Pharmacokinetics of recombinant human basic fibroblast growth factor in rabbits and mice serum and rabbits aqueous humor[1]

Qi ZHANG, Guang-ji WANG[2], Jian-guo SUN

Center of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009, China

KEY WORDS fibroblast growth factor 2; pharmacokinetics; enzyme-linked immunosorbent assay; aqueous humor; rabbits; mice

ABSTRACT

AIM: To study the pharmacokinetics of recombinant human basic fibroblast growth factor (rhbFGF) in rabbits and mice after iv and postocular administration, and the changes of rhbFGF in rabbits aqueous humor after postocular administration. **METHODS:** After iv or postocular administration three doses of rhbFGF in rabbits and mice, rhbFGF concentration in serum and rabbit aqueous humor was determined by enzyme-linked immunosorbent assay. **RESULTS:** Serum concentration-time data of rabbits after iv administration of rhbFGF 1, 2, and 4 μg/kg were fitted to bi-exponential equations with half-lives of 0.9, 0.9, and 0.6 min for $T_{1/2\alpha}$ and 7, 8, and 4.7 min for $T_{1/2\beta}$. Plasma concentration-time data of mice after iv administration of rhbFGF 2.5, 5 and 10 μg/kg were fitted to bi-exponential equations with half-lives of 0.4, 0.6, and 0.9 min for $T_{1/2\alpha}$ and 6, 5, and 7 min for $T_{1/2\beta}$. The AUCs were linearly correlated to doses in both cases (r_{rabbit}=0.997, r_{mouse}=0.999). The serum concentrations of rhbFGF were very low, near to the background after postocular administration of 2 or 5 μg/kg, in both rabbits and mice. The rhbFGF levels in rabbits aqueous humor were higher than control 8 h postdose (P<0.01). **CONCLUSION:** rhbFGF within the examined doses had a linear pharmacokinetics in rabbits and mice. High concentration of rhbFGF was found in rabbits aqueous humor after postocular administration.

INTRODUCTION

Basic fibroblast growth factor (bFGF), a polypeptide mitogen, stimulates the growth and differentiation of a wide variety of cell types derived from the mesodermal and neuroectodermal[1]. bFGF has been purified from various sources and shown to stimulate the proliferation of cells of mesodermal and neuroectodermal origin, including fibroblasts, endothelial cells, astrocytes, oligodendrocytes, neuroblasts, keratinocytes, bovine lens epithelial cells, osteoblasts, smooth muscle cells, and melanocytes[2,3]. The observations also suggested that bFGF might participate in the production of a variety of pathological conditions resulting from uncontrolled cell proliferation and angiogenesis. For example, urine concentrations of the bFGF were obviously high in dogs with bladder cancer compared with normal dogs[4]. The researchers preferred the local application of bFGF though there was no evidence demonstrating bFGF would induce cancer. Recently recombinant human basic fibroblast growth factor (rhbFGF) has been used to heal burn and ulcer on the epidermis in patients. Now it is investigated to repair

[1] Project supported by the National Natural Science Foundation of China, No 39500177.
[2] Correspondence to Prof Guang-ji WANG.
Phn 86-25-8327-1544. Fax 86-25-8530-3260.
E-mail gjwang@mailbox.cpu.edu.cn
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retina after laser photocoagulation.

This article described the pharmacokinetics of rhbFGF in rabbits and mice after iv and postocular administration and rhbFGF changes in rabbits aqueous humor after postocular administration.

MATERIALS AND METHODS

Animals All New Zealand rabbits, weighing 1.8-2.0 kg, and Kun-ming mice, weighing 20-21g, half male and half female, were obtained from Experimental Animal Center of China Pharmaceutical University (Certificate No 97024).

Equipments and reagents Microplate counter (CliniBio 128C), ELISA kit (Lot9918026 R&D system, Wiesbaden, Germany), rhbFGF (No 98070) were supplied by Hainan Biotechnology Institute. Unless otherwise specified, all other chemicals were of analytic grade.

