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Pharmacokinetics of intragastric ipriflavone solid dispersion in rats

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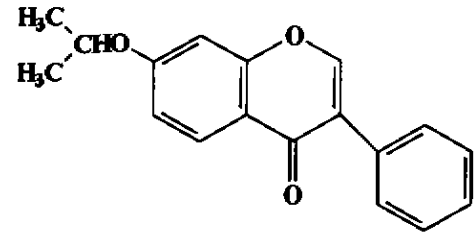
KEY WORDS pharmacokinetics; ipriflavone; isoflavones; high pressure liquid chromatography; biological availability

ABSTRACT

AIM: To evaluate pharmacokinetic behavior of ipriflavone solid dispersion in rats. **METHODS**: The plasma concentrations of ipriflavone in rats were determined by HPLC with UV detector. **RESULTS**: Plasma concentration-time curves after ig ipriflavone solid dispersion 250 mg·kg^{-1} in rats were fitted with one-compartment model. Pharmacokinetic parameters were as follows: $K_e = 0.21$ h^{-1}, $T_{\frac{1}{2}K_e} = 5.19$ h, $K_a = 1.71$ h^{-1}, $T_{\frac{1}{2}K_a} = 0.41$ h, $T_{max} = 0.67$ h, $C_{max} = 429$ μg·L^{-1}, $AUC = 3916$ μg·h·L^{-1}; The relative bioavailability of ipriflavone solid dispersion was 323 %. **CONCLUSION**: Ipriflavone in solid dispersion was absorbed more effectively than that in physical mixture in rats.

INTRODUCTION

Ipriflavone (7-isopropoxyisoflavone, IP), a nonhormonal isoflavone derivative, is currently used in several countries for prevention and treatment of postmenopausal osteoporosis[1]. IP has been shown to be effective in reducing bone turnover rate mainly through an inhibition of bone resorption, and in stimulating bone formation[2-4]. Although IP administered to rats orally was well absorbed[5], it had a poor bioavailability because of its poor water solubility and strong first-pass metabolism[6,7]. It has been reported that the solubility of IP was increased dramatically with being enclosed in β-cyclodextrin, for example, from 0.4 mg·L^{-1} to 13.2 mg·L^{-1} in one minute, and plasma levels were increased 10 - 15 times, in addition, the concentrations of the unchanged IP was high and were detected very easy[8]. The solid dispersion technology has been used widely to enhance the dissolution rate of poorly water soluble medicines by dispersing them into water soluble carriers, and to improve their bioavailability[9-11]. Here we prepared the solid dispersion of IP and studied its pharmacokinetic behavior and relative bioavailability in rats.



7-isopropoxyisoflavone (IP)

MATERIALS AND METHODS

Rats Sprague-Dawley rats (Grade Ⅱ, $n = 20$, 250 ± s 30 g, ♂, Certificate № 008) were supplied by Shanghai Experimental Animal Centre, Chinese Academy Sciences.

Chemicals and drugs IP (98.5 %) was synthesized in the Department of Synthetic Drugs, Shanghai Institute of Materia Medica, Chinese Academy of Sciences; Polyvinylpyrrolidone-k30 (PVP) was purchased from Henan Yuyuan Chemical Industrial

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Co Ltd. Methanol was HPLC grade; Doubly distilled water was used for HPLC with UV detector. All other chemicals were of AR grade.

Preparation of samples IP solid dispersion was prepared as follows: the mixed powder (20 g) of the IP and PVP (1:8) were dissolved in ethanol (100 mL), then the solvent was evaporated. The residue was dried at 25 ℃ in a drier, and then ground and sieved (180 μm). The control sample was the physical mixture with the mixed powder (180 μm) of the IP and the PVP at the same proportion as the solid dispersion.

Standard solution Stock solution of IP was prepared in methanol at the concentration of 1.0 g·L^{-1}, and stored below 4 ℃. Standard solutions were obtained from stock solution diluted in methanol to concentrations of 10, 100, 250, 500, 750, and 1000 μg·L^{-1}.

Chromatography A HPLC method[12] was used and improved for the determination of IP in rat plasma. A Shimadzu HPLC system was used with two LC-10AD pumps, an SPD-10A UV detector, and a C-R6A recorder; The column was Lichrosorb C_{18}, 5 μm, 5 mm × 250 mm (Shanghai Institute of Materia Medica, Chinese Academy of Sciences); The mobile phase was a mixture of methanol and 0.1 mol·L^{-1} acetate buffer (PH 3.0) (70:30, vol/vol), prepared and degassed daily. Chromatography assay was performed at 25 ℃ using a flow rate of 1 mL·min^{-1} with a pressure of 50 kgf·cm^{-2}. Absorption was measured at 250 nm with 0.05 absorption units of full scale (AUFS).

