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Full Length Research Paper

The disposition and pharmacokinetics of *Dioscorea nipponica* Makino extract in rats

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This study was aimed to investigate the disposition and pharmacokinetics of the total saponins of dioscorea (TSD) in rats. Male Sprague-Dawley rats were orally administrated with ^{3}H labeled TSD at a single dose ratio of 80 mg TSD per 1 kg rat. Blood samples and feces were collected at different time points to measure the level of TSD activity. At the final time point, determination of the disposition of TSD in lung, kidney, heart, liver, adrenal, and small intestine were performed. From the blood samples' emission of radioactivity, pharmacokinetic parameters were derived as T1/2 = 33.33 ± 4.48 h, T_{max} = 6.5 ± 0.71 h, AUC = 119400 ± 421097.67, and C_{max} = 2643.33 ± 192.26 dpm/ml. There was 51.609% of ^{3}H labeled substance excreted in 24 h. These results suggested that blood concentration of ^{3}H-TSD was extremely low and the majority of TSD was excreted in the feces. The TSD was extensively distributed to multi-tissues. The radioactivity level was measured to be the highest in the liver, adrenal gland, and wall of the gastrointestinal tract. The radioactivity of TSD was still being detected in blood after 96 h. This showed TSD was excreted *in vivo* very slowly.

Key words: Total saponins of dioscorea, disposition, pharmacokinetics, ^{3}H labeled.

INTRODUCTION

In China, the total saponins of dioscorea (TSD) had long been used as major components in herbal medicines for treatments. Ingredients of TSD include dioscin, pseudo-protodiscin, protodioscin and methyl protodioscin (Figure 1), which were extracted from *Dioscorea nipponica* Makino. TSD is commonly used to treat diverse diseases is common in China as there are a number of bioactive compounds with pharmacologic effects in TSD; such as anti-cancer, immunomodulation, anti-diabetics and ameliorating myocardial ischemia (Chiang et al., 1992; Mi et al., 2002; Yoshikawa et al., 2007). TSD are also the main ingredient in several Chinese medicine like Di'aoxinxue-kang capsule and Weiao Xin capsule, which are used to treat coronary heart disease in China for many years (Liu et al., 2004).

Several studies had previously investigated the metabolism and pharmacokinetics of some pure steroidal saponin in TSD, such as dioscin (Li et al., 2005; Li et al., 2005) and methyl protodioscin (Cao et al., 2007; He et al., 2006). The total saponins have been analyzed and reported as well (Lin et al., 2007). The HPLC experiments were performed by means of a reversed-phase C18 column and a binary mobile phase system consisted of water and acetonitrile under gradient elution conditions *in vitro*.

In addition, due to the amount of TSD being absorbed in human body was few (Lin, 2007), the measurements of pharmacokinetics are difficult to be performed. Thus, it is necessary to establish high sensitive analytical methods for determination of the pharmacokinetics of TSD in circulation. Recently we developed a method to measure

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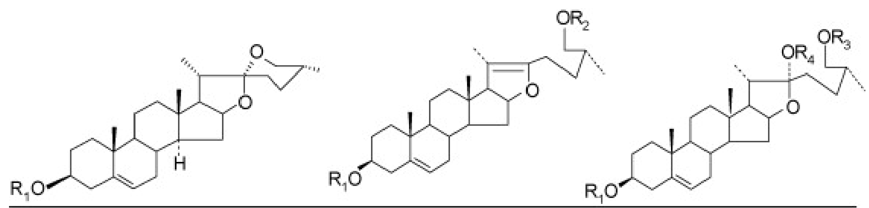

Compounds	R_1	R_2	R_3	R_4	MW
dioscin	Rha(1 →4)[Rha(1 →2)]-Glc				868
pseudoprotodioscin	Rha(1 →4)[Rha(1 →2)]-Glc	Glc			1030
protodioscin	Rha(1 →4)[Rha(1 →2)]-Glc		Glc	H	1048
methyl protodioscin	Rha(1 →4)[Rha(1 →2)]-Glc		Glc	CH_3	1062

Glc =β-D-glucopyranosyl, Rha =α-L-rhamnopyranosyl

Figure 1. Structures of dioscin, pseodoprotodiscin, protodioscin and methyl protodioscin.

