[Chem. Pharm. Bull. 36(11)4560—4566(1988)]

Interactions in Tissue Distribution between Methylphenidate and Pemoline. II. Effects of Methylphenidate or Its Metabolite on Plasma and Tissue Concentrations of Pemoline in the Rat

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

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

(Received April 13, 1988)

Interactions between methylphenidate (MPD) or its metabolite, ritalinic acid (RA), and pemoline in rats were investigated. At 20 min after intravenous administration of 3 mg/kg dose of pemoline as a loading dose, constant infusion at a rate of 139 μg/h of the drug was carried out to give steady-state plasma level. In intravenous administration of 1 mg/kg dose of RA during the constant infusion of pemoline, the plasma concentration of pemoline before and after the administration of RA did not change. Steady-state pemoline also did not affect the pharmacokinetics of RA. On the other hand, in intravenous administration of 1 mg/kg dose of MPD during the constant infusion of pemoline, the plasma concentration of pemoline slightly increased within 15 min after the administration of MPD. Thereafter the concentration of pemoline returned to the level before the superimposition of MPD within 60 min. The liver-, kidney-, lung-, muscle- and blood cell-to-plasma concentration ratios of pemoline at 5 min after the administration of MPD became smaller compared with those of pemoline in the absence of MPD, and those of pemoline at 60 min after the administration were approximately the same as those of pemoline in the absence of MPD. Steady-state pemoline did not affect the pharmacokinetics and tissue distribution of MPD. The increase of plasma concentration of pemoline caused by the superimposition of MPD may result in the displacement of pemoline from tissues and blood cell and/or MPD may inhibit the metabolism of pemoline.

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

Methylphenidate [methyl *threo-dl*-2-phenyl-2-(2′-piperidyl) acetate, MPD] and pemoline (2-imino-5-phenyl-2-oxazolidin-4-one, phenylisohydantoin) are central-nervous-system stimulants, and are used in the treatment of narcolepsy.[1] In recent years, these drugs have been used concomitantly. On the concomitant use of these drugs, it has been recommended[2] that pemoline should be administered once a day, usually in the morning, and MPD twice a day, usually in the morning and afternoon, in order to avoid the side effect of nocturnal insomnia. This was based on the findings[3] that the elimination half-life of pemoline in human plasma is relatively long, about 7—12 h, and that of MPD is short, about 2—3 h. However, it is always necessary to control the dose and schedule of administration of MPD carefully, because of the appearance of severe side effects such as the development of a hallucinatory paranoid state.[1a]

On the other hand, interactions between MPD and several other drugs have been reported,[4] and Garrettson *et al.*[4a] found that MPD is an inhibitor of drug metabolizing enzymes. However, there is no information on the interaction between MPD and pemoline. Therefore, whether disposition of MPD and /or pemoline is changed by the concomitant use of these drugs remains unclear. Several studies have been reported[3a,5] on the pharmacoki-

netics and distribution of MPD and its main metabolite, ritalinic acid [*threo-dl*-2-phenyl-2-(2′-piperidyl) acetic acid, RA], after administration of MPD or RA alone and on those of pemoline in animals after administration of pemoline alone.

In the rat, the elimination half-life of pemoline was longer than that of MPD[5a] and this pattern was similar to that in man. Our approach in this study has been to administer enough pemoline to achieve a steady-state level, and then to examine the change of pemoline, MPD and RA concentrations in plasma and tissues by superimposition of MPD or RA.

Experimental

Materials and Reagents——MPD hydrochloride and RA were kindly supplied by Ciba-Geigy (Japan) Ltd. (Takarazuka), and pemoline by Sanwa Kagaku Kenkyusho Co. (Nagoya). Ethylphenidate hydrochloride was the same as that used previously.[6] 5-Methyl-5-phenylhydantoin was obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals used were of analytical grade.

Experimental Animals——Male Wistar rats, weighing 270 to 320 g, with free access to food and water, were used in this study.

