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Sex Differences in the Kinetic Profiles of d- and l- Methylphenidate in the Brains of Adult Rats

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Sex differences in the kinetic profiles of d- and l- methylphenidate in the brains of adult rats

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Abstract. – OBJECTIVE: Methylphenidate is commonly used in the treatment of Attention Deficit Hyperactivity Disorder and narcolepsy. Methylphenidate is administered as a racemic mixture of the d- and l- threo enantiomers; however, the d-enantiomer is primarily responsible for the pharmacologic activity. Previous studies of the behavioral effects of methylphenidate have highlighted sex differences in the responsiveness to the drug, namely an increased sensitivity of females to its stimulatory effects. These differences may be due to differences in the uptake, distribution, and elimination of methylphenidate from male and female brains. Therefore, we compared the pharmacokinetics of d- and l- threo methylphenidate in the brains of male and female rats.

MATERIALS AND METHODS: Adult male and female Sprague-Dawley rats were injected with 5 mg/kg d, l- threo methylphenidate, and whole brains were collected at various time points following injection. We measured methylphenidate concentrations utilizing chiral high pressure liquid chromatography followed by mass spectrometry.

RESULTS: Females exhibited consistently higher brain concentrations of both d- and l-methylphenidate and a slower clearance of methylphenidate from brain as compared to males, particularly with the active d-enantiomer.

CONCLUSIONS: The increased sensitivity of females to methylphenidate may be partially explained by an increase in total brain exposure to the drug.

Key Words:
Methylphenidate, Brain, Chiral separation, Sex differences, Liquid chromatography-mass spectrometry.

Introduction

Methylphenidate (MPH) is commonly used in children and adolescents for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) and narcolepsy. According to current estimates, 5.9-7.1% of children and 5.0% of adults are affected by ADHD, and MPH is the first line treatment, accounting for a majority of the prescriptions written for the disorder[1-3]. Additionally, increases in off-label use and diversion of MPH have been reported[4-6]. MPH works in the brain by blockade of both the dopamine transporter (DAT) and the norepinephrine transporter (NET), which leads to an increase in both dopamine and norepinephrine in the synaptic cleft. Given the fact that MPH increases extracellular dopamine, it has the potential for abuse[7]. In fact, it is more potent at blocking the DAT than cocaine[8]. However, its limited abuse in the context of clinical use appears to be due to pharmacokinetics, namely its slow, steady uptake in the brain when taken orally[9].

Methylphenidate has two chiral centers; however, only the *threo* enantiomers are used in therapy, as they are more pharmacologically potent[10]. Although the drug is administered as a racemic mixture of the d- and l- *threo* enantiomers, the d-MPH enantiomer is thought to be primarily, if not entirely, responsible for the pharmacologic activity[11-14]. Interestingly, MPH is also subject to stereoselective metabolism. MPH is subject to hydrolysis of the methyl ester linkage via the enzyme carboxylesterase 1 (CES1) to the pharmacologically inactive d- or l- ritalinic acid[11,15]. It is well-recognized the CES1 is considerably more efficient in the de-esterfication of l-MPH relative to d-MPH, resulting in a significantly higher bioavailability of the d- enantiomer[14,15].

Most of the previous studies analyzing the effects of MPH on the brain and behavior have utilized males as subjects. This trend is likely due to the recognized higher prevalence of ADHD in males versus females and the biological complexity of females due to their reproductive cycling[16]. However, both human and animal studies have indicated some important gender differences in ADHD manifestations, as well as responsiveness to psychostimulants[17-20]. For exam-

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ple, Patrick and colleagues reported that females described a significantly greater stimulant effect of MPH than males when asked in a self-report analysis[21]. Interestingly, a number of animal studies also indicate the presence of sex differences in response to MPH. In one study, adult female rats demonstrated increased conditioned hyperactivity to both moderate and high doses of MPH as compared to adult male rats[22]. Additionally, in two recent studies, females demonstrated behavioral sensitization (or increased locomotor responses to subsequent exposure) to MPH whereas males did not[23,24].

