Journal of Medicinal Plants Research Vol. 5(29), pp. 6496-6501, 9 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.400

Full Length Research Paper

Antagonism activity against acute myocardial ischemia of Paeoniflorin and its pharmacokinetic/ pharmacodynamic (PK/PD) modeling in rats

Wenqian Chen[1,2], Yanqing Zhang[1,2,3], Junbo Xie[3], Tianyan Zhou[1,2] and Wei Lu[1,2]*

[1]State Key Laboratory of Natural and Biomimetic Drugs (Peking University), Beijing, 100191 P.R., China.
[2]Department of Pharmaceutics, School of Pharmaceutical Sciences, Peking University, Beijing, 100191 China.
[3]Department of Pharmaceutical Engineering, Tianjin University of Commerce, Tianjin, 300134, China.

Accepted 11 October, 2011

The aim of the present work was to investigate the effects of paeoniflorin (PF) on acute myocardial ischemia in rats. Meanwhile, after intravenous and oral administration of PF, the pharmacokinetic and pharmacodynamic characters of PF in rats were also studied. Plasma concentration of paeoniflorin was determined using a simple and rapid HPLC method. Nonlinear mixed-effect modeling (NONMEM) method was used for pharmacokinetic (PK) and pharmacodynamic (PD) analysis. A two-compartment model with first-order degradation in the absorption compartment was employed for the PK data analysis. The results showed that PF exhibited significant antagonism against acute myocardial ischemia and a PK/PD link model and two IDR models were developed and compared for anticoagulant activity of PF. It was concluded that the integrated PK/PD model could well characterize PK and PD behaviors of PF in rats.

Key words: Paeoniflorin, acute myocardial ischemia, anticoagulant activity, pharmacokinetics, pharmacodynamics.

INTRODUCTION

Paeoniflorin, a principal bioactive component of Paeoniae Rubrae Radix, has been reported to exhibit multiple pharmacological activities, such as neuroprotective (Cao et al., 2010), liver protective (Kim and Ha, 2010) and anti-inflammatory etc. (Tang et al., 2010). It is also one of the major components, which can be detected, in human or animal plasma after oral administration of Paeoniae radix extract and formulations of homologous recipe (Feng et al., 2010; Cheng et al., 2010). As a result, paeoniflorin (PF) has attracted more and more attention in many fields.

In recent years, population pharmacokinetics (PopPK) is widely used to estimate the sources and correlations of variability in drug concentrations among individuals and to analyze complex factors that may influence the pharmacokinetic behavior of a drug (Lahu et al., 2010; Sanchez et al., 2010). Nonlinear mixed-effects modeling (NONMEM) approach (Smith, 2003) is a powerful tool for estimating the distribution of parameters and their relationships with covariates in the population, and provides an alternate way for pharmacokinetic research in natural products. Furthermore, to find the relationship between plasma concentration and drug effect, Pharamacokinetic/Pharmacodynemic (PK/PD) models were proposed in past several decades. Among them, PK/PD link model and indirect response model (IDR) are considered as classical experiential models, and have contributed to the increasing knowledge of some kinds of medicines, such as anticoagulant, antibiotic, antihypertensive drugs, and so on (Cao et al., 2007).

Although, the pharmacokinetic characteristics of purified

*Corresponding author. E-mail: luwei_pk@bjmu.edu.cn. Tel: (+ 8610) 82801717. Fax: (+8610) 82801717.

PF have been investigated in the recent years (Feng et al., 2010; Cheng et al., 2010), little has been known on the population pharmacokinetics (PopPK) of the bioactive compound. Especially, the quantitive relationship between the PF plasma concentration and its pharmacodynamic effect has not been addressed. As a result, in present study, the acute myocardial ischemia animal model induced by pitressin was used to prove the antagonism activity on acute myocardial ischemia of PF after oral administration. Anticoagulant activity of PF after intravenous administration was chosen to develop PK/PD link models of PF.

MATERIALS AND METHODS

Apparatus and reagents

RM-6240 multichannel physiologic recorder was produced by Chengdu instrument factory (Chengdu, China). Prothrombin time kit (ISI=1.1) was from Beijing Shidi scientific instrument Co., Ltd (Beijing, China). S-Plus 6.2 was from insightful company (USA). The HPLC system consisted of a pump (BiSepTM-1100 pump, Unimicro Technologies Inc., USA), and a UV detector (BiSepTM-1100 detector, Unimicro Technologies Inc., USA). An ODS column (Prontosil C18 250 mm×4.6 mm 5 μm, Bischoff Chromatography, German) was used.