Preparation of Solution for Administration——Solution for intravenous injection containing 832 μg/ml of MPD and solution for intravenous injection containing 832 μg/ml of RA were prepared by dissolving the compounds in normal saline solution and normal saline solution containing 0.008 M HCl, respectively. Solution for intravenous injection containing 3 mg/ml of pemoline was prepared by dissolving this compound in 0.04 M NaOH aqueous solution by means of sonication. This solution was used immediately after the preparation, since pemoline in the alkaline solution was gradually decomposed.[5b] Solution for constant infusion of pemoline was prepared as follows: a solution containing 3 mg/ml of pemoline in 0.04 M NaOH aqueous solution was prepared in the same manner as above. Then to 1 ml of this solution, 1 ml of 0.04 M HCl aqueous solution and 9.5 ml of normal saline solution were added. The pH of the mixture was in the range of 6.0—6.5. In a stability test, degradation of the drug in this mixture was not observed for at least 48 h at room temperature (25—26 °C).

Drug Administration and Sample Collection during Constant Infusion of Pemoline——Rats were anesthetized lightly with ether, and polyethylene cannulas were inserted into the right and left femoral veins, and left femoral artery. After the surgery, the rats were left for about 1.5 h in order to recover from anesthesia.

Plasma Preparation: A dose of 3 mg/kg (*i.e.* 17.0 μmol/kg) of pemoline as a loading dose was injected rapidly into the rats *via* the cannula inserted into left femoral vein. At 20 min after the injection, pemoline was infused *via* the cannula inserted into the right femoral vein at a constant rate of 139 μg/h (dosing solution was 0.534 ml/h). At 100 min after starting constant infusion of pemoline, a dose of 1 mg/kg (*i.e.* 4.3 μmol/kg) of MPD or of 1 mg/kg (*i.e.* 4.6 μmol/kg) of RA was injected rapidly *via* the cannula inserted into left femoral vein. In the case of administration of MPD as above, blood samples (*ca.* 0.5 ml) were taken in heparinized micro tubes cooled on ice *via* the cannula inserted into the femoral artery at 20, 10 and 5 min before administration of MPD and 2, 5, 15, 30, 60, 90 and 120 min after administration of MPD. Plasma was immediately separated from the blood with a refrigerated centrifuge at 4300 ***g*** for 10 min at around 0 °C to prevent hydrolysis of MPD. After each blood sampling, an equivalent volume of blood collected from other rats was transfused *via* the venous cannula. In the case of administration of RA as above, blood samples (*ca.* 0.5 ml) were taken at 20, 10 and 5 min before administration of RA and 2, 5, 15, 30, 60, 120, 180 and 240 min after administration of RA, and the transfusion of blood was carried out after each blood sampling. Plasma was immediately separated from the blood in the same manner as above. The plasma sample was divided into two sections. One (20 μl) was used for determination of the plasma concentration of pemoline and the other (0.2 ml) for that of the plasma concentration of MPD or RA. Each plasma sample was stored at −80 °C until analysis.

Blood and Tissue Sample Collection: Intravenous injection of the loading dose of pemoline and constant infusion of pemoline were carried out in the same manner as above. Rats were killed by exsanguination at 100 min after starting constant infusion of pemoline, or a dose of 1 mg/kg of MPD was injected into the rats *via* the cannula inserted into the right femoral vein at 100 min after starting constant infusion of pemoline. In the case of the administration of MPD during constant infusion of pemoline, rats were killed at 5 min or 60 min after the MPD administration. The liver, kidney, lung, muscle and brain were quickly removed. Each tissue was divided into two sections. One was used for determination of the pemoline concentration and the other for that of the MPD concentration. These tissues were transferred into tubes kept on ice and weighed. Blood sample collected was divided into two parts. One was used for determination of the blood concentrations of MPD and pemoline, and the other was centrifuged for the separation of plasma, and the plasma was used for determination of the concentrations of MPD and pemoline. In other experiments, a dose of 1 mg/kg of MPD alone was administered intravenously to rats. Rats were killed by exsanguination at 5 min after the administration, each tissue was removed, and the tissue and blood samples were each divided for determination of MPD and pemoline concentrations. Each plasma, blood and tissue samples

were stored at −80 °C until analysis.

Calculation of Blood Cell Concentration of Pemoline: The blood cell concentration of pemoline was calculated from the blood concentration (C_b), plasma concentration (C_p) and hematocrit value (H_t) using the formula; $[C_b - C_p(1-H_t)]/H_t$.

