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Determination of porcine fibrinogen in rat and dog plasma after intraperitoneal injection of a porcine-derived fibrin glue by fluorescein-labeled assay method: Comparison with isotope-labeled assay method

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ABSTRACT

A sensitive and specific fluorescein isothiocyanate (FITC) label coupled with size-exclusion high-performance liquid chromatography-fluorescence detection (SE-HPLC-FLD) method was developed and validated for the estimation of the pharmacokinetic profiles of porcine fibrinogen after intraperitoneal injection of a porcine-derived fibrin glue (FG) to SD rats and beagle dogs with three single doses. Porcine fibrinogen, the major composition of the FG, was labeled with FITC. The FG containing FITC-labeled porcine fibrinogen was intraperitoneally administered to SD rats at three single dosages (100, 200, 400 mg/kg of porcine fibrinogen), and the collected plasma was then detected by SE-HPLC-FLD method. The present technique was compared to the previously introduced isotope-labeled assay method for the pharmacokinetic studies in SD rats. The pharmacokinetic studies in SD rats showed that the correlation coefficient between the FITC-labeled assay and ^{125}I-labeled assay methods was $r^2 = 0.989$. Thus, this FITC-labeled assay method performed well and demonstrated high concordance with the previous ^{125}I-labeled assay method, suggesting that FITC-labeled assay could substitute the ^{125}I-labeled assay as a method of choice for quantification in beagle dogs. Then the plasma levels of porcine fibrinogen in beagle dogs were studied by the FITC-labeled assay method with three single doses (15, 30, 60 mg/kg of porcine fibrinogen).

The method validation showed that the FITC label coupled with SE-HPLC-FLD method was suitable for the quantification of porcine fibrinogen in plasma samples with satisfactory linear ($r^2 > 0.999$), precision (<12%), accuracy (95.5–104.9%) and recovery (>88%). The results showed linear disposition of porcine fibrinogen at the examined dosage range in SD rats or beagle dogs.

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]. The commonly used FG is derived mainly from human plasma components, which contain 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]. The porcine fibrinogen-based glue, having the advantages of no virus, good film forming ability and rapid operation, has been developed in China since 2000s not only to prevent transmission of human and porcine blood-borne pathogens but also to dramatically reduce production costs of the biomedical material. This kind of porcine-derived FG is composed of porcine fibrinogen, thrombin, and small quantity of factor XIII, fibronectin and calcium chloride [12].

To date, although many studies have implicated a role of human-derived FG in surgical subspecialty settings, there are few published papers regarding integrated data on its pharmacokinetic properties. Only some shallow tissue distribution studies of a human-derived fibrin adhesive agent applied to the incision of stomach, lung, and pericardial space of rats were evaluated in 1986, 1998 and 2000 [13–15]. Recently, the preclinical pharmacokinetics, tissue distribution and excretion profiles of porcine fibrinogen in SD rats after intraperitoneal injection of a porcine-derived fibrin

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glue have been characterized in our previous study [12]. According to the United States Food and Drug Administration (US FDA) requirements, the preclinical pharmacology and toxicology studies should be conducted in two species, a rodent (usually the rat and mouse) and a non-rodent (usually the dog) for all drug candidates, since drugs can behave differently in different species [16]. Thus, the pharmacokinetic study of the porcine-derived fibrin glue in a second species such as the dog was necessitated to characterize the nature of the drug in animals and provide foundation for clinical applications.

Our earlier investigation of porcine fibrinogen in SD rat plasma samples was based on an isotope-labeled assay method, which has been proved to be rapid and sensitive [12]. However, to our knowledge, the isotope-labeled assay method has been frequently applied to the pharmacokinetic studies of small animals such as mice, rats and rabbits, whereas due to the much higher expenses related to radioactive waste management and more risks to the environment, the radioisotope-labeled assay technique has hardly been in the research of large animals (dogs and monkeys) in vivo. Hence, it was necessary to select a suitable label to investigate the plasma levels of porcine fibrinogen in dogs. Fluorescein isothiocyanate (FITC) applied to label and assay polysaccharides and enzymes for pharmacokinetic studies [17–20], was considered to be suitable for the pharmacokinetic study of porcine fibrinogen in dogs. Moreover, size-exclusion high-performance liquid chromatography (SE-HPLC), which has been commonly used for characterization and quantitation of native proteins and their oligomers based on their size difference, could offer a powerful tool to give higher sensitivity, selectivity and repeatability by using fluorescence detector (FLD) [21,22].

