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Interaction of Drugs and Chinese Herbs: Pharmacokinetic Changes of Tolbutamide and Diazepam Caused by Extract of *Angelica dahurica*

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Abstract

The inhibitory effects of *Angelica dahurica* root extract on rat liver microsomal cytochrome P450 and drug–drug interactions were studied.

The 2α- and 16α-hydroxylase activity of testosterone were most strongly inhibited, with 17·2% and 28·5% of their activity remaining, respectively, after oral administration of *A. dahurica* extract at a 1 g kg^{-1} dose. 6β-Hydroxylase activity was also inhibited, with 70% of its activity remaining, under the same conditions. In addition, treatment with the extract inhibited the metabolism of tolbutamide, nifedipine and bufuralol. These results showed that the extract inhibited the various isoforms of cytochrome P450 such as CYP2C, CYP3A and CYP2D1.

The *A. dahurica* extract delayed elimination of tolbutamide after intravenous administration at a 10 mg kg^{-1} dose to rats. Thus, the extract altered the liver intrinsic clearance. It had little effect, however, on the pharmacokinetic parameters of diazepam after intravenous administration at 10 mg kg^{-1}. Since diazepam showed high clearance, it underwent hepatic blood flow rate-limited metabolism. Therefore, the change of intrinsic clearance had little effect on hepatic clearance. However, the C_{max} value after oral administration of diazepam with extract treatment was four times that with non-treatment. It was suggested that the first-pass effect was changed markedly by the extract. High-dose (1 g kg^{-1}), but not low dose (0·3 g kg^{-1}), administration of *A. dahurica* extract increased significantly the duration of rotarod disruption following intravenous administration of diazepam at 5 mg kg^{-1}.

It was concluded that administration of *A. dahurica* extract has the potential to interfere with the metabolism, by liver cytochrome P450, of other drugs.

Cytochrome P450 isoforms contribute to the metabolism of numerous drugs (Wrighton & Stevens 1992). Modifiers of cytochrome P450 activity may cause pharmacokinetic changes in other drugs, resulting in a reduction of efficacy and enhancement of side-effects. Natural herbs contain many compounds that inhibit the activity of cytochrome P450, such as furocoumarins and flavonoids (Letteron et al 1986; Obermeier et al 1995). Recently, furocoumarins were suggested as candidates for causative agents of grapefruit-juice-mediated drug interaction (Fukuda et al 1997; Schmiedlin-ren et al 1997). Furocoumarins, which have been reported to inhibit cytochrome P450 activity (Mäenpää et al 1993), are components of many natural herbs, especially the plants belonging to the Umbelliferae family. The herbs belonging to this family are frequently used in Chinese herbal medicine, and concomitantly with other pharmaceuticals in Asia. One of these herbs, angelicae dahuricae radix, the root of *Angelica dahurica* Benth. et Hook., has been reported to inhibit aniline hydroxylase activity and aminopyrine *N*-demethylase activity in rat liver

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when the hot-water extract of *A. dahurica* root was administered orally (Koizumi et al 1994). In this study, we tried to determine the isoform specificity of the inhibition by *A. dahurica* extract and also to demonstrate the interaction with pharmacokinetic and pharmacodynamic changes of tolbutamide and diazepam as model compounds.

Materials and Methods

Materials

A. dahurica was purchased from Nihonshoyaku (0 Ltd (Tokyo, Japan). *A. dahurica* extract was prepared by immersing *A. dahurica* in a 12-fold (w/w) volume of distilled water and boiling it at 95°C for 60 min, then passing it through a filter (JP100 mesh). The filtrate was evaporated under reduced pressure to about a quarter volume, then spray dried. Testosterone, tolbutamide and nifedipine were purchased from Wako Pure Chemical Industry (Osaka, Japan). 2α-Hydroxytestosterone, 16α-hydroxytestosterone and bufuralol were purchased from Salford Ultrafine Chemicals and Research Ltd (England). 6β-Hydroxytestosterone was obtained from Sumika Chemical Analysis Service Ltd (Osaka, Japan). All other reagents were commercial products and of special or HPLC analytical grade.

