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Pharmacokinetics of methyl protodioscin in rats

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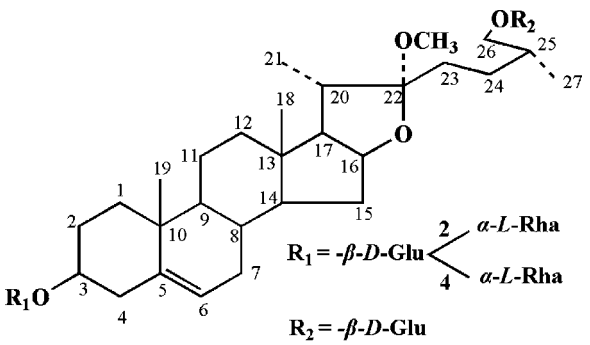
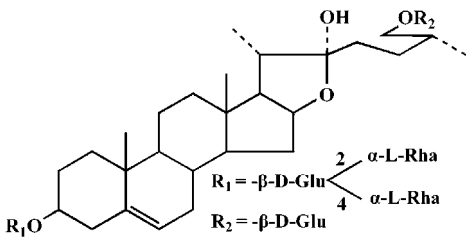
Methyl protodioscin (MPD), a natural furostanol saponin, showed distinct antitumor activity and is distributed in many traditional Chinese medicines. The pharmacokinetics, distribution and excretion of MPD were first investigated after i.v. injection to rats in this study. The dose-dependent pharmacokinetics of MPD were characterized after i.v. injection (20, 40 and 120 mg/kg of MPD) to rats. A good linearity ($r = 0.9989$, $P < 0.05$) was found in the regression analysis of the $AUC_{0\text{-}t}$ -dose. The plasma concentrations of MPD declined rapidly with an elimination half-life ($t_{1/2}$) from 25.56 to 29.32 min. The MPD kinetics was in line with one-compartment model after i.v. injection. 23.43% and 32.86% of MPD was recovered in urine and bile, respectively. The concentrations of MPD in plasma and most examined tissues 5 h after injection were close to or below the Low Limit of Quantification (LLOQ). This indicated that MPD was distributed and eliminated rapidly in rats.

1. Introduction

Methyl protodioscin (MPD), 3-*O*-[α-L-rhamnopyranosyl-(1→2)-{α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl] -22-methoxy-(25*R*)-furost-5-ene-3 β, 26-diol, is a natural furostanol saponin isolated from several traditional Chinese medicinal products (Gonzalez et al. 2003; Hu et al. 1997; Ju and Jia 1992; Shao et al. 1997; Singh and Thakur 1982). It could also be synthesized from diosgenin (Cheng et al. 2003) or dioscin (Li and Yu 2006). All these guaranteed sufficient material for further research and development (R&D) of MPD. Previous studies revealed that MPD showed distinct cytotoxicity to a number of cell lines according to Human Cancer Panel of the National Cancer Institute (NCI) (Hu and Yao 2002, 2003). In recent years, anti-proliferative effect of MPD on the HepG2 cells or K562 cells and its mechanism on the induced cytotoxicity were reported (Liu et al. 2005; Wang et al. 2006). A preclinical pharmacodynamic study on MPD in rats was performed and results showed that the effective intravenous dosage was 20–120 mg/kg.



Methyl protodioscin (MPD)



Protodioscin (PD)

MPD is a promising candidate compound for R&D of new drugs indicated for anticancer therapy. One of the essential steps in R&D for new drugs includes pharmacokinetic study. Some investigations on MPD biotransformation and metabolism (He et al. 2005, 2006a-c) have been fulfilled in our lab, and a LC-MS/MS method (Cao et al. 2007) was established for the quantification of MPD in rat plasma. Based on this method, studies on plasma pharmacokinetics, tissue distribution and excretion of MPD were designed and described in this paper for the first time.

