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Absorption, Distribution, Metabolism, and Excretion of Decursin and Decursinol Angelate from *Angelica gigas* Nakai

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The pharmacokinetics of decursin and decursinol angelate (D/DA) were investigated in male SD rats following oral and intravenous administration. D/DA and metabolites obtained from *in vitro* samples were evaluated by LC/MS. The levels of D/DA and metabolized decursinol in the blood following oral and intravenous administrations declined according to first-order kinetics, with $T_{1/2}$ values of 56.67, 58.01, and 57.22 h, respectively, being observed after administration of a dose of 2 mg/kg body weight. The large intestine was the major site of disposition following oral administration. These data indicate that D/DA is rapidly absorbed from the gastrointestinal tract. In *in vitro* experiment utilizing liver microsomal protein, the major metabolic reaction of D/DA occurred to change decursinol. The cumulative biliary, urinary, and fecal excretions of D/DA in bile duct-cannulated rats was 36.10 ±2.9%, 25.35±3.8%, and 34.20±3.2%, respectively, at 72 h after administration. These results indicate that the absorption of D/DA is almost complete, and that its metabolites are primarily excreted into feces through the bile. These results indicate that D/DA is subject to enterohepatic circulation.

Keywords: Pharmacokinetics, decursin, decursinol angelate, metabolism, *Angelica gigas* Nakai

A. gigas Nakai (Cham-Dang-Gui in Korean) is a Korean traditional herbal medicine that is one of the most popular herbal medicines used in Asian countries, including Korea, Japan, and China. *A. gigas* Nakai is also marketed as a functional food product in Europe and America. *A. gigas* Nakai from Korea has deep purple flowers, whereas strains from Japan and China have white flowers [2]. *A. gigas* Nakai has been studied extensively and found to contain a variety of substances including coumarins [3, 11]. Coumarins are composed of D/DA, which has long been used as a traditional medicine for the treatment of anemia, as a sedative, and as an anodyne or a tonic agent [13]. In addition, *A. gigas* Nakai has been widely used for the treatment of dysmenorrhea, amenorrhea, menopause, abdominal pain, injuries, migraine headaches, and arthritis. Furthermore, *A. gigas* Nakai is known to exert antibacterial and antiamnestic effects, as well as to induce inhibitory effects against acetylcholinesterase, depression of cardiac contraction, and activation of protein kinase C [1, 4, 6, 7, 12]. Many herbal medicines are used in functional food products; however, there is currently a great deal of concern over possible genetic hazards associated with such medicines [5]. Additionally, methods for improving the storage conditions and quality of herbal medicines have recently been evaluated.

The primary objectives of the present study were to determine the absorption, tissue distribution, metabolism, and elimination of D/DA following oral or intravenous (IV) administration to male Sprague–Dawley rats. Additional studies involving jugular vein cannulation were conducted to determine the bioavailability of D/DA following oral administration. Finally, studies using bile duct-cannulated rats were conducted to investigate the metabolism of D/DA as well as its biliary elimination following oral administration.

Materials and Methods

Chemicals

D/DA was extracted from *A. gigas* Nakai with 60% ethanol and determined to have a purity of 95% by HPLC (data not shown). Acetonitrile, methanol, and other reagents were purchased from Sigma (St. Louis, MO, U.S.A.).

Animals and Dosage Forms

Male Sprague–Dawley rats (240±10 g) were used in this study (Samtako, Osan, Korea). The animals were allowed free access to

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food and water, but were fasted overnight prior to treatment. Animals were kept in a temperature-controlled environment (23±2°C) with a 12 h light–dark cycle and a relative humidity of 50±10%. It was found that 2 mg/kg of D/DA would be effective to improve the streptozotocin-induced diabetic rats and the gout of rats, in former experiments (data not shown). For oral administration, D/DA was dissolved or suspended in 1% DMSO at a concentration of 200 mg/kg body weight. For intravenous administration, D/DA was dissolved in 1% DMSO at a concentration of 2 mg/kg (1 ml/min) body weight.

HPLC/MS Analysis

The optimal conditions of D/DA detection were achieved using an HPLC (Agilent Technologies, 1100 series; U.S.A.) column (Agilent Zorbax SB-C18, 250 mm×4.6 mm, 5 μm) and detection at 329 nm using a UV monitor (Agilent Technologies, 1100 series, Photo-Diode Array UV/Vis detector; U.S.A.). The column was held at room temperature and the mobile phase consisted of 70:30 (v/v %) acetonitrile and 0.1% formic acid for absorption, distribution, and excretion. *In vitro* metabolism was analyzed by the gradient method with the mobile phase consisting of acetonitrile and 0.1% formic acid. Chromatography was performed isocratically at a flow rate of 1 ml/min using the same mobile phase described above. Next, ESI/MS analysis was performed on an Agilent Technologies G2708DA electrospray mass spectrometer equipped with an Agilent Technologies Atmospheric Pressure Ionization (API) interface fitted with a hexapole ion guide. The optimal conditions for the analysis of D/DA included pneumatic nebulization with nitrogen (45 psi.) and a counter flow of nitrogen (12 l/min) heated to 350°C for nebulization and desolvation of the introduced liquid.

