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# Pharmacokinetic, Tissue Distribution, and Excretion of Puerarin and Puerarin-Phospholipid Complex in Rats

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**ABSTRACT** Puerarin is a potential therapeutic agent for cardiovascular diseases. But its poor oral bioavailability restricts its clinical application. In present study, as an evaluation of a formulation to improve the bioavailability of the drug, puerarin and its phospholipid complex were given to rats by intragastrically (i.g.) administration to compare pharmacokinetic, tissue distribution, and excretion. Serum samples were obtained at designated times after a single oral dose of 400 mg/kg puerarin or its complex. Tissue samples (heart, liver, spleen, kidney, lung, and brain), urine, and feces were collected and analyzed by a sensitive and specific high performance liquid chromatography (HPLC) method after i.g. administration of puerarin or its phospholipid complex. Compartmental and non-compartmental analyses were applied to the serum concentration versus time data. Pharmacokinetic parameters were calculated using the 3P97 pharmacokinetic software package. An open two-compartment, first-order model was selected for pharmacokinetic modeling. The results showed that after i.g. administration of 400 mg/kg puerarin and its phospholipid complex (equivalent to 400 mg/kg of puerarin), the pharmacokinetic parameters of the two formulations were different. The serum concentrations reached peaks at 0.894 ± 0.521 h and 0.435 ± 0.261 h, respectively, indicating the complex was more readily absorbed in serum than puerarin. The maximum concentrations for puerarin and its complex were 1.367 ± 0.586 mg·L$^{-}$1 and 2.202 ± 1.28 mg·L$^{-}$1 and AUC were 5.779 ± 1.662 mg·h. L$^{-}$1 and 8.456 ± 0.44 mg·h L$^{-}$1, respectively, indicating a higher bioavailability for the complex. The widely distribution characteristics of puerarin and its complex in tissues post-i.g. administration was identical and in a descending order as follows: lung, kidney, liver, heart, spleen, and brain. However, the amount was different. Puerarin distribution was higher in heart, lung, and brain after administering the complex. The cumulative 72 h urinary excretion of puerarin after i.g. administration of puerarin and its complex accounted for 1.05%, 1.11% of the administered dose, respectively. The cumulative feces excretion of puerarin was 32.3% and 25.5%. To sum up, oral administration of puerarin phospholipid complex

modified the pharmacokinetics and tissue distribution of puerarin and it could be an effective oral formulation for puerarin.

**KEYWORDS** Puerarin, Phospholipid, Puerarin phospholipid complex, Serum kinetics, Tissue concentrations, Excretion, HPLC

## INTRODUCTION

Puerarin (Fig.1; Pur) is one of the major active constituents of *Pueraria lobata ohwi*, a traditional Chinese medicine. Research on the clinical application of Pur in recent years shows that Pur, being a vasodilator, has the pharmacological actions of improving microcirculation, lowering blood pressure, slowing down heart rhythm, preventing myocardial ischemia, and restoring blood flow. In addition, it helps resist arrhythmia, inhibit platelet aggregation and thrombosis, prevent atherosclerosis, block β-adrenergic receptor, improve coronary circulation, dilate coronary artery, and restrict the range of coronary artery infarct (Lili et al., 1984, 1985; Xiaoying et al., 1985; Qinglei & Xinran, 1987). Pur is also effective in decreasing blood sugar and serum cholesterol and restricting fever. Pur is clinically used in the treatment of diseases such as angina, myocardial infarction, arrhythmia, hyper-viscous blood, hypertension, cerebral infarction, diabetes, β-hypersensitivity, and retinal artery obstruction, etc. However, the poor solubility ($1.1 \times 10^{-2}$ mol/L), hydrophilicity, and liposolubility of Pur limit its absorption in vivo (Zhenghong et al., 1999; Jianping & Tongying, 1995). So solubilizer is often added to the injection used clinically to increase its solubility. Research indicated that the absorption of Pur in vivo is very poor after oral intake and i.g. administration to rat which diminishes the curative effect (Hong, 1999). Previous pharmacokinetic studies have indicated that Pur is available in intravenous (iv) and oral routes and is rapidly absorbed following oral administration with drug concentrations detectable in the plasma within 30 min. However, the amount absorbed is very small (Xila & Xiuyuan, 1992; Xilu et al., 1991, 1997; Mei et al., 2000). A drug phospholipid complex, integrating one or more natural active components with phospholipid, provides the possibility of improving the oral bioavailability of the drug (Bialecka, 1997; Gatti & Peruua, 1994). In comparison with its original state, the drug's lipophilicity increased, and some physicochemical and biological properties are changed in the complex. By complexing Pur with soy phospholipid, the oral bioavailability is expected to improve. Many aspects of puerarin–phospholipid complex (PPC) have been studied such as interaction of Pur with phospholipid in solid dispersion, permeation of Pur, and its phospholipid complex through rat skin in vitro and bioavailability in rats (Guangxi et al., 2003; Yunshu et al., 2000; Qingguo et al., 2001). But no systemic research has been reported on the pharmacokinetics of the complex, including bioavailability, tissue distribution, and excretion. As part of the preclinical evaluation, a new, rapid and sensitive HPLC method was therefore developed to quantify Pur in serum, tissue, urine, and feces in rats. Then the bioavailability, tissue distribution, and excretion of Pur and its soy phospholipid complex were compared after a single oral dose to rats. Based on the results, we discussed whether oral bioavailability had been improved and whether the tissue distribution and excretion characteristics of Pur have changed after forming a complex with soy phospholipid.