The aim of this paper was to develop a FITC-labeled assay method for the evaluation of the pharmacokinetic profiles of porcine fibrinogen after intraperitoneal injection of a porcine-derived FG to SD rats and beagle dogs with three single doses. With FITC as labeling reagent, a SE-HPLC-FLD method was developed and validated for the determination of porcine fibrinogen in plasma. This labeling and trace assay method used in SD rats was compared to the previously introduced ^{125}I-labeled assay method with the purpose to prove that FITC-labeled assay could substitute the ^{125}I-labeled assay as a method of choice for quantification in beagle dogs.

2. Materials and methods

2.1. Materials

Porcine fibrinogen (purity: >95.0%, MW = 340 kDa) used for FITC-labeling was purified and obtained by Pine & Power Biotech Co. Ltd (Shanghai, China). Fluorescein isothiocyanate isomer I (FITC) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phosphate-buffered saline (PBS), sodium bicarbonate and hydroxylamine were produced by Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Ammonium sulfate (AMS), used for precipitation, was obtained from Shanghai Qianjin Chemical Reagent Factory (Shanghai, China). Milli-Q water was purified using a Symplicity® System (Millipore, Bedford, USA) and was used to prepare all the solutions.

2.2. Preparation of porcine-derived FG containing FITC-labeled porcine fibrinogen

2.2.1. Fluorescence labeling and purification of FITC-labeled porcine fibrinogen

FITC-labeled porcine fibrinogen was prepared by the modified method described in [23]. FITC (140 mg) and porcine fibrinogen (purity: >95.0%, 140 mg) were incubated at room temperature in 10 ml of sodium bicarbonate (0.1 M, pH 9.0) for 4 h with stirring. Addition of hydroxylamine (0.15 M final) stopped the reaction. After dialysis at 4 °C in dark overnight to remove unbound FITC molecules, the product, FITC-labeled fibrinogen was lyophilized. Determined by two chromatographic methods (TLC and HPLC), the fluorescence purity of FITC-labeled porcine fibrinogen was proved to be more than 90%.

2.2.2. Final formulation preparation

The tested porcine-derived FG consisting of solution A and solution B (754 IU/ml thrombin dissolved in 10 mM calcium chloride), supplied by Pine & Power Biotech Co. Ltd (Shanghai, China), was prepared based on the concentration of porcine fibrinogen to be administered. To achieve the final mixture solution for dosing in SD rat and beagle dog experiments, the above FITC-labeled porcine fibrinogen was added to 3 ml of solution A, thus the solution A was made up of 46.67 mg/ml fluorescent porcine fibrinogen, small quantity of factor XIII and fibronectin, which dissolved in 10 mM PBS. In this way, each SD rat in the three single dosage groups received equivalent to 100 mg/kg, 200 mg/kg and 400 mg/kg of porcine fibrinogen containing FITC-labeled porcine fibrinogen, while each beagle dog in the three single dosage groups received equivalent to 15 mg/kg, 30 mg/kg and 60 mg/kg of porcine fibrinogen containing FITC-labeled 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). Male and female beagle dogs (weighing 7–8 kg) were obtained from Experimental Animal Center of Second Military Medical 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 SD rats and beagle dogs 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 and Second Military Medical University.

2.4. SE-HPLC-FLD chromatographic system and conditions

The SE-HPLC-FLD system (Shimadzu, Kyoto, Japan) consisted of two LC-20AD pumps coupled with a model RF-10AXL fluorescence detector, a SIL-20A autosampler and a CTO-20AC column oven. Shimadzu LC-solution software was used for data acquisition and mathematical calculations. The SE separation was achieved on a 300 mm × 7.5 mm, 13 μm, TSK G4000SW column (Tosoh, Tokyo, Japan), and an isocratic mobile phase of 0.1 M PBS (pH 6.8) was delivered at a flow rate of 1 ml/min. FITC-labeled porcine fibrinogen was monitored at λ_{ex} of 492 nm and λ_{em} of 517 nm. The column temperature was 25 °C and the injection volume was 20 μl.

2.5. Validation for fluorescence determination in SD rat and beagle dog plasma by AMS precipitation assay

The amounts of FITC-labeled porcine fibrinogen in biological samples were determined on the basis of the SE-HPLC-FLD peak areas. Fibrinogen was always isolated from heparin-anticoagulated plasma by precipitation with saturated AMS, which can remove free FITC or FITC associated with the fragmented peptide in the fluorescent plasma samples [24,25].

