

Pharmacokinetics and tissue distribution of ergosterol in rats

Ming-Jie Song, Hai-Ying Bao

Engineering Research Centre of Edible and Medicinal Fungi, Ministry of Education, Jilin Agricultural University, Changchun, Jilin 130118, China

Original Research

Abstract: *Phellinus ellipsoideus* is a wood-rotting fungus used medicinally as sanghuang in the north-central area of Fujian Province, China. Ergosterol (ergosta-5,7,22-trien-3 β -ol), one of the best-known bioactive steroids, has been identified as the active constituent of *P. ellipsoideus*. The objective of this study was to investigate the pharmacokinetics and tissue distribution of ergosterol after oral and intravenous administration in rats using high performance liquid chromatography with an external standard. The isolation was achieved with a C18 column using a methanol/water (98:2, v/v) solvent system with a flow rate of 0.8 mL/min at 25°C and a maximum absorption wavelength of 283 nm. The pharmacokinetic profiles of the two routes of administration revealed that ergosterol was absorbed rapidly into the systemic circulation and was widely distributed throughout the body, followed by a rapid elimination phase. The highest concentration of ergosterol was found in lung tissue, followed by spleen and liver. These results will be useful for clinical applications or further study of ergosterol from fungi.

Key words: Ergosterol; Pharmacokinetics; Tissue distribution; HPLC.

Corresponding author:

Prof. Hai-Ying Bao, Engineering Research Centre of Edible and Medicinal Fungi, Ministry of Education, Jilin Agricultural University, Xincheng street 2888, Changchun, Jilin, China. Email: baohaiying2008@126.com

Received: September 21 2015; **Accepted:** November 17 2015; **Published:** November 21 2015

doi: <http://dx.doi.org/10.18088/ejbmr.1.4.2015.pp1-6>

Citation: Song, M., & Bao, H. (2015). Pharmacokinetics and tissue distribution of ergosterol in rats. *European Journal Of BioMedical Research*, 1(4), 1-6. doi:<http://dx.doi.org/10.18088/ejbmr.1.4.2015.pp1-6>

Introduction

Fungi are used in traditional medicines in Asia, Europe and America. There are various classes of primary and secondary metabolites in mushrooms that exhibit biological activity. Steroids are a major chemical component of fungi (1-4) and have been reported to possess anticancer (5,6), antimicrobial (7), diuretic (8), antioxidant (9) and immunosuppressive activities (10).

Ergosterol (ergosta-5,7,22-trien-3 β -ol) is one of the best-known bioactive steroids and exists widely in medicinal fungi, including *Phellinus ellipsoideus* (11), *Phellinus yamanoi* (5), *Hypholoma fasciculare* (12), *Tricholoma mongolicum* (13) and *Lycoperdon pyriforme* (14).

While screening for antitumor agents from fungi in our previous studies, the petroleum ether extract of dried *P. ellipsoideus* fruiting bodies was found to induce significant cytotoxicity in tumor cell lines. Chromatography identified the active constituent as ergosterol (11). *Phellinus ellipsoideus* has been used as the traditional medicine sanghuang in the north-central area of Fujian Province, China, and was first identified scientifically by Cui and Dai and collected from Wanmulin Natural Reserve, Fujian Province (15).

The objective of this study was to describe the pharmacokinetic profile of ergosterol, including its absorption, distribution, metabolism and elimination in plasma, and tissue distribution, in Wistar rats (16). Furthermore, a fast and sensitive method was established to measure ergosterol in plasma and tissue samples using high-performance liquid chromatography (HPLC).

Materials and Methods

Chemicals and Materials

The standard of ergosterol (Fig. 1a) was isolated by our laboratory from *F. ellipsoidea* with 97% purity as determined by HPLC. The procedure isolation and purification of ergosterol was published before (11). Its structure was characterized by chemical and spectroscopic methods (¹HNMR, ¹³CNMR and MS) and compared with literatures (17). Vitamin D₂ (Fig. 1b) was used as external standard which was purchased from Jinsui Co. (Shanghai China). High-pressure liquid chromatography (HPLC)-grade methanol and dichloromethane were purchased from Yuwang Co. (Shandong China). Water was purchased from Wahaha Co. (Hangzhou China). Poloxamer was purchased from BASF Co. (Germany).