Sample Preparation and Analytical Methods——MPD and Its Metabolite: A gas chromatographic-mass spectrometric (GC-MS) procedure[6] using isobutane as a reactant gas was used to determine the plasma and blood concentrations of MPD and the metabolite, RA. Liver and brain were homogenized on ice with 2 volumes of ice-cold normal saline solution, muscle with 3 volumes, and lung and kidney with 4 volumes of the same solution. Then the GC-MS procedure described previously[6] was applied to the analysis of MPD and RA in 1 ml of each homogenate sample. Ethylphenidate hydrochloride was used as an internal standard. The quantitation limits of MPD in plasma, blood and tissue homogenate were 0.5, 0.5 and 5 ng, respectively, and those of the metabolite were 2.5, 2.5 and 7.5 ng, respectively.

Pemoline: A high-performance liquid chromatographic (HPLC) procedure[5b] was used to determine the plasma, blood and tissue concentrations of pemoline. Liver, kidney, lung and brain were homogenized on ice with 2 volumes of ice-cold normal saline solution, and muscle with 3 volumes of the same solution. This HPLC method was based on extraction with dichloromethane, separation on a reversed-phase column, and ultraviolet (UV) detection at 215 nm. 5-Methyl-5-phenylhydantoin was used as an internal standard. The quantitative limits of pemoline in plasma, blood and tissue homogenate were 2, 3 and 20 ng, respectively.

Pharmacokinetic Calculations——The plasma data after intravenous administration of MPD and RA were analyzed based on a two-compartment open model. The value of β was determined from the linear part of the plots by the least-squares method, and the value of α was obtained by the residual method.[7] The steady-state volume of distribution (V_{ss}) and the total body clearance (Cl) were determined according to the formulas given by Gibaldi and Perrier.[7]

Results

Administration of MPD during Constant Infusion of Pemoline

When rats received an intravenous injection of a loading dose of 3 mg/kg of pemoline, followed 20 min later by a constant infusion of the drug at the rate of 139 μg/h, the plasma concentration of pemoline became relatively constant within 90 min after starting the infusion.

Time courses of plasma concentration of pemoline before and after intravenous administration of a 1 mg/kg dose of MPD during constant infusion of pemoline are shown in Fig. 1. The plasma concentration of pemoline slightly increased within 15 min after the superimposition of MPD, and the highest concentration of pemoline was observed at 5 min. Thereafter the concentration of pemoline decreased gradually, and approached the level before the superimposition of MPD within 60 min. From a comparison of the concentration of pemoline before and at 5 min after the superimposition, the rate of increase of pemoline concentration was 0.10 ± 0.04 (mean ± standard deviation (S.D.), $n=6$). Figure 2 shows the tissue-to-plasma concentration ratios of pemoline in the absence of and in the presence of MPD during constant infusion of pemoline. The blood cell-, liver-, kidney-, lung- and muscle-to-plasma concentration ratios of pemoline at 5 min after superimposition of MPD were significantly smaller than those of pemoline in the absence of MPD (Student's t-test; $p<0.05$). However, the tissue-to-plasma concentration ratio in the brain was not changed by the superimposition. From a comparison of the mean tissue-to-plasma concentration ratios of pemoline in the absence of MPD and those of pemoline at 5 min after the superimposition, the rate of decrease of the concentration ratio was the largest in the blood cells, followed by the kidney and muscle (these values were 0.2, 0.17 and 0.13, respectively). The blood cell-, kidney-, lung- and muscle-to-plasma concentration ratios of pemoline at 5 min after superimposition of MPD were significantly smaller than those of pemoline at 60 min after the superimposition ($p<0.05$). The tissue-to-plasma concentration ratios of pemoline in all tissues at 60 min after the superimposition were not significantly different from those of pemoline in the absence of MPD ($p>0.05$).

Plasma concentration of MPD after superimposition of MPD during constant infusion

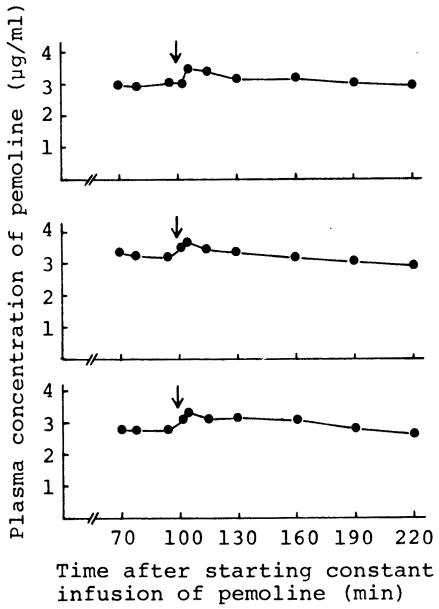

Fig. 1. Time Courses of Plasma Concentration of Pemoline before and after Intravenous Administration of 1 mg/kg Dose of MPD during Constant Infusion of Pemoline into Three Rats

The constant infusion of pemoline was started at 20 min after intravenous administration of pemoline, and MPD was administered at 100 min after starting the infusion of pemoline. The arrow indicates the time at which MPD was administered intravenously.

