Pharmaceutical Research, Vol. 10, No. 1, 1993

Research Article

Enantioselective Pharmacokinetics of *dl-threo*-Methylphenidate in Humans

Nuggehally R. Srinivas,[1,2] John W. Hubbard,[1] Elarien D. Korchinski,[1] and Kamal K. Midha[1,3]

Received October 21, 1991; accepted March 20, 1992

A definitive enantioselective pharmacokinetic evaluation of *dl-threo*-methylphenidate (MPH) was carried out in 11 healthy volunteers, all of whom received, in a randomized crossover design, three oral administrations of MPH: immediate release (IR), slow release (SR), and SR chewed before swallowing (CH). In addition, all subjects received MPH intravenously (IV) on a separate occasion. Both plasma and urine samples were collected for up to 16 hr after each drug administration. Significant enantioselective differences were found in pharmacokinetic parameters such as CL, MRT, Vd_{ss}, AUC_0^∞, and $t_{1/2}$. A profound distortion of the enantiomeric ratio for MPH (d ≫ l) was evident in all plasma samples harvested after oral administration. After IV MPH, however, there was no significant distortion in the plasma d/l ratio until 1.5 hr after dosing, whereafter there was a divergence of the plasma levels of the enantiomers. After oral administration of *dl*-MPH, the absolute bioavailability (*F*) of *d*-MPH was 0.23 and that of *l*-MPH was 0.05. There were no significant differences in renal clearance for *d*- or *l*-MPH after oral or IV administration, although the fraction of the dose excreted unchanged in the urine was significantly greater after IV MPH. These data suggest that enantioselective differences in the pharmacokinetics of oral MPH are the result of enantioselectivity in presystemic metabolism rather than in renal excretion, such that *l*-MPH is preferentially converted into *l*-ritalinic acid. Finally, it was found that chewing the slow release formulation led to a pharmacokinetic profile very similar to that of MPH-IR, suggesting that MPH-SR should not be prescribed for children who chew tablets.

KEY WORDS: enantioselective pharmacokinetics; *dl-threo*-methylphenidate; slow-release methylphenidate; intravenous methylphenidate; immediate-release methylphenidate.

INTRODUCTION

In recent years, numerous articles addressing the issue of chirality in both pharmacokinetic and pharmacodynamic profiles of racemic drugs have shown that failure to recognize stereoselectivity in therapeutic monitoring of racemates may lead to gross misinterpretation of the results (1–6). Although separation and quantitation of optical isomers pose a challenge, the growing awareness of the importance of chirality has provided an impetus for the development of enantioselective assays.

dl-threo-Methylphenidate hydrochloride (MPH; Ritalin) is prescribed widely in North America for the treatment of children with attention-deficit hyperactivity disorder (ADHD). The dosage forms of MPH that have been used in the clinical management of ADHD include both the immediate-release (IR) and the slow-release (SR) formulations. Although MPH-IR has been shown to be clinically beneficial (7,8), the formulation gives an effect of such short duration that many children need to take a second dose before afternoon school. These children usually require the direct supervision of an adult to ensure compliance. An alternative strategy involves use of the MPH-SR dosage form, which is alleged to have a duration of action of approximately 8 hr (8). Thus, a single morning dose of MPH-SR may be sufficient to provide medication for a full school day.

MPH is extensively metabolized to its de-esterified product (9–11), commonly known as ritalinic acid (RA), in both humans and animals. Although, MPH has two centers of chirality, the drug used in therapy comprises only the threo pair of enantiomers, which has been demonstrated to be more potent pharmacologically than the corresponding erythro racemate (12,13). Moreover, *d-threo*-MPH has been shown to be more potent than the *l*-antipode (14–16).

It is only in recent years that procedures have been developed for the analysis of MPH enantiomers in biological fluids (17–20). Pharmacokinetic studies carried out in children with ADHD (17,21) and human adults (18–20,22) have indicated that plasma levels of *d*-MPH at each sampling time

[1] Colleges of Pharmacy and Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada.

[2] Present address: Bristol Myers Squibb Company, Pharmaceutical Research Division, P.O. Box 4755, Syracuse, New York 13221-4775.

[3] To whom correspondence should be addressed at College of Pharmacy, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0 Canada.

0724-8741/93/0100-0014$07.00/0

were invariably greater than those of *l*-MPH following immediate-release MPH. Consequently, the areas under the plasma concentration versus time curves (AUC_0^∞) of *d*-MPH were correspondingly greater than those of the *l*-antipode. These studies suggested that there was pronounced enantioselectivity in presystemic metabolism.

MPH is usually well tolerated by the children under medication with the drug. In a recent case report, however, a boy who chewed the MPH-SR dosage form complained of severe stomach cramps (23). It was suggested that chewing destroyed the waxy matrix of the SR dosage form and compromised the slow-release characteristic of the formulation, leading to an increased rate of input of MPH into the systemic circulation. The present report includes an investigation into the effects of chewing the SR dosage form on the enantioselective pharmacokinetics of MPH.

A four-phase, randomized, crossover study in humans was designed with the following objectives: (i) to describe definitively the enantioselective pharmacokinetics in humans after the administration of intravenous MPH (MPH-IV) and the oral dosage forms MPH-IR and MPH-SR; (ii) to investigate whether or not chewing the MPH-SR dosage form (MPH-CH) altered its slow-release characteristics; (iii) to make comparison of the various pharmacokinetic parameters among MPH-IR, MPH-SR, and MPH-CH drug phases; and (iv) to calculate absolute bioavailability values for both MPH enantiomers after the three oral drug administrations.