In sum, several studies seem to indicate an increased responsiveness of females to MPH. One of these studies was conducted in collaboration with our laboratory, and it revealed that female rats demonstrated more robust sensitization to MPH and increased locomotor activation compared to males[23]. These sex differences could be due to pharmacodynamic and/or pharmacokinetic factors. Since drug effect is directly related to brain concentrations, here, we investigate MPH pharmacokinetics in male and female brains. Specifically, we compare the uptake, distribution, and elimination of d- and l- *threo* MPH in the brains of male and female rats.

Materials and Methods

Animals

Fifty day old rats were chosen for this study in order to follow up on previous behavioral work conducted in collaboration with our laboratory[23]. In that particular study, rats received injections of MPH or saline every other day from P33 through P50; locomotor activity and behavioral sensitization was analyzed throughout this time period, and in females, the most robust effect occurred after day 50 with a dose of 5 mg/kg MPH. As such, we aimed to investigate the pharmacokinetics of MPH in animals at this developmental age (P50) using an identical dose. Furthermore, the 5 mg/kg dose has been utilized in other studies to mimic an "abusive dose" of MPH, and intraperitoneal (IP) administration is believed to mimic the absorption seen in snorting[23,25,26]. As such, 50 day old Sprague-Dawley rats (150-200 g) were injected intraperitoneally with 5 mg/kg d, l- *threo* methylphenidate HCl (Sigma Aldrich, St. Louis, MO, USA) prepared in sterile physiological saline. Animals were sacrificed via decapitation at the following time points post injection: 1 min, 5 min, 10 min, 30 min, 60 min, and 120 min, and an n of 6 to 7 for each sex at each time point was used to ensure appropriate statistical power. Whole brains were flash frozen in liquid nitrogen and stored at –70°C for later use. All animals were housed in an Association for the Assessment and Accreditation of Laboratory Animal Care (AALAC) accredited facility with food and water provided *ad libitum*. All procedures were carried out according to NIH guidelines and were approved by the East Tennessee State University Committee on Animal Care.

Tissue Collection and Sample Preparation

Solid phase extraction was utilized to extract MPH from brain tissue according to our previously validated method[27]; this method allows for extraction recovery of 72-75% for both d- and l-threo methylphenidate and both low (10 ng/mL) and high (100 ng/mL) calibration concentrations.

Liquid Chromatography-Mass Spectrometry

Methylphenidate concentrations were measured using liquid chromatography-mass spectrometry (LC-MS). Since MPH is administered as a racemic mixture of the d- and l- *threo* forms, these enantiomers were separated and measured individually as described previously[27]. This method allowed for a lower limit of detection (LLOD) of 0.5 ng/mL and a lower limit of quantification (LLOQ) of 7.5 ng/mL, with desirable intra- and inter-day precision and accuracy (% RSD and % error were < 15% for every calibration point).

Statistical Analysis

All data were subjected to a noncompartmental analysis utilizing the Phoenix 64/WinNonLin software. Parameters of interest included the area under the curve (AUC), the maximal concentration (C_{max}), the time of maximal concentration (T_{max}), the clearance from the brain (Cl),and the elimination half-life ($T_{1/2}$) from the brain. If standard errors were reported, the results of these analyses were compared using an ANOVA followed by Newman-Keuls Multiple Comparison Tests. Data were considered to be statistically significantly different when $p < 0.05$.

Results

The d- and l- *threo* enantiomers of MPH were measured using LC-MS. Interestingly, females

appeared to exhibit consistently higher brain concentrations of both d- and l- MPH (Figure 1). Additionally, the d-enantiomer appeared to be maintained in the brain at higher concentrations as compared to the l-enantiomer in both females and males (Figure 1). Subsequently, the pharmacokinetic data were analyzed via noncompartmental analysis with Phoenix 64/WinNonLin software. Although the maximal concentrations (C_{max}) of d- and l- MPH were not significantly different in males as compared to females (Figure 2A), the total brain exposure to the drug, as indicated by the area under the curve (AUC), was significantly higher in females versus males (Figure 2B).