Pharmacokinetics of rhbFGF in rabbits after iv and postocular administration Fifteen rabbits were randomly divided into 3 groups of iv rhbFGF 1, 2, and 4 μg/kg, respectively. The other three rabbits were postocular administered rhbFGF 2 μg/kg. Rabbits were fixed conscious on tables and a small polypropylene pipe was inserted into the femoral vein of each rabbit. rhbFGF was dissolved in normal saline and administered via the auricular vein of rabbit for iv or injected into postocular tissues for postocular administration. About 1 mL venous blood was collected from polypropylene pipe before and at 0.5, 2, 5, 8, 10, 15, 20, 30, 45, 60, 90, and 120 min postdose to an ice-cold Eppendorf microtube. Blood samples were solidified and then were centrifuged at 10 000×*g* for 5 min at 4 ºC to separate serum. The high concentration of serum was diluted within the detection range of ELISA kit. All serum was frozen and stored at -75 ºC until assay.

Pharmacokinetics of rhbFGF in mice after iv and postocular administration One hundred and ninety-five mice were randomly divided into several groups according to three different doses of iv 2.5, 5, 10 μg/kg of rhbFGF and serum sampling time points per dose. The design provided five mice per sampling time point for each dose group. Other thirty-three mice were assigned to provide three mice per sampling time point for postocular administration of 5 μg/kg of rhbFGF. Mice were administered rhbFGF via the tail vein or postocular tissues. Blood samples were collected from jugular vein into ice-cold Eppendorf microtubes before and at 0.05, 0.5, 1, 1.5, 2, 3, 5, 8, 10, 12, 15, and 120 min postdose for iv groups and at 0.5, 1, 2, 5, 8, 10, 15, 20, 30, 45 min postdose for postocular groups. Blood sample was solidified and then were centrifuged at 10 000×*g* for 5 min at 4 ºC to separate serum. The serum was diluted within the detection range of ELISA kit. All serum was frozen and stored at -75ºC until assay.

Changes of bFGF in rabbit aqueous humor after postocular administration Eight rabbits were randomly divided into two groups. One group received 10 μg/kg of rhbFGF and the other received the same volume of normal saline by postocular administration. Rabbits were placed consciously in retraining cages respectively. About 100 μL aqueous humor was taken out through cornea at 0.5, 4, 8, 12, 16, 24, and 36 h postdose. Samples were diluted to 200 μL and then stored at -75 ºC until assay.

Sample assay rhbFGF levels of samples were quantitated by enzyme-linked immunosorbent assay kits. The assay was guided by the procedures specified by the manufacture. A series of calibration standard were set up in the microplate.

Data analysis Pharmacokinetic parameters were estimated by program PKBP-N1 based on compartmental analysis. The $AUC_{0\to t}$ was evaluated using the trapezoidal method from time zero to the last measurable blood concentration (C_t). The remaining $AUC_{t\to\infty}$ was estimated by using the following equation: $AUC_{t\to\infty}=C_t/\beta$, where β was the rate constant for the terminal phase. Comparison of the experimental and control group was determined by *t*-test.

RESULTS

Determination of rhbFGF in serum The rhbFGF standard in kit and rhbFGF standard provided by Hainan Biotechnology Institute were regarded as two parallel lines. The precision of intra-assay and inter-assay coefficients of variation (CV) were all within 10 %, and the recovery of rhbFGF from serum was 89 %-120 % over the range of 200 to 3200 ng/L. The minimal detectable level of rhbFGF was 100 ng/L.