Liver Microsomal Incubation and *In Vitro* Metabolism

The rats were sacrificed by cervical dislocation. Preparation of the liver microsomes and biotransformation were performed as described previously [9]. The extra blood from the livers was perfused with saline to remove the blood and then homogenized with four volumes of ice-cold 0.1 M potassium phosphate buffer (pH 7.4). The liver homogenates were then centrifuged at 8,700 rpm for 10 min at 4°C. The supernatants were centrifuged again at 27,000 rpm for 60 min at 4°C. The microsomal pellets were resuspended in 0.1 M potassium phosphate buffer (pH 7.4) containing 20% glycerol. Aliquots of liver microsomes were stored at −80°C until used. The liver microsomes were analyzed as described previously using bovine serum albumin as a standard [10]. The metabolism of D/DA (100 μM) was determined by incubating the samples with 1 mg/ml microsomal protein in 0.1 M phosphate buffer (pH 7.4) at 37°C for 120 min in a reaction mix with a final volume of 500 μl. The reaction was initiated by the addition of NADPH (0.8 mM, final concentration), 10 mM glucose 6-phosphate, and 1 unit of glucose-6-phosphate dehydrogenase to the reaction mixture. The reaction was terminated by the addition of 1 ml of ethyl acetate. After mixing and centrifugation, 500 μl of the organic layer was separated. The organic layer was then dried under a stream of nitrogen gas and analyzed by HPLC/MS.

Quantitative Determination of D/DA in Blood

For evaluation of the D/DA following oral administration, blood samples were collected from a tail vein immediately before administration, and at 0.08, 0.2, 0.3, 0.7, 1, 1.3, 3, 4, 6, 8, 16, 24, 48, and 72 h after oral administration of D/DA at 200 mg/kg body weight to four rats. For intravenous administration, a polyethylene (PE) tube (0.51 mm i.d., 0.94 mm o.d.; Natume, Tokyo, Japan) was inserted into the jugular vein of four rats under 30% urethane anesthesia. D/DA was then administered alone *via* the jugular vein cannula at a dose of 2 mg/kg body weight. Blood samples were collected from all subjects at 0.16, 0.3, 0.5, 0.83, 1.3, 2, 2.67, 4, 8, 24, 36, 60, 72, 84, 96, and 108 h. All heparinized blood samples were immediately cooled on ice and then centrifuged at 1,000 rpm (4°C) for 15 min. The plasma was stored at −70°C until HPLC analysis.

Tissue and Organ Distribution

Four rats were anesthetized with ether and then exsanguinated from the descending aorta using a heparinized disposable plastic syringe at 40 min after oral administration of D/DA. The procedure was conducted at 40 min, because that is when the highest concentration of D/DA in the plasma was observed after oral administration. Twelve major tissues and organs were then sampled by dissection. The organs were blot-dried with paper, and the total weight of each of the tissues and organs was then measured. Next, tissue samples of each organ were homogenized with 3 volumes of 10 mM potassium phosphate buffer solution using a Powergen homogenizer (Fisher Co., U.S.A.). The samples were then centrifuged and the supernatant was stored at −70°C until HPLC analysis.

Urinary, Fecal, and Biliary Excretions

Urine, feces, and bile samples were collected at 0, 1, 3, 5, 8, 10, 24, 36, 48, and 72 h after administration of D/DA at a dose of 200 mg/kg body weight to bile duct-cannulated rats using a metabolic cage (Nalgene Co., U.S.A.). For analysis, 0.3 ml of bile and 1 ml of urine were extracted in 3 ml of ethyl acetate, after which 1 ml of the organic phase was subjected to vacuum evaporation. The residue was then reconstituted with 50 μl of the mobile phase. The feces samples were homogenized in 3-fold distilled water using a Powergen homogenizer, after which the samples were subjected to the same treatment as the urine and bile. All samples were stored at −70°C until HPLC analysis.