Medication and sampling After a fasting for 24 h, rats were divided randomly into two groups, n = 10, respectively, and were given ig 250 mg·kg^{-1} of IP solid dispersion or control that were prepared into suspension with carboxymethyl cellulose sodium (1.0 %) at concentration of 25 g·L^{-1}. Blood samples from the same sober rate, 0.5 mL every time, were collected at 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, and 12 h via caudal vein with heparinized tubes. Plasma was separated, and 0.15 mL of plasma was extracted with 3 mL of ether three times. After centrifugation (2000 × g, 12 min), the solvent was evaporated at 50 ℃ under air flow. The residue was dissolved in 50 μL of methanol and 10 μL was injected onto HPLC column.

Data analysis The concentration-time curves were analyzed with 3P87 program to determine the pharmacokinetic parameters. Comparison of pharmacokinetic parameters between groups was carried out with F-test.

RESULTS

Chromatography There were no endogenous interferences at the retention time of IP (Fig 1). The Tr for IP was 10.6 min. The minimal detection concentration of IP was 5 μg·L^{-1} (S/N ≥3). The calibration curve for IP was linear over the range of 10 − 1000 μg·L^{-1}. Linear equation: $C = 7.57 \times 10^{-2} A + 2.4$ ($r = 0.9993$). The average recoveries of IP were ranged from 92.5 % to 97.1 % (Tab 1). Coefficients of both intra- and inter-day variations (CV) were < 5 % (Tab 1).

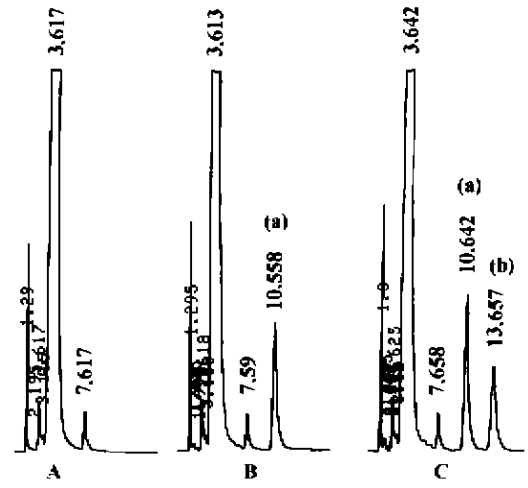

Fig 1. Chromatograms of blank plasma (A), blank plasma with standard solution (B), and sample (C): IP (a), unknown (b).

Tab 1. Average recovery ($\bar{x} \pm s$, n = 5), and coefficients of variation (CV, n = 5) in determination of IP in spiked plasma.

	Added/μg·L^{-1}		
	50	200	800
Recovery	92.5±2.4	95.6±3.2	97.1±2.9
Intra-day CV/%	3.8	4.2	3.6
Inter-day CV/%	4.4	4.1	4.7

Pharmacokinetics The concentration-time

curves (Fig 2) for both IP solid dispersion and control after ig in rats were fitted with one-compartment model. Plasma levels of IP solid dispersion were increased by 1 – 2 times over its control. Some of pharmacokinetic parameters of IP solid dispersion were distinctly different from that of control (Tab 2). AUC was increased from (1212 ± 103) $\mu g \cdot h^{-1} \cdot L^{-1}$ to (3916 ± 236) $\mu g \cdot h^{-1} \cdot L^{-1}$ ($P < 0.01$), C_{max} from (167 ± 34) $\mu g \cdot L^{-1}$ to (429 ± 87) $\mu g \cdot L^{-1}$ ($P < 0.01$), and T_{max} decreased from (1.4 ± 0.5) h to (0.67 ± 0.23) h ($P < 0.01$). The relative bioavailability for IP solid dispersion was increased by 200 % over its physical mixture. This result confirmed that the IP in solid dispersion was released faster and absorbed more effectively than its physical mixture.