Standard paeoniflorin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Propranolol hydrochlorid was obtained from Zhongnuo Pharmaceutical Co., Ltd (Shijiazhuang, China). Posterior pituitary injection was from Nanjing Xinbai Pharmaceutical Co., Ltd (Nanjing, China). Urethane was from Puhua Co., Ltd (Shanghai, China). Methanol and acetonitrile were of HPLC grade. Double-distilled water and all other solvents were of analytical grade.

Animals

Male Sprague-Dawley rats (160 to 220 g) were supplied by Laboratory Animal Center at Health Science Center of Peking University. The rats were fasted overnight before the experiments. All the experimental procedures were performed under the national guidelines on the proper care and use of animals in laboratory research.

Effect on acute myocardial ischemia

24 rats were divided randomly into 3 groups of 8 animals in each. Normal saline (NS), PF and propranolol (PROP) at the dose of 5 $mL \cdot kg^{-1}$, 100 and 1.5mg/kg were orally given to each group, respectively b.i.d for one week. Twenty minutes after last dosage, each rat was anaesthetized by intraperitoneally injection of 20% urethane solution (5 $ml \cdot kg^{-1}$). Then pituitrin solution was injected via tail vein at dose of 0.6 $U \cdot kg^{-1}$. A two-lead electro cardiogram was recorded and both the deviation of ST segment and heart rate (HR) were measured before, and 1, 15, 30, 60, 120, 180, and 300 s after intravenous injection. Then CR, the change rate of ST segment deviation was calculated.

The differences of CR between treatment groups and control group at 15, 30, 120 and 180 s were most obvious, so they were analyzed using T-test. The differences of HR between treatment groups and control group at each time point were analyzed using paired T-test.

Blood sampling and HPLC analysis of paeoniflorin

Under anaesthesia with ether in a glass chamber, rat blood samples (about 0.5 ml) were collected from the eye bottom vein in heparinized 1.5 ml microcentrifuge tubes. The sampling period was 0.17, 0.33, 0.67, 1, 1.5, 2, 3, and 4 h after intravenous injection (n=6), or 0.083, 0.17, 0.33, 0.67, 1, 1.5, 2, 4, and 8 h after oral administration (n=6). The samples were centrifuged at 7000 $r \cdot min^{-1}$ for 10 min, and 200 μl of plasma was separated and kept frozen at −20°C until determination.

The plasma PF concentration was determined by HPLC method. The mobile phase consisted of acetonitrile-water (14:86, v/v) and the flow rate was 1.0 $ml \cdot min^{-1}$. Detection was performed at a wavelength of 230 nm under a constant temperature (20±1°C).

The resulting plasma (200 μl) was mixed with 1.0 ml acetic ether by vortex for 60 s at room temperature. After the mixture was centrifuged at 7000 $r \cdot min^{-1}$ for 10 min, the upper layer was transferred into a clean tube. Then the rest phase was extracted again with acetic ether following the same procedure and the combined organic phase was evaporated to dryness below 50°C in vacuum. The obtained residue was dissolved in 200 μl of methanol. A 20 μl volume of this sample solution was injected into HPLC for analysis. The same sample handling procedure was used to determine the recovery and precision in plasma.

The detection limit of the assay was 32.8 $ng \cdot ml^{-1}$. The PF calibration curve was linear over the range from 0.164 to 16.4 $\mu g \cdot ml^{-1}$ (r^2=0.9994). The method showed good accuracy (>91%) and precision (coefficients of variation <6.4%) at 3 different PF concentrations (0.82, 4.2 and 13.8 $\mu g \cdot ml^{-1}$) for both inter-day and intra-day assay.

Anti-coagulant activity

PF was injected to 8 rats via tail vein at a single dose of 300 $mg \cdot kg^{-1}$. Under anaesthesia with ether in a glass chamber, blood samples (about 0.5 ml) were collected from the eye bottom vein in 1.5 ml microcentrifuge tubes immediately before, and 5, 10, 15, 20, 30, 40, and 60 min after intravenous injection. The freshly drawn blood was mixed thoroughly with 0.9% sodium citrate in a ratio of 9:1. After centrifuged at 3000 $r \cdot min^{-1}$ for 15 min, the plasma was separated and its prothrombin time was detected within 2 h. NS was used as negative control.

