

The mean plasma concentration versus time profiles of ^{125}I-porcine fibrinogen after intraperitoneal injection of porcine-derived FG containing ^{125}I-labeled porcine fibrinogen at three doses are depicted in Fig. 2. The corresponding pharmacokinetic parameters generated by fitting plasma concentration profiles to a noncompartmental model are listed in Table 2.

Dose linearity of the pharmacokinetics over the dosage range examined (100–400 mg/kg) was demonstrated. Following three single intraperitoneal dosing, plasma concentration profiles showed a slow absorption phase with the mean t_{max} of 1.83–5.67 h, which may be caused by the formulation of fibrin glue in vivo and the formidable absorption of macromolecule. The slow terminal elimination half-life ($T_{1/2}$) was about 84.5–96.3 h, which was in good agreement with previous assessments of the fibrinogen concentrates (used in the treatment of afibrinogenemia and hypofibrinogenemia) [16–19]. Some pharmacokinetic parameters might be different from those in the literature [20,21] because of different dosage forms, administration routes, racial type and other reasons. It was considered that the slow absorption and metabolism of fibrin glue may contribute to the healing of the wounds. The C_{max} and $AUC_{0-\infty}$ values of the three doses indicated an apparent dose-proportionality (Fig. 3). The fourfold increase in dosage led to an approximately fourfold increase in C_{max} (35.0 μg/ml versus 111.2 μg/ml) and $AUC_{0-\infty}$ (1393 μg h/ml versus 5919 μg h/ml) (Table 2). Analyzed by ANOVA, though t_{max} seemed to increase with the dosages ($P < 0.05$), there were no significant differences for other parameters including MRT, CL, V_d and $T_{1/2}$ among the three dosages ($P > 0.05$). Thus, the results supported linear rather than non-linear plasma pharmacokinetics of porcine fibrinogen across the investigated dosage range in rats (100–400 mg/kg).

3.4. Tissue distribution

To compare the distribution of porcine fibrinogen in different tissues, the TCA precipitable radioactivity at 2, 8, 24, 72, 168, 240 and 528 h after a single intraperitoneal administration at 200 mg/kg to rats was investigated (Fig. 4). The data were expressed as the percentage of the injected dose per gram of tissue (%ID/g). The results indicated that porcine fibrinogen had a wide distribution in the tissues throughout the whole body within the time course examined. Two hours following administration, most of the analyzed tissues

Table 2
Pharmacokinetic parameters of ^{125}I-porcine fibrinogen after intraperitoneal injection of porcine-derived FG to rats ($n = 6$).

Parameter	Dose of porcine fibrinogen			
	100 mg/kg	200 mg/kg	400 mg/kg	*P* value
$T_{1/2}$ (h)	96.3 ± 10.3	91.6 ± 4.4	84.5 ± 6.7	>0.05
MRT (h)	123.8 ± 16.1	115.5 ± 5.4	113.5 ± 11.4	>0.05
C_{max} (μg/ml)	35.0 ± 10.5	53.4 ± 9.3	111.2 ± 16.4	>0.05
t_{max} (h)	1.83 ± 0.41	3.00 ± 1.10	5.67 ± 0.82	<0.05
$AUC_{0-\infty}$ (μg h/ml)	1393 ± 282	2769 ± 353	5919 ± 820	>0.05
CL (ml/h/kg)	74.51 ± 16.26	73.10 ± 8.24	68.49 ± 6.96	>0.05
V_d (ml/kg)	9402 ± 3146	8415 ± 751	7871 ± 788	>0.05

P value are obtained by evaluating the pharmacokinetic parameters across the three dosage groups using one-way ANOVA followed by Newman–Keuls *t*-test. C_{max} and $AUC_{0-\infty}$ were normalized by the corresponding dosages when conducting comparison between the three dosage groups.