EXPERIMENTAL

Materials

Racemic *dl*-threo-MPH, *d*-threo-MPH, and *l*-threo-MPH were kindly donated by CIBA Geigy, Basel, Switzerland. Chlorphentermine hydrochloride, which served as an internal standard, was kindly donated by Parke Davis, Scarborough, Ontario, Canada. Solvents and all other chemicals were of analytical grade and were used without further purification.

Subjects

Subjects who participated in the study were recruited from students attending classes at the University of Saskatchewan. Each subject was given a complete description of the study design and also informed of possible adverse effects. Selection of the volunteers was contingent upon successful physical examination and medical screening. In addition, each subject was required to answer a questionnaire concerning his medical history. Clinical laboratory tests which included blood chemistry, hematology, and urinalysis were also performed to check for any abnormalities.

Thirteen nonsmoking healthy young men complied with the above requirements and entered the study after signing informed consent forms. These subjects were 18 to 30 years of age and each had an appropriate height-to-weight ratio in accordance with the standards set by the Metropolitan Life Insurance Company Statistical Bulletin, 1983. All subjects were required to refrain from drinking alcoholic beverages from 24 hr prior to each drug administration, during the study, and until 24 hr after the last blood sample was obtained. In addition, consumption of coffee or other caffeine-containing products was not permitted on the day of drug administration and the subjects were required to avoid all other drugs for a period of 1 week prior to the study.

Drug Administrations

The administration of MPH to the healthy volunteers comprised four phases. Each phase was separated by a 1-week washout period. The phases were as follows:

(i) 10 mg intravenous MPH (parenteral Ritalin);
(ii) 40 mg immediate-release MPH (2 × 20-mg tablets, Ritalin) swallowed whole with water;
(iii) 40 mg slow-release MPH (2 × 20-mg tablets, Ritalin-SR) swallowed whole with water; and
(iv) 40 mg slow-release MPH (2 × 20-mg tablets, Ritalin-SR, chewed before swallowing).

Study Design

The study protocol and consent form were reviewed and approved by the University of Saskatchewan President's Advisory Committee on Ethics in Human Experimentation. The three oral phases were administered on three separate occasions in a randomized crossover format. Due to the risk involved in intravenous injection and also to facilitate efficient handling, all subjects received the intravenous administration under close medical supervision on the same day. The subjects were required to fast overnight before each drug administration and for 4 hr afterward. The participants were then provided with lunch, after which the only restriction on food intake was the avoidance of caffeine-containing products. The subjects were confined within the family medicine unit at the Royal University Hospital for at least 8 hr after each drug administration. Upon completion of the protocol, physical examination and clinical tests were mandatory in order to assess the health of the subjects.

Blood Samples

Blood samples (10 to 15 ml) were drawn from the cubital vein into heparinized, evacuated tubes (Vacutainers) without allowing the blood to come into contact with the rubber stopper at any time. The blood collection schedule included a sample taken immediately before drug administration (0 hr) and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, and 16.0 hr after the dosing. After MPH-IV, an additional sample was taken at 0.25 hr. The plasma was separated immediately by centrifugation and stored at −20°C until analysis.

Urine Samples

In each phase of the study, urine samples were collected in Nalgene bottles in six time segments up to 16.0 hr after dosing. At the end of each time segment, the volume and pH of the urine were measured and were subdivided into small aliquots (15 ml). The aliquots were immediately frozen (−20°C) until analysis. A single voiding immediately before the drug administration in each phase served as a control urine, which was subdivided and stored as above.

Analysis of Samples

Enantioselective assay procedures based on GC-ECD

were utilized in the analysis of MPH (17,21) and RA (22). Briefly, MPH and RA were separated from each other by selective extraction procedures. The secondary amino group of MPH was reacted with heptafluorobutyryl-*l*-prolyl chloride (*l*-HPC) to form a pair of diastereomeric amide derivatives, which were then separated on a nonchiral OV-225 GLC capillary column. The concentrated extract containing RA was heated with methanol in the presence of acid to convert the RA back into MPH, which was then reacted with *l*-HPC and analyzed as outlined above. Standard calibration curves in the range 0.14 to 45.0 ng/enantiomer MPH/ml (plasma) and 3.75 to 200.0 ng/enantiomer MPH/ml (urine) and 0.75 to 6.0 μg/enantiomer RA/ml (urine) were constructed separately for the two enantiomers on each day of analysis. The day-to-day performance of the assays were monitored by the analysis of quality-control (QC) samples (analyst blind) in parallel with test samples. The QC samples were prepared in duplicate at two concentrations within the range of the standard curve. The experimentally determined values of the QC samples were required to be within 15% of the nominal values in order for the analytical run to be considered acceptable.

Pharmacokinetic Calculations

Noncompartmental pharmacokinetic parameters such as C_{max}, t_{max}, AUC_0^t, AUC_0^∞, $t_{1/2}$, CL, CL_R, $AUMC_0^\infty$, MRT, Vd_{ss}, and f_e were calculated by standard methods (24). Elimination rate constants (β) were estimated by regressing the last three points of the ln plasma concentration versus time curves. Apparent oral clearance (CL_0) was estimated as the quotient of the oral dose and the corresponding AUC_0^∞. Absolute bioavailability (*F*) was estimated as the quotient of CL and CL_0.

