Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 74 (2003) 747–754
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Neurobehavioral effects of racemic *threo*-methylphenidate and its D and L enantiomers in rats

Steve K. Teo*, David I. Stirling, Steve D. Thomas, Vikram D. Khetani

Celgene Corporation, 7 Powder Horn Drive, Warren, NJ 07059, USA

Received 21 August 2002; received in revised form 31 October 2002; accepted 22 November 2002

Abstract

D,L-Methylphenidate (Ritalin®) is used to treat attention deficit hyperactivity disorder (ADHD) in children. The therapeutic effect is predominantly due to the d enantiomer. Dexmethylphenidate (D-MPH; Focalin®) was therefore developed for its better therapeutic index. The present study determined and compared the acute behavioral toxicity of D,L-MPH, D-MPH and L-MPH in rats after oral dosing. Comprehensive functional observational battery (FOB) evaluations and rota-rod tests were performed 30, 60 and 120 min after dosing. Ten rats/sex/dose were administered a single dose of vehicle, 2, 20, 100 mg/kg D,L-MPH and 1, 10, 50 mg/kg D-MPH or 1, 100, 500 mg/kg L-MPH. There was no mortality. Certain FOB evaluations were statistically significant from vehicle control at any of the time points with most occurring at 60 and 120 min in the high D,L-MPH dose. These included increases in rearing, difficulty in removal from box, arousal, click, tail-pinch and decreases in hind-limb splay distance, hind-limb grip strength and handling reactivity. Behavioral responses were also present at the mid-dose D,L-MPH and high dose D- and L-MPH. Responses in female were significantly different from males in D,L- and L-MPH groups suggesting a sex difference in sensitivity. In the rota-rod test, mean latency to remain on the rod was significantly less for males compared to control given high dose D-MPH and D,L-MPH. In females, latency times were significantly less for high doses of all three compounds. In summary, fewer significant FOBs were seen with D- and L-MPH compared to equimolar doses of D,L-MPH. L-MPH was the least potent in producing FOBs. These results were supported by rota-rod studies.

Keywords: Methylphenidate; Ritalin®; D-Methylphenidate; Focalin®; Dexmethylphenidate; L-Methylphenidate; Neurobehavioral effects; Functional observational battery; Rota-rod

1. Introduction

Methylphenidate is a cyclized derivative of amphetamine with two chiral centers and was originally marketed as a 80:20 mixture of the *erythro*- and *threo*-racemates. Studies have shown that the *threo*-racemate was responsible for the therapeutic central nervous system (CNS) actions of the racemic mixture in treating attention deficit hyperactivity disorder (ADHD), while both racemates were equipotent in producing unwanted hypertensive effects and toxicity (Patrick et al., 1987a; Szporny and Gorog, 1961). The *erythro*-racemate was subsequently removed to improve the therapeutic index and decrease the adverse events. D,L-*threo*-Methylphenidate's (D,L-MPH) CNS stimulant properties are effective in increasing attentiveness in children with ADHD (Kimko et al., 1999; Sunohara et al., 1999; Zuddas et al., 2000). While ADHD is mainly a pediatric and adolescent disorder, it can manifest itself into adulthood necessitating continued treatment (Schachter et al., 2001; Weiss and Hechtman, 1984). The currently marketed D,L-MPH product (Ritalin® its generics and different formulations) is therefore a 50:50 racemic mixture of the D-*threo*- and L-*threo*-MPH enantiomers (Fig. 1).

D,L-MPH resembles amphetamine pharmacologically in its CNS stimulant activity. It is a Schedule II controlled substance under the Drug Enforcement Agency classification system. Whereas amphetamine is a potent CNS and peripheral nervous system stimulant, D,L-MPH possesses more pronounced effects on mental rather than motor activities. It is also similar to neurotransmitters in being a noncatechol-

Abbreviations: D-MPH, D-methylphenidate/dexmethylphenidate; D,L-MPH, D,L-methylphenidate; ADHD, attention deficit hyperactivity disorder.

* Corresponding author. Tel.: +1-732-805-3320; fax: +1-732 805-3616.
E-mail address: steo@celgene.com (S.K. Teo).