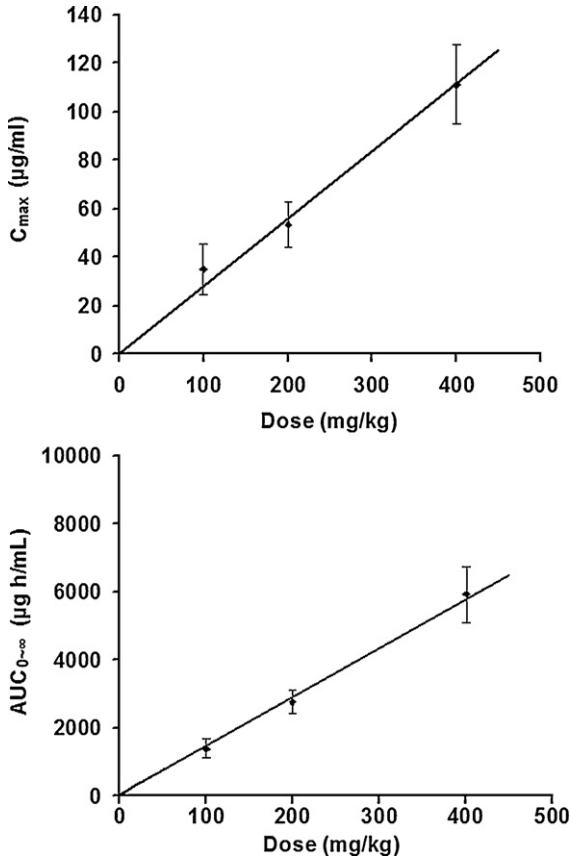

Fig. 3. Profiles of the mean plasma C_{max} and $AUC_{0-\infty}$ after intraperitoneal injection versus the three investigated dosages (100, 200, 400 mg/kg of porcine fibrinogen containing ^{125}I-labeled and unlabeled porcine fibrinogen) in rats ($n = 6$). The r^2 for C_{max} and $AUC_{0-\infty}$ are 0.9821 and 0.9969, respectively.

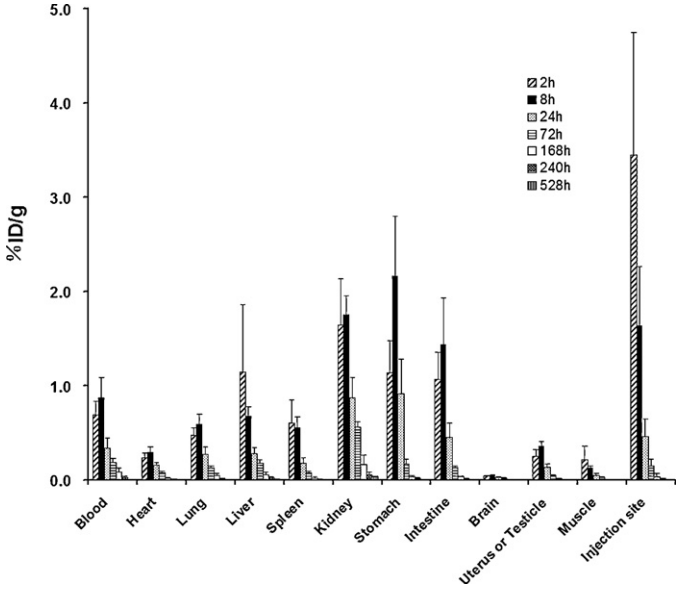

Fig. 4. Tissue distribution of TCA-precipitable radioactivity at 2, 8, 24, 72, 168, 240 and 528 h after a single intraperitoneal administration of fibrin glue to rats (dosage: 200 mg/kg of porcine fibrinogen containing ^{125}I-labeled and unlabeled porcine fibrinogen in rats, $n = 6$).

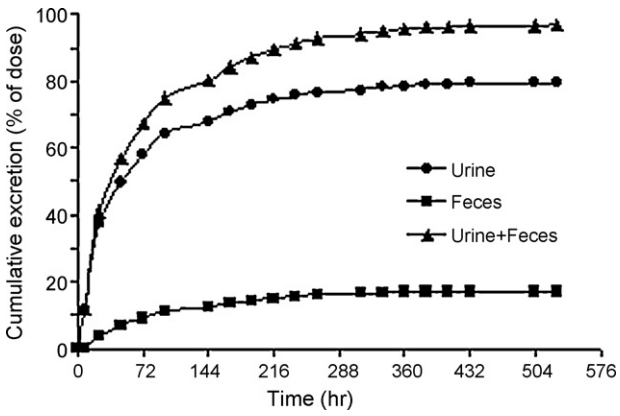

Fig. 5. Cumulative total radioactivity recovered from urine and feces after a single intraperitoneal administration of fibrin glue containing ^{125}I-porcine fibrinogen to rats (dosage: 200 mg/kg of porcine fibrinogen containing ^{125}I-labeled and unlabeled porcine fibrinogen in rats, $n = 6$).

contained significant amounts of radioactivity. In agreement with the previous studies [12–14], the radioactivity in injection site was significantly higher than those in other tissues at the time of initial detection. The radioactivity in most tissues reached the maximum at 8 h after administration and decreased rapidly at 24 h post-dosing. The radioactive fibrinogen showed substantial disposition in liver, kidneys, stomach and intestine. For the clearance organs, kidneys and liver, the radioactivity detected in kidneys were greater than that in liver, which suggested that the toxins or the possible metabolites of fibrinogen have been mainly eliminated by the kidneys.