Statistical Analysis

Comparison of all pharmacokinetic parameters among the three oral drug formulations (MPH-IR, MPH-SR, and MPH-CH) was made separately for either the d- or l-enantiomers using a nested-model analysis of variance (ANOVA) to examine the following effects: phase, treatment, sequence, and subject nested with sequence. ANOVA was followed by the Student–Newman–Keuls multiple-comparison test (SNK). The same model ANOVA-SNK was used to examine amounts recovered from the cumulative 0- to 16-hr urine expressed as percentages of the administered dose.

Student's paired "*t*" tests were employed to test the statistical significance between the d- and the l-enantiomers for the various pharmacokinetic parameters obtained after each formulation.

The significance of the differences between amounts recovered from the cumulative urine (0–16 hr) expressed as the percentage of the administered dose, among either d- or l-enantiomers, was assessed by ANOVA-SNK.

RESULTS

To the best of our knowledge, the present study was the first of its kind to generate data to delineate the pharmacokinetics of MPH enantiomers after separate administrations of MPH-IV, MPH-IR, MPH-SR, and MPH-CH to healthy subjects.

The mean plasma concentration versus time plots for *d*-MPH and *l*-MPH after the administration of MPH-IV or MPH-IR are shown in Fig. 1, and similar plots for the MPH enantiomers after the MPH-SR and MPH-CH drug phases are shown in Fig. 2. Paired *t* tests revealed that, after MPH-IR, there was a significant distortion in the enantiomeric ratio at 0.5 hr, when the first blood sample was taken, and in every sample harvested thereafter until the concentration of *l*-MPH fell below the quantitation limits of the assay. Figure 2 reveals that similar results were obtained after the administration of the other two oral dosage forms MPH-SR and MPH-CH. In sharp contrast, paired *t* tests failed to show any significant enantioselective differences in the blood samples harvested 0.25, 0.5, and 1.0 hr after MPH-IV, but thereafter, there was a progressively widening distortion in the enantiomeric ratio in every sample until *l*-MPH was not quantifiable (Fig. 1).

Table I presents some noncompartmental pharmacokinetic parameters for MPH enantiomers after the administration of MPH-IV. Paired *t* tests revealed that the clearance of the l-enantiomer was significantly greater than that of its d-antipode, despite wide intersubject variation. Moreover, pharmacokinetic parameters such as MRT, Vd_{ss}, AUC_0^∞, and $t_{1/2}$ calculated for *d*-MPH were all significantly greater than the corresponding values for the l-antipode.

After oral administration of MPH-IR, the differences between the enantiomers were significant (paired *t* tests) for all pharmacokinetic parameters except t_{max}, despite wide intersubject variation. Thus, the mean C_{max} of *d*-MPH in plasma was approximately sixfold greater than that of

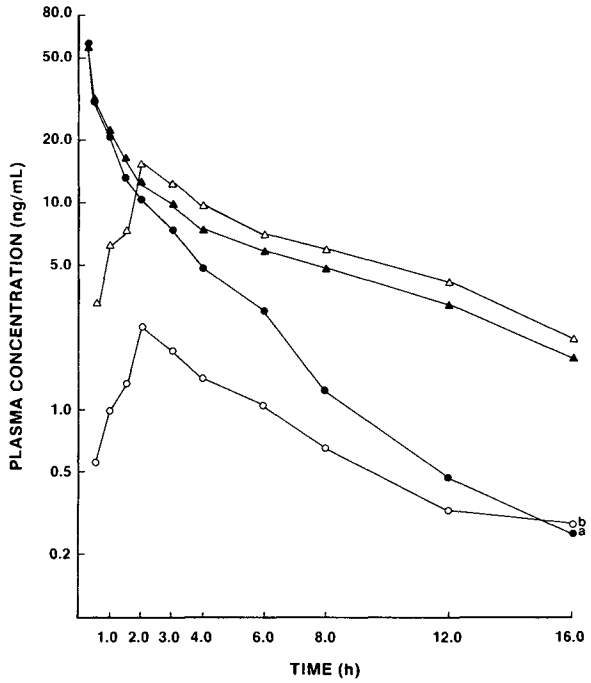

Fig. 1. Mean [n = 13; (a) n = 2; (b) n = 3] plasma concentration versus time plots for *d*-MPH (triangles) and *l*-MPH (circles) after the administration of MPH-IV (filled symbols) or MPH-IR (open symbols).

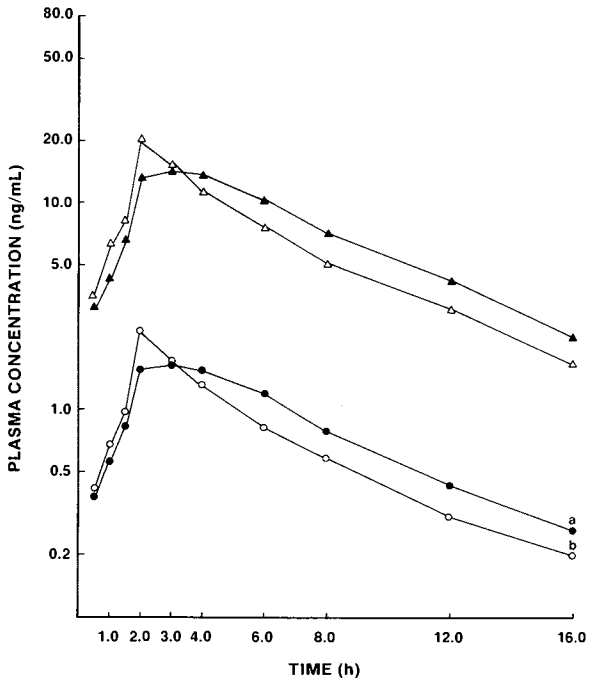

Fig. 2. Mean [n = 13; (a) n = 2; (b) n = 3] plasma concentration versus time plots for *d*-MPH (triangles) and *l*-MPH (circles) after the administration of MPH-SR (filled symbols) or MPH-CH (open symbols).