2. Investigations and results

2.1. Plasma pharmacokinetics study

Dose-dependent pharmacokinetic study of MPD was performed after i.v. administration of 20, 40 and 120 mg/kg of MPD to rats. The results showed that the concentration-time profiles were in line with one-compartment model and an excellent linearity ($r = 0.9989$, $P < 0.05$) was revealed in a regression analysis of the $AUC_{0\text{-}t}$-dose. The mean profiles are shown in Fig. 1 and changes in pharmacokinetic parameters are listed in the Table after injection with 20, 40 and 120 mg/kg of MPD.

Table: Mean (SD) pharmacokinetic parameters of MPD after a single intravenous injection of MPD at three different doses

Parameters	unit	Dose (mg/kg)		
		20[a]	40[a]	120[b]
Ke	1/min	0.0262 ± 0.0014	0.0232 ± 0.0010	0.0237 ± 0.0016
V	l/kg	0.1548 ± 0.0147	0.2172 ± 0.0218	0.2095 ± 0.0159
$t_{1/2}$	min	26.56 ± 1.316	29.91 ± 1.299	29.32 ± 1.906
AUC	μg * min/ml	4997 ± 614.1	7995 ± 651.9	24309 ± 2002.6
CL	ml/min/kg	4.05 ± 0.4822	5.03 ± 0.4106	4.964 ± 0.4154
MRT	min	37.86 ± 2.181	42.62 ± 1.632	43.02 ± 3.679

[a] One-compartment model analysis, mean ± S.D.; n = 6
[b] n = 5

2.2. Tissue distribution study

After i.v. injection of 80 mg/kg of MPD, MPD in rats was rapidly distributed to all selected tissues at 10 min post injection (Fig. 2 and Fig. 3). The concentrations of MPD detected in all selected tissues declined in parallel with those measured in plasma. All of the MPD concentrations in lung exceeded those observed in the other tissues at four sampling-time points for male rats, while the concentrations of MPD in womb were high in female rats. The concentrations of MPD in heart, liver, spleen, kidney and small intestine followed those of lung or womb, and the concentrations in the other tissues were relatively low.

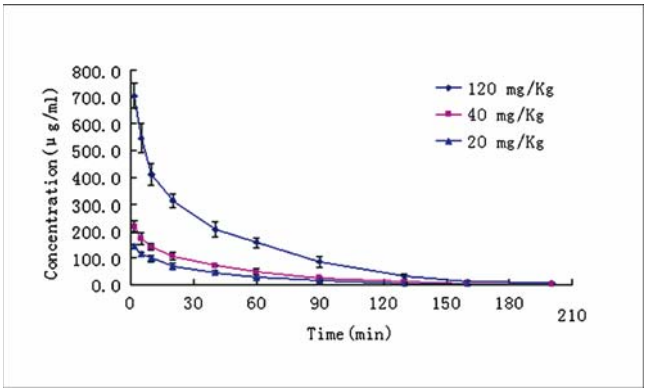


Fig. 1: Mean (SD) plasma concentration-time curves of MPD in rats after a single intravenous injection at three dose of 20, 40 and 120 mg/kg of MPD (n = 6 for 20 and 40 mg/kg; n = 5 for 120 mg/kg)

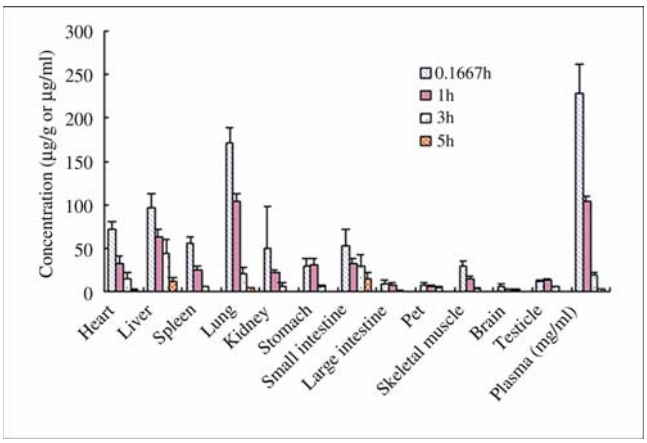


Fig. 2: The concentrations of MPD in the plasma and tissues at 0.1667, 1, 3 and 5 h after an intravenous injection at the dose of 80 mg/kg of MPD to male rats (n = 6)

