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Pharmacokinetics and Brain Distribution of Magnolol in the Rat after Intravenous Bolus Injection

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Abstract

The pharmacokinetics of magnolol in rats was studied after 2, 5, or 10 mg kg^{-1} intravenous bolus injection. Plasma concentration-time profiles of magnolol were fitted by a two-compartment open model. There were no significant differences in the elimination half-life, the total body clearance, steady-state volume of distribution, or mean residence time. The area under the plasma-time curve and area under the moment-time curve of magnolol appears to increase proportionally from a dose of 2 to 10 mg kg^{-1}. These results suggest that magnolol possesses linear pharmacokinetics. Notwithstanding, brain concentration of magnolol showed no significant difference among various regions (cerebral cortex, olfactory bulb, hippocampus, striatum, cerebellum, brain stem and rest of brain) after 10 min of magnolol (5 mg kg^{-1}, i.v.) administration, the mean brain drug concentration was approximately fourfold that of magnolol in plasma.

Magnolol is the major phenolic constituents of *Magnolia officinalis* (Chinese name: Houpo). The stem bark of Houpo has been used as a folk medicine by the Chinese for the treatment of anxiety and nervous disturbance (Chang & But 1986). Apart from possessing antiplatelet (Teng et al 1990), antithrombotic (Teng et al 1991) and antiemetic (Kawai et al 1994) activities, this compound has also been reported to act as a central nervous system depressant as well as a central acting muscle relaxant (Watanabe et al 1983).

We recently reported that the pharmacokinetics of magnolol in rabbits exhibit linearity (Tsai et al 1994). However, there is no information on the pharmacokinetics of magnolol in the brain, the major target of its central effect. In the present study, we evaluate the pharmacokinetics of magnolol by measuring magnolol in rat plasma and parts of brain.

Material and Methods

Chemicals and reagents

Magnolol (Fig. 1) was extracted from magnolia bark (Fujita et al 1973). Identification and purity were compared with authentic compound by ^{13}C NMR (Bruker, Germany) and HPLC coupled with a photodiode-array detector (Tsai & Chen 1992). Acetonitrile and orthophosphoric acid (85%) were obtained from E. Merck (Darmstadt, Germany). Triple-deionized water (Millipore Corp., Bedford, MA, USA) was used in all preparations.

Apparatus and chromatographic

The HPLC system consisted of an autosampler (Model 23, SIC, Tokyo, Japan), a variable wavelength UV-vis detector (Soma, Tokyo, Japan) set at 290 nm, and a chromatographic pump (Model 510, Waters, Milford, MA, USA). Separation was achieved on a reversed-phase COSMOSILE (Nacalai tesque, Kyoto, Japan) 5C18-AR column (4.6 × 250 mm, particle size 5 μm) at room temperature (22–24°C). The mobile phase was acetonitrile-water (70 : 30, v/v, pH 2·5–2·8 adjusted with orthophosphoric acid) at a flow-rate of 1.0 mL min^{-1}.

Blood sampling and treatment for pharmacokinetics

Male Sprague-Dawley rats, 250–300 g, were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.). Only one-quarter (100 mg kg^{-1}, i.p.) of the dose of chloral hydrate was administered during the experimental period when required. Blood samples (0.3 mL) were directly collected from the rat by cardiac puncture and sampled from the same animals at 2·5, 5, 10, 15, 20, 30, 45, 60, and 120 min after administration of magnolol (2, 5, or 10 mg kg^{-1}, n = 5, i.v.). Data from these sample times were used to construct pharmacokinetic profiles by plotting magnolol concentration in plasma against time. Treatment of blood samples was as described previously (Tsai et al 1994). The same sample handling process was used to determine recovery and precision in plasma.

In the brain study, animals were killed by decapitation, and the various brain parts (cerebral cortex, olfactory bulb, hippocampus, striatum, cerebellum, brain stem, and rest of brain) were obtained and weighed. The brain tissues were

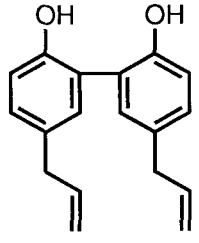

FIG. 1. The chemical structure of magnolol.

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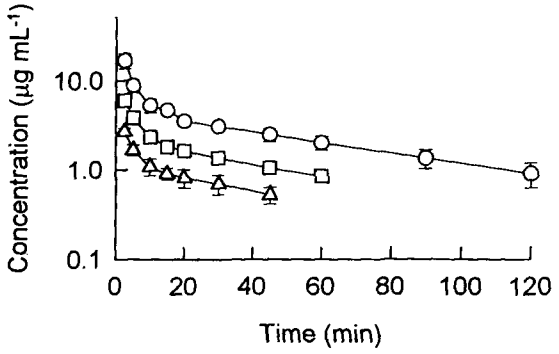

FIG. 2. Mean plasma concentration-time profiles after intravenous administration of magnolol in rats at doses of 2 (△), 5 (□) and 10 (○) mg kg^{-1} (n = 5).

likewise mixed with acetonitrile (10 mL (g tissue)$^{-1}$) to denature proteins. The homogenate, which contained honokiol (1 μg mL^{-1}, as internal standard), was centrifuged at 8000 *g* for 5 min. The supernatant (20 μL) was submitted to HPLC analysis.

Recovery and precision

Plasma samples were mixed with magnolol at concentrations ranging from 1 to 5 μg mL^{-1}. The resulting peak area ratio (magnolol : internal standard) was compared with the peak area of the standards prepared in acetonitrile. Precision over the entire working dose range was determined by replicate analyses of plasma samples (n = 4) mixed with three concentrations (1, 5, or 10 μg mL^{-1}) of magnolol. To determine intra-day variance, quadruplicate assays were carried out on the same samples at different times on the day. Inter-day variance was determined by assaying the samples in quadruplicate on days 1, 2, 4, and 6 after addition of drug. Coefficients of variation (CV) were calculated from these values.