l-MPH, and the mean AUC_0^∞ for *d*-MPH was eightfold higher than that of its antipode. Similar trends were apparent after the administration of MPH-SR or MPH-CH.

Table II provides a comparison of pharmacokinetic parameters of *d*- and *l*-MPH after the three oral administrations. ANOVA-SNK showed that t_{max} values for *d*-MPH and *l*-MPH after MPH-SR were both significantly later than their respective t_{max} values obtained after MPH-IR or MPH-CH. Figure 2 suggests that MPH-CH tends to give higher C_{max} values than MPH-SR. ANOVA-SNK revealed that the tendency was significant for *d*-MPH but not for the l-enantiomer. Furthermore, ANOVA-SNK revealed that the absolute bioavailability (F) of *d*-MPH (overall mean, 0.23) was significantly different from that of *l*-MPH (overall mean, 0.05), although there were no significant differences among the three oral treatments in the bioavailabilities of either enantiomer.

The mean pharmacokinetic parameters for the MPH enantiomers calculated from the urinary data are presented in Table III. The bioavailabilities calculated for the two enantiomers were not significantly different from those calculated from plasma data. Furthermore, the urinary data confirmed that there were no significant differences among the three oral treatments in the bioavailabilities of either enantiomer. There was no significant difference among the enantiomers of MPH in renal clearance (CL_R), irrespective of the type of drug treatment. ANOVA-SNK revealed that the fraction of the dose excreted unchanged in the urine (f_e) after MPH-IV was significantly greater for both *d*- and *l*-MPH compared with corresponding values of f_e obtained after the oral treatments (Table III). There were, however, no significant differences in f_e after MPH-IR, MPH-SR, or MPH-CH.

Table IV showed that after each of the four drug treatments, approximately 40% of the ingested dose of each enantiomer of MPH was recovered in the 0- to 16-hr urine as RA. ANOVA-SNK detected no significant differences in the percentage urinary recoveries of either enantiomer of RA.

Table I. Mean Pharmacokinetic Parameters for Methylphenidate Enantiomers in Plasma After the Administration of 10 mg MPH-IV to Healthy Subjects (n = 11)

	d-MPH	*l*-MPH
CL (L/kg · hr^{-1})	0.40 (0.12)[a]	0.73* (0.28)
MRT (hr)	6.53 (1.62)	2.44* (0.51)
Vd_{ss} (L/kg)	2.65 (1.11)	1.80* (0.91)
AUC_0^∞ (ng/ml hr^{-1})	147.74 (47.91)	88.64* (43.13)
$t_{1/2}$ (hr)	5.96 (1.71)	3.61* (1.12)

[a] Values in parentheses are standard deviations.

* Paired t test: significantly different from the mean value of the corresponding enantiomer ($P < 0.01$).

DISCUSSION

Clinical studies in children with ADHD (25–27) have suggested that maximum therapeutic benefit from *dl*-MPH may be obtained from oral doses of 0.5–0.7 mg/kg, which translates into a dose of 40 mg in a 70-kg adult. Thus, in the present study, oral doses of 40 mg of each oral formulation were administered. For intravenous administration, however, the dose was reduced to 10 mg because bioavailability is presumably maximal after MPH-IV, with a consequent greater risk associated with the use of higher parenteral doses. Moreover, earlier pharmacokinetic data, based on measurement of total *d*- plus *l*-MPH (28–30), suggested that the pharmacokinetics of MPH are essentially linear.

Extensive and Enantioselective Presystemic Metabolism of MPH

Consistent with previous reports in children with ADHD (17,18) and human adults (18–20,22), the data obtained after the three oral drug formulations in the present investigation clearly demonstrated marked enantioselective differences in the plasma concentrations and in the various pharmacokinetic parameters calculated therefrom (Table II).

The inclusion of both intravenous and oral doses in the present study provided the first conclusive evidence of profound enantioselective presystemic metabolism after oral MPH. The oral bioavailability of *d*-MPH (F = 0.23) was significantly greater than that of *l*-MPH (F = 0.05), irrespective of the type of oral dose administered (MPH-IR, MPH-SR, or MPH-CH). Paired t tests revealed that there were no significant differences between plasma levels of the enantiomers until 1.5 hr after iv dosing, although after oral dosing plasma levels of *d*-MPH were significantly higher than those of *l*-MPH at the first sampling time (0.5 hr) and at every sampling time thereafter. Moreover, ANOVA revealed no significant difference in the amounts of *d*- and *l*-MPH recovered in urine samples collected over the first 2 hr after iv dosing with *dl*-MPH. After oral dosing, however, significantly more *d*-MPH than *l*-MPH was recovered in the 0- to 2-hr urine samples (31). Recently, Aoyama *et al.* (20) showed that there was no interconversion between the isomers after