0091-3057/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0091-3057(02)01073-0

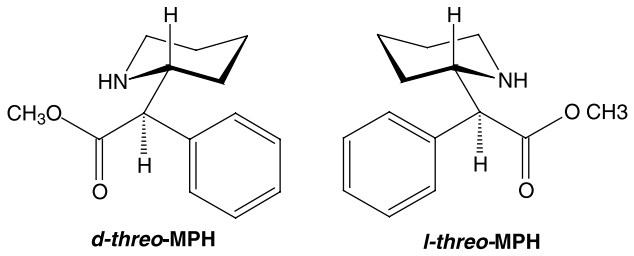

Fig. 1. Structures of D-MPH and L-MPH. D,L-MPH consists of 50% D-MPH and 50% L-MPH.

amine sympathomimetic. As a noncatecholamine sympathomimetic, D,L-MPH functions as a direct and indirect adrenergic agonist. The efficacy of psychostimulants such as D,L-MPH in treating ADHD has been attributed to their ability to release dopamine and block dopamine reuptake at the presynaptic terminal (Greenhill, 1991). In dopamine-depleted neonatal rats, subcutaneous D,L-MPH (1 mg/kg) reversed the induced hyperactivity (Luthman et al., 1989). D,L-MPH's pharmacological actions are most likely due to its ability to increase dopamine levels in the striatum region of the brain. Recent studies have shown that D-MPH (D-*threo*-MPH or dexmethylphenidate) is the pharmacologically active enantiomer. In a rat locomotor activity study using D-, L- or D,L-MPH, greatest activity was seen with D-MPH. The activity of D,L-MPH was intermediate and the L-MPH had the least activity. In addition, depletion of brain catecholamine levels by pretreatment with 6-hydroxydopamine significantly reduced the locomotor response to D-MPH (Patrick et al., 1987b). In rat behavioral studies using various reinforcement patterns, D-MPH was found to be more potent than L-MPH. Little behavioral activity was seen with L-MPH (Eckerman et al., 1991). D-MPH was also found to be 38 times more potent than L-MPH in inhibiting the phenethylamine pump responsible for transporting norepinephrine into peripheral nerve endings with the L-*threo*, D-*erythro* and L-*erythro* being 38-, 380- and 380-fold weaker, respectively (Maxwell et al., 1970). In baboons, D-MPH binds stereoselectively to the dopamine transporter in the brain thereby inhibiting dopamine reuptake (Ding et al., 1996). Follow-up studies using radiolabeled D- and L-*threo*-MPH (L-MPH) in human and baboon brains showed nonspecific binding with L-MPH and specific binding and uptake of D-MPH in the striatum of the basal ganglia. Pretreatment of the baboon with a selective dopamine uptake inhibitor significantly reduced the striatal uptake of labeled D-MPH (Ding et al., 1997). Based on these studies, D-MPH appears to be more neuropharmacologically active than either D,L- or L-MPH and has the potential to provide a better therapeutic index than the currently marketed D,L-MPH. D-MPH was therefore developed as an improved treatment for ADHD. D-MPH was approved by the US Food and Drug Administration (FDA) in 2001 and is sold under the brand name Focalin®, thereby providing physicians with another treatment option for ADHD.

The present study was performed to fulfill FDA regulatory requirements for the approval of D-MPH for ADHD. We determined and compared the acute behavioral toxicity of D,L-MPH and its D and L enantiomers after oral dosing in rats. Comprehensive functional observational battery (FOB) evaluations were performed along with a rota-rod test to determine which enantiomer was responsible for any behavioral effects seen.

2. Materials and methods

2.1. Chemicals

D-, L- and D,L-MPH HCl were manufactured at Celgene (Warren, NJ) with chemical purities ranging from 98% to 100% as assessed by HPLC (Teo et al., 2002). D,L-MPH is a racemate composing of 50:50 ratio of D- and L-MPH. Sterile distilled deionized water was used as the vehicle. All compounds were prepared fresh on the day of the study.

