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Interaction in Tissue Distribution between Methylphenidate and Pemoline. I. Tissue Distribution of Methylphenidate and Its Metabolite in the Rat

HAJIME KOTAKI,* FUTAMI NAKAZATO, TAKAO AOYAMA, YUKIYA SAITOH and FUJIO NAKAGAWA

Hospital Pharmacy, Faculty of Medicine, University of Tokyo, 7–3–1 Hongo, Bunkyo-ku, Tokyo 113, Japan

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Plasma, blood and tissue concentrations of methylphenidate (MPD) and its metabolite, ritalinic acid (RA), were measured after intravenous administration of MPD and RA to rats. After administration of 1 mg/kg dose of MPD, concentrations of MPD in the kidney, lung, brain and liver were remarkably higher than that in plasma. The concentration of RA in the brain was quite low. The mean blood-to-plasma concentration ratios of MPD and RA were 1.13 and 0.884, respectively, while, the mean ratio of RA after administration of 1 mg/kg of RA was 0.895. This finding indicates that the presence of MPD did not affect the blood-to-plasma concentration ratio of RA. After the RA administration, although the highest concentration of RA was found in the kidney, the concentration was only 3-fold that in plasma. From the results of distribution of MPD and RA, and of *in vitro* hydrolysis of MPD in tissues, it was suggested that MPD was hydrolyzed in all tissues examined, and also that the small amounts of RA found in the brain after MPD administration represent the results of nonenzymatic and enzymatic hydrolyses of MPD, and the direct distribution of RA. A two-compartment open model was able to fit the plasma data of MPD and RA after administration of MPD and RA, respectively. The elimination rate constant (β), steady-state volume of distribution (V_{ss}) and total body clearance (Cl) of MPD were approximately 2, 6 and 10 times larger than those of RA, respectively.

Keywords——methylphenidate; ritalinic acid; plasma concentration; blood concentration; tissue concentration; tissue-to-plasma concentration ratio; pharmacokinetics; rat

Methylphenidate (MPD), methyl *threo-dl*-2-phenyl-2-(2-piperidyl) acetate, is a central-nervous-system stimulant used for treatment of attention-deficit disorder with hyperactivity (ADD-H) in children and narcolepsy.[1] It is always necessary to control the dose and schedule of administration of MPD carefully, since administration of large amounts of the drug may increase the frequency and severity of side effects such as the development of a hallucinatory paranoid state.[1a] Pemoline (2-imino-5-phenyl-2-oxazolidin-4-one, phenylisohydantoin), which is a central-nervous-system stimulant, is also used for treatment of narcolepsy. It appears that the duration of the stimulating effect of pemoline is longer than that of MPD.[1a,2] This may be due to the difference in the disposition of these drugs.[3] Interactions between MPD and several other drugs in man have been reported,[4] and Garrettson *et al.* have reported[4a] that MPD is an inhibitor of drug-metabolizing enzymes. In recent years, concurrent use of MPD and pemoline for the treatment of narcolepsy has been recommended.[2] However, there is no report on the interaction between MPD and pemoline. For examination of the interaction between these drugs, information on the disposition of MPD after administration of MPD alone, and on that of pemoline after administration of pemoline alone are necessary. Several studies on the pharmacokinetics of MPD in man, especially in children with ADD-H, and in animals have been reported.[3a,5] Concerning the metabolism and distribution of MPD, it has

been reported[6] that distribution of MPD into rat brain was rapid, the concentration of MPD in rat brain was remarkably higher than that in plasma, and the main metabolite was the deesterified product, ritalinic acid [*threo-dl*-2-phenyl-2-(2-piperidyl)acetic acid, (RA)]. However, detailed studies on the distribution of MPD and its metabolite, RA, in tissues other than the brain and on the pharmacokinetics of RA in rats have not been reported. While, several studies have been done on the distribution, metabolism and pharmacokinetics of pemoline.[3b,c,7]

In the present study, we have examined the plasma, blood and tissue concentrations of MPD and the metabolite, RA, after intravenous administration of MPD and RA to rats.

Experimental

Chemicals——MPD hydrochloride and RA were kindly supplied by Ciba Geigy (Japan) Ltd. (Takarazuka). Ethylphenidate hydrochloride (used as an internal standard for the determination of MPD and RA concentrations) was the same as that used previously.[8] Pentafluoropropionic anhydride (PFPA) was obtained from Pierce Chemical Co. (Rockford, II). All other chemicals used were of analytical grade.