Table II. Comparison of Mean Pharmacokinetic Parameters for Methylphenidate Enantiomers After the Three Oral Formulations in 11 Healthy Subjects

Pharmacokinetic parameter	Treatment			Treatment		
	d-MPH-IR	*d*-MPH-CH	*d*-MPH-SR	*l*-MPH-IR	*l*-MPH-CH	*l*-MPH-SR
t_{max} (hr)	$2.36^{a,*}$	1.95^{a}	3.18^{b}	2.14^{x}	2.14^{x}	3.09^{y}
	$(0.81)^{a}$	(0.15)	(0.64)	(0.64)	(0.64)	(0.70)
C_{max} (ng/ml)	$18.12^{a,b}$	20.75^{a}	16.06^{b}	2.98^{x}	$2.44^{x,y}$	1.85^{y}
	(4.34)	(5.89)	(4.60)	(0.94)	(0.76)	(0.52)
AUC_0^t (ng/ml · hr^{-1})	100.46^{a}	101.86^{a}	116.41^{a}	12.91^{x}	10.68^{x}	12.21^{x}
	(28.50)	(27.21)	(36.66)	(3.82)	(3.14)	(4.04)
AUC_0^∞ (ng/ml · hr^{-1})	120.21^{a}	115.15^{a}	134.36^{a}	14.79^{x}	12.12^{x}	13.69^{x}
	(30.68)	(28.14)	(42.39)	(4.14)	(3.43)	(4.46)
$t_{1/2}$ (hr)	5.69^{a}	5.33^{a}	5.04^{a}	3.93^{x}	3.84^{x}	3.88^{x}
	(1.14)	(1.04)	(0.69)	(0.76)	(0.49)	(0.59)
F	0.22^{a}	0.25^{a}	0.22^{a}	0.05^{x}	0.05^{x}	0.05^{x}
	(0.08)	(0.12)	(0.09)	(0.03)	(0.03)	(0.03)
CL_0 (L/kg · hr^{-1})	1.95^{a}	1.97^{a}	1.75^{a}	16.32^{x}	19.37^{x}	17.26^{x}
	(0.66)	(0.48)	(0.53)	(7.32)	(6.49)	(7.51)

[a] Values in parentheses are standard deviations.

* Superscripts a and b, ANOVA-SNK for *d*-MPH. Superscripts x and y, ANOVA-SNK for *l*-MPH. Means with same superscript letters in row, not significantly different; means with different superscript letters in row, significantly different.

the oral administration of pure *d*-MPH and *l*-MPH separately to healthy humans. Taken together, these data provide firm evidence of profound enantioselective presystemic metabolism after the oral administration of MPH.

The values calculated for CL_R for the MPH enantiomers (Table III) were very low compared to the CL_0 values after the three oral administrations (Table II), which suggested that renal excretion was not a major contributing factor to the rapid and extensive elimination of the MPH enantiomers. Furthermore, the CL_R *d*- and *l*-MPH was not significantly different after iv or oral dosing, showing that the distortion in the plasma d/l MPH ratio was not due to preferential urinary excretion of *l*-MPH.

Examination of the concentrations of RA enantiomers in urine voided in the first 2 hr after oral MPH revealed significantly higher levels of the *l*-RA (data not shown), consistent with the observation of higher plasma levels of *l*-RA over the first few hours (20). It should be noted, however, that after intravenous MPH there was no difference between the urinary concentration of *d*-RA and that of *l*-RA. This demonstrated that the distortion in the enantiomeric ratio in the 0- to 2-hr urinary RA, observed after oral MPH, was attributable to preferential enantioselective presystemic metabolism of *l*-MPH to *l*-RA rather than selective urinary excretion of *l*-RA.

Pharmacokinetics of MPH Enantiomers After MPH-IV

After MPH-IV, the enantiomers were introduced into the systemic circulation in equal proportions. The modest apparent plasma levels of *d*-MPH (96.90 ng/ml) and *l*-MPH (105.14 ng/ml) estimated by extrapolation at 0 hr suggested that the distribution of both isomers was rapid and extensive. This finding was consistent with previous reports (32,33) of rapid and extensive distribution of MPH (measured as d plus l) into the tissues after the administration of iv doses to rats. After the initial rapid distribution, MPH was slowly released from the fatty storage sites and from specific and nonspecific binding sites into the systemic circulation (32). In other words, after IV administration, the drug was gradually exposed to the enantioselective metabolizing enzymes, leading to a gradual distortion in the plasma d/l MPH

Table III. Comparison of the Mean Urinary Pharmacokinetics for Methylphenidate Enantiomers After Intravenous and Oral Administrations

Parameter	d				l			
	IV	IR	SR	CH	IV	IR	SR	CH
CL_r (L/kg · hr^{-1})	$0.005^{a,*}$	0.006^{a}	0.006^{a}	0.007^{a}	0.005^{a}	0.006^{a}	0.006^{a}	0.007^{a}
	$(0.003)^{a}$	(0.003)	(0.003)	(0.003)	(0.003)	(0.004)	(0.003)	(0.005)
F		0.25^{a}	0.25^{a}	0.23^{a}		0.06^{b}	0.07^{b}	0.06^{b}
		(0.09)	(0.09)	(0.13)		(0.09)	(0.09)	(0.11)
f_e	0.013^{a}	0.003^{b}	0.003^{b}	0.003^{b}	0.006^{c}	0.0003^{d}	0.0003^{d}	0.0003^{d}
	(0.005)	(0.005)	(0.005)	(0.005)	(0.003)	(0.0002)	(0.0002)	(0.0002)

[a] Values in parentheses are standard deviations.

