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Pharmacokinetics of Propentofylline and the Quantitation of Its Metabolite Hydroxypropentofylline in Human Volunteers

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Propentofylline (PPF, 3-methyl-1-(5-oxohexyl)-7-propylxanthine) has been reported to be effective for the treatment of both vascular dementia and dementia of the Alzheimer type. The pharmacological effects of PPF may be exerted via the stimulation of nerve growth factor, increased cerebral blood flow, and inhibition of adenosine uptake. The objectives of this experiment are to determine the kinetic behavior of PPF, to identify, and to quantify its metabolite in human. Blood samples were obtained from human volunteers following oral administration of 200 mg of PPF tablets. For the identification and quantification of the metabolite, 3-methyl-1-(5-hydroxyhexyl)-7-propylxanthine (PPFOH), PPFOH was synthesized and identified by gas chromatography/mass spectroscopy (GC/MS) and ^{1}H-nuclear magnetic resonance spectroscopy. The molecular weight of synthesized metabolite is 308 dalton. The PPF and PPFOH in plasma were extracted with diethyl ether and identified by electron impact GC/MS. The plasma concentrations of PPF and PPFOH were determined by gas chromatography/nitrogen phosphorus detector in plasma and their pharmacokinetic parameters were determined. The mean half-life of PPF was 0.74 hr. The areas under the curve (AUCs) of PPF and PPFOH were 508 and 460 ng.hr/ml, respectively. C_{max} of PPF was about 828.4 ng/ml and the peak concentration was achieved at about 2.2 hr (T_{max}). These results indicate that PPF is rapidly disappeared from blood due to extensive metabolism into PPFOH.

Key words : Pharmacokinetics, Propentofylline, Human, Hydroxypropentofylline, Synthesis of Metabolite

INTRODUCTION

Propentofylline (PPF, 3-methyl-1-(5-oxohexyl)-7-propylxanthine) has been reported to be an agent of improving a cerebral microcirculation and to be effective for the treatment of both vascular dementia and dementia of the Alzheimer type (Fugi *et al.*, 1993; Nabeshima, 1995; Meilke *et al.*, 1996). The molecular mechanism of action of PPF is not yet fully elucidated. PPF is a weak antagonist of the adenosine A1 receptor. The treatment of PPF reduces ischemic nerve cell death in the brain in a model of focal ischemia (Parkinson *et al.*, 1994). PPF also decreases brain edema, intraneuronal calcium accumulation (Kadoya *et al.*, 1992), reactive astrocytosis (Deleo *et al.*, 1987), and ameliorate mitochondrial function and the energy state of the ischemic brain (Sasaki *et al.*, 1989). The pharmacological effects of PPF may be exerted via the stimulation of nerve growth factor (Nebeshima, 1995), increased cerebral blood flow (Grome *et al.*, 1996), and inhibition of adenosine uptake (Fredholm *et al.*, 1994).

In contrast to the known pharmacological effects, few clinical pharmacokinetic and metabolism studies of PPF are found and need to be performed in detail. Due to the large hepatic first-pass effects the absolute bioavailability of PPF after oral administration was 4% in rabbits (Kim *et al.*, 1992). A few attempts are made to avoid the first-pass effects of PPF. Minami *et al.* (1995) reported that the plasma concentration of PPF showed large variation among patients with brain disorders and suggested that drug monitoring are important in the evaluation of drug efficacy and prevention of side effects. The objectives of this experiment are to determine the kinetic behavior of PPF in healthy human volunteers and to identify and determine its major

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metabolite in human plasma.

MATERIALS AND METHODS

Medical examination of volunteers

Ten volunteers (age, 22.6±0.53 years; body weight, 64.2±2.33 kg; height, 172.1±1.21 cm) provided blood samples at the designed time intervals. Before drug administration, all volunteers were medically examined by doctors, and received the serological test for hemoglobin, hematocrit, white blood cells, platelets, and red blood cells. Other serum chemical test and urine examination were also made in these volunteers. The volunteers were fasted for 12 hr before administration and established a scalp-vein set in the arm or the back of the hand.