The degree of anticoagulation effect was assessed by measurement of the prothrombin times (PT) which was detected using thromboplastin reagent. 100 μl reconstituted thromboplastin reagent and 100 μl plasma sample were incubated separately at 37°C for 180 s prior to mixing. The time taken for a clot to form following introduction of the thromboplastin reagent into the plasma was recorded. The procedure was repeated for each sample, and the average of the dual results was finally used. The international normalized ratio (INR) was used to assess thrombin activity.

Pharmacokinetic and pharmacodynamic analysis

All data were subsequently processed by nonlinear mixed effects modeling software NONMEM (Version VI, Level 1.0). Parameters were assumed to follow the log-normal distribution across the population, and the residual error was characterized by a combined proportional and additive error model. One- and two-compartmental models were developed and compared using the plasma concentration data of paeoniflorin in i.v. group to assess its disposition characteristics, and then based on the results the better model was used to fit the whole i.v. and p.o. dataset. Finally, the pharmacokinetic model was linked to pharmacodynamic models as follows to fit both PK data of i.v. group and anti-coagulant activity data simultaneously.

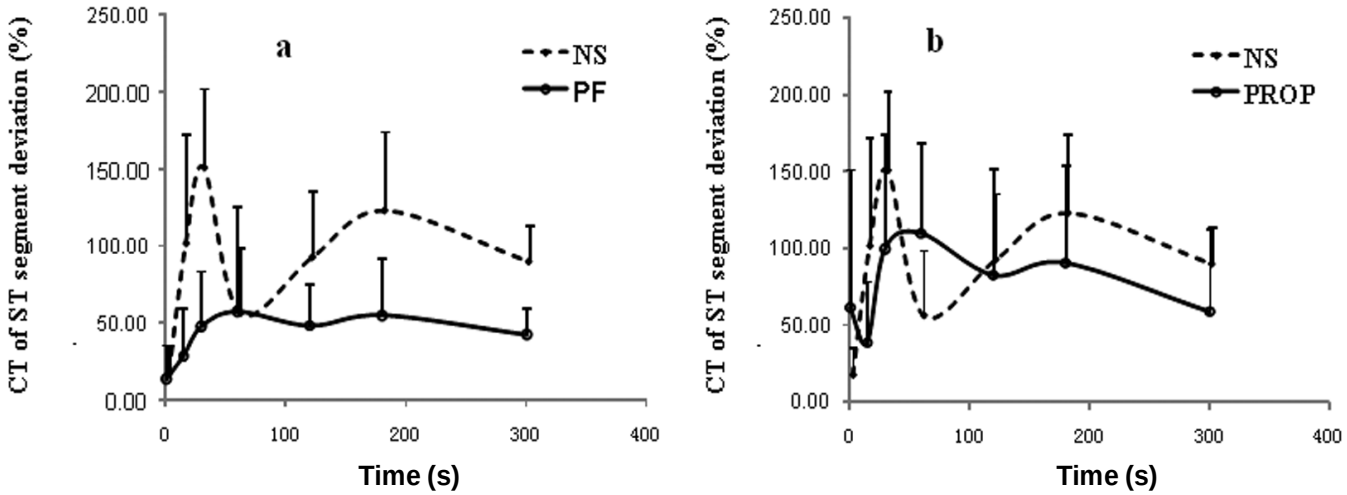

Figure 1. Change rate of ST segment deviation versus time intervals after pituitrin injected for PF (a) and PROP (b) groups. Each point and bar represents the Mean+S.D. (n = 8).