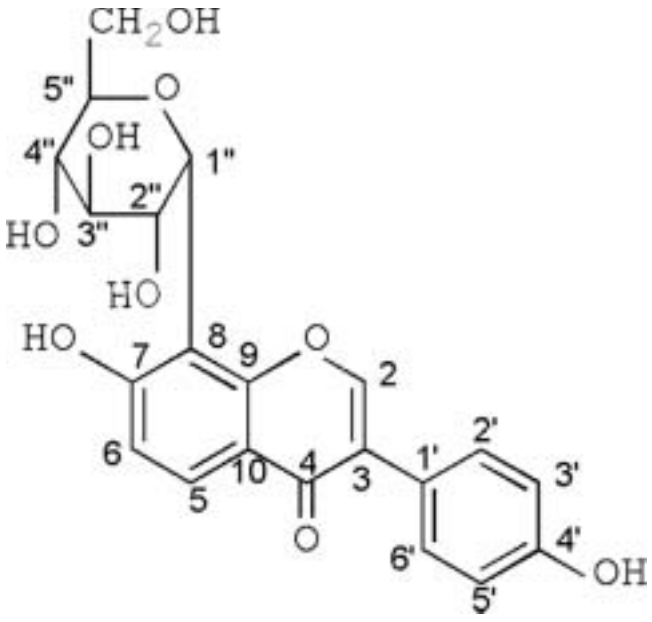


**FIGURE 1** **Chemical Structure of Pur (MW = 416.2).**

## MATERIALS

### Reagents and Equipment

Acetonitrile and methanol were purchased from Burdick & Jackson Company (Muskegon, MI).

Reagents were of HPLC grade. The reference standard of Pur was purchased from the National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China). Pur was purchased from Beijing Union Pharmaceutical Factory (Beijing, China). Soya phospholipid Epikuron 170 was purchased from Degussa Texturant Systems Deutschland GmbH & Co. KG (Hamburg, Germany). Puerarin–soya phospholipid complex was self-made. The 1100 series Agilent HPLC system comprised a G1311 A Quat Pump (Agilent Technologies Inc., Palo Alto, CA, USA), a Rheodyne model 7725i injector (Rheodyne Inc., Rohnert Park, CA, USA) and a G1315B DAD UV detector (Agilent Technologies Inc., U.S.A.). IKA® MS2 Minishaker was purchased from IKA Company (Staufen, Germany). Metabolic cages were purchased from Tecniplast Company (Varese, Italy) and IKA®WERKE ULTRA-TURRAX T8 was purchased from IKA Labortechnik GMBH & CO.KG (Staufen, Germany). Eppendorf centrifuge 5415 D was supplied by Eppendorf AG Company (Hamburg, Germany) and ROTOFIX 32 centrifuge was purchased from Hettich-Zentrifugen GmbH & Co. KG (Tuttlingen, Germany).

## Animals

All the experiments were performed on specific-pathogen-free grade Sprague-Dawley (SD) healthy rats weighing 200 ± 20 g that were purchased from the Experimental Animal Center of Guangzhou Traditional Chinese Medicine University (Guangzhou, China) and the animals were allowed to acclimate for at least one week before being used. Room temperatures were kept at 25 ± 2°C with 12 h light/dark cycles. The Experimental Animal Supervision Office of Guangdong Science and Technology Committee approved the experimental animals with the certificate number of Guangdong Test No. 2002A005. The experiments were carried out in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

# METHODS

## Drug Administration and Sampling

One hundred and twenty healthy rats were divided into two groups randomly. The rats were put on a fast overnight before the experiment while given free access to water. Each group of rats was given a single oral dose of 400 mg/kg of Pur or PPC (equivalent to 400 mg/kg Pur) suspended in water. Three milliliter of blood samples were obtained at 10, 20, 30, 45 min, and 1, 1.5, 2, 4, 6, 8, and 12 h. The serum was separated by centrifugation (3000 rpm, 15 min) after allowing the blood samples to stand for 30 min.