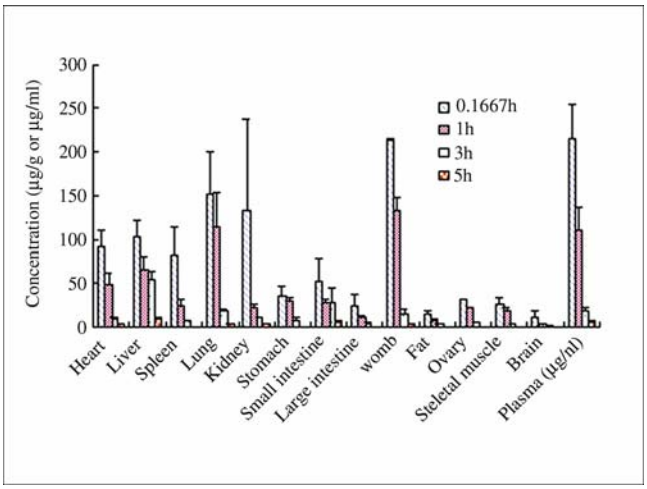


Fig. 3: The concentrations of MPD in the plasma and tissues at 0.1667, 1, 3 and 5 h after an intravenous injection at a dose of 80 mg/kg of MPD to female rats (n = 5)

2.3. Excretion study

Not only MPD but also PD could be detected in urine and bile after i.v. injection of MPD. The cumulative excretion curves of MPD in urine and bile were shown in Figs. 4 and 5, respectively. The cumulative excretion rate of MPD was 23.43% of the dose administered within 48 h, while that of PD was 17.63% in urine. Most of the drug was excreted in urine within 12 h after injection. The cumulative excretion rate of MPD was 32.86% of the injected dose within 24 h, while that of PD was 23.68% in bile. This indicated that MPD was eliminated by biliary and urinary excretion.

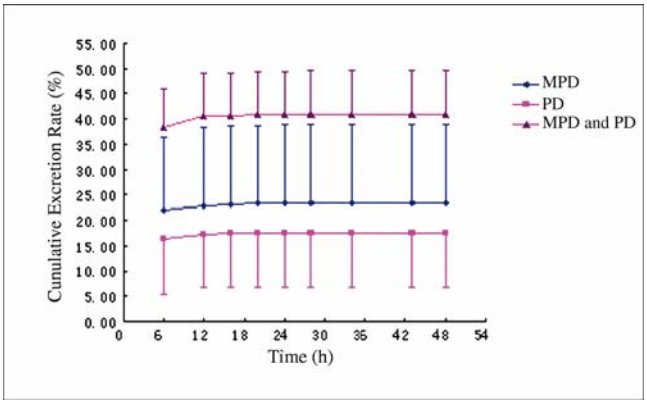


Fig. 4: Cumulative excretion rates of MPD and PD in rat urine after a single i.v. injection of MPD at a dose of 40 mg/kg (n = 10)