3.5. Elimination

Cumulative total radioactivity recovered from urine and feces after intraperitoneal administration of porcine-derived FG containing ^{125}I-labeled porcine fibrinogen to rats (200 mg/kg of porcine fibrinogen) is illustrated in Fig. 5. More than 96.5% of the total radioactivity excreted in urine and feces was detected within 528 h. The amount of radioactivity excreted in urine reached to 50% of the dose within 48 h, and then increased slowly over time. It could be concluded that the urinary excretion was the dominant route of elimination following administration of the porcine-derived fibrin glue, as 79.3% and 17.2% of administered radioactivity were recovered in urine and feces, respectively. The results of distribution and excretion studies show that kidneys play a more important role than liver does in the elimination of foreign proteins from the body. These results are similar to the data in the literature [25,26].

4. Conclusions

In our research, a rapid and sensitive ^{125}I-labeled assay method was developed and validated to evaluate the pharmacokinetics, tissue distribution and excretion of porcine fibrinogen after intraperitoneal injection of porcine-derived fibrin glue to rats. Following a single intraperitoneal injection of fibrin glue, porcine fibrinogen experienced a slow absorption and elimination process. The results showed linear disposition of porcine fibrinogen at the examined dosage range, with C_{max} and $AUC_{0-\infty}$ being proportional to the administered dosages in rats. Porcine fibrinogen had a wide distribution to most of the tissues examined. The findings indicated that the urinary excretion was the major route of elimination following administration of the porcine-derived fibrin glue.

To our knowledge, this is the first report to thoroughly study the plasma pharmacokinetics, tissue distribution and excretion profiles of fibrin glue following intraperitoneal injections to rats. The

present pharmacokinetic study of this new porcine-derived fibrin glue in rats will provide helpful information for the clinical applications.