To study the tissue distribution, 54 rats received an i.g. administration of one of the two drug formulations. Six rats for each formulation were sacrificed at 20 min, 45 min, 60 min, 90 min, 120 min, 240 min, 360 min, and 720 min after drug administration to collect samples of liver, heart, lung, kidney, spleen, and brain. Additional rats were sacrificed predose to provide control serum or tissue for analysis. All urine and feces for each drug formulation were collected separately from three male and three female rats that were housed in individual metabolism cages. Urine and feces were collected quantitatively in polypropylene containers at the following intervals: predose, 0–2, 2–4, 4–6, 6–12, 12–24, 24–36, 36–72 h, and post-dose. Feces were frozen immediately after drying at the end of each collection interval. The weight of the tissue, urine, and fecal samples was recorded before storage. All biological specimens were immediately stored at –20 °C until the time of analysis. Under these conditions Pur was found to be stable in these biological matrices. All tissue, serum samples, and the excretion samples were analyzed by a validated HPLC method described below.

## Serum Preparation

Extraction of Pur from serum involved the addition of 2.0 mL methanol into 500 μL of serum sample (4:1, v/v) in 5 mL glass centrifuging glass tube and vortex mixing for 1 min. After centrifugation at 3000 rpm for 15 min, the supernatant was transferred to a fresh tube and evaporated to dryness under $N_2$. The residue was then dissolved in 200 μL of the chromatographic methanol and centrifuged at 10,000 rpm for 10 min after vortex mixing for 1 min. The final supernatant was transferred into 1.5 ml polypropylene tubes. A 20 μL aliquot of the final supernatant was injected into the analytical column. The three-step sample preparation procedure was employed a priori to ensure optimal purity of the material injected into the analytical column. Simple precipitation/centrifugation may be equally suitable and less expensive.

## Tissue Preparation

Solid tissues were thawed and aliquots of approximately 0.5 g were weighed out for each sample. The specimens were cut in small pieces and homogenized with 2.0 mL of methanol using an ultraturrax T8 (20000 rpm, 3 min) with a S8N-5G head. The leftover on the homogenizer was washed with little methanol after each homogenization and was transferred to the corresponding sample. Calibration standards and quality control samples for Pur analysis were prepared by homogenizing normal tissue in 2.0 mL of methanol and adding known amounts of Pur. Homogenized samples were centrifuged for 15 min at 3000 rpm. The supernatant was evaporated to dryness under $N_2$. The residue was finally dissolved in 200 μL of chromatographic methanol and centrifuged at 10,000 rpm for 10 min after vortex mixing for 1 min. The final supernatant was pipetted into 1.5 mL polypropylene tubes and an aliquot of 20 μL was injected into the analytical column. Pur concentrations in tissue were calculated according to 0.5 g of tissue. Blank samples of all matrices also were extracted to ensure the absence of endogenous interfering peaks.

## Excreta Preparation

Urine samples were thawed at ambient temperature, filtered, and vigorously mixed with methanol (1:4, v/v). A 0.2 g aliquot of each feces sample was thawed, weighed, and added to 2.0 mL methanol for homogenization. The urinary and fecal samples were centrifuged for 15 min at 3000 rpm. The supernatant was evaporated to dryness under $N_2$. The residue was then dissolved in 200 μL of the chromatographic methanol and centrifuged at 10,000 rpm for 10 min after vortex mixing for 1 min. The final supernatant was pipetted into 1.5 mL polypropylene tubes and an aliquot of 20 μL was injected into the analytical column.

## HPLC Conditions

Analysis was performed by HPLC using an Agilent 1100 series HPLC system equipped with a photodiode array detector. Separation was achieved using a Zorbax Eclipse XDB-$C_{18}$ reversed-phase column (250 mm × 4.6 mm i.d., 5 μm particle size), preceded by a guard column (45 mm × 4.6 mm i.d.) filled with $C_{18}$ (5 μm particle size). The mobile phase consisted of acetonitrile and water mixtures, with gradient elution from 10 to 100% acetonitrile over 16 min at a flow-rate of 0.7 mL/min. The mobile phase was filtered through 0.45 μm PTFE membrane (Alltech Associate, Deerfield, IL) and degassed by vacuum sonication prior to use. Briefly, a 20-μL aliquot of the extracted sample (kept at 4 °C) was injected into the column and detected at 250 nm (retention time 8.32 min). The chromatography was carried out at room temperature. Pur concentration in the sample was calculated using an external calibration curve of Pur in methanol. Under the optimal HPLC condition, the blank blood serum did not interfere with the assay. Samples were injected immediately after preparation.

