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Species-dependent plasma metabolism of the ester compound daidzein 7,4′di-succinic acid mon-ester-*O*-ethoxy (DZ5)

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Received January 23, 2006; accepted July 8, 2006

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Pharmazie 62: 574–576 (2007) *doi: 10.1691/ph.2007.8.6021*

Daidzein 7,4′di-succinic acid mon-ester-*O*-ethoxy (DZ5) is an ester-containing compound, which was recently synthesized. The objective of this study was to determine the hydrolysis rate of DZ5 in blood from the rat and the dog. The data showed that the hydrolysis rate of DZ5 in plasma was much more rapid in rats than in dogs following intravenous administration. Moreover, similar results were observed after *in vitro* incubation of DZ5 in the rat or dog plasma. The findings suggested that plasma esterases in the rat plasma have higher activity than in the dog and plasma metabolism of DZ5 is species-dependent.

1. Introduction

Daidzein is one of the major isoflavonoids in soybeans (Fairley et al. 2003). We modified the chemical structure of daidzein and synthesized derivative, named daidzein 7,4′di-succinic acid mon-ester-*O*-ethoxy (DZ5) in order to improve its solubility and pharmacological efficacy. Our pharmacological studies indicated that protective effects of DZ5 on cerebral ischemia, normabaric hypoxia were similar with that of daidzein (data in press).

2. Investigations, results and discussion

In this study, a rapid, simple and sensitive HPLC method was developed and the kinetics of *in vivo* and *in vitro* metabolism of DZ5 in plasma was evaluated by measuring the disappearance of parent compound (DZ5) and the generation of metabolites (DZ4). All the drug concentration data were analyzed using a non-compartmental method to obtain pharmacokinetic parameters (Table, Figs. 1, 2). The data showed that DZ5 was rapidly hydrolyzed *in vivo* to DZ4 following intravenous administration to rats and dogs, which was mainly due to plasma esterase activity (Los et al. 1996). It was found that DZ5 was rapidly meta-

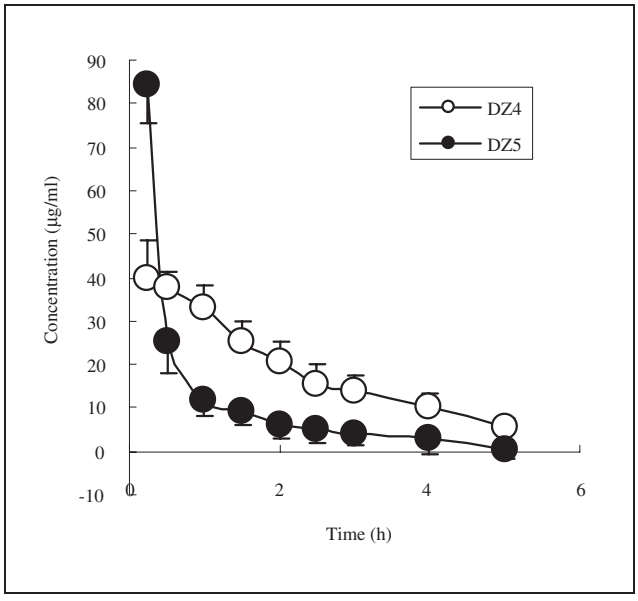
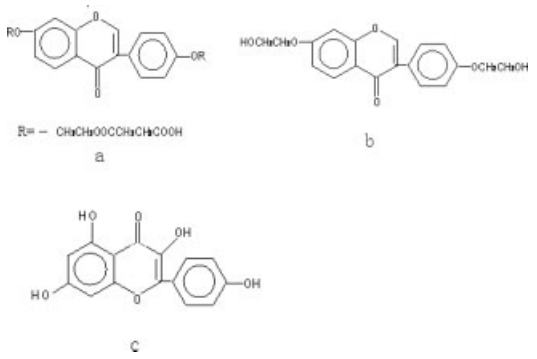


Fig. 1: Mean plasma concentration-time curves of DZ5 and its metabolite DZ4 in rats after a single 200mg/kg intravenous dose of DZ5 (n = 6, Mean ± SD)

Table: Main pharmacokinetic parameters of DZ5 and the primary metabolite DZ4 after intravenous administration of DZ5 to rats (200 mg·kg^{-1}) or dogs (20 mg/kg) (mean ± SD, n = 6)