2.2. Animals

One hundred and twenty male and 120 female CD® [Crl:CD® (SC)IGS BR] rats weighing between 119 and 164 g were obtained from Charles River Laboratories (Portage, MI). They were individually housed in stainless steel wire-mesh cages in an environmentally controlled room at 68–71 °F and 49–69% relative humidity with a 12-h light–dark cycle. They were provided with Rodent Chow #5002 (PMI Nutrition International, St Louis, MO) ad libitum except during the behavioral testing day. Tap water was provided ad libitum. After a 1-week acclimation period, 100 males and 100 females with the best scores on the rota-rod test (longest latency to remain on rod) were selected for the study. They were randomly divided into groups such that the latency to remain on the rod was approximately equal. All animals were euthanized by carbon dioxide inhalation at the end of the study.

2.3. Dose selection

All animals were dosed by oral gavage. Dose groups consisted of vehicle control, 2, 20 and 100 mg/kg D,L-MPH, 1, 10 and 50 mg/kg D-MPH and 1, 100 and 500 mg/kg L-MPH at 10 rats/sex/group. Doses for D,L- and D-MPH were selected based on previous dose ranging and subsequent 90-day toxicity studies in rats (Teo et al., 2002). In these studies, rats were orally dosed with 100 mg/kg/day D,L-MPH and 2, 20 and 50 mg/kg/day D-MPH with significant toxicity observed at the high doses. The low and mid doses for D,L-MPH were chosen to correspond to the same multiples as those for low and mid D-MPH doses. Since D,L-MPH is composed of 50% D-MPH, the corresponding low, mid and high doses are equimolar

Table 1
Significant ($P<.05$ to $P<.01$) measurements of posture, ease of removal, handling reactivity, arousal and hind-foot splay in treated animals compared to control at indicated time points after dosing

Dose (mg/kg)	Sex	Evaluation time (min)	Measurement					
			Posture					
			Sitting/standing		Rearing		Crouched	
0	F	60	10		0		0	
100 D,L			3		6		1	
			Ease of removal					
			Very easy	Easy	Moderately difficult	Flinched	Difficult	Very difficult
0	F	30	10	0	0	0	0	0
100 D,L			2	3	1	1	2	1
500 L			1	4	0	1	3	1
0	M	60	10	0	0	0	0	0
100 D,L			3	3	2	1	1	0
0	F	120	9	1	0	0	0	0
100 D,L			1	7	2	0	0	0
			Handling reactivity					
			Low	Moderately low	Moderately high	High		
0	F	30	9	1	0	0		
100 D,L			1	2	2	5		
500 L			1	2	1	6		
0	M	120	10	0	0	0		
100 D,L			2	8	0	0		
0	F	120	9	1	0	0		
100 D,L			1	7	1	1		
			Arousal					
			Normal	Low	Slightly low	Slightly high	Very high	
0	F	30	9	0	1	0	0	
100 D,L			3	0	0	4	3	
0	M	60	5	1	4	0	0	
100 D,L			2	0	0	7	1	
50 D			3	0	0	7	0	
0	F	60	7	2	1	0	0	
20 D,L			2	0	1	6	1	
100 D,L			2	0	0	1	7	
50 D			2	0	0	7	1	
0	M	120	6	1	3	0	0	
100 D,L			1	0	1	3	5	
0	F	120	5	3	2	0	0	
100 D,L			0	0	1	3	6	
50 D			0	0	0	8	2	
500 L			1	0	0	7	2	
			Hind-foot splay (mm)					
0	F	30	106.3 ± 20.2					
100 D,L			80.8 ± 21.9					
0	F	60	97.9 ± 17.8					
100 D,L			80.5 ± 21.2					
0	F	120	96.7 ± 12.2					
100 D,L			74.6 ± 18.4					

in the D-MPH content. No comprehensive toxicity studies have previously been performed on L-MPH. Doses for L-MPH were therefore chosen based on a maximum tolerated dose (MTD) study. The MTD for L-MPH was found to be 500 mg/kg and was selected as the high dose. The low and mid doses were chosen as the same multiples as D-MPH. The chosen doses are not expected to produce any mortality after single administration. The maximum doses for D,L- and D-MPH are at least 67 times that of a human dose. These produced systemic exposures that were over