References

[1] G. Recinos, K. Inaba, J. Dubose, D. Demetriades, P. Rhee, Local and systemic hemostatics in trauma: a review, Turk. J. Trauma Emerg. Surg. 14 (2008) 175–181.
[2] H.J. Kim, Y.H. Choi, H.M. Kim, The clinical use of fibrin glue: report of 20 cases, Korea Univ. Med. J. 25 (1988) 549–553.
[3] C. Mouritzen, M. Dromer, H.O. Keinecke, The effect of fibrin glueing to seal bronchial and alveolar leakages after pulmonary resections and decortications, Eur. J. Cardiothorac. Surg. 7 (1993) 75–80.
[4] V. Van Velthoven, G. Clarici, L.M. Auer, Fibrin tissue adhesive sealant for the prevention of CSF leakage following transsphenoidal microsurgery, Acta Neurochir. (Wien) 109 (1991) 26–29.
[5] K.C. Lee, S.K. Park, K.S. Lee, Neurosurgical application of fibrin adhesive, Yonsei Med. J. 32 (1991) 53–57.
[6] Z. Gnjidic, D. Tomac, L. Negovetic, T. Gjinolli, Fibrin sealants in the management of cerebrospinal fistulae, Biomed. Prog. 7 (1994) 39–42.
[7] K. Takakura, T. Kubo, Y. Tajika, A. Teramoto, Y. Tagusagawa, Efficacy and safety of the use of BI 91.021.3 (Beriplast) in cranial nerve surgery, Shinryo To Shinyaku 31 (1994) 1808–1817.
[8] S. Athanasiadis, C. Kuhlgatz, I. Girona, Experiences with fibrin adhesives in surgery of the rectum and colon, Zentralbl. Chir. 109 (1984) 1011–1107.
[9] T. Muto, K. Ishibiki, K. Hatakeyama, H. Sasaki, F. Hanyu, S. Nakajima, Y. Maruyama, Y. Moriya, T. Hattori, M. Akita, Y. Sowa, A. Takabayashi, F. Kiba, N. Tomoda, Efficacy and safety of BI 91.021.3 in patients receiving anostomotic surgery, Shinyaku To Rinsho 43 (1994) 2274–2283.
[10] K. Matsumoto, E. Kohmura, A. Kato, T. Hayakawa, Restoration of small bone defects at craniotomy using autologous bone dust and fibrin glue, Surg. Neurol. 50 (1998) 344–346.
[11] G. Dickneite, H. Metzner, T. Pfeifer, M. Korez, G. Witzke, A comparison of fibrin sealants in relation to their in vitro and in vivo properties, Thromb. Res. 112 (2003) 73–82.
[12] S. Tabata, H.G. Eckert, H.M. Kellner, S. Hayashi, K. Fujimoto, Pharmacokinetic study of a fibrin adhesive agent, Beriplast in rats, Oyo Yakuri 31 (1986) 1123–1127.
[13] H. Omiya, Y. Saito, H. Imamura, A. Okamura, The pharmacokinetics of fibrin adhesive agent applied to the rat lung, Jpn. J. Thorac. Cardiovasc. Surg. 46 (1998) 1275–1278.
[14] R. Hattori, H. Otani, H. Omiya, S. Tabata, Y. Nakao, T. Yamamura, M. Osako, Y. Saito, H. Imamura, Fate of fibrin sealant in pericardial space, Ann. Thorac. Surg. 70 (2000) 2132–2136.
[15] L. Tang, A.M. Persky, G. Hochhaus, B. Meibohm, Pharmacokinetic aspects of biotechnology products, J. Pharm. Sci. 93 (2004) 2184–2204.
[16] W. Kreuz, E. Meili, K. Peter-Salonen, A. Dobrkovská, J. Devay, S. Haertel, U. Krzensk, R. Egbring, Pharmacokinetic properties of a pasteurised fibrinogen concentrate, Transfus. Apher. Sci. 32 (2005) 239–246.
[17] C. Négrier, C. Rothschild, J. Goudemand, J.Y. Borg, S. Claeyssens, M.C. Alessi, A.C. Jaffry, C. Teboul, B. Padrazzi, T. Waegemans, Pharmacokinetics and pharmacodynamics of a new highly secured fibrinogen concentrate, J. Thromb. Haemost. 6 (2008) 1494–1499.
[18] C. Fenger-Eriksen, J. Ingerslev, B. Sorensen, Fibrinogen concentrate—a potential universal hemostatic agent, Expert Opin. Biol. Ther. 9 (2008) 1325–1333.
[19] M.J. Manco-Johnson, D. Dimichele, G. Castaman, S. Fremann, S. Knaub, U. Kalina, F. Peyvandi, G. Piseddu, P. Mannucci, Pharmacokinetics and safety of fibrinogen concentrate, J. Thromb. Haemost. 7 (2009) 2064–2069.
[20] Y. Takeda, Studies of the metabolism and distribution of fibrinogen in healthy men with autologous ^{125}I-labelled fibrinogen, J. Clin. Invest. 45 (1966) 103–111.
[21] G.H. Lyman, R.E. Bettigole, E. Robson, J.L. Ambrus, H. Urba, Fibrinogen kinetics in patients with neoplastic disease, Cancer 41 (1978) 1113–1122.
[22] Y.C. Gao, B.H. Gao, H.D. Wang, Study of ^{125}I labeling fibrinogen using Iodogen method, Labeled Immunoass. Clin. Med. 7 (2000) 90–92.
[23] L.C. Knight, A.Z. Budzynski, S.A. Olexa, Radiolabeling of fibrinogen using the iodogen technique, Thromb. Haemost. 46 (1981) 593–596.
[24] J. Tong, Y.L. Li, Y. Feng, Trace study of slow release on gelatin microsphere, Isotopes 11 (1998) 168–171.
[25] Z.P. Hu, H.S. Niu, X.X. Yang, H.F. Li, G.W. Sang, B. Li, Recombinant human parathyroid hormone 1–34: pharmacokinetics, tissue distribution and excretion in rats, Int. J. Pharm. 317 (2006) 144–154.
[26] G. Garibotto, P. Tessari, P. Sacco, G. Deferrari, Amino acid metabolism, substrate availability and the control of protein dynamics in the human kidney, J. Nephrol. 12 (1999) 203–211.