Pharmacokinetics of rhbFGF in rabbits The serum concentration-time profiles of rhbFGF after iv administration to rabbits fitted to bi-exponential equation (Fig 1). The distribution ($t_{1/2\alpha}$) and terminal half-lives ($t_{1/2\beta}$) ranged from 0.6 to 0.9 min, and from 4.68 to 7.81 min, respectively. V_c and C*l* were from 0.023 to 0.033 L/kg, and from 0.011 to 0.015 $L\cdot kg^{-1}\cdot min^{-1}$ respectively. AUCs were linearly increased with doses

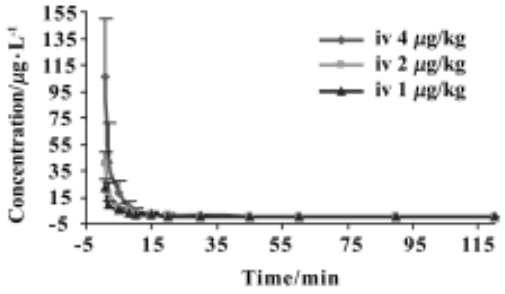

Fig 1. The mean serum concentration-time curves for rhbFGF in rabbits after iv administration. *n*=5. Mean±SD.

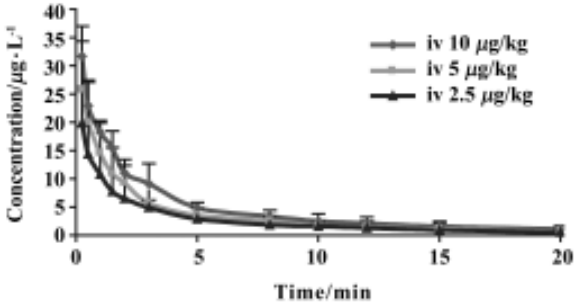

Fig 2. The mean serum concentration-time curves for rhbFGF in mice after iv administration. *n*=5. Mean±SD.

(*r*=0.997) (Tab 1). For postocular administration, little systemic exposure was observed in rabbit serum.

Pharmacokinetics of rhbFGF in mice The serum concentration-time profiles of rhbFGF after iv administration to mice were fitted to bi-exponetial equations (Fig 2). The distribution ($t_{1/2\alpha}$) and terminal half-lives ($t_{1/2\beta}$) ranged from 0.4 to 0.9 min, and from 5.0 to 7 min respectively. V_c and C*l* were from 0.10 to 0.28 L/kg, and from 0.06 to 0.10 L·kg^{-1}·min^{-1} respectively. AUCs were linearly increased with doses (*r*=0.999, Tab 2). For postocular administration of 5 μg/kg, the rhbFGF serum concentration in mice were very low, near to the background.

The change of bFGF in rabbits' aqueous humor after postocular administration At the first sampling time point, bFGF levels were unexpectedly high, reaching 6.8 μg/L and 9.8 μg/L in experimental and control group respectively, and no significant difference was found between them. Later, bFGF levels of control group decreased to about 1 μg/L, and remained over 36 h; while that of experiment group firstly decreased to about 1 μg/L, but then increased to 6.4 μg/L at 8 h postdose, and at that time differences was significant between two groups (P<0.01, Fig 3).

DISCUSSION

This article described a reliable assay method for detecting rhbFGF in serum of rabbits and mice and aqueous humor of rabbits.

The pharmacokinetics of rhbFGF after iv administration in rabbit and mouse were studied at three different doses respectively. Serum concentration-time data were analyzed by a two-compartmental model and pharmacokinetics parameters were presented above and indicated that rhbFGF had a linear pharmacokinetics in dose of 1-4 μg/kg for rabbits and 2.5-10 μg/kg for mice.

Tab 1. Pharmacokinetic parameters of iv rhbFGF in rabbits. *n*=5. Mean±SD.

Dose/μg·kg^{-1}	$T_{1/2\alpha}$/min	$T_{1/2\beta}$/min	K_{10}/min^{-1}	V_c/L·kg^{-1}	C*l* /L, kg^{-1}·min^{-1}	AUC/μg·min·L^{-1}
1	0.9±0.4	7.2±2.7	0.37±0.12	0.033±0.014	0.011±0.003	102±25
2	0.9±0.7	8±5	0.47±0.23	0.030±0.010	0.013±0.004	221±82
4	0.6±0.5	4.7±1.8	0.64±0.19	0.023±0.008	0.015±0.007	377±164

Tab 2. Pharmacokinetic parameters of iv rhbFGF in mice. *n*=5. Mean±SD.