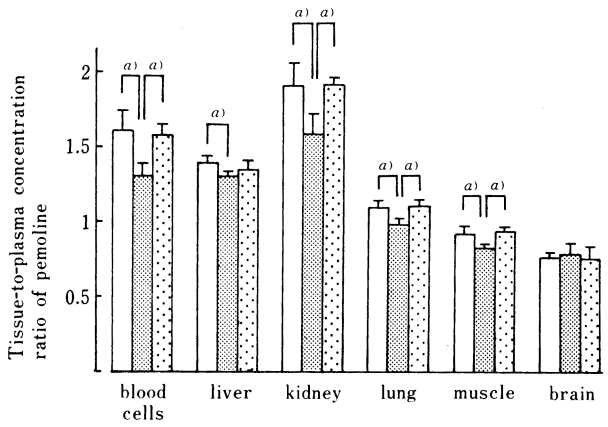

Fig. 2. Comparison of Tissue-to-Plasma Concentration Ratios of Pemoline at 100 min after Starting Constant Infusion of Pemoline into Rats, at 5 min and at 60 min after Superimposition of MPD

□, in the absence of MPD; ▩, 5 min after superimposition of MPD; ⊡, 60 min after superimposition of MPD. For further details, see the legend to Fig. 1. Bars represent mean ± S.D. of four rats. *a*) Significantly different from the tissue-to-plasma concentration ratio of pemoline in the absence of MPD, or from that of pemoline at 60 min after superimposition of MPD ($p<0.05$).

TABLE I. Pharmacokinetic Parameters of MPD and RA after Intravenous Administration of 1 mg/kg of MPD and of 1 mg/kg of RA during Constant Infusion of Pemoline into Rats

Parameter	MPD[a)]	RA[a)]
A (ng/ml)	341.2 ± 47.6	4029.4 ± 636.9
α (h^{-1})	9.707 ± 1.370	5.712 ± 0.588
B (ng/ml)	161.1 ± 21.4	540.7 ± 78.4
β (h^{-1})	1.432 ± 0.280	0.580 ± 0.048
V_{ss} (ml/kg)	3778.0 ± 168.0	619.5 ± 56.4
Cl (ml/kg·min)	114.0 ± 27.1	10.3 ± 1.6

MPD or RA was administered at 100 min after starting the infusion of pemoline. Pharmacokinetic parameters were calculated based on a two-compartment open model.[7)] *a*) Each value is the mean ± S.D. of four rats.

of pemoline declined biexponentially. The results of analysis of the plasma data in terms of a two-compartment open model are summarized in Table I. The values of A, α, B, β, V_{ss} and Cl of MPD were not significantly different from those after administration of MPD alone reported previously,[5a)] which were 344.7 ng/ml for A, 9.138 h^{-1} for α, 150.6 ng/ml for B, 1.325 h^{-1} for β, 3965.7 ml/kg for V_{ss} and 111.5 ml/kg·min for Cl on average ($p>0.05$).

TABLE II. Tissue Concentrations of MPD and RA, and Tissue-to-Plasma Concentration Ratios at 5 min after Administration of 1 mg/kg of MPD Alone to Rats

Tissue	Concentration (ng/ml or ng/g)		Tissue/plasma concentration ratio	
	MPD[a)]	RA[a)]	MPD[a)]	RA[a)]
Blood cells	433.9± 98.9	57.4± 12.7	1.2±0.2	0.8±0.1
Liver	655.1± 91.4	637.2± 75.4	2.3±0.5	9.6±3.4
Kidney	15165.1±1544.6	343.2±106.5	45.8±9.5	4.3±1.2
Lung	7023.8± 676.6	2581.0±393.2	18.6±3.7	37.3±8.0
Brain	1963.9± 300.1	69.9± 23.1	5.8±1.6	0.8±0.3
Muscle	555.0± 105.3	50.3± 17.6	1.6±0.2	0.8±0.2

a) Each value is the mean±S.D. of three to five rats.