Animals, treatment and sampling

Seven-week-old male Sprague–Dawley rats (197·1–266·1 g) were purchased from Charles River (Atsugi, Japan). Rats were housed under controlled conditions (23 ± 2°C, 55 ± 20% relative humidity and 12-h light–dark cycle) and were fed standard laboratory chow with water freely available.

Rats were treated with 1 g kg^{-1} of the *A. dahurica* extract (10 mL, p.o.) and killed 1, 3, 6, 12 or 24 h later. The liver was perfused with 1·15% KCl, and then a microsomal fraction was prepared by ordinary differential ultracentrifugation. Microsomal protein was estimated by the method of Lowry et al (1951).

For the pharmacokinetic interaction experiments, 1 h after treatment with *A. dahurica* extract, tolbutamide (10 mg kg^{-1}, 1 mL, i.v.) or diazepam (5 mg kg^{-1}, 10 mL, p.o. or 10 mg kg^{-1}, 2 mL, i.v.) was administered. Blood samples were taken at 5, 15, 30, 60, 90, 120, 240 and 360 min after the administration of tolbutamide or diazepam, from the femoral artery using cannulated polyethylene tubing (PE-10), which was filled with heparin sodium at a concentration of 100 IU mL^{-1}.

For the pharmacodynamic interaction experiments, rats were treated with the *A. dahurica* extract (1 g kg^{-1}, 10 mL, p.o.), and 1 h later, diazepam was administered intravenously at a dose of 5 mg kg^{-1}. Then skeletal muscle relaxants were tested with the rotarod procedure (below).

All animal experiments were performed in accordance with our institutional guidelines after obtaining the permission of the Laboratory Animal Committee of Tsumura & Co.

Assays of enzyme activity

Testosterone hydroxylase activity, tolbutamide hydroxylase activity, nifedipine oxidase activity and bufuralol hydroxylase activity were measured according to Sonderfan et al (1987), Relling et al (1990), Guengerich et al (1986) and Kronbach et al (1987), respectively, with minor modifications.

The incubation mixture (250 μL for testosterone, 200 μL for others) consisted of potassium phosphate buffer (pH 7·4, 100 mmol L^{-1}), liver microsomes (50 μg for testosterone, 40 μg for tolbutamide or nifedipine, 100 μg for bufuralol), a substrate (200 μmol L^{-1} for testosterone and nifedipine, 1 mmol L^{-1} for tolbutamide and 20 μmol L^{-1} for bufuralol) and an NADPH-generating system containing NADP (0·8 mmol L^{-1}), glucose 6-phosphate (8·0 mmol L^{-1}), glucose 6-phosphate dehydrogenase (1 IU) and magnesium chloride (6 mmol L^{-1}). The incubation was started by the addition of the NADPH-generating system and was continued for 20 min. The metabolites were measured by HPLC under the conditions summarized in Table 1.

Determination of plasma concentrations of tolbutamide and diazepam

To quantify the tolbutamide concentration in rat plasma, perchloric acid (50 μL), acetonitrile (150 μL) and chlorpropamide (as internal standard, 20 μg mL^{-1} in CH_3OH, 50 μL) were added to the plasma sample (50 μL). The mixture was centrifuged at 15 000 rev min^{-1} and 4°C for 10 min. The resulting supernatant (10 μL) was injected into the HPLC system under the conditions described in Table 1.