* Superscripts a–d, ANOVA-SNK embracing both *d*- and *l*-MPH. Means with the same superscript letters in row, not significantly different; means with different superscript letters in row, significantly different.

Table IV. Recoveries of Ritalinic Acid Enantiomers[a] from 0- to 16-hr Total Urine After Intravenous and Three Oral Formulations to Healthy Subjects[b]

	d				l			
Phase	IV	IR	SR	CH	IV	IR	SR	CH
Mean[b]	42.54[m,*]	39.73[m]	37.84[m]	38.21[m]	46.65[n]	44.51[n]	43.28[n]	41.69[n]
	(7.81)[c]	(7.40)	(5.93)	(6.74)	(9.24)	(7.32)	(8.84)	(4.73)

[a] Expressed as the percentage of the administered dose.
[b] $n = 8$.
[c] Values in parentheses are standard deviations.
* Superscripts m and n, ANOVA-SNK embracing both *d*- and *l*-MPH. Means with the same superscript letters in row, not significantly different; means with different superscript letters in row, significantly different.

ratio over time. Thus, only after 1.5 hr did a significant difference in plasma levels of *d*-MPH and *l*-MPH emerge. Thereafter, there was a significant distortion in every sample examined until *l*-MPH fell below the detection limits of the assay.

Examination of the urinary MPH data after MPH-IV revealed no significant difference between the amount of *d*- and the amount of *l*-MPH (paired *t* tests) excreted in the first 2 hr, which is consistent with the observation that plasma levels of the MPH enantiomers were not significantly different up to 1.5 hr after MPH-IV. Moreover, as in the case of plasma, a statistically significant distortion in the d/l ratio of MPH in urine developed over time.

As a result of enantioselective metabolism, the mean CL of *l*-MPH (67.99 L/hr) was 2-fold greater than that of *d*-MPH (36.61 L/hr), and the mean MRT value for *d*-MPH (6.53 hr) was at least 2.5-fold longer than the corresponding value for the l-enantiomer (2.44 hr). Moreover, the Vd_{ss} of *d*-MPH was 1.6-fold greater than that of *l*-MPH, which is consistent with the preferential presystemic metabolism of *l*-MPH. Thus, from the present iv and oral data, it could be argued that the wide intersubject variability in the plasma concentration vs. time curves are attributable to the differences in F values rather than to the variability in Vd as suggested by Hungund *et al.* (34).

Comparison of the Pharmacokinetics for MPH Enantiomers After the Three Oral Administrations

Table II shows that there were no significant differences in AUC_0^∞, AUC_0^t, $t_{1/2}$, or F for either enantiomer after administration of the three oral doses. These data are consistent with an earlier report by Patrick *et al.* (35), who showed that there were no significant differences in AUC_0^∞ values for MPH (d plus l) between a single dose of MPH-SR (20 mg) and two doses of MPH-IR (10 mg) given 5 hr apart. Further, the F value of 0.27 calculated for total *dl*-MPH from the present MPH-IR data (Table II) was found to be consistent with the value of 0.31 (0.10–0.52; $n = 5$) reported for the racemic drug in children with ADHD (36).

The present study revealed that chewing the slow release product produced significant shortening of t_{max} and increases in C_{max}, with the result that these parameters for both enantiomers became indistinguishable from those obtained after MPH-IR. Thus, the t_{max} values of both enantiomers after MPH-CH or MPH-IR were significantly shorter than corresponding values obtained after MPH-SR (37), and there was no significant difference in C_{max} after MPH-CH and MPH-IR (Table II). These data suggest that children who chew the slow-release MPH may indeed be at risk of side effects, particularly if the administered dose of MPH-SR is increased compared with the child's regular dose of MPH-IR.

In a previous study on the enantioselective pharmacokinetics of MPH after the administration of MPH-SR to children with ADHD (21), there was no significant difference in plasma levels of *d*-MPH at 4.5, 6.0, and 8.0 hr after administration, which was taken as evidence of sustained plasma levels of the active enantiomer. In the present study, there was no significant difference between the plasma levels of either enantiomer at 2, 3, and 4 hr after dosing, although there was no such evidence of sustained levels at later time points. Nevertheless, plasma levels of *d*-MPH at 6 and 8 hr after MPH-SR were significantly higher than their corresponding values after MPH-IR (ANOVA-SNK). Thus the longer t_{max}, lower C_{max}, and relatively high plasma levels at the later sampling times provide evidence of a slow-release formulation.

Comparison of the pharmacokinetic parameters calculated after MPH-IR in the present study with those obtained from an earlier study in children with ADHD (17) indicated apparent discrepancies in $t_{1/2}$ values for both enantiomers. For example, $t_{1/2}$ values were longer for *d*-MPH (5.69 hr) than *l*-MPH (3.93 hr) in the present study; on the contrary, $t_{1/2}$ values calculated for *d*-MPH (3.10 hr) were shorter than those for *l*-MPH (5.59 hr) in the previous MPH-IR study (17). Two possible explanations for this discrepancy are as follows: Practical and ethical considerations permitted the collection of blood samples only up to 8 hr in the study with children, whereas the collections were made up to 16 hr in the present study in order to define clearly the elimination kinetics of both MPH enantiomers. Thus, the present data suggest that the relatively long $t_{1/2}$ for *l*-MPH reported in the earlier study was an experimental artifact.