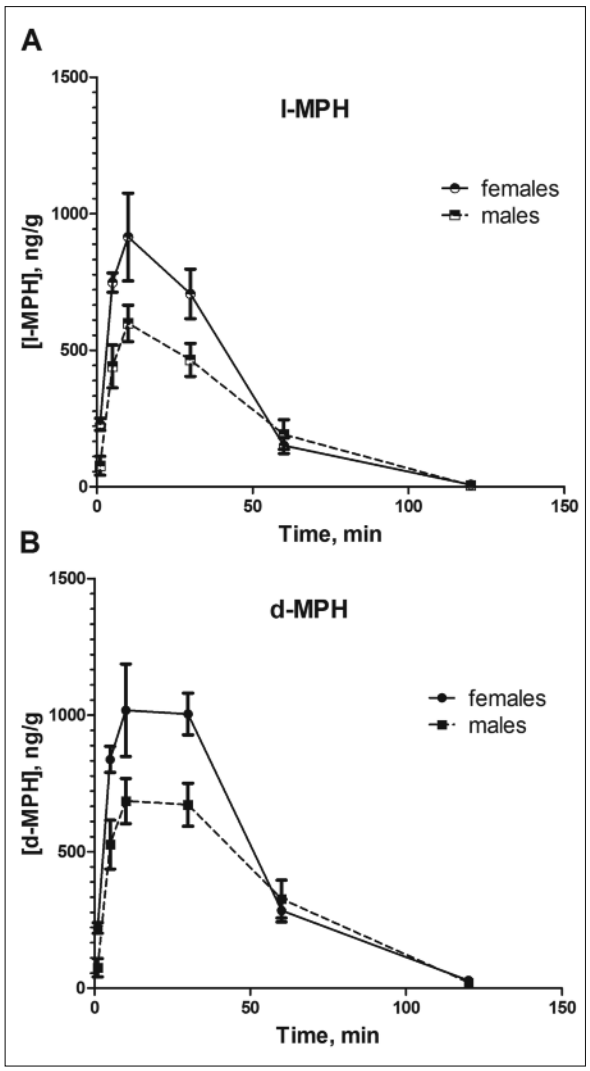

Figure 1. Pharmacokinetics of ***A***, l- and ***B***, d- methylphenidate (MPH) following intraperitoneal injection of 5 mg/kg MPH in females (solid line) and males (dotted line). Data are expressed as the mean ± SEM, n = 6-7.

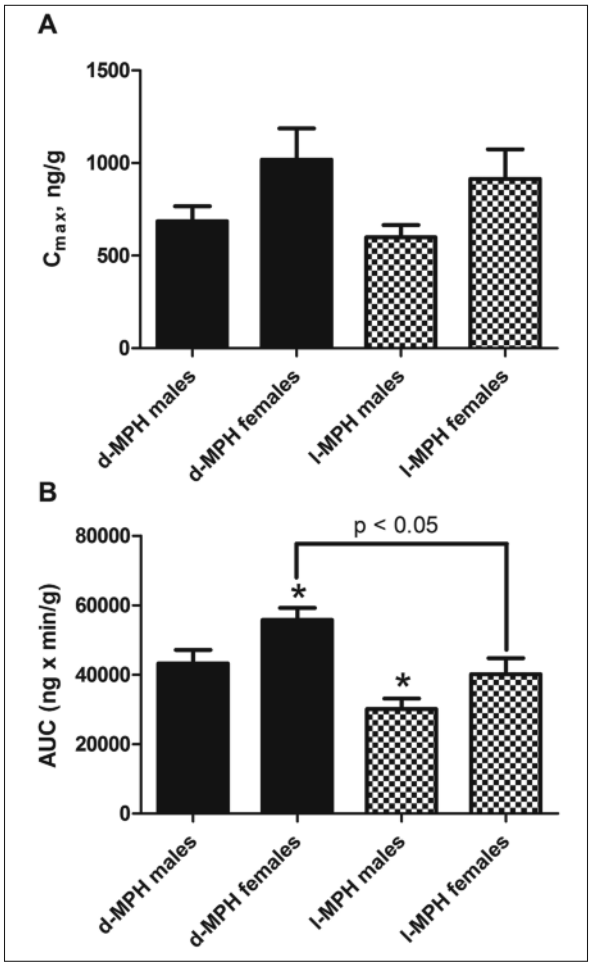

Figure 2. ***A,*** Maximal concentrations of d- and l-MPH (C_{max}) following injection of 5 mg/kg MPH. No statistically significant differences were achieved. ***B,*** Total brain d- and l- MPH exposure as calculated by the area under the curve (AUC) in males versus females. $^{*}p < 0.05$ versus the AUC of d-MPH in males (n = 6); One way ANOVA followed by Newman-Kuels multiple comparisons test.