	Rat		Dog	
	DZ5	DZ4	DZ5	DZ4
T1/2(h)	1.115 ± 0.225	2.260 ± 0.967	1.461 ± 0.119	2.200 ± 0.492
AUC_{0-t} (μmol × h/ml)	102.675 ± 13.735	281.611 ± 27.997	55.935 ± 1.197	16.650 ± 26.084
$AUC_{0-\infty}$ (μmol × h/ml)	104.435 ± 14.114	329.505 ± 44.918	57.457 ± 1.123	20.029 ± 32.090

bolized to DZ4 in the rat plasma, and after 30 min the content of DZ4 in the plasma exceeded that of DZ5, with 70% of DZ5 converted into DZ4 within 6 h, while the metabolism rate from DZ5 to DZ4 was relatively low in dogs, with only 25% of DZ5 converted into DZ4 within 6 h.



Chemical structures of DZ5 (a), DZ4 (b) and I.S. (c).
DZ4 – Daidzein 7,4′dioxy-ethoxy
DZ5 – Daidzein 7,4′di-succinic acid mon-ester-*O*-ethoxy

In order to confirm these findings, an *in vitro* incubation experiment of DZ5 was carried out in rats and dogs plasma. The result showed that DZ5 hydrolysis resulted in the formation of an equivalent amount of a primary metabolite DZ4 by cleavage of the ester group, with the total amount of DZ5 and DZ4 remaining basically unchanged. Other metabolites were not determined in the experiment. The study also indicated that the concentration of DZ5 in rat plasma gradually decreased while the concentration of DZ4 gradually increased and after 3 h nearly 90% of DZ5 was metabolized to DZ4, which suggested a high esterase activity in rat plasma. In Beagle dog, about 15% of DZ5 was rapidly metabolized to DZ4 within 15 min and then metabolism reached a saturation state, with the content of DZ4 and DZ5 remaining basically unchanged, suggesting the relatively low activity of esterases in dog plasma (Fig. 3). This result was in agreement with the result of *in vivo* experiment and confirmed reports about significant species dependence in intestinal esterase activity towards *p*-nitrophenyl acetate (Van Gelder et al. 2000). The rapid hydrolysis of DZ5 in rats is also in agreement with reports on other ester-containing compounds, such as clevidipine, which showed marked species differences in the *in vitro* hydrolysis rate, in the order rat > dog > human (Ericsson et al. 1999).

In summary, the findings in the present study confirmed that the activity of esterases is higher in the rat plasma.

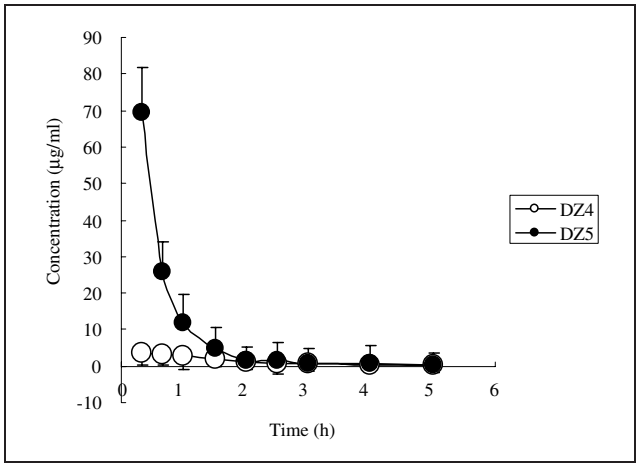


Fig. 2: Mean plasma concentration-time curves of DZ5 and its metabolite DZ4 in dogs after a single 20 mg/kg intravenous dose of DZ5 (n = 6, Mean ± SD)

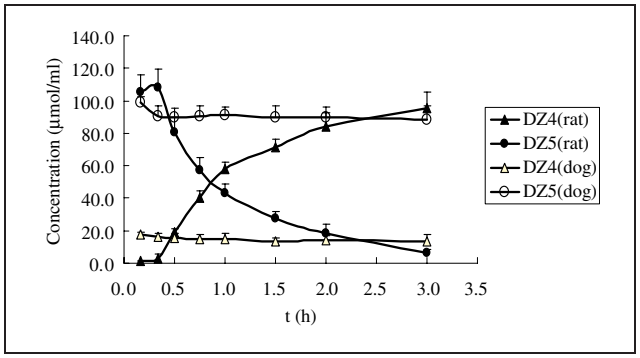


Fig. 3: Mean concentration-time of DZ5 and the primary metabolite DZ4 in plasma from the rat and dog. Each values was the mean ± SD (n = 6)

3. Experimental

3.1. Reagents and chemicals

DZ4 and DZ5 were obtained from Shenyang Pharmaceutical University (Shenyang, China), with the internal standard kaempferol from the Institute for Drug Control of Liaoning Province. Methanol and acetonitrile were of HPLC grade and some other reagents of analytical grade were from Yuwang reagent Company (Shandong, China).