Table 2
Significant ($P<.05$ to $P<.01$) measurements of approach, touch, click, tail pinch and thermal responses and fore-limb grip strength and body temperature in treated animals compared to control at indicated time points after dosing

Dose (mg/kg)	Sex	Evaluation time (min)	Measurement			
			Approach response			
			No reaction	Slight reaction	Freeze or flinch	
0	M	120	9	1	0	
100 D,L			2	8	0	
50 D			2	7	1	
0	F	120	10	0	0	
20 D,L			3	7	0	
100 D,L			3	4	3	
50 D			3	5	2	
500 L			0	9	1	
			Touch response			
			No reaction	Slight reaction	Freeze or flinch	Exaggerated
0	M	120	9	1	0	0
500 L			2	8	0	0
			Click response			
			No reaction	Slight reaction	Freeze or flinch	Exaggerated
0	F	60	2	7	1	0
100 D,L			0	1	7	2
			Tail pinch response			
			No reaction	Slight reaction	Freeze or flinch	Exaggerated
0	F	60	8	2	0	0
20 D,L			1	9	0	0
			Thermal response (s)			
0	M	30	6.3 ± 2.4			
100 D,L			29.9 ± 19.7			
50 D			19.7 ± 11.2			
500 L			34.9 ± 18.0			
0	F	30	7.7 ± 2.0			
100 D,L			44.7 ± 21.1			
0	F	60	9.7 ± 6.4			
100 D,L			41.9 ± 21.8			
50 D			31.2 ± 16.7			
500 L			35.6 ± 20.7			
0	M	120	7.0 ± 2.9			
50 D			14.8 ± 6.2			
0	F	120	5.5 ± 2.1			
100 D,L			20.9 ± 15.9			
50 D			24.6 ± 17.7			
500 L			21.1 ± 16.4			
			Forelimb grip strength (kg)			
0	F	30	475.0 ± 417.5			
100 D,L			256.6 ± 208.0			
0	F	60	363.7 ± 222.6			
100 D,L			216.6 ± 104.7			
0	F	120	590.6 ± 227.2			
100 D,L			326.3 ± 126.0			
			Body temperature (°C)			
0	F	30	37.2 ± 2.6			
100 D,L			38.8 ± 1.0			

10 times higher than those typically achieved in children (Teo et al., 2002).

2.4. FOB evaluation

Test compounds were administered once on Study Day 1 by oral gavage at a dose volume of 10 ml/kg based on the most recent body weight. Rats were approximately 7 weeks old at the start of dosing. Cage-side observations were made throughout the study. Body weights were measured upon arrival, on Study Day 1 prior to dosing, as part of the FOB evaluation and prior to necropsy on Study Day 2. Testers were blinded to treatment group during FOB evaluations. FOB testing was conducted at approximately 30, 60 and 120 min after dosing. Each rat was observed for a minimum of 3 min in a black plexiglass, open-field observation box measuring 20 × 20 × 8 in. Parameters evaluated and the methods used have previously been described (Moser et al., 1988). These included home cage (posture, involuntary motor movements, palpebral closure), ease of removal from box, handling reactivity, lacrimation/salivation, palpebral closure (drooping eyelid), piloerection, exophthalmus (eyeball protrusion), open field (rearing, urination/defecation, involuntary motor movements, gait, mobility, arousal, vocalizations, respiration, stereotypy), sensorimotor (approach, touch, click, tail-pinch, thermal and pupil responses, air righting reflex, hind-foot splay) and grip strength measurements. Ease of removal was recorded based on the intensity of a rat's reaction ranging from 'very easy' to 'very difficult.' Handling reactivity was measured ranging from 'docile' to 'hyperactive.' Following the FOB measurements, body weight and temperature (by embedded microchip) were recorded.