TABLE III. Tissue Concentrations of MPD and RA, and Tissue-to-Plasma Concentration Ratios at 5 min after Administration of 1 mg/kg of MPD at 100 min after Starting Constant Infusion of Pemoline to Rats

Tissue	Concentration (ng/ml or ng/g)		Tissue/plasma concentration ratio	
	MPD[a)]	RA[a)]	MPD[a)]	RA[a)]
Blood cells	421.4± 87.4	62.0± 9.5	1.3±1.2	0.8±0.1
Liver	698.1± 6.2	566.5± 95.0	2.2±0.2	8.6±1.0
Kidney	15823.3± 943.2	288.7± 39.1	48.8±2.4	4.4±0.6
Lung	7648.3±1142.2	2801.8±441.2	23.4±2.3	42.7±4.2
Brain	2144.4± 233.1	52.5± 24.5	6.6±0.6	0.8±0.3
Muscle	491.9± 60.5	32.1± 3.9	1.5±0.3	0.5±0.1

a) Each value is the mean±S.D. of three to five rats.

Tables II and III show the tissue concentrations of MPD and the metabolite, RA, and the tissue-to-plasma concentration ratios at 5 min after intravenous administration of MPD alone (Table II) and those of MPD and RA at 5 min after superimposition of MPD (Table III). There was no significant difference between the blood cell-, liver-, kidney-, lung-, brain- and muscle-to-plasma concentration ratios of MPD after the superimposition and those of MPD after administration of MPD alone, or between the tissue-to-plasma concentration ratios of RA in their tissues after the superimposition and those of RA after administration of MPD alone ($p > 0.05$).

Administration of RA during Constant Infusion of Pemoline

Time courses of plasma concentration of pemoline before and after intravenous administration of 1 mg/kg dose of RA during constant infusion of pemoline are shown in Fig. 3. No change of plasma concentration of pemoline by superimposition of RA was observed. The plasma concentration of RA after the superimposition declined biexponentially. The results of analysis of the plasma data are summarized in Table I. The values of A, α, B, β, V_{ss} and Cl of RA were not significantly different from those after administration of RA alone described previously,[5a)] which were 3820.3 ng/ml for A, 5.696 h^{-1} for α, 590.2 ng/ml for B, 0.638 h^{-1} for β, 619.5 ml/kg for V_{ss} and 10.5 ml/kg·min for Cl on average ($p > 0.05$).

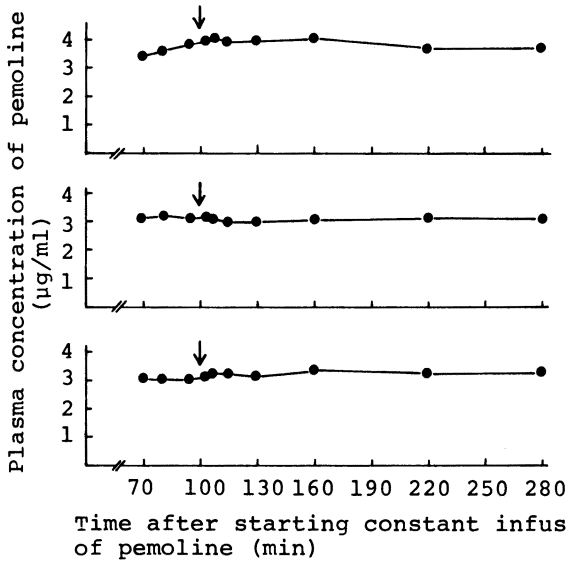

Fig. 3. Time Courses of Plasma Concentration of Pemoline before and after Intravenous Administration of 1 mg/kg Dose of RA during Constant Infusion of Pemoline into Three Rats

The constant infusion of pemoline was started at 20 min after intravenous administration of pemoline, and RA was administered at 100 min after starting the infusion of pemoline. The arrow indicates the time at which RA was administered intravenously.