Dose/μg·kg^{-1}	$T_{1/2\alpha}$/min	$T_{1/2\beta}$/min	K_{10}/min^{-1}	V_c/L·kg^{-1}	C*l* /L, kg^{-1}·min^{-1}	AUC/μg·min·L^{-1}
2.5	0.4±0.4	6±7	0.64±0.36	0.10±0.06	0.06±0.04	63±37
5	0.6±0.4	5.0±1.3	0.46±0.06	0.16±0.05	0.080±0.027	85±24
10	0.9±0.6	7±5	0.38±0.19	0.28±0.08	0.100±0.000	113±15

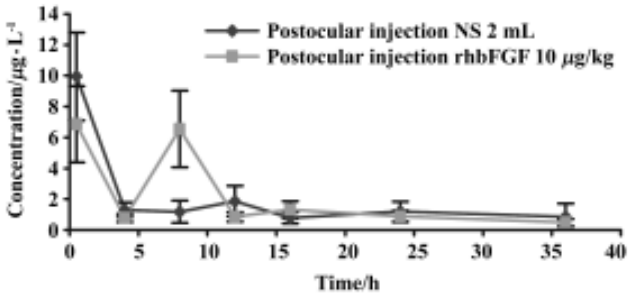

Fig 3. The mean aqueous humor concentration-time curves for bFGF in rabbits after a postocular injection of NS or rhbFGF. *n*=4. Mean±SD.

Endogenous bFGF exists extensively in various tissues of rabbit and mice, and has homology among different species. For rabbit, endogenous bFGF had little effect on pharmacokinetic experiment for its level is low and individual variation is small. But for the mice, endogenous bFGF level is high and varies apparently among the mice whose blood could not be sampled sequentially for pharmacokinetic study, so mice must be selected strictly by controlling their weight, sex, *etc* to minimize the individual variation.

The rhbFGF levels in the serum of rabbits and mice after postocular administration were very low, approaching the endogenous bFGF levels, so concentration-time data could not be analyzed by equations. It has been demonstrated that bFGF and bFGF receptor existed in retina of human and animals, and participated in retinal development and differentiation[5,6]. bFGF plays an important role in wound repair in the retina after laser photocoagulation[7,8]. So we concluded that postocular injection of rhbFGF had a local effect on postocular tissues.

In order to investigate the process of rhbFGF in eyes, we determined bFGF levels in rabbit aqueous humor which were taken out through rabbit cornea after postocular administration. bFGF is combined with heparin sulfate proteoglycan and glycosaminoglycans, located in membrane of many kinds of cells as a stored factor[9,10], and can be released when stimulated[11,12]. So we could infer that when the needle was used to take out the aqueous humor through the cornea, the stimulated tissues such as the cornea and the ciliary body, may release endogenous bFGF and thus bFGF levels both in experimental and control group were substantially increased.

In general, the ocular drug will experience two phases after injection[13,14]. First, part of the drug reached and precipitated the related tissues leading to initial higher drug levels in aqueous humor. Then the precipitates start to disappear, suggesting the second phase, elimination of the drug. The fate of rhbFGF seems to be in accordance with this rule. After the release of endogenous bFGF, bFGF levels decreased to a relatively low state. The control group maintained such a steady level over 36 h, while in the experimental group, rhbFGF permeated into the eyes and entered rabbits aqueous humor slowly, and then reached the peak concentration 8 h postdose. rhbFGF in eyes were degraded or transferred, and bFGF levels in aqueous humor also decreased to a low level.

Aqueous humor could be used specially to determine the medicine levels in eyes, but it could not be sampled frequently for its secretion rate is slow and its content is small. On the other hand, the ciliary body that create aqueous humor also secrete endogenous bFGF[15] which might disturb the determination of rhbFGF in aqueous humor. So, available data only describe the rough process of rhbFGF after postocular administration in eyes, further investigation is needed to verify the assumption above.

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