Animals——Male Wistar rats, weighing 270 to 310 g, were used. They had free access to food and water before experiments.

Blood Sample Collection——Rats were anesthetized lightly with ether, and polyethylene cannulas were inserted into the femoral artery and vein. A dose of 1 mg/kg (*i.e.* 4.3 μmol/kg) of MPD was administered intravenously. Blood samples (*ca.* 0.5 ml) were collected in heparinized tube cooled on ice *via* the arterial cannula at 2, 5, 15, 30, 60, 90, 120, 180, 240 and 300 min after administration. After each blood sampling, an equivalent volume of blood collected from other rats was transfused *via* the venous cannula. Plasma (0.2 ml) was immediately separated from the blood by centrifugation with a refrigerated centrifuge at 4300 *g* for 10 min at around 0 °C to prevent the hydrolysis of MPD, and stored at −80 °C until analysis. A dose of 1 mg/kg (*i.e.* 4.6 μmol/kg) of RA was administered intravenously, and blood samples (*ca.* 0.5 ml) were collected as above at 2, 5, 15, 30, 60, 120, 180 and 240 min after administration. Plasma (0.2 ml) was separated from the blood in the same manner as above, and stored at −80 °C until analysis.

Tissue Sample Collection——A dose of 1 mg/kg of MPD or of 1 mg/kg of RA was administered intravenously to rats. Rats were sacrificed by exsanguination at an appropriate time after administration. The liver, kidney, lung and brain were removed quickly. The tissues were transferred to tubes kept on ice and weighed. The blood sample was divided into two portions. One (0.2 ml) was used for the determination of blood concentration of MPD and RA, and the other was centrifuged for the separation of plasma. The plasma (0.2 ml) was used for determination of the concentration of MPD and RA. All tissue, blood and plasma samples were stored at −80 °C until analysis.

***In Vitro* Hydrolysis**——Liver, lung, kidney and brain pooled from three rats were homogenized on ice with 4 volumes of ice-cold 0.01 M phosphate buffer solution (pH 7.4) containing 0.15 M KCl. A 1 ml aliquot of each homogenate was transferred to a 10-ml glass-stoppered test tube. Each homogenate sample was preincubated in an incubator at 37 °C for 5 min, then 25 μl of normal saline solution containing 20 μg/ml of MPD was added and incubation was started at 37 °C. The incubation was terminated at 0.5, 1, 3 and 5 h by placing the mixture in liquid nitrogen. The mixture was stored at −80 °C until analysis.

Determination of MPD and Metabolite Concentrations——MPD and RA concentrations in plasma were determined according to the gas chromatographic-mass spectrometric (GC-MS) procedure using isobutane as a reactant gas, as described in the previous paper.[8] This method was based on the extraction of MPD with cyclohexane, derivatization of MPD with PFPA, extraction of RA with 2-propanol from the salt-saturated aqueous phase, conversion from RA to the parent drug by treatment with a mixture of methanol and sulfuric acid (2 : 1), followed by derivatization with PFPA, and separation on a glass column packed with 2% OV-17 Chromosorb W. Liver and brain were homogenized on ice with 2 volumes of ice-cold normal saline solution, and lung and kidney were homogenized with 4 volumes of the same solution. Then the GC-MS procedure described previously[8] was applied to the analysis of MPD and RA in 1 ml of each homogenate sample.

Pharmacokinetic Analysis——For calculation of the pharmacokinetic parameters, the plasma concentration data for individual animals after intravenous administration of MPD and RA were fitted to the equation, $C_t = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$ where C_t is drug concentration at time t, A and B are ordinate axis intercepts, and α and β are the corresponding first-order disposition rate constants. The logarithmic plasma concentration of MPD or RA after administration of MPD or RA was plotted against time after administration. The value of β was calculated from the linear part of the plots by the least-squares method, and the value of α was calculated by the method of residuals.[9] The steady-state volume of distribution (V_{ss}) and the total body clearance (Cl) were determined in accordance with the formulas given by Gibaldi and Perrier.[9]