Discussion

It has been reported[4] that MPD causes an increase in the plasma concentration of several other drugs such as primidone, phenytoin, phenobarbital, imipramine and desmethylimipramine in man. The mechanism was suggested to involve the inhibition of hepatic drug-metabolizing enzyme systems.[4] In the present study, it was found that MPD increased the plasma concentration of pemoline in rats. Further, it was found that MPD altered the tissue distribution of pemoline (Fig. 2). Two main reasons for this may be considered. One is displacement of pemoline from the blood cell and tissues by MPD. This displacement might be a difference in binding affinity of tissue proteins for the two drugs. The other possible reason is inhibition of metabolism of pemoline by MPD, that is, although plasma concentration of pemoline is elevated by the inhibition at 5 min after the superimposition of MPD, the reequilibrium between the plasma and tissue of pemoline might be not still attained by that time. Further studies on the mechanism of the interaction are in progress. Although it is of interest that the brain-to-plasma concentration ratio of pemoline was not changed by the administration of MPD, the significance of this finding remains equivocal. Perel *et al.*[8] have reported in an abstract that the blood concentration of thiopental after intravenous administration of thiopental to dogs significantly increased within 10 min after concomitant administration of MPD and thereafter the level of thiopental returned to the original value within 20 to 40 min. This finding is similar to our results on MPD and pemoline. On the other hand, although studies on the plasma-protein binding of MPD and RA in rats have not been reported, it has been found[9] that the bound fraction of MPD to human plasma protein and human albumin was relatively low, 16.2 and 12%, respectively, and that of RA to human albumin was 9%. In our preliminary studies of the plasma-protein binding of pemoline by the ultrafiltration technique, it was shown that the fraction of the drug bound to plasma protein of rats was relatively low, 15.5—16.6%, in the range of 0.5—5 μg/ml of pemoline. Therefore, it is considered that the contribution of plasma-protein binding of MPD and RA to the disposition of pemoline may be minor.

References

1) *a*) Y. Honda, Y. Hishikawa and Y. Takahashi, *Curr. Ther. Res.*, **25**, 288 (1979); *b*) Y. Honda and Y. Hishikawa,

ibid., **27**, 429 (1980); *c*) D. D. Daly and R. E. Yoss, *Mayo Clin. Proc.*, **31**, 620 (1956); *d*) R. E. Yoss and D. D. Daly, *Neurology*, **9**, 171 (1959).
2) Y. Honda, S. Iijima, Y. Hishikawa and F. Nakagawa, *Jpn. Pharmacol. Ther.*, **14**, 229 (1986); Y. Honda, personal communication.
3) *a*) W. Wargin, K. Patrick, C. Kilts, C. T. Gualtieri, K. Ellington, R. A. Mueller, G. Kraemer and G. R. Breese, *J. Pharmacol. Exp. Ther.*, **226**, 382 (1983); *b*) N. P. E. Vermeulen, M. W. E. Teunissen and D. D. Breimer, *Br. J. Clin. Pharmacol.*, **8**, 459 (1979); *c*) K. Nishihara, Y. Kohda, Y. Saitoh, F. Nakagawa and Y. Honda, *Ther. Drug Monit.*, **6**, 232 (1984).
4) *a*) L. K. Garrettson, J. M. Perel and P. G. Dayton, *J. Am. Med. Assoc.*, **207**, 2053 (1969); *b*) R. N. Wharton, J. M. Perel, P. G. Dayton and S. Malitz, *Am. J. Psychiatry*, **127**, 1619 (1971).
5) *a*) H. Kotaki, F. Nakazato, T. Aoyama, Y. Saitoh and F. Nakagawa, *Chem. Pharm. Bull.*, **36**, 3190 (1988); *b*) T. Aoyama, H. Kotaki, Y. Saitoh and F. Nakagawa, *J. Chromatogr.*, **430**, 351 (1988); *c*) J. L. Segal, R. F. Cunningham, P. G. Dayton and Z. H. Israili, *Drug Metab. Dispos.*, **4**, 140 (1976); *d*) B. A. Faraj, Z. H. Israili, J. M. Perel, M. L. Jenkins, S. G. Holtzman, S. A. Cucinell and P. G. Dayton, *J. Pharmacol. Exp. Ther.*, **191**, 535 (1974).
6) K. Nakajima, H. Kotaki, Y. Saitoh and F. Nakagawa, *Chem. Pharm. Bull.*, **34**, 1701 (1986).
7) M. Gibaldi and Perrier, "Drugs and Pharmaceutical Science, Vol. 1. Pharmacokinetics," 1st ed., Marcel Dekker, Inc., New York, 1975, pp. 45—86, 175—185.
8) J. M. Perel, L. Brand, S. Heiber and L. C. Mark, *Clin. Res.*, **20**, 411 (1972).
9) B. L. Hungund, J. M. Perel, M. J. Hurwic, J. Sverd and B. G. Winsberg, *Br. J. Clin. Pharmacol.*, **8**, 571 (1979).