3.2. Collection and storage of samples

Venous blood samples from rats were collected into heparinized plastic tubes. Immediately chilled, and the plasma was separated by centrifugation at 4 °C within 15 min. The plasma was shock frozen and stored at −20 °C until the assay.

3.3. Sample preparation

Frozen plasma samples were thawed in an ice-water bath. An amount of 200 μl plasma (100 μl plasma for rats) was added to 50 μl internal standard solution (20 μg/ml) and vortex-mixed for 1–2 s. An amount of 400 μl ice-cold methanol was added and the solution was mixed again. After 15 min at 4 °C the mixture was centrifuged to separate precipitated proteins. The supernatant was transferred into the sampler vials, and 20 μl were injected into the chromatograph.

3.4. Chromatography

The HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC-10AT pump, an SPD-10A UV detector set at 250 nm. Separation was performed using a prepacked stainless-steel column (200 × 4.6 mm I.D.) with Hypersil C_{18} 5 μm silica. The mobile phase consisted of 400 ml water, 500 μl 85% orthophosphoric acid, 500 ml methanol, 100 ml acetonitrile and was pumped at a flow-rate of 1.0 ml · min^{-1}. The system was used at room temperature.

3.5. Method validation

The calibration curve was linear over the range 0.1–50.0 μg/ml in dog plasma. The average extraction recoveries were 76.6% (DZ5), 75.1% (DZ4) and the within-day and between-day precisions were less than 10.93%. The assay was applied to the analysis of samples from a pharmacokinetic study.

3.6. Stability of plasma samples

The stock solutions of internal standard, Kaempferol (500 μg/ml), DZ5 and DZ4 (1 mg/ml) were prepared in methanol. All solutions were stored at 4 °C. The stability of DZ5 and DZ4 in plasma was evaluated for one month at −20 °C, and for three freeze-thaw cycles. The validation criteria were calculated using commonly accepted statistical procedures.

3.7. Pharmacokinetics study and data analysis

All animal studies were performed according to the Guidelines for the Care and USE of Laboratory Animals that was approved by the Committee of Ethics of Animal Experimentation of Shenyang Pharmaceutical Uni-

versity. Wistar rats (male, 240–300 g), beagle dogs (male, 10–15 kg) were provided by the Animal Center of Shenyang Pharmaceutical University (Shenyang, China). Animals were housed in a room with controlled temperature and humidity, and had free access to food and water. They were fasted overnight before the experiments. Four groups (6 rats or dogs/group) were randomly assigned to receive DZ5 intravenously via jugular vein (rats)/fore-leg vein(dogs); the injection volume was 0.5 ml (rats)/2 ml (dogs). Blood samples were drawn in heparinized tubes at 0, 15, 30, 60, 90, 120, 150, 180, 240, 300 min after administration. The obtained plasma samples were immediately separated and stored frozen at −20 °C until analysis. The plasma concentration-time data were analyzed by noncompartmental analysis with 3p97 software, a practical pharmacokinetic program (the Chinese Society of Mathematical Pharmacology).

3.8. Plasma metabolism of DZ5

The same concentrations (100 μg/ml final concentration) of DZ5 were incubated with rats plasma or dogs plasma for 180 min. Metabolism studies were performed under linear conditions at 37 °C. An amount of 200 μl plasma were drawn in tubes at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180 min, respectively. Each sample was added to 50 μl internal standard solution (20 μg/ml) and vortex-mixed for 1–2 s. The obtained plasma samples were immediately added 0.4 ml ice-cold methanol to stop the reaction and to extract the DZ5 and DZ4. After 15 min at 4 °C the mixture was centrifuged to separate precipitated proteins. The supernatant was transferred into the sampler vials, and 20 μl were injected into the chromatograph.

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