Oral administration of propentofylline tablet to volunteers

Two tablets of propentofylline (PPF, total 200 mg propentofylline, provided kindly from HD Pharmaceutical Inc.) were orally taken to volunteers fasted for 12 hours with about 200 ml of drinking water. The interval of blood sampling between volunteers was 2 min to consider blood collection time. Blank blood was withdrawn prior to the administration of PPF. After the oral administration of PPF, 8 ml of blood was collected at 0.5, 1, 1.33, 1.67, 2, 2.33, 2.67, 3, 4, 5, and 6 hr and centrifuged to obtain plasma. The plasma was stored at -70°C until the assay. At the end of blood withdrawing, antibiotics (amoxacillin, 500 mg capsule) were given to the volunteers to prevent a possible infection.

Determination of propentofylline in human plasma

The thawed plasma (1 ml) was added to a 15 ml centrifuge tube. To the tube, 1N-sodium hydroxide (0.2 ml) was added and caffeine (10 ppm×20 μl) was used as internal standard. After vortex-mixing, 5 ml of distilled diethyl ether was added and shaken on a shaker for 20 min. The centrifuged tubes were located in a freezer (-25°C, about 5 min) for the separation of organic layer. The organic layer was evaporated on drying block (Dri-Block, Techne Inc., Princeton, NJ, USA) by purging of nitrogen, and dried in a desiccator with P_2O_5/KOH. The residue dissolved in 100 μl of methanol was applied to gas chromatography/nitrogen phosphorus detector (GC/NPD) by automatic liquid sampler (HP 7673A).

Synthesis of 3-methyl-1-(5-hydroxyhexyl)-7-propylxanthine (PPFOH)

PPFOH was synthesized from PPF (from Sigma) by the reduction with sodium borohydride in methanol. Briefly, 156 mg of PPF was dissolved in 2 ml of methanol and 80 mg of sodium borohydride was reacted with PPF at 4°C and stirred overnight. The progress of the reaction was monitored by a thin layer chromatography. The reaction mixture was extracted with methylene chloride. The organic layer was washed three times with saturated sodium chloride. The layer was dried by filtration on filter paper covered with magnesium sulfate, and stored in a desiccator until the crystal formation of PPFOH. The structure of PPFOH was identified by NMR and GC/MS.

Instruments used for propentofylline and its metabolite analysis

Gas chromatograph/nitrogen phosphorus detector: A gas chromatograph (HP 5890A) connected to HP capillary column Ultra-2 (cross-linked 5% phenylmethylsilicone; 25 m×0.2 mm×0.11 μm film thickness) was used. The flow rate of carrier gas (He) was 1.08 ml/min. The split ratio was 10:1 and septum purge was in the rate of 5 ml/min. Gas flow rates for the detector of air, hydrogen, and helium (make-up) were 100, 3.5, and 30 ml/min, respectively. Both the injector and detector were set at 300°C. The gradient oven temperature was used: 180°C (0 min)/10°C/220°C (0 min)/3°C/280°C (1 min).

Gas chromatograph/mass spectroscopy/electron impact (GC/MS/EI): HP 5890A GC was coupled with HP 59970 B mass selective detector. Ionization potential was 70 eV. Transfer line temperature was 300°C. GC condition in the EI mode was very similar to that mentioned in gas chromatograph/ nitrogen phosphorus detector.