Results

Plasma Concentrations of MPD and RA after Intravenous Administration

Time courses of plasma concentration of MPD and its metabolite, RA, after intravenous administration of 1 mg/kg of MPD to rats are shown in Fig. 1(a). Plasma concentration of MPD declined biexponentially after administration. The metabolite, RA, was detected in the first blood sample withdrawn from each rat at 2 min after administration of the parent drug. Maximal plasma concentration of RA was observed at 60 min after administration and the mean concentration was 124 ng/ml. The half-life of RA in the terminal phase was 70 min on average. Time courses of plasma concentration of RA after intravenous administration of 1 mg/kg of RA to rats are shown in Fig. 1(b). Plasma concentration of RA also declined biexponentially. A two-compartment open model proved adequate to describe the plasma data of MPD mentioned above and RA. The results of analysis of the data in terms of this model are summarized in Table I. The elimination rate constant (β), steady-state volume of distribution (V_{ss}) and total body clearance (Cl) of MPD were approximately 2,6 and 10 times larger than those of RA, respectively. Mean half-life of RA in the elimination phase after RA administration was 65 min. This value was in good agreement with the value obtained from the results of MPD administration as mentioned above.

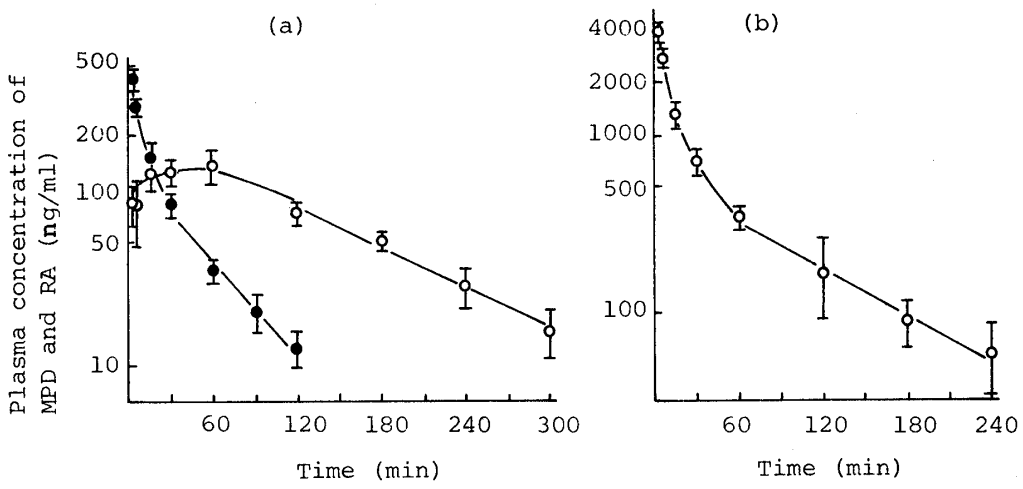

Fig. 1. Time Courses of Plasma Concentration of MPD and RA in Rats after Intravenous Administration of 1 mg/kg of MPD (a) and of 1 mg/kg of RA (b)

●, methylphenidate; ○, ritalinic acid. Each point represents the mean value of four rats and the vertical bar indicates the standard deviation.

TABLE I. Pharmacokinetic Parameters of MPD and RA after Intravenous Administration of 1 mg/kg of MPD and of 1 mg/kg of RA to Rats

Parameter	MPD Mean ± S.D.	RA Mean ± S.D.
A (ng/ml)	344.7 ± 42.1	3820.3 ± 358.5
α (h^{-1})	9.138 ± 1.308	5.696 ± 0.691
B (ng/ml)	150.6 ± 25.5	590.2 ± 49.8
β (h^{-1})	1.325 ± 0.137	0.638 ± 0.104
V_{ss} (ml/kg)	3965.7 ± 607.0	619.5 ± 56.4
Cl (ml/kg·min)	111.5 ± 16.9	10.5 ± 2.3

Each value is the mean ± S.D. of four rats.