Rats

Wistar rats (Equal numbers of males and females, 200-220g) were supplied by the experimental animal research center of Jilin University. The animals were kept in an environmentally controlled breeding room on a 12 h light/12 h dark cycle (light on at 8:00 am) at ambient temperature (23-25°C) and 60% relative humidity with unlimited access to food and water for 7 days before starting the experiment. They were fasted for 12 h and had free access to water before drug administration.

Instrumentation

Analysis was performed with an Agilent 1100 series HPLC system equipped with an UV spectrophotometric detector, a quaternary pump and a thermostatically controlled column apartment.

Chromatographic separations were performed with a 5 μ m ZORBAX Eclipse XDB-C18 (4.6 \times 150 mm) maintained at 25 $^{\circ}$ C. The mobile phase was a 98:2 (v/v) mixed solution of methanol and water at the flow rate of 0.8 mL/min. 10 μ L were injected into HPLC system for analysis. The system pressure was 46 bar. The monitoring wavelength was 283 nm.

Preparation of standard samples

The stock (120 μ g/mL) and working solutions (12 μ g/mL) of ergosterol were prepared by dissolving an accurately weighed quantity of ergosterol in dichloromethane and serial dilution with the same solvent. The stock (150 μ g/mL) and working solution (15 μ g/mL) of vitamin D₂ were prepared in the same way. All the solutions were stored at 4 $^{\circ}$ C.

Calibration Curve

The calibration curve was constructed by plotting the peak area versus the concentration of the calibration standard. Calibration samples were prepared by spiking plasma samples and tissue mixtures with ergosterol standard solution. The final concentrations of ergosterol in the calibration samples were 0.009, 0.037, 0.150, 0.600, 1.200 and 2.400 μ g/mL. Quality control (QC) samples containing 0.009 (low), 0.150 (medium) and 2.400 μ g/mL (high) of ergosterol were also prepared in the same way. To each plasma samples (40 μ L), dichloromethane (240 μ L) was added, and tissue samples (0.2g) was homogenized with dichloromethane (2 ml). Then mixture was vortex mixed for 1 min and centrifuged at 10,000rpm for 5 min. A 10 μ L portion of the substratum was subjected to HPLC analysis.

Precision and accuracy

The intra-day and inter-day accuracy and precision were evaluated by replicative analysis of five sets of samples spiked with QC samples at three concentration levels of ergosterol (0.009, 0.150 and 2.400 μ g/mL) within a day or during three consecutive days. The accuracy was expressed as bias, obtained by calculating the percentage of difference between the measured and spiked concentration over that of the spiked value, whereas the precision was denoted by using the relative standard deviation (RSD%).

Extraction recovery

The absolute recovery of the extraction was determined by comparing the peak area obtained from the samples with peak areas obtained by the direct injection of pure ergosterol standard solutions in the mobile phase at three different concentration levels. The quantification of the chromatogram was performed by using peak area ratios of ergosterol to standard.

Pharmacokinetic Study

Experiments were performed on 60 rats that were randomly divided into 12 groups (n=5). For plasma samples preparation, ergosterol aqueous solution was intravenously injected by vena caudalis of rats in 3 groups with three dose (25mg/kg, 12.5mg/kg and 6.25mg/kg) and orally administered in 3 groups with three dose (50mg/kg, 25mg/kg and 12.5mg/kg) in average. Tissue samples were prepared in 6 groups by intravenous injection at a

single dose (12.5 mg/kg) and oral administration at a dose of 25 mg/kg.

Blood samples (approximately 0.3 mL) were collected from orbital vein at control (0 min) and 0.08, 0.25, 0.50, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24 h after the administration. The sample was transferred to a 1.5mL centrifuge tube with heparin sodium and centrifuged at 10000rpm for 10 min immediately. The plasma was separated into another clean tube and frozen at -20 $^{\circ}$ C until analysis. Next 240 μ L dichloromethane was added into the plasma sample and vortex mixed for 1min. Then the mixture was centrifuged at 10000rpm for 5 min. At last, 10 μ L of the substratum was injected into the HPLC for analysis with external standard method.

Rats in three groups were sacrificed at 10min, 1h and 3h (0.5h, 1h and 3h) respectively after the intravenous administration (oral administration). Subsequently, the brain, heart, lung, liver, spleen and kidney were immediately removed, washed in normal saline and blotted dry with filter paper. An accurately weighed amount of the soft tissue samples (0.2g) was individually homogenized with dichloromethane (2 ml). Then the mixture was centrifuged at 10000rpm for 5 min. At last, 10 μ L of the substratum was injected into the HPLC for analysis.