^{1}H Nuclear magnetic resonance spectroscopy (^{1}H

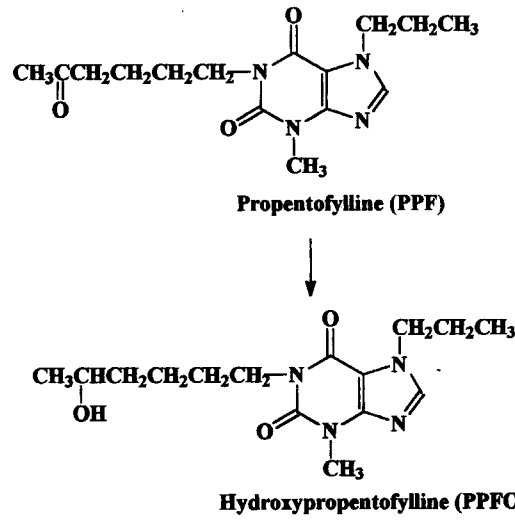

Fig. 1. The chemical structures of propentofylline (PPF) and its metabolite hydroxypropentofylline (PPFOH).

NMR): ^{1}H NMR spectra were recorded on a Varian Gemini 300 spectrometer. Chemical shifts (δ) in ppm and coupling constants in Hz are presented.

RESULTS AND DISCUSSION

Determination of PPF and PPFOH in human plasma

PPF and PPFOH (as shown in Fig. 1) were determined in plasma obtained from human volunteers. The retention times of PPF and PPFOH were about 20.6 and 20.7~20.9 min, respectively. No interfering peak was observed at this time as shown in Fig. 2 (a). Detection limit of PPF was around 5 ng/ml as shown in the chromatogram of Fig. 2 (b).

Synthesis and identification of PPFOH

PPFOH was synthesized from authentic drug PPF. PPF was reduced to PPFOH with sodium borohydride. This reaction was monitored by a thin layer chromatography. Rf of PPF was 0.80 and Rf of PPFOH was 0.56 when chloroform:methanol (10:1) of developing solvent was used. The purity (>95%) of PPFOH was