To quantify the diazepam concentration, the plasma sample (100 μL) was applied to BondElut C8 (Varian), which was conditioned with 1 mL of methanol and 1 mL of distilled water. After a wash with 1 mL of 40% methanol, diazepam was eluted with 1 mL of methanol. The elutant was evaporated to dryness and resuspended in 25% acetonitrile (200 μL) and a 10-μL sample was injected into the

Table 1. Conditions for the analysis of hydroxytestosterone, tolubutamide, 4-hydroxytolbutamide, oxidized nifedipine, 1′-hydroxy bufuralol and diazepam by HPLC.

	Column	Mobile phase	Detection
Hydroxy testosterone	Symmetry C18 4·6 × 75 mm waters	$CH_3CN : CH_3OH : H_2O$ (2 : 50 : 48)	UV 254 nm
Tolbutamide and 4-hydroxy tolbutamide	Nova Pack C18 3·9 × 150 mm waters	CH_3CN : 10 mM CH_3COO-NaHCl pH 4·3 (27 : 73)	UV 230 nm
Oxidized nifedipine	Symmetry C18 4·6 × 75 mm waters	CH_3OHH_2O (50 : 50)	UV 254 nm
1′-Hydroxy bufuralol	CAPCELL PAK CN SGRO 4·6 × 250 mm Shiseido	20 mmol L^{-1} $NaClO_4$-HCl pH 2·5 : CH_3CN (70 : 30)	Ex 252 nm Em 302 nm
Diazepam	Symmetry C18 4·6 × 75 mm waters	CH_3CN : 0·1% phosphoric acid (26 : 74)	UV 230 nm

For all systems column temperature was 40°C and flow rate was 1 mL min^{-1}.

HPLC system under the conditions described in Table 1.

Rotarod procedure

The rotarod procedure was carried out according to the method of Dunham & Miya (1957) with a minor modification. In brief, rats were placed on a narrow rotating cylinder (9 cm diameter, 8 rev min^{-1}) and the time until the animal fell onto a padded platform (180 s maximum) was measured. Before the experiment, rats were trained to walk the rotarod.

Data analysis

Pharmacokinetic evaluation was performed by non-compartmental analysis of the plasma concentration–time data. Differences between means were detected using Student's *t*-test, Aspin-Welch *t*-test or Kruskal–Wallis test with Student's multiple-comparison test.

Results

Inhibition of enzyme activity

The effects on testosterone metabolism of treatment with *A. dahurica* extract are shown in Figure 1. The inhibition of 2α-hydroxylase activity was most potent and significant 1–24 h after administration of the extract, with 17·2–68·7% of activity remaining. 16α-Hydroxylase activity was also inhibited. The inhibition of 16α-hydroxylase was significant 1–6 h after administration with 28·5–39·8% of activity remaining. 6β-hydroxylase activity was inhibited as well. A significant inhibition was observed at 6 h after administration of extract, with 70% of its activity remaining.

The effects of *A. dahurica* extract on tolbutamide hydroxylase, nifedipine oxidase and bufuralol hydroxylase activity are shown in Figure 2. Tolbutamide hydroxylase activity was significantly inhibited 1–6 h after administration of extract, with 41·8–63·0% of its activity remaining (Figure 2A). Nifedipine oxidase activity was significantly inhibited 3–6 h after administration of extract, with 61·4–71·4% of its activity remaining (Figure 2B). Bufuralol hydroxylase activity was significantly inhibited 1–6 h after administration of extract, with 34·4–43·1% of its activity remaining (Figure 2C).