Results and Discussion

In Vitro Metabolism Study

The *in vitro* characteristics of D/DA after incubation with the liver microsomes of rats are shown in Fig. 1. The compound was more hydrophilic than D/DA, with a retention time of 13.162 min (Fig. 1). The parent and primary metabolite were detected after microsomal incubation of D/DA. HPLC/MS revealed the presence of one major peak

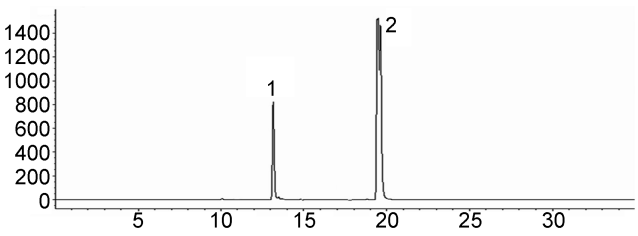

Fig. 1. HPLC chromatogram of D/DA, following microsomal incubation in the presence of NADPH.
Chromatogram for the rat liver microsomal incubation products (peak 1) of D/DA. Peak 2: D/DA standard.

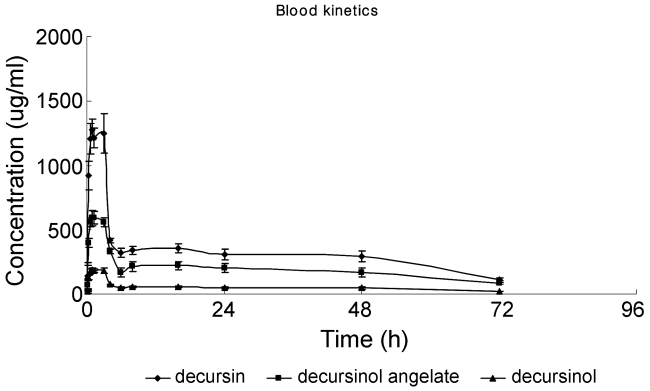

Fig. 2. Concentration in plasma after a single oral administration of D/DA at a dose of 200 mg/rat.
Points and bars are the mean values and standard deviations for data from four rats.

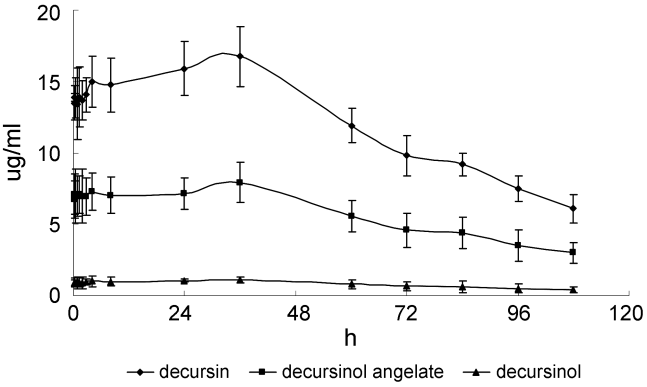

Fig. 3. Concentration in plasma after an intravenous administration of D/DA at a dose of 2 mg/rat.
Points and bars are the mean values and standard deviations for data from four rats.

(decursinol), with the sample containing *quasi*-molecular ions at *m/z*=247 ($[M+H]^+$) and 269 ($[M+Na]^+$). The chemical structure of decursinol was identified by LC/MS [2]. Although we currently do not have an explanation for these results, further metabolism of D/DA may be possible in these microsomes. Knowledge of the proposed mass of the metabolite of D/DA will be helpful in future studies conducted to evaluate the *in vivo* metabolism of D/DA.

Pharmacokinetics

The plasma concentration-versus-time profiles of D/DA and metabolized decursinol following oral and intravenous administrations of 200 and 2 mg/kg of the compound to Sprague–Dawley rats are shown in Fig. 2 and 3. Pharmacokinetic parameters, such as the maximum time (t_{max}, h), maximum concentration (C_{max}, μg equiv. of D/DA/ml), elimination half-lives ($t_{1/2}$, h), and AUC_{36-108} (area under the blood concentration–time curve from 36 to 108 h) derived from the plasma curve analysis are shown in Table 1. After administration of a single oral dose of D/DA, the concentrations of D/DA and metabolized decursinol in the plasma reached maxima of 1,278.26, 592.16, and 186.75 μg/ml in male rats at 1, 1.3, and 1 h after oral administration, respectively. Thereafter, the concentration of D/DA in the plasma declined and approached the limit of quantification, 72 and 108 h after oral and intravenous administration, respectively. The elimination half-lives ($t_{1/2}$, h) of D/DA and decursinol in the plasma for male rats after intravenous administration were estimated to be 56.67, 58.01, and 57.22 h, respectively, using first-order kinetics. Based on the ratio of AUC PO to AUC IV in the plasma, the absorption rate of D/DA in male rats was estimated to be >95%. These results indicate that most of the D/DA and metabolized decursinol administered was absorbed from the gastrointestinal tract, after which it was eliminated from the body. These findings indicate that D/DA is poorly and slowly absorbed across the gut and *via* the blood following oral and intravenous administrations. In addition, the oral and intravenous administration data demonstrated that D/DA is readily extracted during systemic circulation by the plasma. This slow absorption results in extensive excretion in the feces. These results indicate that D/DA shows promise for use as an antitumor agent owing to its long half-life in the plasma [8].