The concentrations of ergosterol in plasma samples and tissue samples were expressed as mean \pm standard deviation (SD). The mean concentration-time curve was constructed by the sample data. The pharmacokinetic parameters for ergosterol were evaluated by analyzing the data of plasma concentration-time profiles, which was calculated by the pharmacokinetic software, 3P97 (Chinese Pharmacological Society, China). The absolute bioavailability of ergosterol expressed as $F = \text{AUC}_{\text{po}} \cdot \text{Div} / \text{AUC}_{\text{iv}} \cdot \text{Dpo} \times 100\%$.

Results

Isolation and purification of ergosterol

The purity of ergosterol was above 97% as determined by HPLC in our published paper (11). The characteristics of ergosterol are: white needles from petroleum ether, mp. 153-155 $^{\circ}$ C, ¹H NMR (CDCl₃, 500 MHz) 3.64(1H, m, H-3), 5.57(1H, dd, H-6), 5.38(1H, s, H-7), 0.63 (3H, s, H-18), 0.95 (3H, s, H-19), 1.04 (3H, d, H-21), 5.17 (1H, dd, H-22), 5.46 (1H, dd, H-23), 0.92 (3H, s, H-25), 0.83 (3H, s, H-27), 0.82 (3H, s, H-28); ¹³C NMR (CDCl₃, 125 MHz) 39.09 (C-1), 32.00 (C-2), 70.46 (C-3), 38.38 (C-4), 139.78 (C-5), 119.59 (C-6), 116.29 (C-7), 141.35 (C-8), 46.26 (C-9), 37.03(C-10), 21.25 (C-11), 40.40 (C-12), 42.38 (C-13), 54.56 (C-14), 22.99 (C-15), 28.27 (C-16), 55.75 (C-17), 12.04 (C-18), 16.28 (C-19), 40.80 (C-20), 21.10 (C-21), 135.56 (C-22), 131.98 (C-23), 42.83 (C-24), 33.09 (C-25), 19.64 (C-26), 19.94 (C-27), 17.59 (C-28).

Optimization of the chromatographic conditions

The HPLC parameters were compared to develop accurate, valid and optimal chromatographic conditions, including: mobile phase (methanol, methanol-water and acetonitrile-water solutions); column type (Agilent ZORBAX Eclipse XDB-C18 column 4.6 \times 150 mm, 5 μ m, Agilent ZORBAX Eclipse XDB-C18 column 4.6 \times 250 mm, 5 μ m and Agilent Venusil MP-C18 column 10 \times 250 mm, 10 μ m); wavelength (265 and 283 nm); column tem-

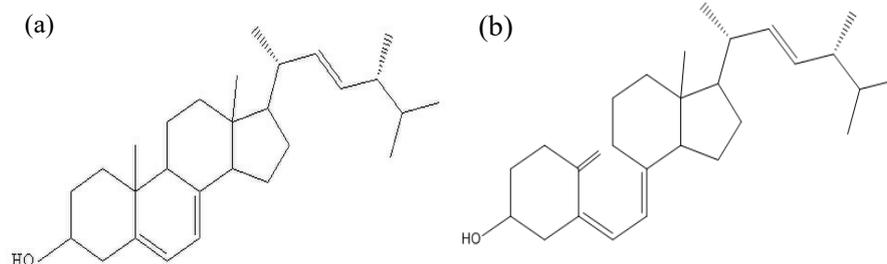


Figure 1. Chemical structures of ergosterol (a) and vitamin D₂ (b).