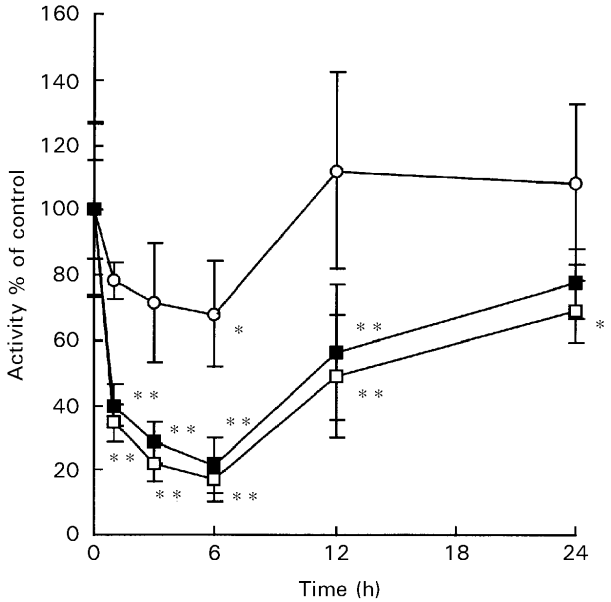

Figure 1. The changes of testosterone hydroxylase activities in rat liver microsomes after oral administration of *A. dahurica* extract at a dose of 1 g kg^{-1}. Control values were as follows: 2α-hydroxylase activity (□), 1·63 ± 0·43 nmol min^{-1} (mg protein)$^{-1}$; 6β-hydroxylase activity (○), 3·31 ± 0·50 nmol min^{-1} (mg protein)$^{-1}$; 16α-hydroxylase activity (■), 2·10 ± 0·56 nmol min^{-1} (mg protein)$^{-1}$. Each value represents the mean ± s.d. of six animals. *$P < 0·05$, **$P < 0·01$ vs control.