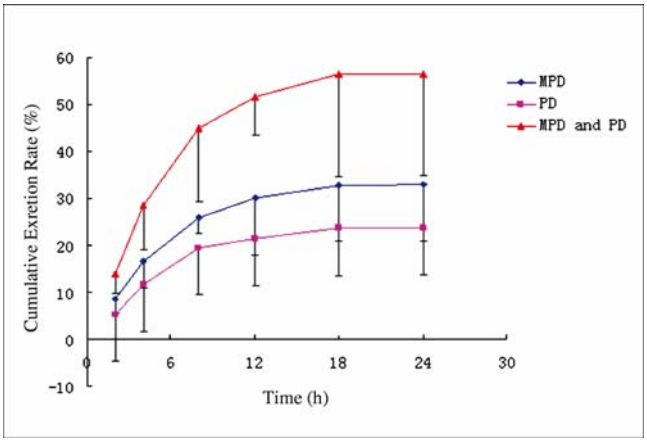


Fig. 5: Cumulative excretion rates of MPD and PD in rat bile after a single i.v. injection of MPD at a dose of 40 mg/kg (n = 6)

3. Discussion

The plasma kinetic property was in line with the known one-compartment model after i.v. injection of MPD to rats. The elimination half-live was 26–29 min, which suggested that MPD was rapidly eliminated. By comparing the plasma peak levels and the AUCs among the three i.v. doses, it was found that the C_{max} and AUC increased in parallel with the increase in concentration of dose. In order to obtain more kinetic characteristics of MPD in rats, the urinary excretion and biliary excretion of MPD were also studied. As a result, more than 23% and 32% of MPD was detected in urine and bile, respectively. The tissue distribution of MPD in main organs was also evaluated in order to understand whether there was any tissue accumulation of MPD. It was found that MPD was rapidly distributed in the organs and the tissue concentrations of MPD were vibrated along with the plasma levels, and the concentrations of MPD in most tissues at 5 h after injection were close to or below the LLOQ. The results indicated that there was no MPD accumulation in examined tissues in rats.

It was concluded from this study that MPD is rapidly distributed and eliminated in rats after i.v. injection. Biliary excretion and urinary excretion were the two main elimination pathways. This study provides a base for further R&D study of MPD.

4. Experimental

4.1. Materials

MPD (purity 98.7%) was isolated from *Dioscorea hypoglauca* and supplied by the Key Lab for Research and Development of Traditional Chinese Medicine and Natural Products, Research Institute of Tsinghua University in Shenzhen. 17α-Ethinylestradiol (internal standard, IS, purity≥ 98%) was purchased from Sigma (St. louis, Mo, USA). HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany) and Yuwang Co. Ltd. (Shandong, China), respectively. Water was doubly distilled in the laboratory. Sprague-Dawley rats (sanitary grade, 220 ± 20 g) were purchased from the Experimental Animal Center of Zhongshan University and were kept in an animal room at constant temperature (23 ± 2 °C) and humidity (55 ± 10%) with 12 h of light per day and were allowed access to water and food *ad libitum*. All the animal experiments were approved by the Ethical Committee of Zhongshan University and conducted according to the National Research Council's guidelines.

4.2. Equipment

A Finnigan LCQ HPLC-MS/MS system (Thermo Electron Corporation, San Jose, CA, USA) was used with Xcalibur 1.3 software. The HPLC-MS/MS system consisted of SCM1000 vacuum degasser, P2000 LC pump, AS3000 autosampler and LCQ Advantage MAX ion trap tandem mass spectrometer with electrospray ionization (ESI) interface.

4.3. Plasma pharmacokinetics

For the dose-dependent pharmacokinetic investigation, different i.v. doses (20, 40 and 120 mg/kg) of MPD were administered to three groups of male rats (n = 6 for 20 and 40 mg/kg; n = 5 for 120 mg/kg). Blood samples (200 μl) were taken from the external jugular vein of rats at 0 min (preinjection) and 2, 5, 10, 20, 40, 60, 90, 130, 160, 200 min post injection, and transferred to heparinized tubes during which rats were allowed to move, eat and drink freely.The plasma samples were obtained by centrifugation at 3000 rpm for 10 min and stored at −20 °C until analysis.