Based on the pharmacokinetic model created by the noncompartmental analysis, several other parameters were calculated including the time of maximal concentration (T_{max}), the half-life of MPH in the brain ($T_{1/2}$), and the clearance of MPH from the brain (Cl). (Table I). Since the data are theoretical, standard errors could not be calculated. Interestingly, the $T_{1/2}$ for either enantiomer did not appear to be substantially different between the sexes. Additionally, the T_{max} for both d- and l- in males and females was 10 min. However, there did appear to be some sex differences that should be noted; females exhibited a slower Cl of MPH as compared to males, particularly with the d-enantiomer (88.45 g/min versus

Table I. Theoretical pharmacokinetic parameters as calculated through pharmacokinetic modeling. Values for half-life (T ½), time of maximal concentration (T max), and clearance (Cl) from the brain are presented.

	l-MPH Males	l-MPH females	d-MPH males	d-MPH females
$T_{1/2}$ (min)	13.55	14.33	17.52	17.62
T_{max} (min)	10	10	10	10
Cl (g/min)	165.68	124.24	144.22	88.46

Values for half-life ($T_{½}$), time of maximal concentration (T_{max}), and clearance (Cl) from the brain are presented.

144.22 g/min). Finally, when comparing the enantiomers, the $T_{1/2}$ appeared to be slightly longer and the Cl slightly less for d-MPH as compared to l-MPH.

Discussion

In this study, we examined the brain pharmacokinetics of d- and l- *threo* MPH in male and female rats following intraperitoneal injection of 5 mg/kg MPH. Interestingly, we discovered the females had consistently higher brain concentrations of both d- and l- MPH than males, resulting in a significantly higher overall brain exposure to MPH (as represented by the AUC). This was accompanied by a notable decrease in the rate of clearance of MPH from the brain for both enantiomers, but most profoundly with the pharmacologically active d-enantiomer. The reason for the sex differences in the pharmacokinetics of MPH is currently unclear. To date, there is little known regarding the extent to which transporters contribute to movement of MPH across the blood-brain barrier; however, one study indicates that a carrier-mediated process is at least partially responsible[28]. This carrier is saturable and is also responsible for the uptake of amphetamine and β-phenethylamine. In addition, one study indicated that d-MPH is a weak substrate of P-glycoprotein[29]. Given the fact that transporters are at least partially responsible for access of methylphenidate to the brain, sex differences in the levels of these transport proteins may contribute to the differences observed in this study. Another possible explanation for the increased levels of MPH in females as compared to males would be sex differences in the metabolism of methylphenidate. As noted earlier, methylphenidate is subjected to hydrolysis via the CES1 enzyme; if males have higher CES1 activity than females, this would result in lower bioavailability of MPH and thus lower brain concentrations. Regardless, these results may at least partially explain the previous findings that females are more sensitive to the psychostimulant effects of MPH. Specifically, in a study completed in conjunction with our laboratory, females demonstrated robust locomotor sensitization in response to 5 mg/kg MPH, whereas males did not.

In this study, we also quantified the levels of d- and l- *threo* MPH separately. Of note, the AUC for d-MPH was also significantly greater than the AUC for l-MPH in both males and females, thereby indicating greater exposure to the more active enantiomer. This is likely due to the enhanced bioavailability of this enantiomer due to preferential hydrolysis of l- *threo* MPH by CES1, which in rodents is active in both the liver and the plasma[14,15]. Brain concentrations found in this study (725 ± 87.0 at 10 min and 552.7 ± 60.2 at 30 min, pooled d- and l-MPH, male and female data) utilizing LC-MS were similar to previous reports employing other methodologies; Levant and colleagues used an ELISA-based assay to find that adult rats at postnatal days 42 and 70 had brain concentrations of 985 ± 44 ng/g and 1006 ± 32 ng/g, respectively, 20 min following subcutaneous administration of 5 mg/kg MPH[30]. In that particular study, the enantiomers were not quantified separately, and multiple time points were not examined. A few other studies have examined brain concentrations of MPH in rats, yet the doses and/or methods of administration used in these studies were vastly different, making direct comparisons difficult[31-35].