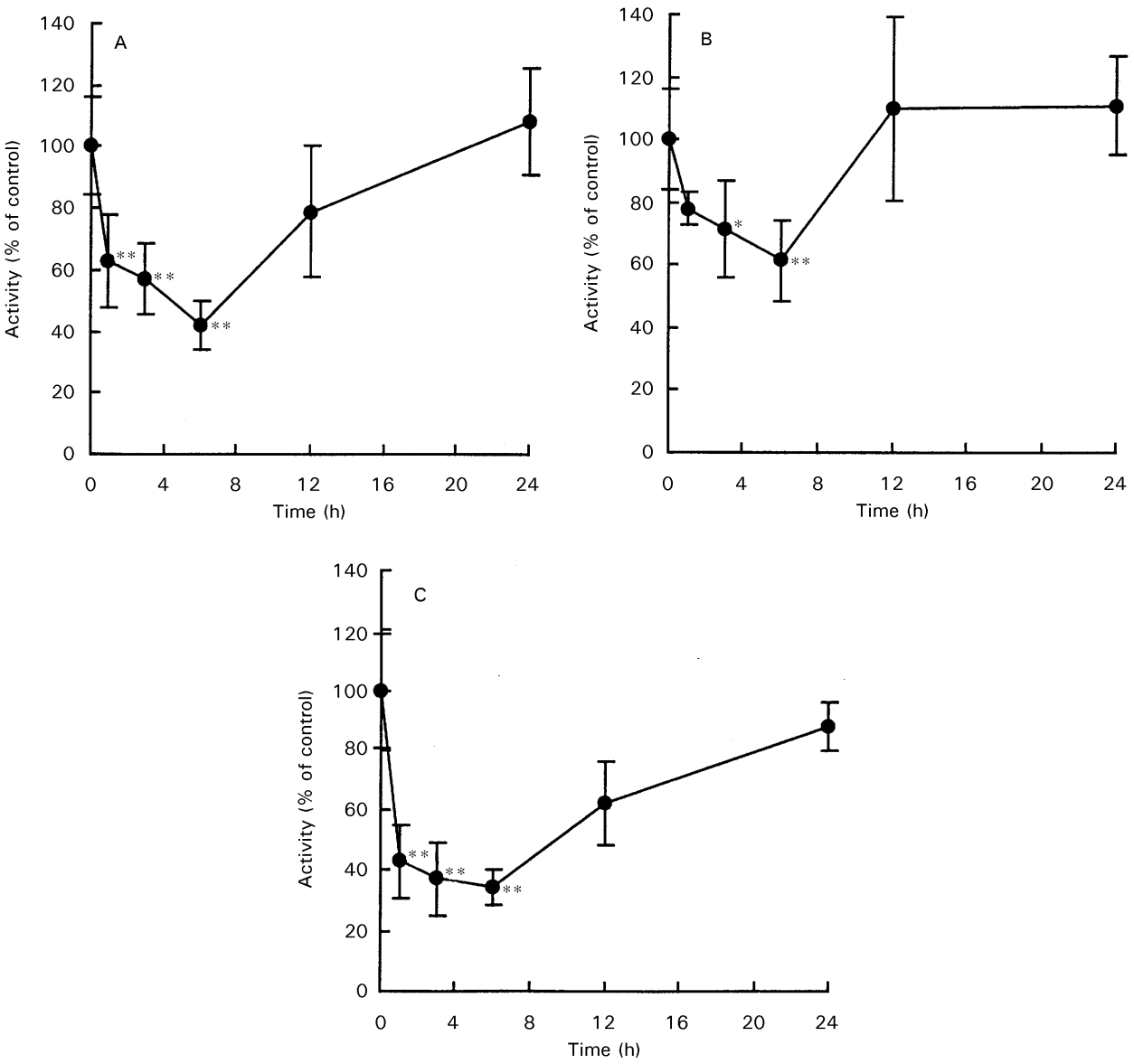

Figure 2. The changes of tolbutamide hydroxylase activity (A), nifedipine oxidase activity (B) and bufuralol 1′-hydroxylase activity (C) in rat liver microsomes after oral administration of *A. dahurica* extract at a dose of 1 g kg^{-1}. Control values were as follows: tolbutamide hydroxylase activity, 0·364 ± 0·058 nmol min^{-1} (mg protein)$^{-1}$; nifedipine oxidase activity, 3·59 ± 0·57 nmol min^{-1} (mg protein)$^{-1}$; and bufuralol 1′-hydroxylase activity, 1·36 ± 0·23 nmol min^{-1} (mg protein)$^{-1}$. Each value represents the mean ± s.d. of six animals. *$P < 0·05$, **$P < 0·01$ vs control.

Pharmacokinetic interaction study

Plasma concentrations and pharmacokinetic parameters of tolbutamide with or without *A. dahurica* treatment in rats are shown in Figure 3 and Table 2. The AUC, $t_{\frac{1}{2}}$, Vd and CL of the non-treated group were 238 ± 36·9 μg h mL^{-1}, 4·33 ± 0·70 h, 0·265 ± 0·0369 L kg^{-1} and 0·714 ± 0·108 mL min^{-1} kg^{-1}, respectively. These values were comparable with those of previous reports (Matthew & Houston 1990). Treatment with *A. dahurica* extract increased the AUC, $t_{\frac{1}{2}}$ and CL value 2·5, 2·3 and 0·45 fold, respectively.

The effects of *A. dahurica* extract on the plasma concentration of diazepam after oral (5 mg kg^{-1}) and intravenous (10 mg kg^{-1}) administration in rats were studied (Figure 4, 5 and Table 3). When diazepam was administered orally, most of the pharmacokinetic parameters could not be calculated in the non-treated group because the plasma concentration of diazepam was below the detection limit at all sampling time points, except for 2 h. Only C_{max} was calculated (23·0 ± 12·4 ng mL^{-1}). In the *A. dahurica*-treated group, the plasma concentrations of diazepam were sufficient for profil-