Each of the fluorescent plasma samples (100 μl) was purified by precipitation with 20% saturated AMS (20 °C). After standing

(4 °C) for 30 min, the precipitate was collected by centrifugation at 3000 × *g* for 10 min, and then dissolved in 100 μl of water for analysis. Calibration curves consisted of nine standard concentrations of fluorescently labeled porcine fibrinogen spiked in SD rat and beagle dog plasma: equivalent to 0.51–204.00 μg/ml and 0.49–196.00 μg/ml, respectively. For the analysis method, QC samples at three concentrations containing 1.02, 10.20 and 163.20 μg/ml in SD rat plasma and 0.98, 9.80 and 156.80 μg/ml in beagle dog plasma, and the lower limit of quantification (LLOQ) samples including 0.51 μg/ml in SD rat plasma and 0.49 μg/ml in beagle dog plasma were analyzed to assess the accuracy and precision. Intra-day precision was evaluated by analyzing QC samples in five replicates over 1 day, while inter-day precision was evaluated from the analysis of each control once on each of 5 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 levels of QC biological samples finally obtained against those originally untreated. Five aliquots of each of the low and high QC samples were prepared to determine the stability. The freeze-thaw stability was evaluated after three freeze (−80 °C)-thaw (ambient) cycles. Two levels of QC samples were thawed and kept at room temperature for 4 h in the short-term temperature stability test. The post-preparative stability was determined after keeping the samples at room temperature for 2 h. The long-term stability was analyzed after keeping the plasma samples frozen at −80 °C for 20 days.

2.6. Pharmacokinetic studies

2.6.1. Pharmacokinetic study in SD rats

Three single doses (equivalent to 100 mg/kg, 200 mg/kg and 400 mg/kg of porcine fibrinogen containing FITC-labeled porcine fibrinogen) of solution A and an equivalent volume of solution B were intraperitoneally injected to SD rats through duplex syringes, similar to the use in clinical abdominal surgery. The SD 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 plasma samples were measured after precipitation with 20% saturated AMS. The result of porcine fibrinogen concentration in each time phase was expressed as μg/ml, calculated by standard curve equation.

2.6.2. Pharmacokinetic study in beagle dogs

Three single doses (equivalent to 15 mg/kg, 30 mg/kg and 60 mg/kg of porcine fibrinogen containing FITC-labeled porcine fibrinogen) of solution A and an equivalent volume of solution B were intraperitoneally injected to beagle dogs through the duplex syringes. The beagle dogs were randomly assigned to three groups ($n = 6$ per group). Blood samples (approximately 200 μl each) were collected from saphenous vein into heparinized tubes before administration and at 0.5, 1, 2, 4, 6, 8, 12, 24, 72, 168, 240 h post-dose. The plasma was isolated by centrifugation for 10 min at 3000 × *g*. Plasma samples were stored at −80 °C until analyzed.

2.7. Data analysis

The concentration versus time profiles were 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.

3. Results and discussion

3.1. Fluorescence labeling

Considering that the labeling process is based on the atom exchange, chemical reaction and physicochemical treatments, the biological activity of labeled fibrinogen was one of the main factors to be taken into account. To investigate the influence of labeling on biological activity of fibrinogen, the labeled porcine fibrinogen–thrombin binding experiment was carried out. Compared with the positive control group (unlabeled porcine fibrinogen) and the negative control group (normal saline), the thrombin-induced fibrin clot formation was observed in the FITC-labeled porcine fibrinogen group, which indicated that the FITC-labeled porcine fibrinogen possessed the intrinsic biological activity of porcine fibrinogen.

Since FITC and FITC-labeled solutions stored at light environment and room temperature tend to have reduced fluorescence due to fluorescence quenching, the spontaneous reduction of fluorescence cannot be avoided in vitro or in vivo. As described by Dong [26], the concentration of FITC-labeled porcine fibrinogen solution residues, which stored in the dark at room temperature after the intraperitoneal administration, was synchronously analyzed and regarded as substitutes of original concentrations for calibration.