Tissue Concentration of MPD and RA after Intravenous Administration

Plasma and tissue concentrations of MPD and the metabolite at 30, 60 and 120 min after intravenous administration of 1 mg/kg of MPD, and the tissue-to-plasma concentration ratios of the compounds are shown in Table II. Concentrations of MPD in all tissues examined were higher than those in plasma at each time. The highest concentration of MPD was found in the kidney. This tissue had the highest tissue-to-plasma concentration ratio of MPD, followed by the lung, brain and liver. The concentration of MPD in blood was also higher than that in the plasma (data not shown). The blood-to-plasma concentration ratio of MPD was fairly constant, and the value was 1.13 ± 0.01 (mean $\pm$ standard deviation (S.D.), $n=26$) in the range of plasma MPD concentration of 9.6—510 ng/ml. The metabolite, RA, was found in all tissues examined, and the concentrations in all tissues except for the brain were higher than those of RA in plasma at each time (Table II). The lung had the largest tissue-to-plasma concentration ratio of RA, and the ratio became smaller with time. The blood-to-plasma concentration ratio of RA was fairly constant, and the value was 0.884 ± 0.10 ($n=16$) in the range of plasma RA concentration of 42—151 ng/ml. Plasma and tissue concentrations of RA at 60 and 180 min after intravenous administration of 1 mg/kg of RA, and the tissue-to-plasma concentration ratios of RA are shown in Table III. The highest concentration was found in the kidney, followed by the liver and lung. In the brain, the concentration was low at each time. The blood-to-plasma concentration ratio of RA was 0.895 ± 0.15 ($n=21$) in the range of RA plasma concentration of 72—4142 ng/ml. This value was agreement with the blood-to-plasma concentration ratio of RA after administration of the parent drug as mentioned above.

TABLE II. Plasma and Tissue Concentrations of MPD and RA after Intravenous Administration of 1 mg/kg of MPD to Rats, and Tissue-to-Plasma Concentration Ratios of MPD and RA

Time (min)	Concentration (ng/ml or ng/g)[a]		Tissue/plasma ratio[a]	
	MPD Mean ± S.D.	RA Mean ± S.D.	MPD Mean ± S.D.	RA Mean ± S.D.
Plasma				
30	71.2 ± 11.9	118.5 ± 30.2	—	—
60	33.8 ± 15.5	124.0 ± 23.5	—	—
120	16.3 ± 8.1	71.5 ± 10.6	—	—
Liver				
30	288.2 ± 156.3	581.8 ± 233.8	4.4 ± 1.3	4.7 ± 2.0
60	143.3 ± 54.8	309.4 ± 97.8	4.5 ± 0.7	3.0 ± 0.4
120	77.8 ± 42.0	222.7 ± 14.7	4.9 ± 1.6	3.2 ± 0.4
Kidney				
30	3125.7 ± 889.1	503.6 ± 81.4	43.6 ± 8.1	4.6 ± 2.0
60	1182.9 ± 608.6	415.4 ± 151.5	34.5 ± 5.7	4.1 ± 1.0
120	510.9 ± 226.9	383.6 ± 70.6	33.8 ± 1.0	5.4 ± 1.2
Lung				
30	2276.9 ± 669.7	1576.8 ± 439.6	31.6 ± 5.2	15.7 ± 2.7
60	1208.7 ± 621.4	837.2 ± 247.2	34.5 ± 5.7	7.5 ± 1.3
120	438.8 ± 72.6	486.5 ± 173.8	29.6 ± 6.7	6.7 ± 1.9
Brain				
30	664.5 ± 71.8	54.4 ± 10.7	9.1 ± 0.8	0.5 ± 0.1
60	249.3 ± 94.7	47.5 ± 4.9	7.6 ± 1.2	0.4 ± 0.0
120	122.3 ± 42.9	52.4 ± 6.9	7.8 ± 0.8	0.7 ± 0.1

a) Mean ± S.D. of three to five rats.

TABLE III. Plasma and Tissue Concentrations of RA after Intravenous Administration of 1 mg/kg of RA to Rats, and Tissue-to-Plasma Concentration Ratios of RA

Tissue	Time (min)	RA concentration[a] (ng/ml or ng/g) Mean ± S.D.	Tissue/plasma ratio[a] Mean ± S.D.
Plasma	60	615.3 ± 341.4	—
	180	114.2 ± 40.6	—
Liver	60	1007.0 ± 581.2	1.6 ± 0.1
	180	155.9 ± 33.6	1.4 ± 0.2
Kidney	60	1932.3 ± 1007.4	3.2 ± 0.5
	180	428.8 ± 315.9	3.4 ± 2.1
Lung	60	681.5 ± 189.5	1.0 ± 0.2
	180	197.7 ± 112.0	2.2 ± 2.1
Brain	60	18.3 ± 8.3	0.03 ± 0.01
	180	5.0 ± 1.5	0.05 ± 0.00

a) Mean ± S.D. of three rats.