## Preparation of Standard Solutions

A stock standard solution of Pur (2.5 mg/mL) was prepared by weighing out the appropriate amount of Pur and dissolving it by ultrasonication in methanol. More diluted stock solution (500 μg/mL) was made by diluting the initial stock standard solution with methanol. Standard solutions at different concentrations for determination of calibration curves and quality control samples were also prepared. The stock solutions were protected from light and stored at 4°C.

## Linearity and Precision

Calibration curves of Pur in serum, tissue, and excretion were established over the concentration ranges as shown in Table 3. Six data points were utilized to construct the curves. Calibration standards were prepared by adding known amounts of stock solution to normal serum, normal tissue homogenized in methanol, or normal excretion homogenized in methanol. Peak areas versus the corresponding Pur concentrations were plotted. The detection and quantification limits were estimated by repeated analysis of the blank samples of serum, tissue homogenates (heart, liver, spleen, brain, lung, and kidney), and excretion homogenates (urine and feces). Precision (intra-day and inter-day variation) was evaluated by analyzing three replicate serum, tissue samples, and excretion samples at the following concentrations: 0.25, 2.0, and 8.0 μg/mL for serum; 0.625, 2.5, and 10.0 μg/mL for liver; 0.625, 2.5, and 20.0 μg/mL for heart; 1.25, 5.0, and 15.0 μg/mL for kidney; 0.3125, 1.25, and 5.0 μg/mL for brain and spleen; 1.25, 10.0, and 250.0 μg/mL for lung; 2.50, 50.0, and 200.0 μg/mL for urine, and 2.50,

40.0, and 1000.0 μg/mL for feces. The variability was expressed as the relative standard deviation (RSD). To be acceptable, the RSD value should be less than 15% of the corresponding mean value at all concentrations.

## Recovery

The recovery of Pur from serum, brain, heart, liver, spleen, lung, kidney, urine, and feces was determined by spiking an equal amount of Pur into the corresponding blank sample and methanol. Recoveries at three concentration levels were studied in triplicate for each type of sample. Percentage recovery was calculated by comparing the absolute responses (peak area) of Pur in sample extracts to the absolute responses (peak area) of non-extracted standards (Pur in methanol for serum, tissue, and excretion, respectively).

## Pharmacokinetic Parameters

The pharmacokinetic parameters for Pur in serum were estimated by appropriate compartmental methods. All parameters were determined from the sample collection times and the assayed concentrations at these times. Concentration values below the lower limit of quantification were set to zero. The goodness of fit and the most appropriate model were determined by assessing the randomness of the scatter of actual data points around the fitted function. Serum concentrations were plotted against time and the pharmacokinetic calculations were performed using the standard software package program 3P97 (developed by Chinese Pharmacological Society). The following parameters were estimated: maximal plasma concentration (Cmax), time to maximal plasma concentration (Tmax), plasma half life ($T_{1/2}$), area under the plasma concentration time curve (AUC), total plasma clearance (Cls), apparent distribution volume (V/F), etc. Mean residence time (MRT) and variance of residence time (VRT) were calculated by non-compartmental method of statistical quadrature. The distribution of Pur in each tissue sample was expressed as the Ct/Cp ratio (concentration of Pur per gram of each tissue divided by the concentration of Pur in serum). The Student's *t*-test was used to analyze differences between two groups. The difference in two groups of data with *p*-value of less than 0.05 or 0.01 was considered significant. The data were presented as means ± SD.

The cumulative urinary or fecal excretion ratios (% of dose) were calculated as follows:

$$\frac{\text{Cumulative Pur excretion}}{\text{Dose of Pur}} \text{X100}$$

# RESULT AND DISCUSSION

## Assay Method

The Zorbax Eclipse XDB-$C_{18}$ column and the simple mobile phase used were found to be appropriate for the analysis of Pur. The retention times for Pur were around 8.2–9.4 min. In Fig. 2, the chromatograms obtained for drug-free rat samples (serum, tissues, and excretion) and post-dose rat samples obtained after oral administration of drugs are shown. No peaks with retention times similar to those of Pur were present in blank rat samples. Different ratios with different gradient elution and flow-rates of the mobile phase were studied in order to shorten the retention times of Pur and separate it from the endogenous impurities. The optimal separation was obtained from gradient elution from 10% acetonitrile in water to 100% acetonitrile over 16 min, with the flow-rate at 0.7 mL/min. The results of the recoveries and variabilities of the HPLC assay for Pur are summarized in Tables 1 and 2. The data indicate that the assay method was reproducible within the same day and between different days; RSDs were less than 15% for all sample types over the concentration ranges assayed. The results of linear regression analysis are listed in Table 3 and showed that the correlation coefficients of the calibration curves for all sample types were ~0.99.