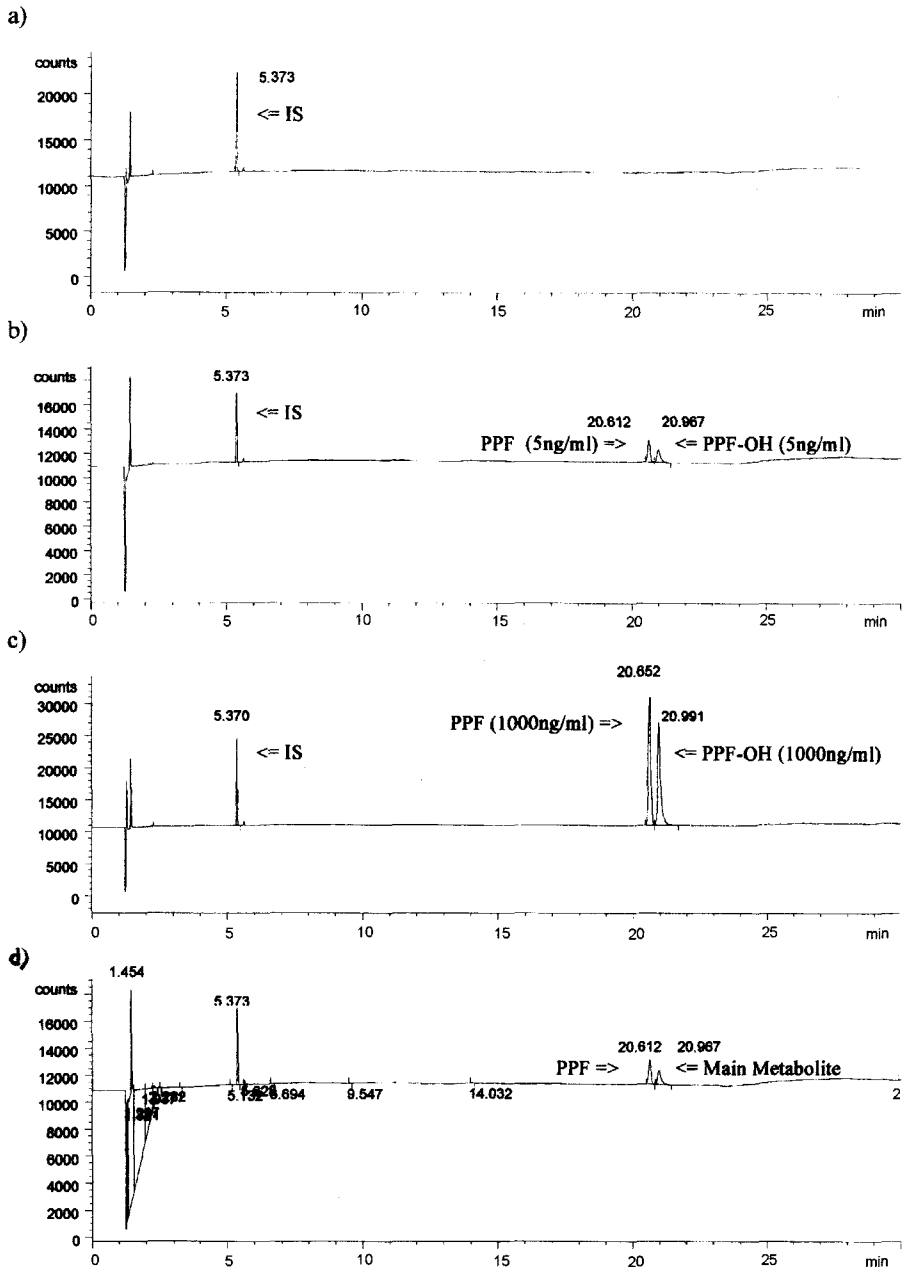

Fig. 2. GC/NPD chromatograms of (a) blank plasma with internal standard, (b) the plasma spiked authentic PPF and PPFOH (5 ng/ml, respectively), (c) the plasma spiked authentic PPF and PPFOH (1000 ng/ml, respectively), and (d) the plasma of a volunteer obtained after oral administration of PPF tablets (200 mg).

made with gas chromatography/nitrogen phosphorus detector, and monitored by mass spectroscopy/electron impact, and nuclear magnetic resonance spectroscopy. Mass spectra of PPF (Fig. 3, I) and trimethylsilylated PPF (Fig. 3, II) were interpreted and molecular weight of PPF was 306 dalton. The observed characteristic ions of PPF were 249 {M^+-CH_3COCH_2 (57)}, 221 {M^+-$CH_3COCH_2CH_2$(85)}, 208 {M^+-98; cleavage of 1-(5-oxohexyl)}, and 166 {M^+-98-42; cleavages of 1-(5-oxohexyl) and 7-propyl groups}. The molecular weight of trimethylsilylated PPF was 378 {306+TMS (72)}. The other characteristic ions were 208 {cleavage of 1-(5-oxohexyl) group}, 248 {M^+-CH_3-$CH_2CH_2CH_3$}, and 363 {M^+-15}.

