Gastrointestinal absorption of paeoniflorin in germ-free rats

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Abstract

The influence of intestinal bacterial metabolism on the gastrointestinal absorption of paeoniflorin was studied using Wistar germ-free and conventional rats.

Plasma concentration profiles of paeoniflorin in germ-free and conventional rats after its oral administration at a dose of 2 mg/kg were compared. In conventional rats, the mean plasma concentration of paeoniflorin rapidly reached Cmax (32.7 ng/ml) at 15 min and decreased ; the mean value of 0.7 ng/ml at 240 min after administration. In germ-free rats, paeoniflorin was rapidly absorbed as well as in conventional rats, but the plasma concentration was kept at the nearly constant level around 25 ng/ml until 240 min after the administration. There were significant differences in AUC and tmax values between germ-free and conventional rats, while the difference of Cmax values was not found. AUC value in germ-free rats was two times larger than that in conventional rats.

These findings suggests that paeoniflorin orally administered is rapidly absorbed from upper part of the gastrointestinal tract, but the more than half of paeoniflorin which must be absorbed from intestine is degraded to some metabolites by the intestinal bacteria.

Key words gastrointestinal absorption, paeoniflorin, germ-free rat, paeony root.

Abbreviations EIA, enzyme immunoassay ; HPLC, high performance liquid chromatography ; Cmax, peak plasma concentration ; tmax, time to Cmax ; AUC, area under plasma concentration-time curve ; $t_{1/2}$, apparent half-life.

Introduction

Paeoniflorin is a monoterpene glucoside and contained in paeony roots which are composed of Kampo medicines in Japan and China. Paeoniflorin has been shown to have several pharmacological actions[1-3] and therapeutic effects of paeony roots are explained by the pharmacological actions of paeoniflorin.[4] Our previous reports[5,6] suggest that the oral bioavailability of paeoniflorin is extremely low, which is shown by its poor gastrointestinal absorption, and its active metabolites produced in the intestine by gut microflora may be involved in the pharmacological actions.

In the present paper we report the influence of intestinal bacterial metabolism on the absorption of paeoniflorin using germ-free and conventional rats.

Materials and Methods

Materials : Paeoniflorin was supplied by Technical Department in our laboratories. β-Galactosidase was purchased from Boehringer Mannheim (Mannheim Germany). Bovine serum albumin and 7-β-D-galactopyranosyloxy-4-methylcoumarin were purchased from Sigma, physiological saline from Fusou Pharmaceutical Industry (Osaka Japan), Freund's complete adjuvant from Difco and goat anti-rabbit IgG (Marcella 10) for enzyme-immunoassay from

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Dainippon Pharmaceutical Industry (Osaka Japan). All other reagents were of special or HPLC analytical grade obtained from Wako Pure Chemical Industry (Osaka Japan).

Animals, treatments and sampling : Male Wistar germ-free and conventional rats (8 weeks old) were purchased from Clea Japan, Inc (Tokyo Japan). Conventional rats were fed standard laboratory chow with water *ad libitum*. Germ - free rats were maintained in an isolator cage under sterilized condition, and autoclaved water and germ-free laboratory chow were freely available. Animals were fasted overnight prior to the experiment after accommodation for a week. Germ-free condition was checked before and after the experiment.

Paeoniflorin (2 mg/kg) was dissolved in physiological saline and given orally to three germ-free and six conventional rats after sterilizing with membrane filter (MILLIPORE). Blood sample (100 μl) was taken at an appropriate time interval from femoral vein after being cannulated with a polyethylene tubing (PE-10), which was filled with sodium heparin at concentration of 100 int. units/ml, under ether anesthesia. The plasma was collected by centrifugation and stored at -20°C until analysis.

Determination of paeoniflorin : The determination of paeoniflorin in rat plasma was carried out by EIA according to the previous paper.[5]

Pharmacokinetic analysis : Pharmacokinetic analysis was carried out by model independent methods. The Cmax and the tmax were determined from the individual profile by inspection. AUC from zero to 240 min after oral administration was calculated by the trapezoidal rule. The $t_{1/2}$ was calculated by $\ln 2/\lambda$ (λ ; terminal elimination rate constant). Statistical analysis was performed by Mann-Whitney U test with the level of significance at 0.05.

Results and Discussion

When drugs are orally administered, they encounter the intestinal microflora during absorption from intestine. There is the further possibility that the drugs meet the intestinal microflora by enterohepatic circulation via bile, when administered intravenously or subcutaneously. The microfloral metabolism involves not only drugs but also food and endogenous substances and hydrolysis of glucuronide, reductions of double bond, aldehyde, ketone and alcohol and other reactions are performed by bacteria.[7,8] Little is known about the metabolism by intestinal microflora, though a large number of metabolic studies by liver, kidney, lung and oher organs are conducted.

Methods in studying the effects of intestinal flora include the uses of germ-free rats in comparison with conventional rats, germ - free rats infected certain bacteria (gnotobiote), and conventional rats treated with various antibiotics (pseudogerm-free) and the incubation with caecal contents or feces *in vitro*. Metabolism of flavonoid compounds in germ-free rats has been reported by Griffiths and Barrow[9] and they have suggested the advantage of the use of germ-free rats. We have reported that glycyrrhizin is not converted to glycyrrhetic acid, an aglycone of glycyrrhizin, in the absence of intestinal bacteria by the use of germ-free and gnotobiote rats.[10] Thus, the study of germ-free rats gives the distinct evidence for the influence of bacterial metabolism on the absorption of paeoniflorin.