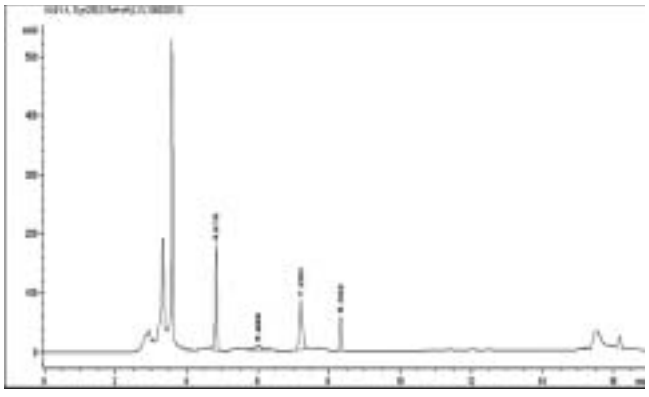

**FIGURE 2** **Typical Chromatogram of Post-dose Serum Specimen Obtained After Oral Administration of Pur or Its Phospholipid Complex (400 mg/kg) in Rat.**

**TABLE 1** Recoveries of Pur from Serum, Tissue, and Excretion

| | Recovery(%): mean ± SD($n$ = 3) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Serum | Heart | Liver | Spleen | Lung | Kidney | Brain | Urine | Feces |
| High | 98.4 ± 6.79 | 98.4 ± 2.98 | 93.2 ± 9.1 | 105.3 ± 2.80 | 107.8 ± 8.36 | 101.4 ± 4.11 | 104.1 ± 2.65 | 97.0 ± 5.33 | 102.4 ± 2.30 |
| Medium | 98.4 ± 6.91 | 99.3 ± 6.21 | 95.6 ± 4.61 | 103.9 ± 4.65 | 94.6 ± 2.17 | 99.9 ± 5.06 | 97.1 ± 3.60 | 102.8 ± 3.78 | 93.6 ± 3.38 |
| Low | 100.4 ± 4.37 | 104.8 ± 6.55 | 106.7 ± 6.06 | 96.9 ± 5.42 | 104.4 ± 3.37 | 98.3 ± 9.40 | 103.7 ± 7.35 | 95.6 ± 1.67 | 94.0 ± 6.42 |

**TABLE 2** Inter-day and Intra-day Variabilities of the HPLC Assay for Pur

| | RSD (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Serum | | Heart | | Liver | | Spleen | | Lung |
| | Inter-day | Intra-day | Inter-day | Intra-day | Inter-day | Intra-day | Inter-day | Intra-day | Inter-day |
| High | 6.7 | 4.1 | 2.3 | 2.4 | 1.6 | 6.4 | 6.8 | 5.1 | 2.0 |
| Medium | 7.8 | 8.6 | 8.2 | 6.5 | 4.3 | 9.2 | 4.8 | 6.8 | 5.7 |
| Low | 7.7 | 9.1 | 7.6 | 5.2 | 3.7 | 7.4 | 5.2 | 8.1 | 7.8 |

| | RSD (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Kidney | | | Brain | | Urine | | Feces | |
| | Intra-day | Inter-day | Intra-day | Inter-day | Intra-day | Inter-day | Intra-day | Inter-day | Intra-day |
| High | 11.6 | 5.5 | 4.5 | 1.9 | 2.5 | 3.0 | 10.7 | 8.3 | 4.7 |
| Medium | 7.7 | 5.7 | 4.6 | 5.4 | 4.2 | 5.3 | 7.7 | 4.4 | 0.3 |
| Low | 7.2 | 8.4 | 8.6 | 6.2 | 6.9 | 5.6 | 6.5 | 12.3 | 5.2 |