2.5. Rota-rod test

A rota-rod test was performed after the FOB evaluation based on previously described methods (Schafer et al., 1995). In the selection phase of the study, all 120 male and 120 female rats were given 1 trial on the morning of Study Day 0 and again in the afternoon. The 100 males and 100 females with the best scores (longest latency to remain on the rota-rod) were retained for the definitive testing. These rats were randomly divided into 10 groups of 10 males and 10 females each. On Study Day 1, approximately 30 min after compound or vehicle control administration, they were tested in the FOB followed by the rota-rod. In the rota-rod study, rats were placed on the rod moving at a constant speed of 5 rpm and rotated in the direction opposite to the animals. The time elapsed up to 60 s or until the rat fell was recorded. All animals were tested at 30, 60 and 120 min after dosing.

2.6. Statistical analysis

All statistical analyses were performed using SAS (Cary, NC). For FOB categorical data (all endpoints except thermal response, grip strength, body weight, body temperature), each treatment group was compared to the control group using a chi-square test with a Bonferroni correction. Results were reported at the .05 and .01 significance levels. Endpoints were analyzed using a one-tailed test. For FOB continuous data (thermal response, grip strength, body weight, body temperature, rota-rod), a repeated measures analysis was performed using time as a regression variable. If there was no significant ($P>.05$) group by period interaction, a Dunnett's test was used to compare the control group mean over all time periods to each treatment group mean over all time periods. If the interaction term was significant, each time period was analyzed separately as follows. Endpoints were tested by Levene's test to assess for homogeneity of group variances. If the test was not significant ($P>.01$), a Dunnett's test was used to compare each treatment group with the control. If Levene's was significant, comparisons with the control group was made using Welch's *t*-test with a Bonferroni correction. Results of all pairwise comparisons were reported at .05 and .01 significance levels. All endpoints were analyzed using two-tailed tests.

Table 3
Rota-rod response times (mean ± S.D.)

Time (min)	Dose (mg/kg)	Compound	Response time (s)	
			Male	Female
30	0	Control	55 ± 15	54 ± 13
	1	D-MPH	56 ± 13	40 ± 22
	10	D-MPH	42 ± 23	45 ± 22
	50	D-MPH	26 ± 18[a]	14 ± 14[a]
	1	L-MPH	50 ± 17	48 ± 20
	100	L-MPH	49 ± 20	43 ± 20
	500	L-MPH	35 ± 22	13 ± 14[a]
	2	D,L-MPH	40 ± 23	50 ± 20
	20	D,L-MPH	37 ± 25	39 ± 17
	100	D,L-MPH	19 ± 14[a]	25 ± 23[a]
60	0	Control	55 ± 14	53 ± 16
	1	D-MPH	55 ± 16	48 ± 19
	10	D-MPH	52 ± 17	40 ± 34
	50	D-MPH	36 ± 26[a]	24 ± 20[a]
	1	L-MPH	56 ± 11	51 ± 15
	100	L-MPH	50 ± 17	48 ± 20
	500	L-MPH	42 ± 23	21 ± 20[a]
	2	D,L-MPH	53 ± 12	44 ± 22
	20	D,L-MPH	52 ± 17	32 ± 23
	100	D,L-MPH	23 ± 14[a]	27 ± 22[a]
120	0	Control	56 ± 11	58 ± 8
	1	D-MPH	55 ± 16	50 ± 22
	10	D-MPH	54 ± 10	55 ± 8
	50	D-MPH	44 ± 22[a]	19 ± 19[a]
	1	L-MPH	56 ± 14	55 ± 11
	100	L-MPH	57 ± 9	52 ± 14
	500	L-MPH	47 ± 20	45 ± 20[a]
	2	D,L-MPH	58 ± 6	49 ± 19
	20	D,L-MPH	60 ± 0	42 ± 22
	100	D,L-MPH	36 ± 20[a]	41 ± 23[a]

Times were based on latency to remain on rotating rod.
[a] Statistically significant $P<.01$.