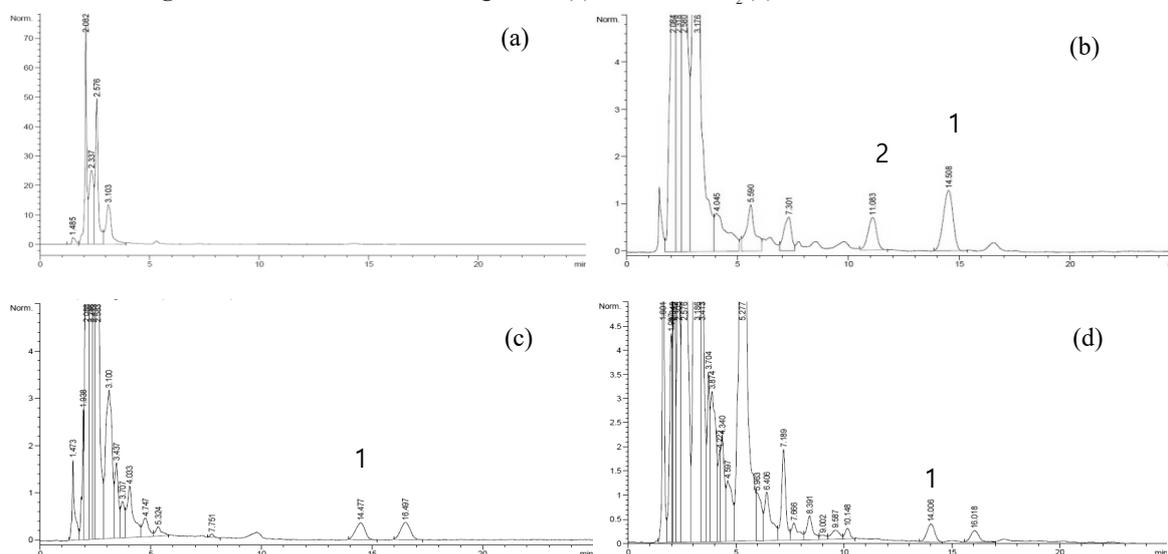


Figure 2. Representative HPLC chromatograms of (a) rat plasma; (b) rat plasma spiked with ergosterol and vitamin D₂; (c) a rat plasma sample obtained 15 min after intravenous injection of ergosterol at a dose of 6.25 mg/kg; and (d) a rat plasma sample obtained 15 min after oral administration of ergosterol at a dose of 50 mg/kg. Peak identifications: 1 ergosterol, 2 vitamin D₂.

Table 1. Calibration curves, correlation coefficients and linear ranges of ergosterol in rat plasma and tissue samples (n = 3).

Biological samples	Calibration curves	Correlation coefficients (r ²)	Linear ranges (µg/ml)
Plasma	y = 15.680x + 2.978	0.999	0.009-2.400
Liver	y = 9.604x + 2.145	0.996	0.009-2.400
Heart	y = 7.496x + 2.392	0.997	0.009-2.400
Spleen	y = 9.986x + 2.450	0.996	0.009-2.400
Lung	y = 11.150x + 2.245	0.995	0.009-2.400
Kidney	y = 9.398x + 1.922	0.996	0.009-2.400
Brain	y = 8.390x + 2.607	0.997	0.009-2.400

perature (20, 25 and 30°C); and flow rate of the mobile phase (0.5, 0.8 and 1.0 mL/min). Based on these comparisons, the samples were separated by HPLC using an Agilent ZORBAX Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm) with a methanol/water (98:2, v/v) solvent system at a flow rate of 0.8 mL/min at 25°C and a maximum absorption wavelength of 283 nm. Optimum peak shapes and high responses were obtained for ergosterol and Vitamin D₂ under these conditions. Vitamin D₂ was chosen as the external standard for its similarity in structure with the analyte (Fig. 1).

Selectivity

Representative chromatograms from the blank rat plasma, blank rat plasma spiked with the analyte and external standard and plasma sample at 15 min after intravenous injection of 6.25 mg/kg of ergosterol are shown in Figure 2c, and the plasma sample at 15 min after oral administration of 50 mg/kg of ergosterol is shown in Figure 2d.

Endogenous plasma did not interfere with the elution of ergosterol and vitamin D₂. Peak identification: (1) ergosterol, (2) vitamin D₂. The retention times of ergosterol and vitamin D₂ were 14.3 and 11.1 min, respectively, with a total run time of less than 20 min.

Calibration curves

The representative regression equation for the calibration curve (y = ax + b) was determined as the peak area (y) versus analyte concentration (x). Linearity was determined using linear regression weighted by 1/x². The results of the calibration curves, correlation coefficients and linear ranges of ergosterol in plasma and tissues are listed in Table 1.

Accuracy, precision and extraction recovery

The intra- and inter-day precision (RSD, n = 5) for the ergosterol-spiked control samples at 0.009, 0.150 and 2.400 µg/mL varied from 1.57 to 9.94%. The correspon-

Table 2. The recovery, precision and accuracy (n = 5) of the assay method.