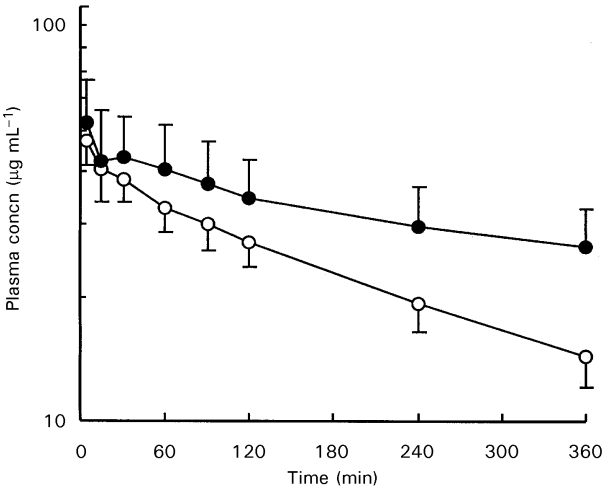

Figure 3. Plasma concentrations of tolbutamide after intravenous administration of 10 mg kg^{-1} in rats with (●) or without (○) *A. dahurica* extract treatment (1 g kg^{-1}, p.o.). Each value represents the mean ± s.d. of six animals.

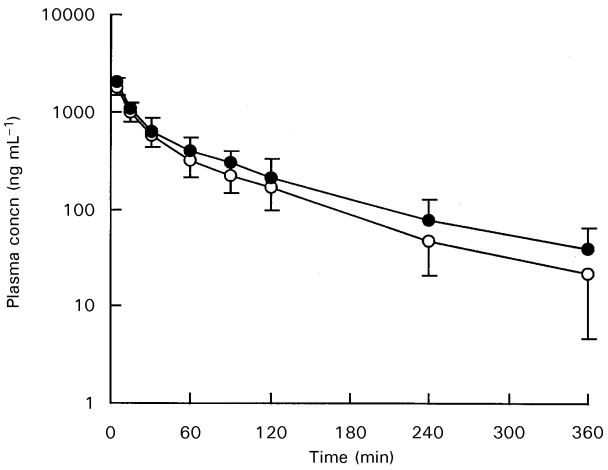

Figure 5. Plasma concentrations of diazepam after intravenous administration of 10 mg kg^{-1} in rats with (●) or without (○) *A. dahurica* extract treatment (1 g kg^{-1}, p.o.). Each value represents the mean ± s.d. of six animals.

ing. The C_{max} value (92·1 ± 50·3 ng mL^{-1}) of the extract-treated group was significantly larger than that of the non-treated group.

When diazepam was administered intravenously, however, *A. dahurica* treatment had a tendency to delay the elimination of diazepam, although no significant difference in the pharmacokinetic parameters was observed. The AUC, $t_{\frac{1}{2}}$, Vd and CL values of the non-treated group were 1006 ± 283 ng h mL^{-1}, 1·00 ± 0·330 h, 10·5 ± 3·08 L kg^{-1} and 176·4 ± 49·0 mL min^{-1} kg^{-1}, respectively. These values were comparable with those of previous reports (Klotz et al 1976).

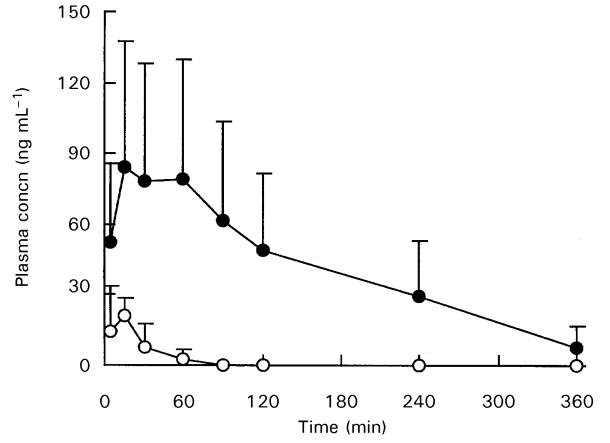

Figure 4. Plasma concentrations of diazepam after oral administration of 5 mg kg^{-1} in rats with (●) or without (○) *A. dahurica* extract treatment (1 g kg^{-1}, p.o.). Each value represents the mean ± s.d. of six animals.