3. Results

No animals died during the course of the study. There were several FOB evaluations that were statistically significant ($P < .01$) from controls at one or more of the three time points evaluated with most occurrences observed in the 100 mg/kg D,L-MPH dose group. Most occurrences in general were at 60 and 90 min (Tables 1 and 2). Major behavioral responses were also seen at the mid dose of D,L-MPH and high doses of D- and L-MPH. The significant ($P < .05$) FOB findings consisted of increases in rearing/crouched posture, difficulty in removal from box, arousal; approach response, click, tail-pinch and thermal responses compared to control. Decreases in hind-limb splay distance, hind-limb grip strength and handling reactivity along with slight reaction to touch and an increase in body temperature in females dosed with 100 mg/kg D,L-MPH were also observed. From the FOB results in D,L- and L-MPH, females appeared to respond significantly differently from males, suggesting they are more sensitive to both compounds. In the rota-rod test, mean latency to remain on the rod was significantly shorter compared to control for males given high dose D- and D,L-MPH. In females, latency times were significantly shorter for the high doses of all three compounds (Table 3). No statistical differences in body weight were observed after the FOB measurements.

4. Discussion

Even though D,L-MPH has been used for over 40 years, no comprehensive and comparative neurobehavioral studies have been performed on it and its enantiomers even though their behavioral effects in animals and humans are well known. Limited studies comparing the enantiomeric behavioral effects of intraperitoneally administered D-, L- and D,L-MPH were conducted in rats using fixed and concurrent variable interval reinforcement schedules (Eckerman et al., 1991). Another study investigated the locomotor stimulant properties of the three compounds (Patrick et al., 1987b). While both studies show that the D-MPH is the behaviorally active enantiomer, no measurements or comparisons were made on any acute FOB and rota-rod endpoints. FOB and rota-rod tests are behavioral studies commonly used to assess for neurotoxicity. The behavioral end-points measured represent sensory, motor and integrative outputs of the central, peripheral and autonomic nervous systems and are therefore good surrogate measures of compound-induced changes in nervous system integrity and function (Kulig and Jaspers, 1996).

Our present studies show that all three compounds produced behaviors consistent with the actions of a psychostimulant. The high D,L- and D-MPH doses of 100 and 50 mg/kg are 67 and 100 times that of the maximum human daily dose, respectively. The FOB and rota-rod testing time-points of 30, 60 and 120 min were chosen based on previous studies showing that maximum plasma concentrations of D- and L-MPH were achieved at 30 min (Teo et al., 2002). The differences observed in this study can be attributed to the psychostimulant action. For example, the difficulty in handling the animals (ease of removal and handling reactivity) was significantly greater after high doses of L- and D,L-MPH. Although rats given the high dose D-MPH also tended to be more difficult to handle, the finding was not statistically significant. Based on these and other similar data, L-MPH had the least psychostimulant potency (Table 1). The greatest number of statistically significant changes in the FOB were from D,L-MPH. Since D,L- and D-MPH are equimolar in D-MPH content, this increased incidence suggests an interaction between the D and L enantiomers in producing the psychostimulant actions. Metabolic interaction where one enantiomer modulates the metabolism of the other thereby raising its plasma levels and stimulant action is possible. Recent in vitro studies using human hepatic microsomes though did not show significant inhibition of all three compounds against major cytochrome *P*-450 isoform-specific substrates (unpublished data). It is possible that after an oral dose of D,L-MPH in rats, there is enantioselective first-pass metabolism of one enantiomer over the other giving rise to higher plasma levels of one enantiomer. Our studies however show similar plasma levels of both enantiomers but significantly higher plasma levels of the major metabolite L-ritalinic acid (L-RA) compared to D-ritalinic acid (D-RA) (unpublished data). Some of the significant FOB observations could therefore be due to D-RA although other chiral metabolites were not analyzed for. The significant FOBs seen with D,L-MPH could also be due to the additive effects of the individual D and L enantiomers' psychostimulant properties. The preponderance of significant findings at 60 and 120 min post-dose suggests that a metabolite(s) is mediating the behavioral effects (Table 1). In a rat toxicology study, maximum plasma levels of L- and D-RA were observed 45–120 min after dosing with 1 and 25 mg/kg D-MPH and 50 mg/kg D,L-MPH (unpublished data). Intracerebroventricular studies using RA and other metabolites however showed no pharmacological activity indicating that parent methylphenidate, particularly D-MPH is the active compound producing the significant FOBs (Faraj et al., 1974; Patrick et al., 1981). It is not known why female rats were found to be more sensitive to certain FOB measurements after dosing with L- and D,L-MPH compared to males (Table 1). It is also not known if there is a sex-specific side-effect profile in humans taking D- or D,L-MPH as most ADHD patients are males. Since the high doses of D- and D,L-MPH are equimolar in D-MPH content, they therefore have the same potency in reducing the response times on the rota-rod. It is not known why only females dosed with 500 mg/kg L-MPH had a reduced response time but the higher doses used further confirm the lower potency compared to the other two compounds.