Tissues	Concentration ($\mu\text{g}/\text{mL}$)	Absolute recovery (Mean \pm SD, %)	Intra-day		Inter-day	
			Precision (RSD%)	Accuracy (bias%)	Precision (RSD%)	Accuracy (bias%)
Plasma	0.009	95.40 \pm 1.59	6.29	7.91	7.36	-0.21
	0.150	94.79 \pm 2.51	4.54	4.78	4.85	5.26
	2.400	94.14 \pm 2.12	8.05	-4.46	3.56	-2.06
Liver	0.009	96.96 \pm 1.66	8.88	-1.76	4.11	7.34
	0.150	96.58 \pm 2.07	7.73	-0.07	3.36	4.27
	2.400	96.49 \pm 1.98	8.29	0.93	8.65	1.63
Heart	0.009	95.58 \pm 2.06	7.72	6.69	3.48	3.75
	0.150	96.21 \pm 2.21	7.74	0.96	1.85	3.01
	2.400	95.35 \pm 2.57	8.15	3.74	4.87	0.37
Spleen	0.009	96.17 \pm 1.17	6.40	9.95	3.65	9.05
	0.150	95.91 \pm 3.15	4.57	4.91	4.88	8.21
	2.400	97.19 \pm 2.41	9.42	5.20	4.51	4.39
Lung	0.009	96.28 \pm 1.65	5.71	-4.78	8.14	8.27
	0.150	96.58 \pm 2.55	6.80	0.94	3.49	7.70
	2.400	95.82 \pm 1.97	8.08	-6.72	9.03	2.16
Kidney	0.009	95.15 \pm 2.16	7.70	7.02	3.12	5.01
	0.150	96.44 \pm 2.55	6.66	-0.42	5.46	5.37
	2.400	95.38 \pm 2.95	6.56	1.45	7.63	-1.77
Brain	0.009	96.44 \pm 2.42	9.16	6.09	3.19	3.75
	0.150	97.35 \pm 1.63	9.45	4.05	4.34	3.01
	2.400	95.48 \pm 2.57	9.94	2.95	1.57	2.46

ding intra- and inter-day accuracy (bias, n = 5) ranged from -4.78 to 9.95% (Table 2). The absolute recoveries of ergosterol from plasma were greater than 95%, indicating that most of the ergosterol in the plasma samples was extracted with no obvious interferences in the chromatogram.

Pharmacokinetics

The HPLC method was applied successfully to determine the pharmacokinetics of ergosterol following intravenous injection of 6.25, 12.5 or 25 mg/kg and oral administration of 12.5, 25 or 50 mg/kg. The pharmacokinetic parameters were calculated with 3P97 software using a compartmental analysis. The plasma concentration-time profile of ergosterol is shown in Figure 3. The corresponding pharmacokinetic parameters are presented in Tables 3 and 4. The distribution processes were fitted to a two-compartment model.

The results indicated that ergosterol was eliminated rapidly after intravenous injection; the half-lives ($t_{1/2}$) were 0.27, 0.26, and 0.23 h for doses of 6.25, 12.5 and 25 mg/kg, respectively (Table 3). After oral dosing, ergosterol concentrations increased rapidly within the first hour, and decreased substantially from 1 to 3 h, followed by a gradual decrease thereafter. At doses of 12.5, 25 and 50 mg/kg, the maximum plasma concentrations (C_{max}) were 0.23, 0.46 and 0.91 $\mu\text{g}/\text{mL}$ with times of peak concentration (T_{max}) of 0.84, 0.84 and 0.86 h, respectively (Table 4; Fig. 3).

Tissue distribution

Tissue distribution of ergosterol was evaluated 10 min, 1 h and 3 h after intravenous dosing (12.5 mg/kg) and 0.5, 1 and 3 h after oral dosing (25 mg/kg). The results (Table