There were no significant differences in CL_R for *d*- or *l*-MPH after oral or intravenous MPH, although the fraction of the dose of both enantiomers excreted unchanged in the urine (f_e) was significantly greater after intravenous MPH. There were no significant differences in F values calculated from the plasma or urinary data for either enantiomer after MPH-IR, MPH-SR, or MPH-CH. Although the cumulative urinary RA data did not provide supportive evidence of

enantioselectivity in the presystemic metabolic process, it should be realized that the amounts of *d*-RA found in urine were approximately 150-fold greater than those of *d*-MPH and the amounts of *l*-RA were approximately 1700-fold greater than those of *l*-MPH. Hence, even a 10-fold difference in the levels of urinary MPH enantiomers will not be reflected as a significant difference in the corresponding recoveries of the RA enantiomers in 0- to 16-hr urine. This finding was found to be consistent with the report of Aoyoma *et al.* (20).

CONCLUSION

In conclusion, the results of the study confirmed the extensive presystemic metabolism of both MPH enantiomers and, further, provided conclusive evidence for presystemic metabolism of *l*-MPH in humans. Although no side effects were reported after chewing MPH-SR in the present adult study, the results suggest that the slow-release product should not be administered to children who tend to chew their tablets.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Medical Research Council of Canada for PG-34 and the University of Saskatchewan for the Ph.D. Scholarship to N.R.S. The authors also wish to thank Ms. G. Rauw for excellent technical assistance during the clinicals.

REFERENCES

1. E. J. Ariens. Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology. *Eur. J. Clin. Pharmacol.* 26:663–668 (1984).
2. J. W. Hubbard, D. Ganes, H. K. Lim, and K. K. Midha. Chiral pharmacology and its consequences for therapeutic monitoring. *Clin. Biochem.* 19:107–112 (1986).
3. J. Caldwell, S. M. Winter, and A. J. Hutt. The pharmacological and toxicological significance of the stereochemistry of drug disposition. *Xenobiotica* 18:59–70 (1988).
4. D. E. Drayer. Problems in therapeutic drug monitoring: The dilemma of enantiomeric drugs in man. *Ther. Drug Monit.* 10:1–7 (1988).
5. K. Williams and E. Lee. Importance of drug enantiomers in clinical pharmacology. *Drugs* 30:333–354 (1985).
6. E. J. Ariens. *Stereochemistry and Biological Activity of Drugs,* Blackwell Scientific, Oxford, London, 1983.
7. V. I. Douglas, R. G. Barr, M. E. O'Neill, and B. G. Britton. Short-term effects of methylphenidate on the cognitive, learning and academic performance of children with attention deficit disorder in the laboratory and the classroom. *J. Child Psychol. Psychiat.* 27:191–211 (1986).
8. D. Whitehouse, U. Shah, and F. B. Palmer. Comparison of sustained release and standard methylphenidate in the treatment of minimal brain dysfunction. *J. Clin. Psychiat.* 41:282–285 (1980).
9. B. A. Faraj, Z. H. Israili, J. M. Perel, M. L. Jenkins, S. G. Holtzman, S. A. Cucinell, and P. G. Dayton. Metabolism and disposition of methylphenidate-14C: Studies in man and animals. *J. Pharmacol. Exp. Ther.* 191:535–547 (1974).
10. B. A. Faraj and M. L. Jenkins. Two new metabolites of methylphenidate. *Pharmacologist* 15:155 (1973).
11. H. Egger, F. Bartlett, R. Dreyfuss, and J. Karliner. Metabolism of methylphenidate in dog and rat. *Drug Metab. Dispos.* 9:415–423 (1981).
12. R. M. Ferris, F. L. M. Tang, and R. A. Maxwell. A comparison of the capacities of isomers of amphetamine, deoxypipradol and methylphenidate to inhibit the uptake of tritiated catecholamines into rat cerebral cortex slices, synaptosomal preparations of rat cerebral cortex, hypothalamus and striatum and into adrenergic nerves of rabbit aorta. *J. Pharmacol. Exp. Ther.* 181:407–416 (1972).
13. R. M. Ferris and F. L. M. Tang. Comparison of the effects of the isomers of amphetamine, methylphenidate and deoxypipradol on the uptake of 1-[3H]-norepinephrine and [3H]-dopamine by synaptic vesicles from rat whole brain, striatum and hypothalamus. *J. Pharmacol. Exp. Ther.* 210:422–428 (1979).
14. R. Rometsch. U.S. Patent 2,838,519 (1958).
15. R. A. Maxwell, E. Chaplin, S. Batmanglidj Eckhardt, J. R. Soares, and G. Hite. Conformational similarities between molecular models of phenethylamine and of potent inhibitors of the uptake of tritiated norepinephrine by adrenergic nerves in rabbit aorta. *J. Pharmacol. Exp. Ther.* 241:152–158 (1970).
16. K. S. Patrick, R. W. Caldwell, R. M. Ferris, and G. R. Breese. Pharmacology of the enantiomers of threo-methylphenidate. *J. Pharmacol. Exp. Ther.* 241:152–158 (1987).
17. N. R. Srinivas, D. Quinn, J. W. Hubbard, and K. K. Midha. Stereoselective disposition of methylphenidate in children with attention-deficit disorder. *J. Pharmacol. Exp. Ther.* 241:300–306 (1987).
18. H. K. Lim, J. W. Hubbard, and K. K. Midha. Development of enantioselective gas chromatographic quantitation assay for dl-threo-methylphenidate in biological fluids. *J. Chromatogr.* 378:109–123 (1986).
19. T. Aoyoma, H. Kotaki, and Y. Saitoh. Gas chromatographic-mass spectrometric analysis of threo-methylphenidate enantiomers in plasma. *J. Chromatogr.* 494:420–423 (1989).
20. T. Aoyoma, H. Kotaki, Y. Honda, and F. Nakagawa. Kinetic analysis of enantiomers of threo-methylphenidate and its metabolite in two healthy subjects after oral administration as determined by a gas chromatographic-mass spectrometric method. *J. Pharm. Sci.* 79:465–469 (1990).
21. J. W. Hubbard, N. R. Srinivas, D. Quinn, and K. K. Midha. Enantioselective aspects of the disposition of dl-*threo*-methylphenidate after the administration of a sustained-release formulation to children with attention deficit-hyperactivity disorder. *J. Pharm. Sci.* 78:944–947 (1989).
22. N. R. Srinivas, J. W. Hubbard, and K. K. Midha. Enantioselective gas chromatographic assay with electron capture detection for dl-ritalinic acid in plasma. *J. Chromatogr.* 530:327–336 (1990).
23. R. B. Rosse and W. L. Licamele. Slow-release methylphenidate: Problems when children chew tablets. *J. Clin. Psychiat.* 48:525 (1984).
24. M. Rowland and T. N. Tozer. *Clinical Pharmacokinetics: Concepts and Applications,* Lea and Febiger, Philadelphia, 1989.
25. M. D. Rapport, G. J. Du Paul, G. Stoner, B. K. Birmingham, and G. Masse. Attention deficit disorder with hyperactivity: Differential effects of methylphenidate on impulsivity. *Pediatrics* 76:938–943 (1985).
26. M. M. Seberchts, S. E. Shaywitz, B. A. Shaywitz, P. Jatlow, G. M. Anderson, and D. J. Cohen. Components of attention, methylphenidate dosage and blood levels in children with attention deficit disorder. *Pediatrics* 77:222–228 (1986).
27. S. S. Kupietz, B. G. Winsberg, E. Richardson, S. Maitinsky, and N. Mendell. Effects of methylphenidate dosage in hyperactive reading-disabled children. I. Behaviour and cognitive performance effects. *J. Am. Acad. Child Adolesc. Psychiat.* 27:70–77 (1988).
28. B. G. Winsberg, S. S. Kupeitz, J. Sverd, B. L. Hungund, and N. L. Young. Methylphenidate and dose plasma concentrations and behavioural response in children. *Psychopharmacology* 76:329–332 (1982).
29. W. Wargin, K. Patrick, C. Kilts, C. T. Gualtieri, K. Ellington, R. A. Mueller, G. Kraemer, and G. R. Breese. Pharmacokinetics of methylphenidate in man, rat and monkey. *J. Pharmacol. Exp. Ther.* 226:382–386 (1983).
30. S. A. Shaywitz, R. D. Hunt, P. Jatlow, D. J. Cohen, G. Young, N. Pierce, G. M. Anderson, and B. A. Shaywitz. Psychopharmacology of attention deficit disorder: Pharmacokinetic, neuroendocrine and behavioural measures following acute and