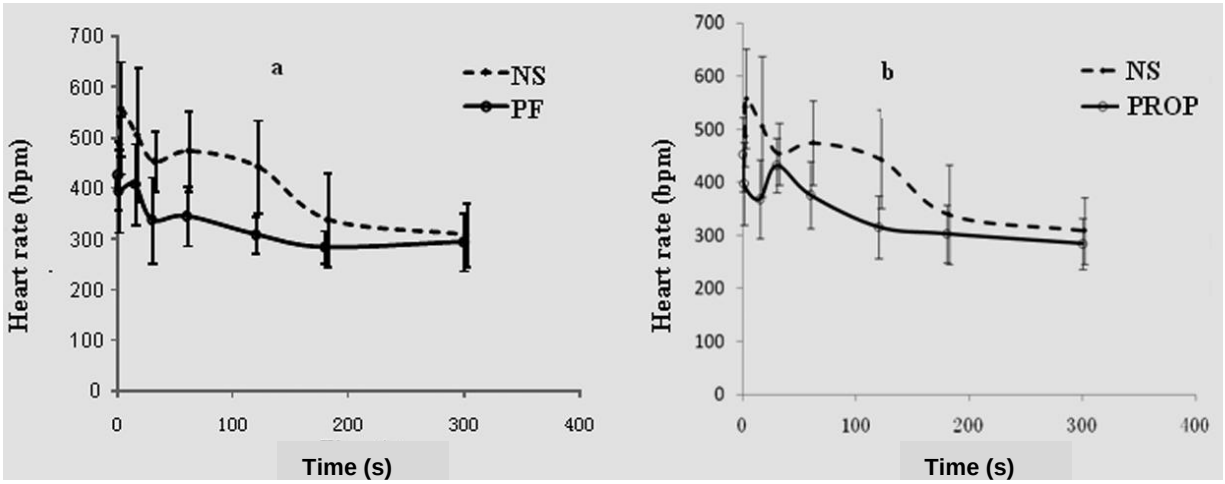

Figure 2. Heart rate versus time intervals after pituitrin injected for PF (a) and PROP (b) groups. Each point and bar represents the Mean±S.D. (n = 8).

RESULTS

Effect of PF on acute myocardial ischemia

The change rates of ST segment deviation (CR) and heart rate (HR) versus time for PF and propranolol (PROP) groups compared with normal saline (NS) group were plotted in Figures 1 and 2, respectively.

Anti-coagulant activity and PK/PD analysis of PF

A two-compartment model was used to fit the data obtained from the i.v. and p.o. administration. Based on this PK model, the estimated values of clearances and volumes of distribution were used to determine the parameters in PK/PD model.

Population pharmacokinetic parameters of the i.v. model, and i.v. and p.o. model are listed in Table 1. The relative standard errors (RSE) for the estimation were acceptable, with a range of 7.6 to 24.5%.

DISCUSSION

According to T-test, CR of PF group at 15 and 30 s were significantly lower than those of the NS group on the level of $P<0.05$. There was also difference between the two groups at 180 s on the level of $P<0.1$. For PROP group, CR at 15s was significantly lower than NS group on the

Table 1. Parameter estimates of PK models.

Parameter	i.v. model		i.v. and p.o. model	
	Value (%R.S.E.)	Inter-individual variability (%CV)	Value (%R.S.E.)	Inter-individual variability (%CV)
CL1 / $L \cdot h^{-1}$	0.450(7.60)	7.30	0.464(7.80)	13.6
V1 / L	0.149(10.7)	9.86	0.152(13.2)	26.7
CL2 / $L \cdot h^{-1}$	0.0679(9.06)	0.00795	0.656(10.3)	-
V2 / L	0.131(17.2)		0.106(19.0)	$1.35e^{-5}$
Ka0 / h^{-1}			0.141(24.5)	-
Ka1 / h^{-1}			0.0128(15.2)	33.5