Conclusions

In summary, we have found significant sex differences in the pharmacokinetics of MPH; namely, females have a higher overall brain exposure to MPH as compared to males, especially with the d-enantiomer. Additionally, the elimination of MPH from the brain as represented by the clearance appears to be substantially slower in females. Future studies are needed to determine the reason for the sex-related differences, but they could be related to variance in metabolism between the sexes or differences in the rate of transport of methylphenidate across the blood brain barrier. Nonetheless, these data may explain many of the previously documented sex differences in the responses to this psychostimulant indicating an increased sensitivity of females to the drug.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Chai G, Governale L, McMahon AW, Trinidad JP, Staffa J, Murphy D. Trends of outpatient prescription drug utilization in US children (2002-2010). Pediatrics 2012; 130: 23-31.
2) Willcutt EG. The prevalence of DSM-IV attention-deficit/hyperactivity disorder: a meta-analytic review. Neurotherapeutics 2012; 9: 490-499.
3) Getahun D, Jacobsen SJ, Fassett MJ, Chen W, Demissie K, Rhoads GG. Recent trends in childhood attention deficit/hyperactivity disorder. JAMA Pediatr 2013; 167: 282-288.
4) Advokat C. What are the cognitive effects of stimulant medications? Emphasis on adults with attention-deficit/hyperactivity disorder (ADHD). Neurosci Biobehav Rev 2010; 34: 1256-1266.
5) Advokat C, Vinci C. "Do stimulant medications for attention deficit/hyperactivity disorder (ADHD) enhance cognition?" in Current Directions in ADHD and Its Treatment. Rijeka InTech (Croatia) 2012: 125-156.
6) Olfson M, Blanco C, Wang S, Greenhill LL. Trends in office-based treatment of adults with stimulants in the United States. J Clin Psychiatry 2013; 74: 43–50.
7) Wise RA. Brain reward circuitry: insights from unsensed incentives. Neuron 2002; 36: 229-240.
8) Volkow ND, Wang GJ, Fowler JS, Fischman M, Foltin R, Gatlety SJ, Logan J, Wong C, Figgord A, Hitzemann R, Pappas N. Methylphenidate and cocaine have a similar in vivo potency to block dopamine transporters in the human brain. Life Sci 1999; 65: 7-12.
9) Volkow ND, Swanson JM. Variables that affect the clinical use and abuse of methylphenidate in the treatment of ADHD. Am J Psychiatry 2003; 160: 1909-1918.
10) Ferris RM, Tang FLM. Comparison of the effects of the isomers of amphetamine, methylphenide and deoxypipradol on the uptake of 1-[3H]-norepinephrine and [3H]-dopamine by synaptic vesicles from the rat whole brain, striatum and hypothalamus. J Pharmacol Exp Ther 1979; 210: 422-428.
11) Patrick KS, Caldwell RW, Ferris RM, Breese GR. Pharmacology of the enantiomers of threo-methylphenidate. J Pharmacol Exp Ther 1987; 241: 152-158.
12) Ding YS, Fowler JS, Volkow ND, Dewey SL, Wang GJ, Logan J, Gatley SJ, Pappas N. Chiral drugs: comparison of the pharmacokinetics of [11C]d-threo and l-threo-methylphenidate in the human and baboon brain. Psychopharmacology 1997; 131: 71-78.
13) Kimko H, Cross JT, Abernethy DR. Pharmacokinetics and clinical effectiveness of methylphenidate. Clin Pharmacokinet 1999; 37: 457-470.
14) Markowitz J, Patrick K. Differential pharmacokinetics and pharmacodynamics of methylphenidate enantiomers. Does chirality matter? J Clin Psychopharmacol 2008; 28: 54-61.
15) Sun Z, Murry DJ, Sanghani SP, Davis WI, Kedishvili NY, Zou Q, Hurley TD, Bosron WF. Methylphendiate is stereoselectively hydrolyzed by human carboxylesterase CES1A1. J Pharmacol Exp Ther 2004; 310: 469-476.
16) Hasson R, Fine JG. Gender differences among children with ADHD on continuous performance tests: a meta-analytic review. J Atten Disord 2012; 16: 190-198.
17) Dafny N, Yang PB. The role of age, genotype, sex, and route of acute and chronic administration of methylphenidate: a review of its locomotor effects. Brain Res Bull 2006; 68: 393-405.
18) Sonuga-Barke EJS, Coghill D, Markowitz JS, Swanson JM, Vanderberghe M, Hatch SJ. Sex differences in the response of children with ADHD to once-daily formulations of methylphenidate. J Am Acad Child Adolesc Psyciatry 2007; 46: 701-710.
19) Rucklidge JJ. Gender differences in attention-deficit/hyperactivity disorder. Pyschiatr Clin North Am 2010; 33: 357-373.