4.4. Tissue distribution

Forty four rats were divided equally into four groups (6 male and 5 female per group) at random. Each group of rats received an i.v. dose (80 mg/kg) of MPD. At 0.1667, 1, 3 and 5 h after administration, blood samples were collected from the jugular vein of eleven rats in each group after which they were sacrificed. The heart, liver, spleen, lung, kidney, stomach, small intestine, large intestine, skeletal muscle, fat, brain, testicle, womb and ovary were immediately removed. Specimens were rinsed, dried and then stored in polypropylene tubes at −20 °C until analysis.

4.5. Excretion

A single i.v. injection of 40 mg/kg of MPD was given to ten rats (5 males and 5 females). After injection, the rats were individually housed in metabolism cages. Urine was collected at time intervals of 0–6, 6-12, 12–16, 16–20, 20–24, 24–28, 28–34, 34–43 and 43–48 h. Another six rats (3 males and 3 females) were anesthesised and then a cannula was implanted surgically into their bile duct. The bile was collected separately between 0–2, 2–4, 4–8, 8–12, 12–18 and 18–24 h after i.v. injection of MPD (40 mg/kg). The urine and bile samples were collected and stored at −20 °C until analysis.

4.6. Sample treatment

In brief, protein was precipitated by the addition of 800 μl of methanol following the addition of 50 μl of IS (520.0 μg/ml) to 50 μl of plasma sample. For the tissue distribution experiment, 0.5 g of heart tissue or 0.3 g weight of spleen tissue were homogenized in 1 ml of methanol, and 1.0 g weight of the other tissues were homogenized in 2 ml of methanol, respectively. Then 2.5 ml of acetonitrile were added following the addition of 100 μl of IS (127.5 μg/ml) to 0.5 ml of various tissue homogenate for protein precipitation. After protein precipitation, 2.0 ml of supernatant were dried under a nitrogen stream at 37 °C. At last, 200 μl of methanol were used for dissolving each of the residues. For the excretion experiment, urine and bile were purified and condensed with ProElut C18 cartridges (100 mg/1 ml; DIKMA). The SPE (Kuniko et al. 2007) microcolumn was washed with methanol (1 ml) and afterward equilibrated with water (1 ml). Samples (500 μl urine or 50 μl bile) were mixed with 50 μl of IS (127.5 μg/ml) and subsequently loaded onto the microcolumn. Salts and weakly bound analytes were removed by washing with water (1 ml). Other bound analytes were eluted with methanol (1 ml) and then dried under a nitrogen stream at 37 °C. At last, 200 μl of methanol was used for dissolving the residues.

4.7. Drug assay

The concentrations of MPD in biological samples were determined by LC-MS/MS (Cao et al. 2007) using 17α-ethinylestradiol as internal standard (IS). After pretreatment, samples (plasma, tissues, bile and urine) were injected into a reversed phase C18 column using a mobile phase consisting of methanol/water (72:28, v/v). Drug and IS were detected using an LCQ Advantage MAX ion trap mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA). Ion acquisition was performed in selective reaction monitoring (SRM) positive mode by monitoring the transition of m/z 1085.7 → 1053.7 for MPD, and in selective ion monitoring (SIM) negative mode by monitoring the deprotonated ion m/z 295.5 for IS. The assay was linear in the range of 2.024–270 μg/ml. In addition, protodioscin (PD, a major metabolite of MPD) was determined and semi-quantified (Gao 2007) according to the calibration curve of MPD in urine and bile samples because it has similar structure and MS response with MPD.

4.8. Data process of plasma pharmacokinetics

Pharmacokinetic parameters for MPD in plasma were calculated using Practical Pharmacokinetic Program-Version 97 (3P97, Chinese Pharmacological Association, Beijing, China). The maximum concentration in plasma (C_{max}) was obtained from observed data. The area under the concentration-time curve ($AUC_{0\text{-}t}$) was calculated by the trapezoidal rule. Other parameters such as: excretion rate constant (K_e), apparent volume of distribution (V), elimination half-life ($t_{1/2}$), clearance (CL) and mean residence time (MRT) were obtained directly from the results of 3P97 software.

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