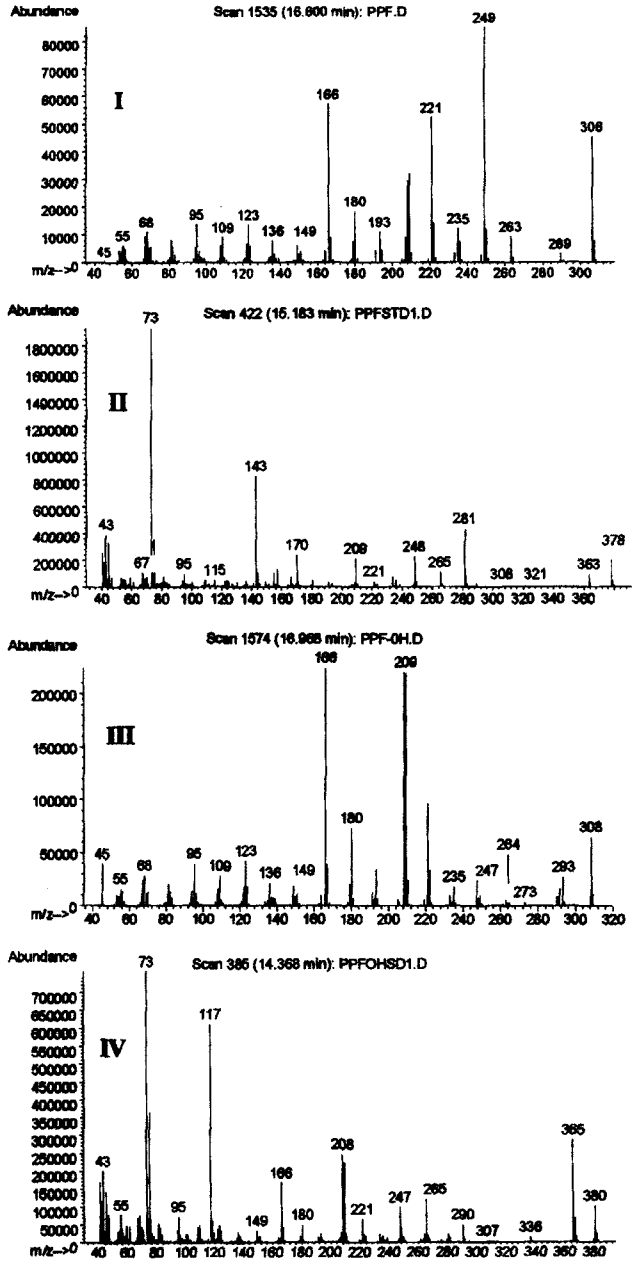

Fig. 3. Mass spectra of propentofylline and synthesized hydroxypropentofylline as its metabolite: (I) Mass spectrum of PPF; (II) Mass spectrum of trimethylsilylated PPF; (III) Mass spectrum of PPFOH; (IV) Mass spectrum of trimethylsilylated PPFOH.

The mass spectra of synthesized PPFOH and its trimethylsilylated derivative were indicated in Fig. 3, III and Fig. 3, IV, respectively. M^+ of PPFOH and trimethylsilylated PPFOH are 308 and 380 {308+TMS (72)}, respectively. The characteristic ions of m/z 293 (M^+-15), 208 {cleavage of 1-(5-oxohexyl) group}, 264 (cleavage of 7-propyl group) were observed (Fig. 3, III). In mass spectrum of trimethylsilylated PPFOH (Fig. 4, IV), the observed characteristic ions were 365 (M^+-15), 208 {cleavage of 1-(5-oxohexyl) group}, and 117 {CH_3CH-OTMS}.

PPF: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95 (t, J=7.4, 3H, $NCH_2CH_2CH_3$), 1.64~1.70 (m, 4H, CH_3COCH_2-CH_2CH_2N), 1.91 (sextet, 2H, $NCH_2CH_2CH_3$), 2.14 (s, 3H, CH_3CO), 2.50 (t, 2H, CH_3COCH_2), 3.58 (s, 3H, N-CH_3), 4.02 (t, 3H, $CH_2CH_2CH_2N$), 4.24 (t, 3H, $NCH_2CH_2CH_3$), 7.53 (s, 1H, vinyl CH).

PPFOH: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.94 (t, 3H, $NCH_2CH_2CH_3$), 1.17 (d, J=6.2, 3H, CH_3CH(OH)), 1.35~1.75 (m, 6H, CH_3CH(OH) $CH_2CH_2CH_2CH_2N$), 1.89 (sextet, 2H, $NCH_2CH_2CH_3$), 3.47 (s, 1H, OH), 3.57 (s, 3H, N-CH_3), 3.76~3.82 (m, 1H, methine), 4.01 (t, 2H, $CH_2CH_2CH_2N$), 4.24 (t, 2H, $NCH_2CH_2CH_3$), 7.53 (s, 1H, vinyl CH).