Estimates on the incidence of ADHD have ranged from 3–5% to 7–16% of school age children in the United States (DSM-IV, 1994; Rowland et al., 2001; Barbaresi et al., 2002). Many of these children still manifest ADHD symptoms into adulthood (Schachter et al., 2001; Weiss and Hechtman, 1984). While D,L-MPH is largely effective, some patients experience significant side-effects including appetite suppression, weight loss, insomnia, headache, tachycardia and stomach upset (Greenhill, 1992). Some children exhibit behavioral rebound with D,L-MPH withdrawal at the end of the school day exhibiting the hyperactivity and other symptoms that are the hallmark of ADHD. Advances in stereo-specific manufacturing have enabled the development of chirally pure versions of racemic drugs through a process that removes the inefficacious L-MPH enantiomer. The lack of enantiomeric inversion between the enantiomers in body fluids makes it a viable drug with little decrease in therapeutic activity upon dosing (data not shown). Studies in juvenile rats with motor hyperactivity induced by 6-hydroxy-dopamine lesioning showed that both D- and D,L-MPH inhibited the activity while L-MPH did not. D-MPH was found to be 3.3-times more potent than D,L-MPH in reducing the locomotor hyperactivity while pretreatment of the lesioned rats with L-MPH significantly reduced the motor inhibiting effects of D-MPH (Davids et al., 2002). D-MPH was developed as a potential improved treatment for ADHD and has been shown in clinical trials to be efficacious in treating ADHD with fewer side-effects than D,L-MPH (Celgene internal document). Adverse events associated with prolonged human use of D,L- and D-MPH include, insomnia, nausea, weight loss, fever and abdominal pain (Novartis Ritalin® package insert, 2002; Novartis Focalin® package insert, 2002). Phase III clinical trials have shown that the nervousness and tachycardia seen with D,L-MPH use is absent with D-MPH and illustrate the superior safety profile of D-MPH (manuscript in preparation). Significant rat behaviors observed with D,L-MPH such as increases in the difficulty in removal from box, arousal, click and tail-pinch responses could represent a rodent manifestation of the nervousness in humans.

In conclusion, comparative neurobehavioral studies showed that fewer significant behavioral effects were seen with D- and L-MPH compared to equimolar doses of D,L-MPH. L-MPH was the least potent in producing the FOBs; this is consistent with the lower overall neuropharmacological activity of the L enantiomer compared to the d enantiomer. The psychostimulant properties of D- and D,L-MPH appear to be responsible for the observed FOBs. These results were supported by the rota-rod studies.

References

Barbaresi W, Katusic S, Colligan R, Oankratz V, Weber K, Mrazek D, et al. How common is attention-deficit/hyperactivity disorder? Incidence in a population-based cohort in Rochester Minnesota. Arch Pediatr Adolesc Med 2002;156:217–24.

Davids E, Zhang K, Tarazi F, Baldessarini R. Stereoselective effects of methylphenidate on motor hyperactivity in juvenile rats induced by neonatal 6-hydroxydopamine lesioning. Psychopharmacology 2002; 160:92–8.

Diagnostic and statistical manual of mental disorders (DSM-IV). 4th ed. Washington (DC): American Psychiatric Association; 1994.

Ding Y, Fowler J, Volkow N, Logan J, Gatley S, Sugano Y. Carbon-11-D-*threo*-methylphenidate binding to dopamine transporter in baboon brain. J Nucl Med 1996;36:2298–305.

Ding Y, Fowler J, Volkow N, Dewey S, Wang G, Logan J, et al. Chiral drugs: comparison of the pharmacokinetics of [^{11}C]D-*threo*- and L-*threo*-methylphenidate in the human and baboon brain. Psychopharmacology 1997;131:71–8.