Pharmacodynamic interaction study

To confirm the pharmacodynamic interaction of *A. dahurica* extract treatment with diazepam, the effect of skeletal muscle relaxants was tested with the rotarod procedure. High-dose (1 g kg^{-1}), but not low-dose (0·3 g kg^{-1}) *A. dahurica* extract treatment increased significantly the duration of rotarod disruption following intravenous administration of diazepam at a dose of 5 mg kg^{-1} (Figure 6). Administration of *A. dahurica* extract alone had no effect on rotarod performance (data not shown).

Discussion

Oral administration of *A. dahurica* extract, 1 g kg^{-1}, which is relatively high for use in man, inhibited rat liver testosterone hydroxylase activity. CYP2C11-mediated 2α- and 16α-hydroxylase activity of testosterone were the most strongly

Table 2. Pharmacokinetic parameters of tolbutamide after intravenous administration at a dose of 10 mg kg^{-1} in rats.

Treatment	AUC (μg h mL^{-1})	$t_{\frac{1}{2}}$ (h)	Vd (L kg^{-1})	CL (mL min^{-1} kg^{-1})
Non-treatment	238 ± 36·9	4·33 ± 0·70	0·265 ± 0·0369	0·714 ± 0·108
A. dahurica extract treatment (1 g kg^{-1}, p.o.)	580 ± 230*	9·76 ± 2·89**	0·257 ± 0·0683	0·323 ± 0·117**

Values represent the mean ± s.d. (n = 5). *$P < 0·05$, **$P < 0·01$ vs non-treatment.

Table 3. Pharmacokinetic parameters of diazepam after intravenous or oral administration in rats.

Treatment	C_{max} (ng mL^{-1})	AUC (ng h mL^{-1})	$t_{1/2\lambda z}$ (h)	Vd or Vd/F (L kg^{-1})	CL (mL min^{-1} kg^{-1})
Diazepam (10 mg kg^{-1}, i.v.)					
Non-treatment	–	1006 ± 283	1·00 ± 0·33	10·5 ± 3·08	176·4 ± 49·0
A. dahurica extract treatment (1 g kg^{-1}, p.o.)	–	1639 ± 534	1·26 ± 0·41	8·58 ± 1·78	114·3 ± 50·2
Diazepam (5 mg kg^{-1}, p.o.)					
Non-treatment	23·0 ± 12·4	–	–	–	–
A. dahurica extract treatment (1 g kg^{-1}, p.o.)	92·1 ± 50·3*	318 ± 206	1·97 ± 1·01	74·9 ± 71·9	633·4 ± 833·5

Values represent the mean ± s.d. (n = 5). *$P < 0·05$ vs non-treatment. –, not calculated.

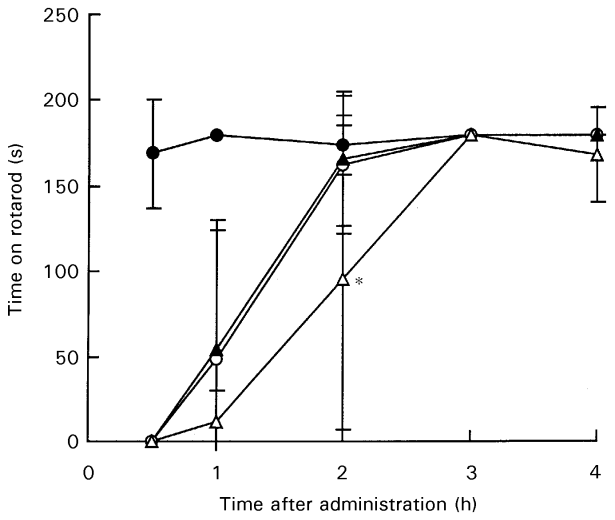

Figure 6. The time-course for the effect of diazepam on rotarod performance of rats with or without *A. dahurica* extract treatment. Control (●); non-treatment (○); *A. dahurica* extract treatment (▲, 0·3 g kg^{-1}, p.o.); *A. dahurica* extract treatment (△, 1 g kg^{-1}, p.o.). Each value represents the mean ± s.d. of eight animals. *$P < 0·05$ vs non-treatment.