3.2. Validation for fluorescence determination in SD rat and beagle dog plasma by AMS precipitation assay

Fig. 1 shows the representative chromatograms of blank plasma, blank plasma sample spiked with FITC-labeled porcine fibrinogen and plasma sample obtained from SD rats and beagle dogs following dose with porcine-derived FG. It can be seen that there was no significant interference resulting from blank plasma under this condition, thus, the developed method was found to be selective for determination of porcine fibrinogen in SD rat and beagle dog plasma. The retention time of FITC-labeled porcine fibrinogen was 13.1 min. Calibration curves for the plasma assay were constructed by plotting the peak area (y) against the nominal concentrations (x) of calibration standards. The regression equations were $y = 2.04x + 0.056$ ($r^2 = 0.9991$) in SD rat plasma and $y = 2.05x + 0.01$ ($r^2 = 0.9992$) in beagle dog plasma. The absolute recoveries in the matrices were more than 88%. Table 1 shows a summary of intra- and inter-day precision and accuracy of LLOQ and QC samples in SD rat and beagle dog plasma. The intra- and inter- day precision were less than 9.5% and the accuracy was from 95.5% to 104.9% for QC samples. The precision and accuracy of LLOQ were below 12% and 104%. After three freeze-thaw cycles, the concentrations of analytes in SD rat and beagle dog plasma deviated less than ±15% from their nominal concentrations (0.68–6.5%). In the short-term stability, long-term stability and post-preparative stability test, the relative errors for two levels of QC samples were within ±6.07%. Accordingly, it was expected that the present method would be applicable to the pharmacokinetic studies of porcine fibrinogen containing FITC-labeled porcine fibrinogen in SD rats and beagle dogs.

3.3. Comparative pharmacokinetics in SD rats using FITC-labeled assay and ^{125}I-labeled assay methods

The mean plasma concentration versus time profiles of porcine fibrinogen containing FITC-labeled porcine fibrinogen after intraperitoneal injection to SD rats at three doses, superposed with the previously introduced result (^{125}I-labeled assay method), are depicted in Fig. 2. The corresponding pharmacokinetic parameters generated by fitting plasma concentration profile to a non-compartmental model are listed in Table 2. The slow terminal

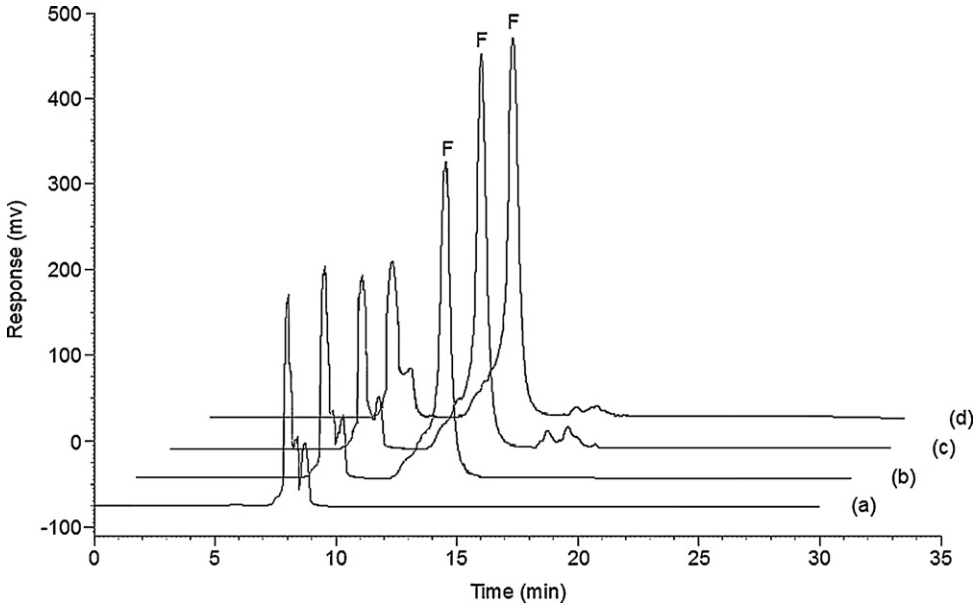

Fig. 1. SE-HPLC-FLD chromatograms of (a) SD rat blank plasma, (b) SD rat blank plasma spiked with FITC-labeled porcine fibrinogen (F) (50 μg/ml), (c) SD rat plasma at 2 h after the dose of 400 mg/kg, and (d) beagle dog plasma at 2 h after the dose of 30 mg/kg.

Table 1
Intra- and inter-day precision and accuracy of porcine fibrinogen containing FITC-labeled porcine fibrinogen assay in SD rat and beagle dog plasma ($n = 5$).