Distribution Study

The distribution of D/DA in the tissues and organs after administration of a single oral dose of 200 mg/kg is shown in Table 2. D/DA was rapidly distributed to all of the tissues and organs within 40 min of administration. As

Table 1. Pharmacokinetic parameters after a single oral or intravenous administration of D/DA.

Route of administration	Dose (mg/rat)	Dosing compound	t_{max} (h)	C_{max} (μg/ml)	$t_{1/2}$[a] (h)	AUC (μg h/ml)
PO	200	Decursin	1	1,278.26		8,258.22
		Decursinol angelate	1.3	592.16		4,220.26
		Decursinol	1	186.75		1,244.85
IV	2	Decursin		14.99	56.67	203.17
		Decursinol angelate		7.26	58.01	99.29
		Decursinol		1.12	57.22	13.54

[a]Elimination half-lives for intravenous administration were calculated using the concentrations for 36–108 h, respectively.

Table 2. Tissue distribution of D/DA in male rats after oral administration.

Tissue	Concentration (μg/tissue) at 40 min, mean±SD	
	Decursin	Decursinol angelate
Lung	91±35.26	42.3±4.60
Kidney	780.9±38.35	433.4±63.50
Heart	97.1±25.81	130.6±37.19
Spleen	1,127.1±40.88	466.9±54.91
Liver	1,953.2±57.86	928.2±57.12
Stomach	1,981.2±37.88	923±48.04
Muscle	174.3±16.48	156.8±13.93
Skin	684.8±21.71	393.4±25.46
Testicles	1,219.7±90.86	660.6±83.58
Pancreas	576.4±81.20	391.3±81.53
Large intestine	2,974.4±19.19	1,412.2±77.34
Duodenum	696.7±14.16	429±47.34
Total	18,724.5	

Mean and SD are calculated for four rats at each time point.

shown in Table 2, these high concentrations in the excretory and metabolizing organs indicate that excretion and metabolism began immediately after absorption. Based on the concentrations of D/DA in the tissues and organs in male rats, it is expected that D/DA is slowly eliminated from tissue organs in rats in a manner similar to its elimination from plasma.

Excretion Study

Following oral administration, the cumulative excretion of D/DA *via* urine, feces, and bile was examined for 72 h using metabolic cages. The urinary, biliary, and fecal excretion data from the bile duct-cannulated rats are shown in Fig. 4. When D/DA was administered orally, >90% of the administered dose was excreted 2 days after administration. The cumulative excretions of D/DA in the urine, feces, and bile for 72 h after administration accounted for 25.35±3.8%, 34.20±3.2%, and 36.10±2.9%, respectively. These findings indicate that 95.65±0.46% of the total dose of D/DA administered was excreted within 72 h.

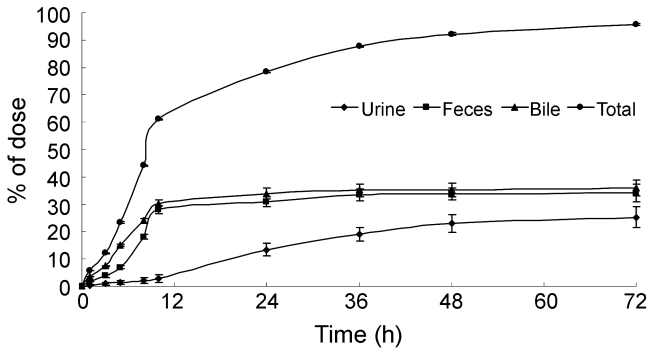

Fig. 4. Cumulative urinary, fecal, and bile excretions after oral administration of D/DA to rats.
Each point represents the mean of four rats.

In conclusion, these data show that D/DA was slowly absorbed across the gut lumen following oral and intravenous administrations. These data also indicate that, following oral and intravenous administrations, greater than 90% of D/DA was absorbed and metabolized. There was no notable accumulation of D/DA in the whole blood or tissues.

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