TSD in rat plasma with liquid chromatography tandem multi-stage mass spectrometry *in vitro* (Lin et al., 2007). However, no one has ever reported that the signal intensities of TSD obtained in positive in infinitesimal *in vivo*. There are some advantages about radioactive labeled, such as sensitivity which can have infinitesimal solution.

In this study, we developed a specific radioactive assay and characterized the pharmacokinetic parameters of TSD in rats was characterized after a single oral administration dose. To understand the characterization of the disposition and pharmacokinetics of the ^{3}H –TSD, this study utilized SD rats by orally administering the rats with a single dose of ^{3}H –TSD. Here, we report the pharmacokinetic activities in blood, tissue distribution and excretion studies of the herb in rats.

MATERIALS AND METHODS

Drug preparation

TSD powder (the content of total saponins was 90.2% by HPLC, product No.19990618) was obtained from Pharmaceutical Corp of Di'ao group (Chengdu, China). ^{3}H labeled TSD was placed with compared items under microwave stimulation exchange method (^{3}H labeled twice, pure by silicon) by China Nuclear Science Academy (Beijing). The purity of radioactive substance was 63.3%, and the ^{3}H concentration was 1 mci/800 mg (according to pre-tests, the minimal and optimal concentration were selected). The radioactive substance was kept at -20°C before use. Methylene dichloride, dimethylbenzene, perchloric acid, hydrogen peroxide (analytically pure) and PPO scintillator liquid were used as reagents in the experiment. The rats were fed with ^{3}H labeled TSD at a single dose of 80 mg/kg body weight of the rat (radioactive concentration 250 µci/ml).

Method validation

A standard curve of ^{3}H–TSD was obtained by adding ^{3}H–TSD to 0.2 ml of blood in a series of dilution: 0, 1250, 2500, 5000, 10000, 20000, 40000, and 80000 dpm. The equation shows (in results section) the relationship between radioactive dosage and dpm. The relative standard deviation (R.S.D.) was used to report the precision. The lower limit of quantization (LLQ) was assessed by analyzing 8 samples of blood.

Animals

The animal study has approved by ethic committee of Sun Yat-Sen University. Male Sprague-Dawley rats, weighing 230±15 g were purchased from the Animal Center of Sun Yat-Sen University (Guangzhou, China). Rats were housed under constant temperature and humidity using sterile bedding control room with a 12-hour in dark to12-hour in light cycle. They were given free food and water. The rats were fasted overnight before administration with a single dose of ^{3}H-TSD.

Blood sample collection (pharmacokinetic)

The rats had been under anesthetized prior to blood taking. The blood samples in amount of 150 µl blood with anticoagulant were collected from each rat by the puncture of the retro-orbital sinus. This was performed at 0 (predose), 0.5, 1, 2, 4, 6, 8.5, 12, 24, 36, 48, 72, 96 and 120 h after oral administration of ^{3}H-TSD.

Tissue distribution study

At the time point 120 h, the rats were then sacrificed by decapitation. The lung, kidney, heart, liver, adrenal gland, and small intestine were immediately removed. The tissue amples of (100 mg) were minced and homogenized in methanol (0.5 ml). These tissues were collected and stored at –80°C for further analysis.

Excretion study

The animals were housed in metabolic cages and were given free food and water during the course of the experiment. Pooled urine from each rat was collected from 0, 0 - 2, 2 - 4, 4 - 6, 6 - 8.5, 8.5 - 12, 12 - 24, 24 - 48, 48 - 96 h after administration, fecal samples (50 mg) were dried homogenized and stored at –20°C until analysis. The ^{3}H-TSD in the urinary and fecal excretion of the rats was determined.

Radioactive analysis

For radioactive analysis, 0.3 ml perchloric acid and 0.2 ml H_2O_2 (hydrogen peroxide) methylbenzene were put into the blood. The blood was then incubated at 80°C for 50 min. The resulted solution (200µl) was added to PPO scintillator liquid and measured with the LCD reader Beckman Ls6500II (USA).