Pharmacokinetics of PPF

The principle kinetic parameters of PPF were represented in Table I. The mean plasma half-lives of PPF and PPFOH in human volunteers were 0.74 hr and 0.79 hr, respectively after oral administration of PPF. The areas under the curve (AUCs) of PPF and PPFOH were 508 and 460 ng.hr/ml, respectively. C_{max} of PPF was about 828.4 ng/ml and the peak concentration was achieved at about 2.2 hr (T_{max}). The mean plasma concentrations of PPF and PPFOH observed from volunteers were shown in Fig. 4 (n= 10). Time intervals to collect blood samples in Fig. 4 were decided from preliminary experiment (data not shown). The reasons that plasma concentrations of PPF were not detected in early phase (0~1 hr in Fig. 4) and large variation of PPF plasma concentrations

Table I. Pharmacokinetic parameters of propentofylline (PPF) and hydroxy propentofylline (PPFOH) in volunteers after oral dose of PPF tablet (200 mg)

	PPF[a]	PPFOH[a]
$AUC^{0-\infty}$, ng.hr/ml	508.5 ±101.1	460.1 ± 93.1
$AUMC^{0-\infty}$, ng.(hr)^2/ml	1206.1 ±270.8	1438.4 ±282.5
C_{max}, ng/ml	828.4 ±311.4	269.2 ± 61.9
$T_{1/2}$, β, hr	0.74± 0.08	0.79± 0.07
T_{max}, hr	2.18± 0.18	2.49± 0.18

[a]Mean±SE of 10

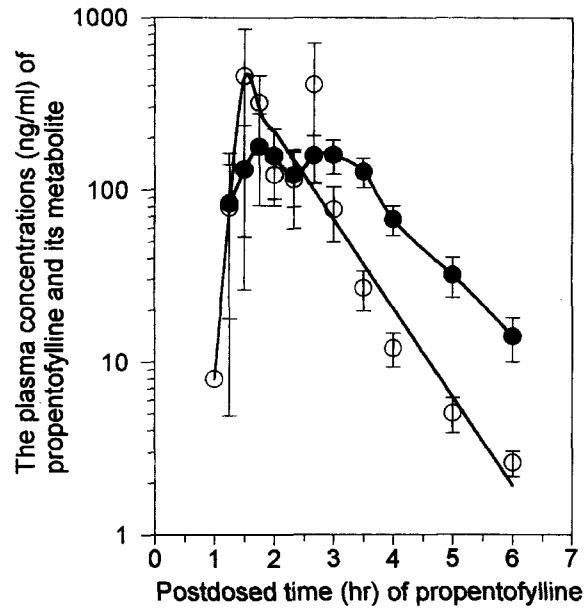

Fig. 4. The mean plasma concentrations of propentofylline and its metabolite hydroxypropentofylline from 10 volunteers (see Materials and Method section for time intervals). The plasma disappearance curve of PPF (solid line with open circles) was represented by curve-fitting (○—○: Propentofylline, ●—●: Hydroxypropentofylline).

among volunteers may be related to the dissolution rates of PPF tablets, the metabolism of PPF, and/or the sensitivity of PPF to the detector. Many drugs have been reported to show unusual peak pattern after oral administration (Shim and Lee, 1991). The unusual peak patterns may suggest the complexity in the absorption of drugs after oral administration. The plasma concentrations of PPFOH, one of the PPF main metabolite, were higher than those of PPF from 3 to 6 hr.

Taken together, PPFOH, one of the main metabolites of PPF, was synthesized and identified by GC, GC/MS, and ^{1}H-NMR. The PPFOH metabolite in human plasma was determined based on the synthesized PPFOH for authentic compound. The principle kinetic parameters of PPF were determined after oral administration. These data indicate that PPF is rapidly disappeared from human plasma due to extensive metabolism to PPFOH. The pharmacokinetic study of PPFOH and other several metabolites structurally unidentified remain to be further investigated.

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