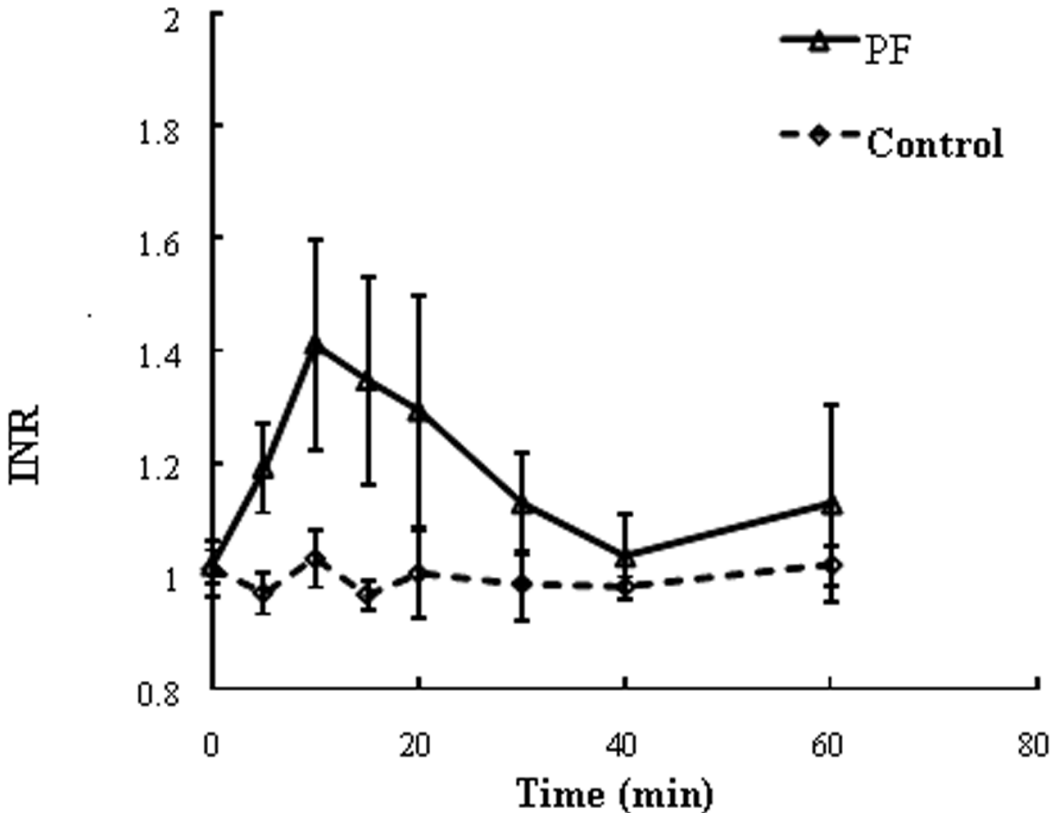

Figure 3. INR of rat plasma sample versus time after PF injected (solid line) and in control group (dashed line). Each point and bar represents the mean±S.D. (n = 8).

level of P<0.1. Both PF and PROP group can significantly restrain the increase of HR according to paired T-test on the level of P<0.001 and P<0.05, respectively. In present study, we established the acute myocardial ischemia model. Although the deviation of ST segment in rat electro cardiogram makes great differences between each individual, PF group shows significant antagonism against acute myocardial ischemia induced by pituitrin.

Usually the intensity of drug effect is not coincident with its concentration in blood, and often reaches to peak later than blood concentration with contrary situation now and then. Therefore, pharmacodynamic model seems more applied in the clinical therapy than the model describing drug concentration-time profiles. In this study, anticoagulant effect of PF arose gradually and faded away after injected directly into the blood, so there was a time delay before it works (Figure 3).

It has been confirmed that some anticoagulants can restrain certain coagulant factors directly, inhibit production of vitamin-K, influent metabolizing of

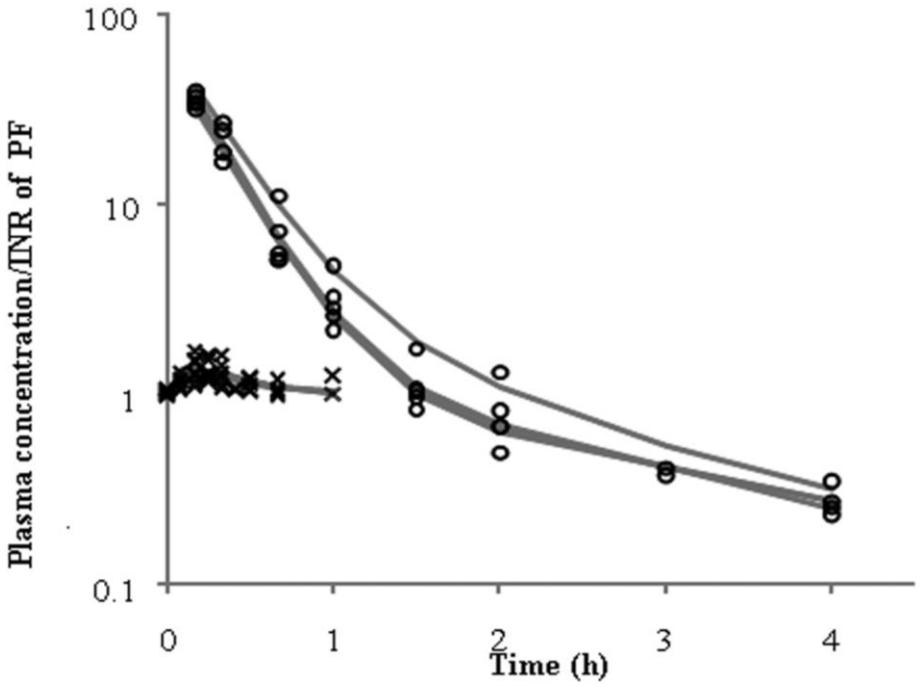

Figure 4. Semi-log plots of observed plasma concentration(○) or INR(×) and their predicted (line) values versus time in Kin-stimulate IDR model.