Hydrolysis of MPD in Tissue Homogenate

Hydrolysis of MPD in the liver, kidney, lung and brain homogenates at 37 °C was examined. MPD was degraded according to a pseudo-first-order rate process in all tissue homogenates. The pseudo-first-order rate constants in the liver, kidney, lung and brain homogenates were 4.64×10^{-3}, 1.61×10^{-3}, 2.33×10^{-3} and 7.25×10^{-4} min^{-1}, respectively. That in 0.1 M phosphate buffer solution (pH 7.4) as the control was 4.89×10^{-4} min^{-1}. The deesterified product, RA, was found in all tissue homogenate samples at each time, and the sum (as amount of MPD) of the amounts of MPD and RA was approximately equal to the initially added amount of MPD at each time.

Discussion

The data reported here demonstrate that tissue accumulation of MPD is larger than that of RA. This finding is reflected in the fact that the steady-state volume of distribution (V_{ss}) of MPD calculated on the basis of the two-compartment open model was larger than that of RA. The tissue-to-plasma concentration ratios of RA at each time after administration of the parent drug were larger in all tissues examined than those of RA after RA administration. These results suggest that MPD is hydrolyzed in the tissues. Further, from the results of *in vitro* hydrolysis experiments, it is suggested that MPD was hydrolyzed in tissues and the hydrolyzed of MPD in tissues occurred by way of a nonenzymatic process as well as an enzymatic process. Although the tissue-to-plasma concentration ratio of RA after MPD administration was largest in the lung, the *in vitro* hydrolysis rate of MPD was largest in the liver. This discrepancy is considered to have arisen because the metabolism of MPD *in vivo* may occur extensively in the liver, since *threo-dl*-2-(*p*-hydroxyphenyl)-2-(2-piperidyl)acetic acid (*p*-OH RA), its methyl ester and the glucuronide conjugate of *p*-OH RA have been found as metabolites of MPD other than RA in rat urine,[6a] although metabolism of MPD in *in vitro* system used in this study occurred only by a hydrolytic process.

The lung-to-plasma concentration ratio of RA after MPD administration was remarkably larger than that after RA administration. Further, in our preliminary examination, the lung-to-plasma concentration ratio of RA at 5 min after MPD administration was about 40. One reason for this difference of the ratio may be that MPD might be subject to first-pass metabolism in the lung, but this requires further investigation.

RA, which has little or no pharmacological activity,[10] was found at low levels in the

brain at each time after MPD administration. The brain-to-plasma concentration ratio of RA after MPD administration was less than 0.7. These results support the data of Faraj *et al.*[6a] Segal *et al.*[6b] suggested that small amounts of RA found in the brain after intravenous administration of MPD may represent the result of nonenzymatic hydrolysis of administered MPD. However, our data show that RA found in the brain represents not only the result of nonenzymatic hydrolysis of MPD in the brain, but also the result of enzymatic hydrolysis of MPD and direct distribution of RA.

The blood-to-plasma concentration ratio of RA after the RA administration was in agreement with that of RA after the MPD administration. This finding indicates that the presence of MPD did not affect the blood-to-plasma concentration ratio of RA.

It has been reported[3] that the elimination half-life of MPD in plasma after oral administration of MPD to normal adults was relatively short, 2—3 h, and that of pemoline was long, about 7—12 h. In the rat, as shown in the present study, the half-life of MPD in plasma was also relatively short, about 30 min. While there is no information on the elimination half-life of pemoline in rats, in our preliminary experiments, the half-life of pemoline in plasma after intravenous administration at a dose of 0.5 mg/kg was relatively long, 3.1 ± 0.4 h (mean $\pm$ S.D., $n=4$). Therefore, rats may be suitable as an experimental animal for study on the interaction between MPD and pemoline. On the basis of the present results, further studies on the interaction of MPD or RA and pemoline are in progress in rats. These results will be reported elsewhere.

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