Eckerman D, Moy S, Perkins A, Patrick K, Breese G. Enantioselective behavioral effects of *threo*-methylphenidate in rats. Pharmacol Biochem Behav 1991;40:875–80.

Faraj B, Israili Z, Perel J, Jenkins M, Holtzman S, Cucinell S, et al. Metabolism and disposition of methylphenidate-^{14}C: studies in man and animals. J Pharmacol Exp Ther 1974;191:535–47.

Greenhill L. Pharmacologic treatment of attention deficit hyperactivity disorder. In: Shaffer D, editor. The psychiatric clinics of North America, vol. 15, #1. Philadelphia: Harcourt Brace Jovanovich; 1992. p. 1–7.

Kimko H, Cross J, Abernethy D. Pharmacokinetics and clinical effectiveness of methylphenidate. Clin Pharmacokinet 1999;37:45–70.

Kulig B, Jaspers R. Assessment techniques for detecting neurobehavioral toxicity. In: Niesink R, editor. Neurobehavioral toxicology and addiction: food, drugs and environmental textbook, vol. 1. Heerlen, Netherlands: Open University; 1996. p. 71–112.

Luthman J, Fredriksson A, Lewander T, Jonsson G, Archer T. Effects of D-amphetamine and methylphenidate on hyperactivity produced by neonatal 6-hydroxydopamine treatment. Psychopharmacology 1989;99: 550–7.

Maxwell R, Chaplin E, Batmanglidj Eckhardt S, Soares J, Hite G. 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 1970;173: 158–65.

Moser V, McCormick J, Creason J, MacPhail R. Comparison of chlordimeform and carbaryl using a functional observational battery. Fundam Appl Toxicol 1988;11:189–96.

Novartis Ritalin package insert, 2002. East Hanover, NJ.

Novartis Focalin package insert, 2002. East Hanover, NJ.

Patrick K, Kilts C, Breese G. Synthesis and pharmacology of hydroxylated metabolites of methylphenidate. J Med Chem 1981;24:1237–40.

Patrick K, Muller R, Gualtieri C, Breese G. Pharmacokinetics and actions of methylphenidate. In: Meltzer H, editor. Psychopharmacology: the third generation of progress. New York: Raven Press; 1987a. p. 1387–95.

Patrick K, Caldwell R, Ferris R, Breese G. Pharmacology of the enantiomers of *threo*-methylphenidate. J Pharmacol Exp Ther 1987b;24: 152–8.

Rowland A, Umbach D, Catoe K, Stallone L, Long S, Rabiner D, et al. Studying the epidemiology of attention-deficit hyperactivity disorder: screening method and pilot results. Can J Psychiatry 2001;46:931–40.

Schachter H, Pham B, King J, Langford S, Moher D. How efficacious and safe is short-acting methylphenidate for the treatment of attention-deficit disorder in children and adolescents? A meta-analysis. Can Med Assoc J 2001;165:1475–88.

Schafer G, York R, Terrill J. The effect of both psychoactive drugs and conditioning on rota-rod performance. Toxicologist 1995;15:246.

Sunohara G, Malone M, Rovet J, Humphries T, Roberts W, Taylor M. Effect of methylphenidate on attention in children with attention deficit hyperactivity disorder (ADHD): ERP evidence. Neuropsychopharmacology 1999;21:218–28.

Szporny L, Gorog P. Investigations into the correlations between mono-

amine oxidase inhibition and other effects due to methylphenidate and its stereoisomers. Biochem Pharmacol 1961;8:263–8.

Teo S, Stirling D, Thomas S, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of D-methylphenidate and D,L-methylphenidate in Sprague–Dawley rats. Toxicology 2002;179:183–196.

Weiss G, Hechtman L. Hyperactive children grown up: ADHD in children. Adolescents and adults. 2nd ed. New York: Guilford Press; 1984.

Zuddas A, Anciletta B, Muglia P, Cianchetti C. Attention-deficit/hyperactivity disorder: a neuropsychiatric disorder with childhood onset. Eur J Paediatr Neurol 2000;4:53–62.