chronic treatment with methylphenidate. *Pediatrics* 69:688–694 (1982).
31. N. R. Srinivas, J. W. Hubbard, E. D. Korchinski, and K. K. Midha. Stereoselective urinary pharmacokinetics of dl-threo-methylphenidate and its major metabolite in humans. *J. Pharm. Sci.* 81:747–749 (1992).
32. K. S. Patrick, K. R. Ellington, and G. R. Breese. Distribution of methylphenidate and p-hydroxymethylphenidate in rats. *J. Pharmacol. Exp. Ther.* 231:61–65 (1984).
33. J. Gal, B. J. Hodshon, C. Pintauro, B. L. Flamm, and A. K. Cho. Pharmacokinetics of methylphenidate in the rat using single-ion monitoring GLC-mass spectrometry. *J. Pharm. Sci.* 66:866–869 (1977).
34. B. L. Hungund, J. M. Perel, M. J. Hurwic, J. Sverd, and B. G. Winsberg. Pharmacokinetics of methylphenidate in hyperkinetic children. *Br. J. Clin. Pharm.* 8:571–576 (1979).
35. K. S. Patrick, A. B. Straughn, E. J. Jarvi, G. R. Breese, and M. Meyer. The absorption of sustained-release methylphenidate formulations compared to an immediate-release formulation. *Biopharm. Drug Dispos.* 10:165–171 (1989).
36. Y. M. Chan, J. M. Swanson, S. S. Soldin, J. J. Thiessen, S. M. Macleod, and W. Logan. Methylphenidate hydrochloride given with or before breakfast. II. Effects on plasma concentrations of methylphenidate and ritalinic acid. *Pediatrics* 72:56–59 (1983).
37. B. Birmaher, L. L. Greenhill, T. D. Cooper, J. Fried, and B. Naminski. Sustained release methylphenidate: Pharmacokinetic studies in ADDH males. *J. Am. Acad. Child Adolesc. Psychiat.* 28:768–772 (1989).