Sample matrix	Spiked concentration of fibrinogen (μg/ml)	Intra-day precision (R.S.D., %)	Inter-day precision (R.S.D., %)	Accuracy (mean ± S.D., %)	Absolute recovery (mean ± S.D., %)
Rat plasma	0.51	11.21	10.57	103.6 ± 7.3	88.3 ± 4.9
	1.02	7.65	8.04	104.9 ± 5.9	90.2 ± 4.6
	10.20	8.38	7.82	98.1 ± 8.1	91.7 ± 3.6
	163.20	7.40	6.69	97.6 ± 7.2	90.3 ± 5.7
Dog plasma	0.49	10.35	9.07	99.3 ± 9.6	89.1 ± 6.4
	0.98	9.22	9.54	98.4 ± 8.4	88.9 ± 5.2
	9.80	6.98	6.32	95.5 ± 7.5	91.4 ± 4.1
	156.80	8.12	7.26	95.9 ± 8.3	92.5 ± 5.1

elimination half-life ($T_{1/2}$) was about 83.06–97.75 h, which was in good agreement with our previous report [12]. For this analytical method, the area under the plasma concentration versus time curve ($AUC_{0-\infty}$) and the maximum plasma concentration (C_{max}) increased with the dose, showing apparent dose-dependent relationship ($r^2 > 0.99$). Analyzed by ANOVA, though t_{max} seem to increase with the dosages ($P < 0.05$), there were no significant differences for other parameters including $T_{1/2}$, MRT, CL and V_d among the three dosages ($P > 0.05$).

The porcine fibrinogen concentration data obtained by the two independent trace analytical methods (FITC-labeled and ^{125}I-labeled) for SD rat plasma samples were compared. Fig. 3 describes the correlation in a scatter plot between the results of the ^{125}I trace assay and FITC trace assay for the mean plasma concentrations at each time after three doses. As all points were concentrated around this line and no strong outliers could be observed, the two methods showed in average and within the scope of the measurements a good correlation. The equation for the linear regression through all

Table 2
Pharmacokinetic parameters of porcine fibrinogen containing FITC-labeled fibrinogen after intraperitoneal injection of fibrin glue to SD rats and beagle dogs ($n = 6$).

Parameter	Animal							
	Rat				Dog			
	Dose				Dose			
	100 (mg/kg)	200 (mg/kg)	400 (mg/kg)	*P* value	15 (mg/kg)	30 (mg/kg)	60 (mg/kg)	*P* value
$T_{1/2}$ (h)	97.75 ± 13.26	88.51 ± 6.11	83.06 ± 8.46	>0.05	97.61 ± 12.46	87.16 ± 8.95	86.37 ± 6.63	>0.05
MRT (h)	127.66 ± 20.53	111.60 ± 7.94	110.16 ± 13.12	>0.05	118.40 ± 21.28	114.60 ± 12.79	114.94 ± 9.38	>0.05
C_{max} (μg/ml)	35.34 ± 9.40	58.24 ± 5.48	117.56 ± 15.74	>0.05	42.20 ± 6.87	70.06 ± 7.33	150.35 ± 7.17	>0.05
t_{max} (h)	2.00 ± 0.00	3.00 ± 1.10	5.00 ± 1.10	<0.05	4.33 ± 0.82	4.67 ± 1.03	5.33 ± 1.03	>0.05
$AUC_{0-\infty}$ (μg h/ml)	1481.4 ± 357	2938.1 ± 503.1	6233.1 ± 941.8	>0.05	2244.7 ± 370.4	4262.6 ± 592.5	8742.5 ± 855.2	>0.05
CL (ml/h/kg)	71.08 ± 18.0	69.85 ± 12.56	67.91 ± 7.76	>0.05	6.76 ± 1.10	6.94 ± 1.22	6.83 ± 0.89	>0.05
V_d (ml/kg)	9269.5 ± 3486.1	8256.9 ± 1261.6	7714.9 ± 600.6	>0.05	802.3 ± 58.8	797.6 ± 98.7	786.3 ± 59.0	>0.05

P values 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. $T_{1/2}$, slow terminal elimination half-life; MRT, mean residence time; C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; $AUC_{0-\infty}$, area under the plasma concentration versus time curve; CL, total clearance; V_d, volume of distribution.