Fig. 1 shows the plasma concentration profiles of paeoniflorin after its oral administration at a dose of 2 mg/kg in germ-free and conventional rats. In conventional rats, the mean plasma concentration of paeoniflorin rapidly reached Cmax (32.7 ng/ml) at 15 min and decreased ; the mean value of 0.7 ng/ml at 240 min after administration. This observation agrees

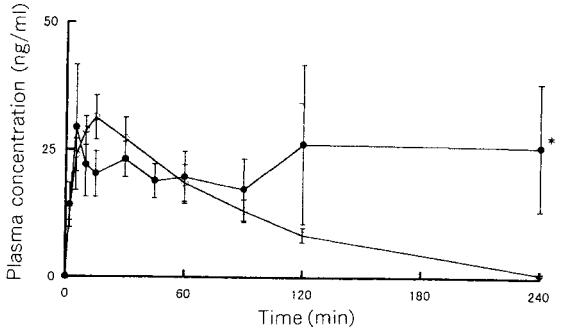

Fig. 1 Plasma concentration profiles of paeoniflorin after its oral administration at a dose of 2 mg/kg in germ-free (●) and conventional (○) rats. Each value represents mean±S.E. of 3 germ-free and 6 conventional rats. *$p<$ 0.05 : significantly different from conventional rat

Table I Pharmacokinetic parameters after oral administration of paeoniflorin at a dose of 2 mg/kg in germ-free and conventional rats.

Rat	n	Cmax (ng/ml)	tmax (min)	AUC_{0-240} (ng·min/ml)	$t_{1/2}$ (min)
Conventional	6	32.7±4.1	10±1.8	2799.7±376.6	40.2±6.1
Germ-free	3	42.3±11.5	130±60.8*	5528.2±1461.8*	−

− : not calculated
Each value represents mean±S.E.
*$p<0.05$: significantly different from conventional rat

with the previous report.[5] In germ-free rats, paeoniflorin was rapidly absorbed as well as in conventional rats, but the plasma concentration was kept at the nearly constant level around 25 ng/ml until 240 min after the administration. Significant difference of plasma concentrations between germ-free and conventional rats at 240 min after oral administration was observed. Pharmacokinetic parameters are listed in Table I. There are marked differences ($p<0.05$) in AUC and tmax values between germ-free and conventional rats, while the difference in Cmax values was not found. The mean AUC value in germ-free rats was two times larger than that in conventional rats. In the preceding papers,[5,6] we have reported the absorption and excretion of paeoniflorin in conventional rats. Since approximately 50 % of the dose was excreted in urine after intravenous administration, little being excreted in bile and feces, paeoniflorin absorbed was mostly excreted in urine. The oral bioavailability of paeoniflorin was very low (3-4 %) when calculated by AUCs after its oral and intravenous administrations and also 2 % by urinary excretions. This seemed to be induced by poor absorption from the intestine, because paeoniflorin was not metabolised in the lung, liver and by small intestinal enzymes and had a low intestinal permeability *in vitro*. Accordingly, the microfloral metabolism in gastrointestine has a major role in the elimination process of paeoniflorin after its oral administration, based on the low excretion in the feces. In general, plasma concentration profile of the orally administered drug and its pharmacokinetic parameters consist of absorption and elimination processes and are defined as hybrid parameters. The pharmacokinetic difference of paeoniflorin in both processes between germ-free and conventional rats is considered to be negligible, based on its poor bioavailability, low gastrointestinal permeability as mentioned above, and similar Cmax values obtained in this study, though there is no experimental evidence to support that. Thus, the discrepancy of the plasma concentration profiles in the present study between two groups may be attributable to the presence or absence of intestinal bacteria.

In conclusion paeoniflorin orally administered is rapidly absorbed from the upper part of the gastrointestinal tract, but more than half of the paeoniflorin which must be absorbed from the intestine is degraded to some metabolites such as paeonimetaboline I, II and III[11] by the intestinal bacteria. It is interesting that intestinal bacterial metabolism extensively affects the pharmacokinetics of paeoniflorin, similarly with glycyrrhizin.[12]

和文抄録

Paeoniflorinの消化管吸収に及ぼす腸内細菌の影響を，無菌ラットと通常ラットで検討した。Paeoniflorin 2 mg/kgを無菌および通常ラットに経口投与し，血漿中濃度推移を比較した。通常ラットでは，平均の血漿中濃度は投与後15分で速やかにCmax (32.7 ng/ml) に達し，その後減少し，240分には平均0.7 ng/mlの濃度となった。無菌ラットでは，paeoniflorinは通常ラットと同様に速やかに吸収されたが，投与後240分までほぼ25 ng/mlの一定の濃度を推持した。両者におけるAUCとtmax値には有意な差がみられたが，Cmax値に差は認められなかった。無菌ラットにおけるAUC値は，通常ラットのそれより2倍大きかった。これらの結果は，経口投与されたpaeoniflorinは速やかに消化管上部から吸収されるが，吸収されるべきpaeoniflorinの半分以上が腸内細菌によっていくつかの代謝物に代謝されることを示唆している。

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