inhibited, followed by CYP3A- and CYP1A-mediated 6β-hydroxylase activity. In addition, *A. dahurica* extract inhibited the metabolism of nifedipine, which was metabolized at CYP3A (Guengerich et al 1986), bufuralol, which was metabolized at CYP2D1 (Mimura et al 1994) and tolbutamide. Tolbutamide methyl hydroxylation, which is the major pathway of metabolism of tolbutamide in-vivo (Thomas & Ikeda 1966; Tagg et al 1967) and in-vitro (McDaniel et al 1969) in rats, is thought to involve a CYP2C enzyme. These results suggested that treatment with *A. dahurica* extract inhibited the various isoforms of cytochrome P450, such as CYP2C, CYP3A and CYP2D1.

As a next step, we demonstrated the pharmacokinetic and pharmacodynamic interaction of *A. dahurica* extract using tolbutamide and diazepam as model compounds. According to in-vivo (Schwartz et al 1967) and in-vitro data (Neville et al 1993), diazepam is mainly metabolized by CYP2C11- and CYP2D1-mediated *N*-demethylation, CYP3A2-mediated 3-hydroxylation and CYP2D1-mediated 4'-hydroxylation in rats. All of these isozymes were inhibited by treatment with *A. dahurica* extract.

It has been reported that grapefruit juice has no effect on drug disposition after intravenous administration in man (Kupferschmidt et al 1995; Lundahl et al 1997), and does not alter liver CYP3A4 activity as measured by the erythromycin breath test (Lown et al 1997). These reports suggested that the effect of grapefruit juice is mediated through inhibition of gut wall, and not liver, metabolism. However, the *A. dahurica* treatment delayed the elimination of tolbutamide after intravenous administration in the rat. This result, and the result of the ex-vivo study, showed clearly that *A. dahurica* treatment altered the hepatic intrinsic clearance of tolbutamide in the rat. Treatment with *A. dahurica* extract had little effect on the pharmacokinetic parameters of diazepam after intravenous administration. This contrariety can be explained by the difference in the clearances of the two compounds. Since the clearance of diazepam was greater than that of tolbutamide, diazepam underwent blood flow rate-limited metabolism. Therefore, the change of intrinsic clearance had little effect on hepatic clearance. Meanwhile, the C_{max} value after oral administration of diazepam with *A. dahurica* treatment was four times that without the treatment. It was suggested that the first-pass effect was markedly changed by *A. dahurica* treatment.

In the case of grapefruit juice-mediated drug interaction, 6′,7′-dihydroxybergamottin, GF-I-1 and GF-I-4 have been suggested as candidate causative agents (Fukuda et al 1997; Schmiedlin-ren et al 1997). In addition, the furocoumarins, including imperatorin, showed differential inhibition of coumarin 7-hydroxylase activity in mouse and human liver microsomes (Mäenpää et al 1993). The main components of *A. dahurica* are furocoumarins, such

as byak-angelicol, byak-angelicin, imperatorin, isoimperatorin, oxypeucedanin and oxypeucedanin hydrate (Fujiwara et al 1980). In preliminary experiments, byak-angelicin, one of the furocoumarins, was found to exert an inhibitory effect on tolbutamide hydroxylation. It is, therefore, likely that the furocoumarin components of *A. dahurica* extract are responsible, at least in part, for its inhibition of drug metabolism. However, the possibility cannot be excluded that other components contained in *A. dahurica* are responsible for the inhibitory effect since *A. dahurica* contains many components in addition to furocoumarins. In conclusion, administration of *A. dahurica* has the potential to affect the metabolism of other drugs by liver cytochrome P450.

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