20) Dow-Edwards D. Sex differences in the effects of cocaine abuse across the life span. Physiol Behav 2010; 100: 208-215.

21) Patrick KS, Straughn AB, Minhinnett RR, Yeatts SD, Herrin AE, DeVane CL, Malcolm R, Janis GC, Markowitz JS. Influence of ethanol and gender on methylphenidate pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther 2007; 81: 346-353.

22) Wooters TE, Dwoskin LP, Bardo MT. Age and sex differences in the locomotor effect of repeated methylphenidate in rats classified as high or low novelty responders. Psychopharmacology 2006; 188: 18-27.

23) Brown RW, Hughes BA, Hughes AB, Sheppard AB, Perna MK, Ragsdale WL, Roeding RL, Pond BB. Sex and dose-related differences in methylphenidate adolescent locomotor sensitization and effects on brain-derived neurotrophic factor. J Psychopharmacol 2012; 26: 1480-1488.

24) Chelaru M, Yang P, Nachum D. Sex differences in the behavioral response to methylphenidate in three adolescent rat strains (WKY, SHR, SD). Behav Brain Res 2012; 226: 8-17.

25) Bradberry CW. Dynamics of extracellular dopamine in the acute and chronic actions of cocaine. Neuroscientist 2002; 8: 315-22.

26) Dela Peña I, Kim HJ, Sohn A, Kim BN, Han DH, Ryu JH, Shin CY, Noh M, Cheong JH. Prefrontal cortical and striatal transcriptional responses to the reinforcing effect of repeated methylphenidate treatment in the spontaneously hypertensive rat, animal model of attention-deficit/hyperactivity disorder (ADHD). Behav Brain Funct 2014; 10: 17.

27) Combs C, Hankins, E, Copeland, C, Brown, S, Pond, BB. Quantitative determination of d- and l- enantiomers of methylphenidate in brain tissue by liquid chromatography-mass spectrometry. Biomed Chromatogr 2013; 27: 1587-1589.

28) Pardridge WM, Connor JD. Saturable transport of amphetamine across the blood-brain barrier. Experientia 1973; 29: 302-304.

29) Zhu HJ, Wang JS, DeVane CL, Williard RL, Donovan JL, Middaugh LD, Gibson BB, Patrick KS, Markowitz JS. The role of the polymorphic efflux transporter P-glycoprotein on the brain accumulation of d-methylphenidate and d-amphetamine. Drug Metab Dispos 2006; 34: 1116-1121.

30) Levant B, Zarcone TJ, Davis PF, Ozias MK, Fowler SC. Differences in methylphenidate dose response between periadolescent and adult rats in the familiar arena-novel alcove task. J Pharmacol Exp Ther 2011; 337: 83-91.

31) Gal J, Hodshon BJ, Pintauro C, Flam BL, Cho AK. Pharmacokinetics of methylphenidate in the rat using single-ion monitoring CLC-mass spectrometry. J Pharmaceut Sci 1977; 66: 866-9.

32) Patrick KS, Ellington KR, Breese GR. Distribution of methylphenidate and p-hydroxymethylphenidate in rats. J Pharmacol Exp 1984; 231: 61-65.

33) Aoyama T, Kotaki H, Sawada Y, Iga T. Pharmacokinetics and pharmacodynamics of methylphenidate enantiomers in rats. Psychopharmacology 1996; 127: 117-122.

34) Aoyama T, Yamamoto K, Kotaki H, Sawada Y, Iga T. Pharmacodynamic modeling for change of locomotor activity by methylphenidate in rats. Pharm Res 1997; 14: 1601-1606.

35) Thai DL, Yurasits LN, Rudolph GR, Perel JM. Comparative pharmacokinetics and tissue distribution of the d-enantiomers of para-substituted methylphenidate analogs. Drug Metab Dispos 1999; 27: 645-650.