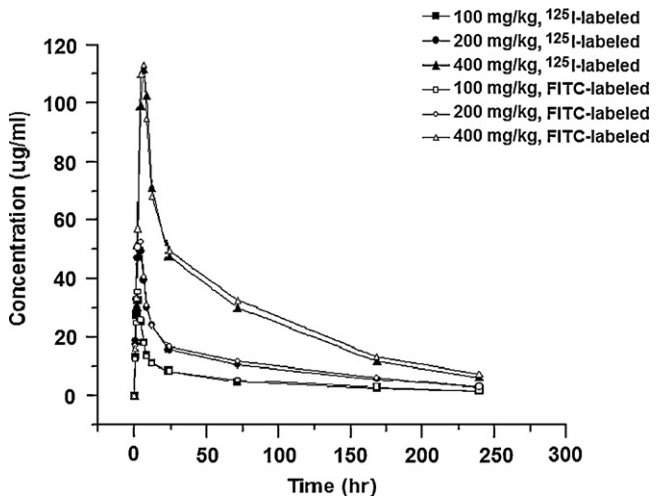

Fig. 2. Plasma concentration–time profiles of porcine fibrinogen containing ^{125}I-labeled porcine fibrinogen or FITC-labeled porcine fibrinogen after intraperitoneal injection of fibrin glue to SD rats ($n = 6$). Three investigated dosages are 100, 200, 400 mg/kg of porcine fibrinogen containing ^{125}I-labeled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

points ($n = 66$) was $y = 1.03x + 0.30$ with the linear regression coefficient ($r^2 = 0.989$). The paired samples t-test was used to compare the levels measured with the two methods. No significant difference and strong correlation were shown (P value = 0.169).

Based on the validation results, it was possible to apply successfully both trace assays and assess porcine fibrinogen levels after intraperitoneal injection of porcine-origin FG to SD rats. Both methods used were reliable, easy and fast to perform, and provide adequate accuracy and precision for the quantitative determination of porcine fibrinogen in SD rat plasma. In this study the FITC trace assay performed well and demonstrated high concordance with the ^{125}I trace method, suggesting that FITC trace assay could substitute the ^{125}I trace as a method of choice for quantitation. Compared with the use of radioisotope in large animal models, the use of fluorescence can further reduce the expenses related to radioactive waste management and the risk to the environment. Thus, the FITC trace

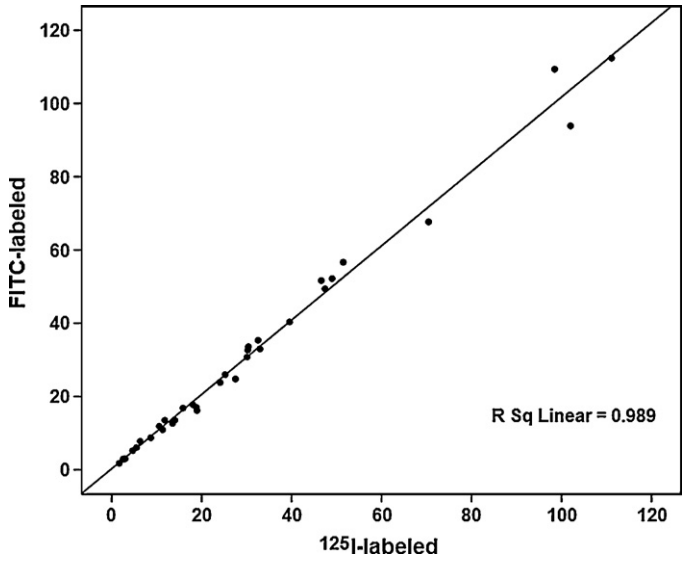

Fig. 3. Correlation plot between the determined plasma concentration obtained with the ^{125}I trace assay (x-axis) and the FITC trace assay (y-axis) at each time after three doses.