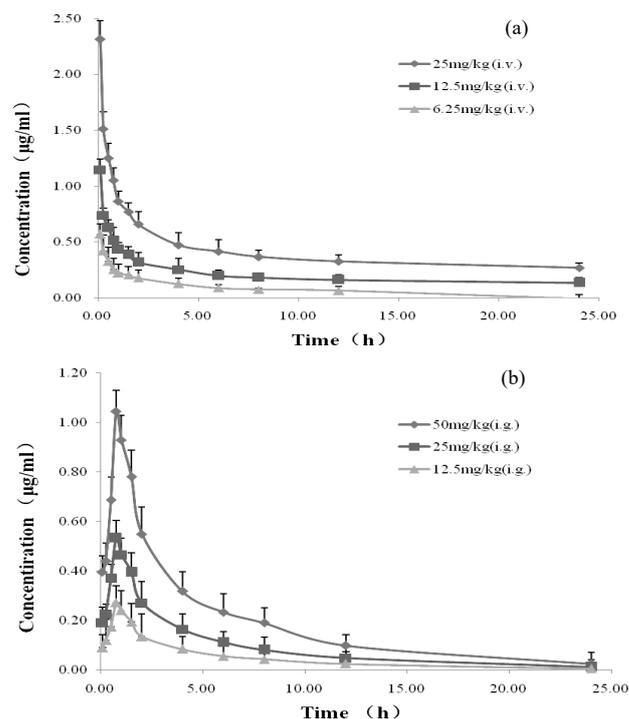


Figure 3. (a) Plasma concentration-time profile after intravenous injection of ergosterol at the dose of 25, 12.5 or 6.25 mg/kg (n = 5). (b) Plasma concentration-time profile after oral administration of ergosterol at the dose of 50, 25 or 12.5 mg/kg (n = 5).

5; Fig. 4) indicated that ergosterol was rapidly and widely distributed throughout the body, including the liver, heart, spleen, lung, kidney and brain. Ergosterol was transported to most organs within 10 min of intravenous administration. The ergosterol concentration in rat tissues is shown in Table 5. The highest concentration of ergosterol was detected in lung tissue, approximately 15-fold higher than

Table 3. The pharmacokinetic parameters of ergosterol in rats following intravenous injection (n = 5).

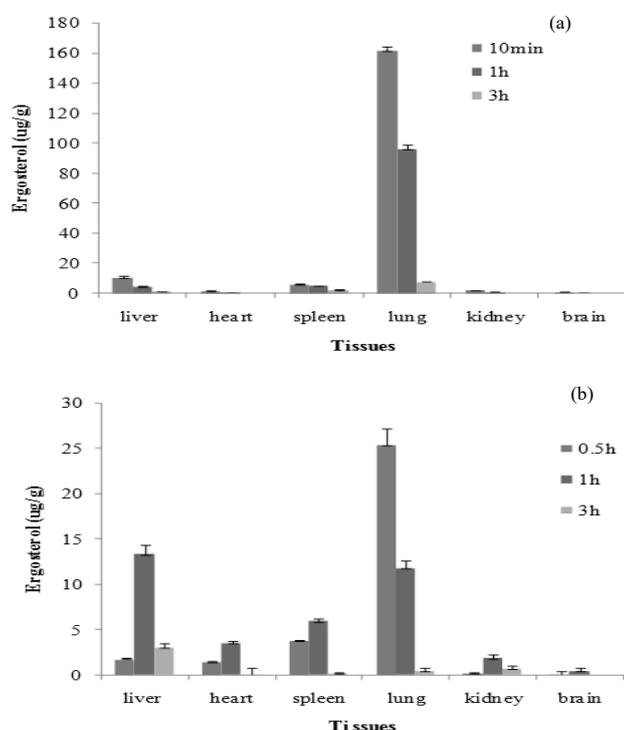
Parameters	6.25 mg /kg	12.5 mg /kg	25 mg /kg
$t_{1/2}$ (h)	0.23±0.04	0.26±0.03	0.27±0.05
V_2 (L/mg)	13.59±2.39	19.84±4.24	20.20±4.02
CL_2 (L/mg/h)	15.21±2.62	16.32±2.79	15.72±3.18
AUC_{0-t} (µg/ml*h)	1.76±0.07	4.62±0.16	9.34±0.29
AUC_{0-inf} (µg/ml*h)	1.83±0.11	5.56±0.13	11.37±0.26

Table 4. The pharmacokinetic parameters of ergosterol in rats following oral administration (n = 5).

Parameters	50 mg /kg	25 mg /kg	12.5 mg /kg
T_{max} (h)	0.86±0.07	0.84±0.02	0.84±0.04
C_{max} (µg/ml)	0.91±0.11	0.46±0.07	0.23±0.02
$t_{1/2}$ (h)	0.53±0.05	0.52±0.03	0.52±0.04
AUC_{0-t} (µg/ml*h)	4.60±0.13	2.23±0.06	1.13±0.03
AUC_{0-inf} (µg/ml*h)	4.95±0.10	2.36±0.05	1.20±0.02
V_2/F (L/mg)	51.86±4.73	48.86±3.82	49.70±4.47
CL_2/F (L/mg/h)	15.73±1.43	15.29±2.15	15.33±1.76

Table 5. The concentration (µg /g) of ergosterol in rat tissues after administration (n=5).