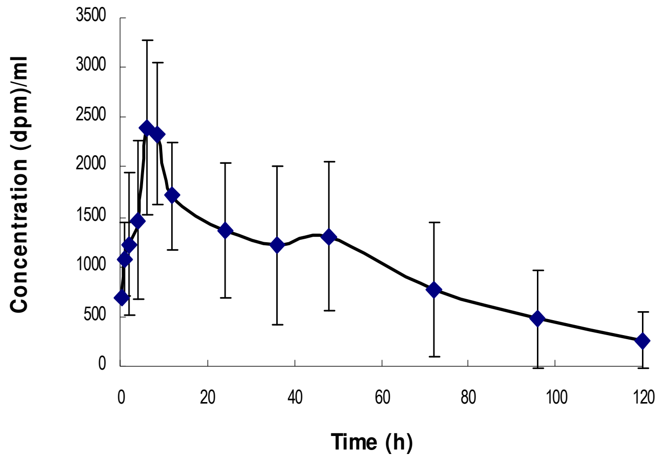

Figure 2. Mean whole blood radioactive concentration-time profiles of the TSD ^{3}H labeled after oral administrations to rat (n = 6).

Table 1. The drug pharmacokinetic parameters X ± SD, n = 6.

PK parameters	X ± SD
Dose (µCi/kg)	1000 µci/Kg
t_{max} (h)	6.5 ± 0.71
C_{max} (dmp/mL)	2643.33 ± 192.26
Half time (h)	33.33 ± 4.48
AUC_{0-t} (h*dmp/mL)	119400.00 ± 21097.67
AUC_{0-inf} (h*dmp/mL)	131701 ± 21837.25
Volume (L/kg)	$1.09*10^{8} \pm 4.02*10^{7}$
CL/F(L/h/kg)	20126667 ± 4333248.70

Explanation: one time feeding (the dose is 80 mg/Kg), 14 point was be observed about 120 h collected parameter of pharmacokinetic. AUC: area under the concentration of blood and time; $t_{1/2}$: half time dose of decline; C_{max}: the biggest concentration of blood.

Statistic analysis

For statistic analysis, the software of WinNonlin (Pharsight Corporation, CA USA) was used.

RESULTS

Validation

The equation below shows the relationship between radioactive dosage and dpm. A standard curve of the ^{3}H-TSD. A good linearity ($r = 0.995$, $P < 0.05$) was found in the regression analysis of the AUC_{0-t}-dose plot.

$$Y = 190.02X - 271.71 \quad R = 0.9991$$

Pharmacokinetic studies

TSD in blood concentration for individual rats were analyzed by compartmental analysis using the WinNonlin (Pharsight Corporation, CA USA). Area under the curve (AUC_{0-t}) was calculated using the linear-trapezoidal rule, with extrapolation to infinity (AUC0–∞) from the last detectable concentration. The absolute oral bioavailability (F) was determined as F = (AU Coral/AUCI.V.) × 100%, using mean AUC values for the oral dose. T-test with $P < 0.05$ was taken as significant. The concentration-time profiles of the administration was measured (Figure 2) and pharmacokinetic parameters are listed (Table 1). The results showed that the radioactivity and pharmacokinetic parameters were low, due to the extremely low blood concentration after the oral dose of 80 mg ^{3}H-TSD per kilogram of rats, the radioactivity and pharmacokinetic parameters shown is low.

Tissue distribution study

As shown in Figure 3 the ^{3}H-TSD in the adrenal glands, small intestine, and liver were higher when compared with other organs at the 120th h. The highest level of TSD was found in adrenal glands and intestinal contents (Figure 3). The obtained data indicates that oral TSD could retained in the adrenal tract and intestinal tract for an extended period and absorbed TSD is widely distributed around the body.