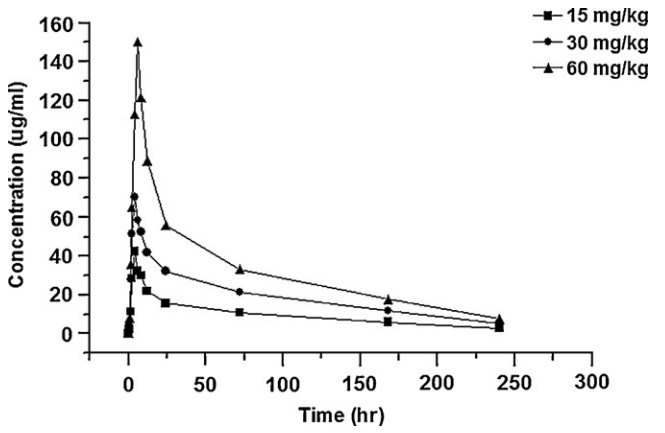

Fig. 4. Plasma concentration–time profiles of porcine fibrinogen containing FITC-labeled porcine fibrinogen after intraperitoneal injection of fibrin glue to beagle dogs ($n = 6$). Three investigated dosages are 15, 30, 60 mg/kg of porcine fibrinogen containing FITC-labeled porcine fibrinogen.

assay was more suitable for the determination of porcine fibrinogen in beagle dog plasma.

3.4. Pharmacokinetic study in beagle dogs

Fig. 4 shows the plasma concentration–time curves of porcine fibrinogen containing FITC-labeled porcine fibrinogen after intraperitoneal injection at three doses to beagle dogs. The pharmacokinetic parameters performed by a noncompartmental model are summarized in Table 2.

Dose linearity of the pharmacokinetics over the dosage range examined (15–60 mg/kg) was demonstrated. Following three single intraperitoneal dosing, plasma concentration profiles showed a slow absorption phase with the mean t_{max} of 4.33–5.33 h, which might be caused by the formulation of fibrin glue in vivo and the formidable absorption of macromolecule. The average terminal half-life ranging from 86.37 to 97.61 h. The C_{max} and $AUC_{0-\infty}$ values of the three doses indicated an apparent dose-proportionality ($r^2 = 0.9932$ for C_{max}, $r^2 = 0.9994$ for $AUC_{0-\infty}$). The fourfold increase in dosage led to an approximately fourfold increase in C_{max} (42.20 μg/ml versus 150.35 μg/ml) and $AUC_{0-\infty}$ (2244.7 μg h/ml versus 8742.5 μg h/ml) (Table 2). Analyzed by ANOVA, there were no significant differences for the parameters including $T_{1/2}$, MRT, t_{max}, CL and V_d among the three dosages in beagle dogs ($P > 0.05$). Thus, the results supported linear rather than non-linear plasma pharmacokinetics of porcine fibrinogen containing FITC-labeled porcine fibrinogen across the investigated dosage range in beagle dogs (15–60 mg/kg). It can be seen that the pharmacokinetic parameters, $T_{1/2}$ and MRT, of porcine fibrinogen in beagle dogs after intraperitoneal injection of fibrin glue were consistent with those in SD rats, while the CL and V_d values were significantly lower in beagle dogs than in SD rats, which might result from racial difference.

4. Conclusion

In this paper, the FITC label coupled with SE-HPLC-FLD method was developed and validated to evaluate the pharmacokinetic of porcine fibrinogen after intraperitoneal injection of porcine-origin fibrin glue. Based on the validation results, it was possible to successfully apply assay and assess of porcine fibrinogen in SD rats. The pharmacokinetic studies in SD rats showed that the FITC trace assay performed well and demonstrated high concordance with the ^{125}I trace method, suggesting that FITC trace assay could substitute the ^{125}I trace as a method of choice for quantitation in beagle dogs. The

FITC trace assay method was then fully validated with adequate accuracy, precision and selectivity for the quantitative determination of porcine fibrinogen in beagle dog plasma. After three single administrations, plasma concentration profiles showed a slow absorption phase elimination proceeding. The results showed linear disposition of porcine fibrinogen at the examined dosage range in SD rats or beagle dogs. The present pharmacokinetic study of this new porcine-origin fibrin glue in SD rats and beagle dogs will provide helpful information for the clinical applications. Owing to the relatively lower cost of waste management and less risk to the environment, the proposed FITC label coupled with SE-HPLC-FLD method could be adopted as a wide range of choice for quantitative analysis of macromolecules in rat or dog plasma. As for the determination in various biological matrices such as tissues, urine and feces by using this FITC trace assay method, some theoretical and technical bottlenecks remain to be solved. For instance, the large number and complexity of fluorescent substances in tissues and excreta can interfere with the detection results. Compared with the overall application of isotopic probe in drug absorption, distribution, metabolism and excretion study, further investigations of this FITC label coupled with SE-HPLC-FLD method are necessitated for the determination of biomacromolecular drugs.

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