Biological samples		Liver	Heart	Spleen	Lung	Kidney	Brain
Intravenous administration	10 min	10.82±1.24	1.62±0.30	6.04±0.91	161.27±3.43	1.89±0.18	0.97±0.71
	1 h	4.43±0.48	0.68±0.35	5.14±0.24	96.12±3.03	0.92±0.13	0.20±0.58
	3 h	1.24±0.40	ND	2.46±0.48	7.81±0.39	ND	ND
Oral administration	0.5 h	1.72±0.23	1.37±0.16	3.75±0.18	25.35±1.88	0.14±0.17	0.07±0.33
	1 h	13.32±1.01	3.47±0.33	5.87±0.34	11.76±0.93	1.82±0.39	0.40±0.42
	3 h	3.01±0.55	0.04±0.69	0.22±0.07	0.39±0.37	0.63±0.38	ND

**Figure 4.** Tissue distribution of ergosterol in rats after intravenous injection (a) at dose of 12.5 mg /kg and oral administration (b) at dose of 25 mg /kg (n = 5).

in other tissues, followed by spleen and liver. The elimination of ergosterol was rapid. Following oral adminis-

tration, ergosterol was absorbed rapidly and its concentration in lung peaked at 30 min, while concentrations in other organs peaked at 60 min. Samples collected 3 h after administration indicated that ergosterol cleared gradually. The results suggested that ergosterol, a small, fat-soluble molecule, was easily absorbed into the blood stream and that the tissue concentration of ergosterol was related to blood circulation. First, ergosterol in the blood flowed into the right atrium through the liver, then it completed pulmonary circulation and finally redistributed into other tissues, such as the spleen. Since lungs receive the largest blood flow volume, the highest concentration of ergosterol was detected in lung tissue 10 min and 0.5 h after intravenous and oral administration, respectively, followed by liver and spleen.

Discussion

The HPLC method was established successfully and used to study the pharmacokinetics of ergosterol in Wistar rats. Ergosterol is a steroid and one of the major bioactive constituents of fungi. The HPLC method described provides higher resolution, baseline stability and ionization efficiency. By developing this method further, the ergosterol assay could be adapted for use with other biological samples, such as urine and feces.

Ergosterol is found in all fungi and its biological activity, in particular antitumor activity, has been reported in the literature (5-10). However, only two studies were

found on its pharmacokinetics (18,19), which reported a T_{max} in rat plasma of about 8 h, while we demonstrated a T_{max} of about 0.8 h. By examining the T_{max} and $t_{1/2}$ of ergosterol, our results indicate that ergosterol is absorbed and eliminated rapidly in plasma and its concentration in plasma is dose-dependent. The absolute bioavailability of ergosterol was 24.46%. In a tissue distribution study, Yi reported (20) that *Flammulina velutipes* sterols, consisting of ergosterol and others, were distributed mostly in the liver and spleen and eliminated rapidly. However, we found that ergosterol was rapidly and widely distributed in rat tissues, including liver, heart, spleen, lung, kidney and brain, but especially lung tissue. These results will be useful for clinical applications and further study of ergosterol extracted from fungi. This is the first report on the pharmacokinetics of ergosterol in rats using both intravenous and oral routes of administration. Further experiments will be conducted that combine enzymes or receptors in rats with ergosterol and examine the mechanisms of its antitumor activity.

Acknowledgments

The study was supported by National Science Foundation of China (31270088) and Program for Changjiang Scholars and Innovative Research Team (No.IRT1134).