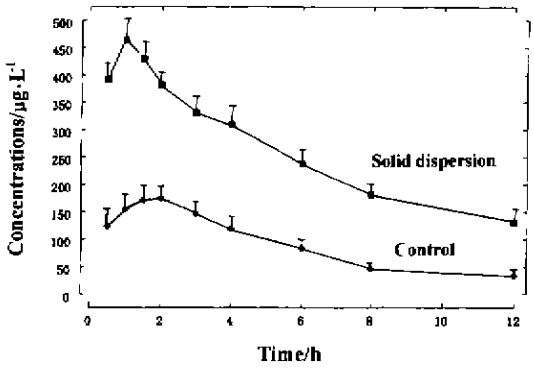

Fig 2. IP concentrations of plasma in rats, $n = 10$, $\bar{x} \pm s$.

Tab 2. Pharmacokinetic parameters of IP solid dispersion and control following a single ig dose of 250 mg·kg^{-1} in rats. $n = 10$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

Parameter	Solid dispersion	Physical mixture
K_e/h^{-1}	0.21 ± 0.07	0.19 ± 0.19
K_a/h^{-1}	1.71 ± 0.23	1.84 ± 0.31
$T_{\frac{1}{2}K_a}/h$	0.41 ± 0.11	0.38 ± 0.09
$T_{\frac{1}{2}K_e}/h$	5.19 ± 1.51	4.92 ± 1.36
T_{max}/h	0.67 ± 0.23	1.4 ± 0.5
$C_{max}/\mu g \cdot L^{-1}$	429 ± 87^c	167 ± 34
AUC/$\mu g \cdot h \cdot L^{-1}$	3916 ± 236^c	1212 ± 103

DISCUSSION

IP is metabolized exclusively in the liver by elimination, or oxidation of the isopropyl group and hydroxylation of the beta-ring followed by conjugation with glucuronic and/or sulphuric acid. In rats 7-hydroxyisoflavone (M1) was the main metabolite of IP, while in man 7-(1-carboxy-ethoxy)-isoflavone (M5)[5]. In the present study, an unknown metabolite which could be M1 was detected out (Fig 1).

The previous reported HPLC method[12] for the determination of IP and its metabolites in rates plasma involved deproteinization followed by injection onto a C_{18} reversed-phase column with mobile phases acetate buffer 0.05 mol·L^{-1} (pH 3.0): acetonitrile: methanol (40:35:25, vol:vol:vol) for IP and acetate buffer 0.05 mol·L^{-1} (pH 3.0): acetonitrile : methanol : phosphoric acid (50:25:25:0.1, vol:vol:vol:vol) for its metabolites. The detection limits for IP and M1 in rates plasma were 20, 20 ng·mL^{-1}, respectively. With improved method, IP and its main metabolite in rats plasma were simultaneously determined.

The systemic absorption of most drug products consists of a succession of rate processes[13] including (1) disintegration of the drug product and subsequent release of the drug; (2) dissolution of the drug in an aqueous environment; and (3) absorption across cell membrane into the systemic circulation. In the process of drug disintegration, dissolution, and absorption, the rate at which drug reaches the circulatory system is determined by the slowest step in the sequence. For drugs that have very poor aqueous solubility, the rate at which the drug dissolution (dissolves) is often the slowest step, and therefore exerts a rate-limiting effect on drug bioavailability. IP poorly dissolves in water, artificial gastric juice, or artificial intestinal juice, and had a poor bioavailability. The dissolving rate of IP could have a rate-limiting effect on its bioavailability. The solid dispension method is a useful technology for increasing dissolution of drugs with a poor solubility in water, and for improving their bioavailability[9-11]. The pharmacokinetic evaluation of IP solid dispersion in rats provided a useful information for further studies on its oral preparation in clinical trial in future.

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1035 – 1038

依普黄酮固体分散体在大鼠的药物动力学评价

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关键词 药物动力学；依普黄酮；异黄酮类；高压液相色谱法；生物利用度

目的：评价依普黄酮固体分散体在大鼠体内的药物动力学行为. **方法**：测定它的药物动力学参数和相对生物利用度，采用高压液相色谱法测定大鼠血浆中依普黄酮的浓度. **结果**：大鼠灌胃依普黄酮固体分散体 250 $mg \cdot kg^{-1}$，其血药浓度－时间曲线符合一室模型，药物动力学参数为：$K_e = 0.21\ h^{-1}$，$T_{\frac{1}{2}K_e} = 5.19$ h，$K_a = 1.71\ h^{-1}$，$T_{\frac{1}{2}K_a} = 0.41$ h，$T_{max} = 0.67$ h，$C_{max} = 429\ \mu g \cdot L^{-1}$，AUC = 3916 $\mu g \cdot h \cdot L^{-1}$，相对生物利用度是 323 %. **结论**：依普黄酮固体分散体与依普黄酮的物理混合物比较，在大鼠体内有更多被吸收.

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