Table 2. Parameter estimates of Kin-stimulate IDR model.

Parameter	Value (%R.S.E.)	Inter-individual variability (%CV)
CL1 / L·h^{-1}	0.449 (7.66)	7.05
V1 / L	0.146 (10.6)	7.67
CL2 / L·h^{-1}	0.0668 (13.1)	—
V2 / L	0.126 (34.3)	26.5
Kin	9.32 (20.4)	36.2
Kout	9.32 (20.4)	36.2
SC50/μg·ml^{-1}	3.5×10^{4} (47.1)	—
Smax	99.0 (46.7)	—

haematoblast, combine with Ca^{2+}, or act indirectly on the route of blood coagulation, whereas detailed mechanism of PF anticoagulant is still unfamiliar. Shown as the plots of predict and observed data (Figure 4), K_{in}-stimulate IDR model can describe PK/PD profiles of PF well and truly. Parameter estimates of Kin-stimulate IDR model are listed in Table 2. On the viewpoint of mechanism, it can be explained by K_{in}-stimulate IDR model more convincingly than by other model. The biological signal (INR) generates from thrombin, the value of which was just contrary to the activity of coagulant factors. When PF works on the factors in some process and inhibit its activity, the signal will increase. Correspondingly, this correlation was implemented by stimulating the production of response (K_{in}) in the model.

ACKNOWLEDGEMENTS

This work was supported by a grant from National Natural Science Foundation (Grant No. 30472165) and The State Key Laboratory of Natural and Biomimetic Drugs (Grant No. 26870507728).

REFERENCES

Cao BY, Yang YP, Luo WF, Mao CJ, Han R, Sun X, Cheng J, Liu CF (2010). Paeoniflorin, a potent natural compound, protects PC12 cells from MPP+ and acidic damage via autophagic pathway. J. Ethnopharmacol., 131: 122-129.

Cao YG, Liu XQ, Chen YC, Hao K, Wang GJ (2007). Warfarin maintenance dose adjustment with indirect pharmacodynamic model in rats. Eur. J. Pharm. Sci., 30: 175-180.

Cheng YS, Peng C, Wen FY, Zhang H (2010). Pharmacokinetic comparisons of typical constituents in white peony root and sulfur fumigated white peony root after oral administration to mice. J. Ethnopharmacol., 129: 167-173.
Feng C, Liu M, Shi XW, Yang W, Kong DZ, Duan KF, Wang QA (2010). Pharmacokinetic properties of paeoniflorin, albiflorin and oxypaeoniflorin after oral gavage of extracts of Radix Paeoniae Rubra and Radix Paeoniae Alba in rats. J. Ethnopharmacol., 130: 407-413.
Kim ID, Ha BJ (2010). The effects of Paeoniflorin on LPS-induced liver inflammatory reactions. Arch. Pharm. Res., 33: 959-966.
Lahu G, Hunnemeyer A, Diletti E, Elmlinger M, Ruth P, Zech K, McCracken N, Facius A (2010). Population Pharmacokinetic Modelling of Roflumilast and Roflumilast N-Oxide by Total Phosphodiesterase-4 Inhibitory Activity and Development of a Population Pharmacodynamic-Adverse Event Model. Clin. Pharm., 49: 589-606.
Sanchez JL, Dominguez AR, Lane JR, Anderson PO, Capparelli EV, Cornejo-Bravo JM (2010). Population pharmacokinetics of vancomycin in adult and geriatric patients: comparison of eleven approaches. Int. J. Clin. Pharmacol. Ther., 48: 525-533.
Smith MK (2003). Software for non-linear mixed effects modelling: a review of several packages. Pharm. Stat., 2: 69-73.
Tang NY, Liu CH, Hsieh CT, Hsieh CL (2010). The anti- inflammatory effect of Paeoniflorin on cerebral infarction induced by ischemia-reperfusion Injury in Sprague-Dawley Rats. Am. J. Chin. Med., 38: 51-64.