Data analysis

The concentration of magnolol was computed by comparing the peak-area ratio of magnolol and internal standard of each sample against standard solutions of known concentrations. All data were subsequently processed by the computer program PCNONLIN (version 4.2, SCI Software Inc., Lexington, KY, USA). Statistical analyses were by Student-Newman-Keuls test with the level of significance set at $P < 0.05$.

Results

The recoveries of magnolol from rat plasma were found to be 107·2, 103·39, and 97·32% for concentrations 1, 2, and 5 μg mL^{-1}, respectively. The detection limit for magnolol, at a signal-to-noise ratio of 3, was 0·05 μg mL^{-1} in rat plasma. The reproducibility of the method can be defined by examining both intra-day and inter-day variabilities. The intra-day CV values for magnolol at concentrations of 1, 5, and 20 μg mL^{-1} were 4·44, 2·78, and 0·78%, respectively, and the inter-day CV values for magnolol at the same concentrations were 5·32, 1·87, and 0·37%, respectively.

The pharmacokinetic models (one- and two-compartment) were compared according to the Akaike's information criterion (AIC) (Yamoaka et al 1978) and Schwartz criterion (SC) (Schwartz 1978), with minimum AIC and SC values regarded as the best representation of the plasma concentration-time course data. A two-compartment open model with elimination from a central compartment was proposed and validated through the program to explain the apparent biphasic disposition of magnolol in plasma after an intravenous bolus injection (Fig. 2). The pharmacokinetic parameters, as derived from these data and calculated by PCNONLIN program, are shown in Table 1.

Brain concentrations after 10 min of magnolol administration (5 mg kg^{-1}, i.v.) are shown in Table 2. The mean brain:plasma concentration at 10 min after magnolol administration was approximately fourfold that in plasma.

Discussion

When the intravenous bolus dose of magnolol was increased from 2 to 10 mg kg^{-1}, there were no significant differences in the apparent total body clearance (CL), and the elimination half-life ($t_{\frac{1}{2}\beta}$), steady-state volume of distribution (Vd_{ss}), and mean residence time (MRT). The area under the curve (AUC) and area under the moment vs time curve (AUMC) of magnolol in rat appears to increase proportionally in the dose range of 2–10 mg kg^{-1}. These results indicate that the pharmacokinetics of magnolol in rats are linear. As in our previous report, magnolol exhibits linear pharmacokinetics (Tsai et al 1994). In contrast, other commonly used Chinese herbal medicines, glycyrrhetinic acid (Tsai & Chen 1991), asarone (Tsai et al 1992a), and glycyrrhizin (Tsai et al 1992b), show nonlinear pharmacokinetic phenomena. In nonlinear pharmacokinetics, CL, $t_{\frac{1}{2}\beta}$, and Vd_{ss} increased

Table 1. Estimates of pharmacokinetic parameters using a two-compartment open model with elimination from the central compartment, when a dose of 2, 5, or 10 mg kg^{-1} magnolol was intravenously administered in rats.

Parameter	2 mg kg^{-1}	5 mg kg^{-1}	10 mg kg^{-1}
$t_{\frac{1}{2}\alpha}$ (min)	2·24 ± 0·27	2·58 ± 0·40	2·47 ± 0·68
$t_{\frac{1}{2}\beta}$ (min)	54·15 ± 5·14	49·05 ± 5·96	49·58 ± 6·81
CL (mL min^{-1} kg^{-1})	22·01 ± 1·58	27·05 ± 0·83	25·28 ± 1·98
AUC (μg min mL^{-1})	91·50 ± 6·33	173·56 ± 8·84*	400·56 ± 31·83**
Vd_{ss} (mL kg^{-1})	1491 ± 136	1619 ± 93	1453 ± 123
AUMC (μg min^2 mL^{-1})	6516 ± 1161	10663 ± 1664*	19426 ± 3431**
MRT (min)	68·73 ± 6·98	63·85 ± 6·50	58·14 ± 4·63

Data are expressed as mean ± s.e.m. (n = 5). *Significantly different ($P < 0.05$) from the 2 mg kg^{-1} dose. **Significantly different ($P < 0.05$) from both the 2 and 5 mg kg^{-1} doses.

Table 2. Mean brain magnolol concentration in rats 10 min after magnolol administration (5 mg kg^{-1}, i.v.).

Region	Concn (μg g^{-1})
Brain stem	15·74 ± 1·65
Cerebellum	14·85 ± 1·28
Striatum	14·62 ± 1·40
Hippocampus	13·41 ± 1·10
Cortex	12·61 ± 1·18
Olfactory bulb	11·69 ± 1·16
Rest of brain	12·81 ± 0·61

Data are expressed as mean ± s.e.m. ($n = 5$).

significantly with increases in the dose (Shargel & Yu 1993), and administration of a large dose may lead to retardation of a drug's elimination and prolongation of its effects.

Based on these studies, we suggest that the much higher brain concentration of magnolol may be due to its effects on regional circulation, as magnolol is thought to exhibit central effects (Watanabe et al 1983; Chang & But 1986; Kawai et al 1994). Whether the much higher capability of magnolol to bind with brain tissue implies a novel form of activation or inactivation requires further elucidation.

In conclusion, the pharmacokinetics of magnolol (2, 5 or 10 mg kg^{-1}, i.v.) could be characterized by the two-compartment model. The dose-related results indicate that the pharmacokinetics of magnolol in rat are linear. Magnolol distributes evenly in the brain regions following an intravenous bolus injection.

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