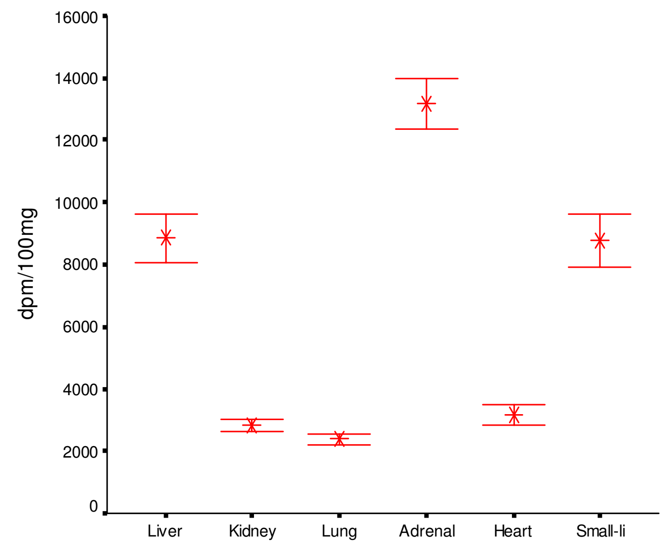

Figure 3. Dose of radioactive in tissues of body (X ± SD). Cumulative excretion of radioactivity in organ after single dosage of ^{3}H TSD labeled drug (the dose is 80 mg/Kg) to the SD rats. After120 h, radioactivity is still detected in organs and extensively distributed in many tissues and organs.

Excretion study

The radioactivity of ^{3}H-TSD was collected and was detected in feces at different time points. The cumulative radioactivity amount was 51.6% after 24 h administration (Table 2). There was no radioactivity found in urine within

Table 2. Excretion of radioactive substance (n = 6).

Time interval (h)	Excretion (%)	Accumulative (%)
0 - 2	0.00302	0.00302
2 - 4	10.8539	10.8579
4 - 6	9.11602	19.9653
6 - 8.5	11.0407	31.0057
8.5 -12	10.3551	41.3608
12 - 24	10.2482	51.6090
24 - 48	1.7656	53.3746
48 - 96	1.6402	55.0158

Cumulative excretion of radioactivity in feces after single dosage of ^{3}H-TSD (80 mg/Kg) to the SD rats; tests of radioactivity were conducted at different time intervals.

24 h (Table 2).

DISCUSSION

The pharmacokinetics of oral TSD to rat is characterized by long t_{max} and $t_{1/2}$. Drugs that undergo entero-hepatic recycling are often characterized by a long half-life and having multiple peaks in the concentration–time profile. In the present study, TSD was found to have a long half-life in rat which reveals that ^{3}H-TSD absorption *in vivo* is very low in content, but quick in rate, and it reaches peaks within 4 to 6 h. After 96 h, traces of radioactivity can still be detected in blood. At various time points, however, the dpm proportion is only 2% of the total given amount. The result is similar to others in the literature (Ma et al., 2002; Li, 2005).

The repeated tests within 100 h through the entero-hepatic recycling into the blood of TSD time curve show many peaks. These peaks distort the calculation of pharmacokinetic parameters and the results suggest that entero-hepatic recycling is important.

^{3}H-TSD is expeled slowly from rat. It can even be traced in liver, adrenal gland, and intestinal tract, but little in the heart. The long t_{max} is a special pharmacokinetic characteristic of ^{3}H-TSD by oral administration, as even at the 120 h time point, the level of ^{3}H-TSD remained high in the adrenal gland, intestinal, and liver contents. This may be an important factor resulting from the continuous absorption of the drug from the intestinal tract. Our results shows that ^{3}H-TSD was mainly excreted in the feces up to 40 - 50% in 12 h, but it could not be found in urine.

After 120 h the rat tissues were tested for radioactivity. Data reveals that the liver, kidney, and heart have traces of radioactivity. Liver, adrenal gland, and intestines have higher rates of absorption. It is very interesting that less absorption of TSD in the heart help treat heart diseases. It is possible that TSD acts upon the endocrine system to meditate the cardiovascular functions. Through *in vitro* study of the dissected heart organ, we applied TSD and discover no improvement on cardiovascular functions after application of TSD. On the contrary, we detected toxic side effect which explains TSD in its original form has no direct pharmaceutical benefit. However, its metabolites can trigger bioactivity (Mitchell et al., 1979; Han, 1999).

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