**TABLE 3** Calibration Curve of Pur in Spiked Rat Biological Materials ($n$ = 3)

| | Regression equation | R | Linear range (μg/mL) |
|---|---|---|---|
| Serum | A = 88.267C – 3.6348 | 0.9994 | 0. 25 ~ 8.0 |
| Liver | A = 48.176C + 10.858 | 0.9993 | 0.625 ~ 10.0 |
| Heart | A = 48.266C – 1.1729 | 0.9996 | 0.625 ~ 20.0 |
| Kidney | A = 69.212C – 14.539 | 0.9979 | 1.25 ~ 15.0 |
| Brain | A = 76.128C – 6.1583 | 0.9993 | 0.3125 ~ 5.0 |
| Spleen | A = 69.058C – 6.7792 | 0.9984 | 0.3125 ~ 5.0 |
| Lung | A = 35.968C + 39.528 | 0.9996 | 1.25 ~ 250.0 |
| Urine | A = 62.542C + 50.293 | 0.9997 | 2.5 ~ 200.0 |
| Feces | A = 27.285C + 175.62 | 0.9999 | 2.5 ~ 1000.0 |

The detection limits of the assay were found to be 83 ng/mL for serum, 208 ng/mL for heart, 140 ng/mL for liver, 104 ng/mL for spleen and brain, 417 ng/g for lung and kidney, and 833 ng/mL for urine and feces. The estimated limits of quantification were 250 ng/mL for serum, 625 ng/mL for heart, 420 ng/mL for liver, 938 ng/mL for spleen and brain, 1250 ng/mL for lung and kidney, and 2500 ng/mL for urine and feces. These limits of detection and quantitation were validated, obtaining coefficients of variation <15%.

## Pharmacokinetics of Pur and Its Phospholipid Complex

The poor oral bioavailability of Pur is mainly due to its poor water solubility and poor intestinal membrane permeability. The use of phospholipid complex in drug delivery is a technique adopted to increase bioavailability and enhance the pharmacological actions of the active constituent. In the present study, in order to observe its effect on bioavailability, a phospholipid complex was used in the oral formulation of Pur because of its proven success in promoting the absorption of a variety of natural substances, including silybin (Peyne et al., 1987), dilichol (Kimura, 1986), saponins from *Centella asiatica* (Bombardelli et al., 1992), herba epimedii total flavonoids (Yongnan et al., 2002), baicalin (Jianmei et al., 1999), diclofenac (Khazaeinia & Jamili, 2003) and piroxicam (Elkheshen et al., 2001). After the administration of a single dose of either Pur or PPC to the rats, the serum Pur levels were analyzed over a 12 h period. Figure 3 shows the mean serum concentration–time profiles after oral administration at the dose of 400 mg Pur/kg. The pharmacokinetic parameters calculated from the data are summarized in Table 4. The concentration–time profiles were best described by an open two-compartment model. Following oral administration, the serum Pur concentrations increased sharply, reaching *C*max within 0.4–1 h post-dose. It showed that Pur was rapidly absorbed following oral administration which was consistent with the literature. For PPC, *C*max value for the complex was 1.61 times that of the pure Pur. The Tmax of the complex was shortened by 2.06 times. Although the *C*max and Tmax of the complex were better than that of Pur, the difference may not be statistically significant when the standard deviations are considered. The complex exhibited more rapid and

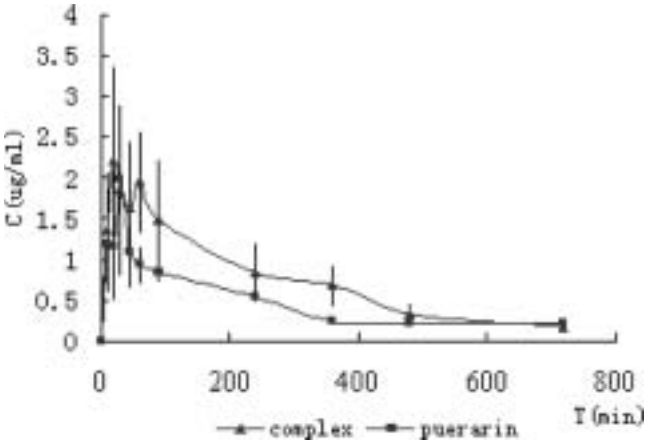


**FIGURE 3** **Serum Concentration-time Profiles of Pur After Oral Administration at the Dose of 400 mg/kg (*n* = 5) in Rats. Error Bars Covered by Symbol Are Not Shown.**