References

- Ohsawa T, Yukawa M, Takao C, Murayama M, Bando H. Studies on constituents of fruit body of *Polyporus umbellatus* and their cytotoxic activity. *Chem Pharm Bull.* 1992; 40: 143-147.
- Zheng SZ, Yang HP, Ma XM, Shen XW. Two new polyporusterones from *Polyporus umbellatus*. *Nat Prod Res.* 2004; 18: 403-407.
- Zhou WW, Lin WH, Guo SX. Two new polyporusterones isolated from the sclerotia of *Polyporus umbellatus*. *Chem Pharm Bull.* 2007; 55: 1148-1150.
- Sun Y, Yasukawa K. New anti-inflammatory ergostane-type ecdysteroids from the sclerotium of *Polyporus umbellatus*. *Med Chem Lett.* 2008; 18: 3417-3420.
- Liang W, Bao HY. Antitumor active constituent in fruiting body of *Phellinus yamanoi* against Hepatoma H22 cell. *Mycosystema.* 2011; 30(4): 630-635.
- Lee WY, Park Y, Ahn JK, Park SY, Lee HJ. Cytotoxic activity of ergosta-4,6,8(14),22-tetraen-3-one from the sclerotia of *Polyporus umbellatus*. *Bull Korean Chem Soc.* 2005; 26: 1464-1466.
- Charles L. Cantrell, Mohamed S. Rajab, Scott G. Franzblau, Frank R.

Fronczek, Nikolaus H. Fiseher. Antimycobacterial Ergosterol-5,8-endoperoxide from *Ajuga remota*. *Planta Med.* 1999; 65: 732-734.

- Zhao YY, Xie RM, Chao X, Zhang YM, Zhao Y, Lin RC, Sun WJ. Bioactivity directed isolation, identification of diuretic compounds from *Polyporus umbellatus*. *J Ethnopharmacol.* 2009; 126: 184-187.
- Kim, S.W., Park, S.S., Min, T. J., Yu, K.H. Antioxidant Activity of Ergosterol Peroxide (5,8-Epidioxy-5a,8a-ergosta-6,22E-dien-3b-ol) in *Armillariella mellea*. *Bull. Korean Chem. Soc.* 1999; 20(7): 819-823.
- Fujimoto H, Nakamura E, Okuyama E, Ishibashi M. Six immunosuppressive features from an ascomycete, *Zopfiella longicaudata*, found in a screening study monitored by immunomodulatory activity. *Chem Pharm Bull.* 2004; 52: 1005-1008.
- Liu HB, Bao HY, Cui BK. Chemical constituents of *Fomitiporia ellipsoidea* fruiting bodies. *Mycosystema.* 2011; 30(3): 459-463.
- Yan Ding, Bao Haiying, Bau Tolgor, Li Yu, Young Ho Kim. Antitumor Components from *Naematoloma fasciculare*. *Microbiol Biotechnol.* 2009; 19(10): 1135-1139.
- Tong CL, Bao HY, Bau Tolgor. Chemical constituents and antibacterial activity of petroleum ether extract from fruit bodies of *Tricholoma mongolicum*. *Mycosystema.* 2010; 29(4): 619-624.
- K kahlos, T seppanen-Laaksa, R hilitunen. Occurrence of some compounds in *Lycoperdon pyriforme*. *Planta Medica.* 1989; 55: 621.
- Cui BK, Dai YC. A new species of *Fomitiporia* (Basidiomycota) from Wanmulin Nature Reserve, Fujian Province. *Mycotaxon.* 2008; 105: 343-348.
- ChongKL, JunSC, Joon SB. Effects of Fluvastatin on the Pharmacokinetics of Repaglinide: Possible Role of CYP3A4 and P-glycoprotein Inhibition by Fluvastatin. *Korean J Physiol Pharmacol.* 2013; 17(3): 245-251.
- Zhang XQ, Yin ZQ, Ye WC. Chemical constituents from fruiting bodies of *Ganoderma lucidum*. *Chinese Traditional and Herbal Drugs.* 2005; 36(11): 1601.
- Wang Lili. The Study of *Polyporus umbellatus*'s Quality Specification in Chinese Pharmacopoeia and Pharmacokinetics study of Ergosterol in *P. umbellatus*. Northwest university. 2011
- Zhao YY, Cheng XL, Liu R, Ho CC, Wei F, Yan SH, Lin RC, Zhang Y, Sun WJ. Pharmacokinetics of ergosterol in rats using rapid resolution liquid chromatography atmospheric pressure chemical ionization multistage tandem mass spectrometry and rapid resolution liquid chromatography/tandem mass spectrometry. *J Chromatogr B.* 2011; 879(21): 1945-1953.
- Yi C, Fu M, Cao X, Tong S, Zheng Q, Firepong CK, Jiang X, Xu X, Yu J. Enhanced oral bioavailability and tissue distribution of a new potential anticancer agent, *Flammulina velutipes* sterols, through liposomal encapsulation. *J Agric Food Chem.* 2013; 61(25): 5961-5971.