**TABLE 4** **Pharmacokinetic Parameters for Pur and PPC After Oral Administration at a Dose of 400 mg/kg in Rats (*n* = 5)**

| Pharmacokinetic parameters | Pur | Pur complex |
|---|---|---|
| A (μg/ml) | 4.309 ± 0.918 | 6.672 ± 7.230 |
| α ($h^{-1}$) | 2.638 ± 3.058 | 7.874 ± 1.502 |
| B (μg/ml) | 0.800 ± 0.454 | 0.675 ± 0.600 |
| β ($h^{-1}$) | 0.202 ± 0.061 | 0.122 ± 0.08 |
| Ka ($h^{-1}$) | 6.665 ± 9.86 | 13.213 ± 15.31 |
| V/F(c)(mg/(μg/ml)) | 206.14 ± 113.79 | 155.55 ± 85.19 |
| $T_{1/2\alpha}$ (h) | 0.861 ± 0.687 | 1.232 ± 1.61 |
| $T_{1/2\beta}$ (h) | 3.703 ± 1.224 | 4.67 ± 1.59 |
| $T_{1/2Ka}$ (h) | 0.428 ± 0.407 | 0.257 ± 0.404 |
| $K_{21}$ ($h^{-1}$) | 0.484 ± 0.331 | 0.663 ± 0.351 |
| $K_{10}$ ($h^{-1}$) | 0.461 ± 0.261 | 0.38 ± 0.36 |
| $K_{12}$ ($h^{-1}$) | 0.948 ± 1.573 | 1.77 ± 0.673 |
| AUC ((μg/ml)*h) | 5.797 ± 1.662 | 8.456 ± 0.44* |
| CL(s) (mg/h/(μg/ml)) | 74.892 ± 26.786 | 56.737 ± 18.81 |
| T (peak)(h) | 0.894 ± 0.521 | 0.435 ± 0.261 |
| C (max)(μg/ml) | 1.367 ± 0.586 | 2.202 ± 1.28 |
| $MRT_{0-\infty}$(h) | 5.503 ± 1.39 | 6.562 ± 2.89 |
| $VRT_{0-\infty}$(ml) | 36.718 ± 18.93 | 52.69 ± 51.74 |

Note: Comparison between two groups, *$P > 0.05$.

greater absorption. As seen in the serum curves, the complex showed higher serum levels than Pur. The extent of Pur absorption was greater for the complex as seen from the significantly increased $AUC_{(0-T')}$ values, rising from 5.797 ± 1.662 μg/mL *h to 8.456 ± 0.44 μg/mL *h, $P < 0.05$. The AUC values showed that by formulation as a phospholipid complex, the bioavailability of Pur in rats was 1.46-fold higher. $T_{1/2\alpha}$, $T_{1/2\beta}$, and $MRT_{0-\infty}$ were prolonged while the $T_{1/2Ka}$, CL(s) were decreased compared to Pur showing that the absorption time of PPC has been increased. GI retention time was improved and elimination rate slowed when compared with Pur. Double peaks in the concentration–time profile of the complex may be, on the one hand, due to the possible enterohepatic recirculation; on the other hand, an absorption time lag after the first absorption might exist because of the phospholipid and more time was needed for Pur in the complex to be released and absorbed, and thus the second absorption peak formed. Phospholipid is a component of the cell membrane with strong affinity with the cell surface, thus it can stimulate Pur to integrate with the cells and improve the absorption and bioavailability. Based on these results, it might be feasible to develop an oral Pur preparation which is more convenient than the i.v. form.

## Tissue Distribution

Understanding the pharmacokinetics after oral administration of Pur and its phospholipid complex is crucial to evaluate the effect of the complexing on Pur. High percentages of tissue distribution and penetration into target tissues, especially heart and brain, are essential for an effective vasodilator. Furthermore, the issues of whether PPC can serve as an oral formulation of Pur and why the complex is more effective are addressed by studying the pharmacokinetics and tissue distribution.

In general, the results of time-courses of mean tissue concentrations of Pur following a single oral dose of Pur and its phospholipid complex and the corresponding time-courses of tissue/serum ratios showed that there was no significant difference in the tissue distribution of Pur in rats following oral administration of Pur and the complex except in the lung, heart, and liver. Pur was all rapidly distributed into different tissues. The ratios of Pur level to serum in the brain, heart, liver, spleen, and kidney were constant at various times of measurement (20, 45, 60, and 90 min), suggesting that these tissues are in the same compartment as the plasma which is in the central part of the two-compartmental pharmacokinetic model. The concentration of Pur in lung, heart, and liver were higher after oral administration of the complex than that after Pur administration. Meanwhile, the ratios of Pur level to serum in the liver and kidney were much lower and those in the brain and heart were remarkably increased at 120 and 480 min,

respectively, after the administration of the complex. This result was in accordance with pharmacokinetic research that the elimination of Pur slowed after complexing with the phospholipid. The increase in the distribution of Pur in heart and brain suggests the complex may be useful in cardiovascular and cerebrovascular diseases.

An important parameter for pharmacokinetic analyses of a drug is the AUC which represents the total drug exposure integrated over time. The AUC is traditionally the relationship between time and plasma concentration, but can also be applicable to concentration of drug in tissues. It is the best estimate of drug delivery and an indicator of response. Comparision of the AUC of tissues after oral administration of Pur to that after administration of PPC provides an estimate of the target characteristic of the complex. Tissue bioavailability was higher than serum bioavailability in the lung, showing the highest comparative value of 2.51- and 3.11-fold higher than the AUC in serum after oral administration of Pur and complex, respectively. AUCs after the administration of Pur and complex were all in the order of lung > kidney > liver > heart > spleen > brain which indicated that the patterns of tissue distribution for Pur and the complex were identical. It was important to note that by complexing with the phospholipid, the AUC of Pur in lung, heart, and brain were increased remarkably by 1.81, 1.77, and 3.62 times. It was estimated that one of the reasons that the complex was able to improve permeability to cross the blood–brain barrier (BBB) was its higher lipophilicity compared with Pur despite concentrations were still very low.

Among the tissues, lung presented the highest level of Pur followed by kidney, liver, heart, spleen, and the brain. In addition, the lung, liver, and heart showed a multi-peak pharmacokinetic profile. This phenomenon may be attributed to capillary occlusion in the lung (Payne et al., 1987). This phenomenon would lead to an initial high concentration in the lungs, followed by a fast decline as the blood flow removed the cochleae particles. This initial lung uptake and fast release seemed to condition Pur distribution profiles of other organs, creating multi-peak, plateau-shaped profiles in liver and spleen. On the other hand, the multi-peak profile found in lung, liver, and heart could indicate possible enterohepatic recirculation and changes in the vehicle stability. Previous studies indicate (Xiuyuan, 1979; Li et al., 2001) that biliary excretion of Pur is below 4% after i.v. which illustrated that there was enterohepatic recirculation in the excretion of Pur but it was not the main excretion route. As to the more remarkable multi-peak phenomenon in the complex, it was hypothesized that this second peak was related to a later release to the blood of free Pur from its phospholipid complex after its degradation in the tissues. Pur in blood would then undergo a redistribution process leading to a second peak in the tissues. The foundation of this hypothesis was the different tissue uptake mechanisms for complex and free molecules. The physical stability of the complex and the enterohepatic recirculation may explain the presence of several peaks in the Pur tissue concentration–time profile; however, the relative contribution of each mechanism remains to be solved.

## Urinary and Fecal Excretion

The results of cumulative percentages of Pur eliminated in urine and feces over the 72 h period following oral administration of Pur and its phospholipid complex showed that the patterns of excretion after oral administration of Pur or its phospholipid complex were the same. By oral route, Pur was mainly excreted in feces. When Pur was orally administered, only unchanged compound was excreted in feces and experimental data also suggested that C-glycoside Pur was partially hydrolyzed to aglycon in the body but mainly excreted in the urine as unchanged Pur (Yasuda et al., 1995). The percentage of unchanged Pur excreted in feces in rats was highest in the first 24 h, accounting for 98.4% and 99.3% of the total excretion for Pur and the complex, respectively. Much smaller quantities of Pur were excreted in the two subsequent 24 h periods. Urinary elimination was a minor route of elimination. The unchanged Pur was present in very low concentrations and represented only 1.05% and 1.11% up to 72 h after administration. The total excretion of Pur in the excreta was 33.30% and 26.61% at 72 h after administration. The amount and pattern of Pur excretion were similar to the findings of other investigators (Xiuyuen, 1979). The phospholipid complex did not change the excretion characteristics of Pur. The remaining Pur from the oral administration could be lost by decomposition in the GI lumen, irreversibly binding to food or other contents of the intestines or metabolized.

## CONCLUSION

In conclusion, orally administered Pur and its phospholipid complex were rapidly absorbed, widely distributed, and readily eliminated from plasma and tissues of rat. The relative bioavailability of Pur given as a high single dose could be increased nearly 1.46 fold by its incorporation into phospholipid formulations as PPC. In both formulations, Pur was rapidly distributed into different tissues and the distribution characteristics of Pur and its complex were similar. By complexing with the phospholipid, the distribution of Pur in lung, heart, and brain could be improved. Research showed that Pur was mainly excreted in feces after oral intake and the phospholipid complex did not change the excretion characteristics of Pur. Studies on the pharmacokinetics and disposition of the complex after oral administration to rats have yielded very positive results and encouraged its clinical development.

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