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Commentary

Potential for Underestimation of d-Methylphenidate Bioavailability Using Chiral Derivatization/Gas Chromatography

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ABSTRACT

A tenable hypothesis is presented which explains disparities between older oral dl-MPH bioavailability data generated using chiral derivatization-gas chromatography versus more recent findings using chiral liquid chromatography. These disparities persist in current literature. The gas chromatographic methods found that the absolute bioavailability of d-MPH is 23% and that of l-MPH is 5% (i.e., 82% as the active d-isomer), while liquid chromatographic methods consistently report that approximately 99% of circulating MPH is d-MPH. Older methods used perfluoroacylated S-prolyl derivatizing agents which have a history of imprecision due to the susceptibility of the prolyl S-configuration to isomerize to the R-enantiomer. Accordingly, any R-prolyl impurity in the chiral derivatization reagent yields the (R,R,R)-MPH-prolyl diastereomer which, in being related as the opposite enantiomer of (S,S,S)-prolyl-MPH, co-elutes with l-(S,S)-MPH. This results in overestimation of the percent l-MPH at the expense of underestimating d-MPH. Unless compelling reasons exist to justify use of any chiral discriminators, less complex and less costly achiral analysis of plasma MPH appears appropriate for d-MPH quantitation since 99% exists as d-MPH. However, simultaneous plasma monitoring of d-MPH and l-MPH may be warranted when alterations in first-pass hepatic metabolism by carboxylesterase 1 (CES1) occurs. For example, (a) with transdermal dl-MPH delivery; (b) in cases of concomitant dl-MPH and a CES1 inhibitor, e.g., ethanol, which elevates l-MPH and d-MPH concentrations; (d) in forensic studies of intravenous or intranasal dl-MPH abuse; (e) were dl-MPH to be formulated as a free base sublingual product; or (f) as emerging advances in dl-MPH gene-dose effects warrant isomer correlations.

We reevaluated the extent to which immediate-release (IR) dl-methylphenidate (dl-MPH) is subject to enantioselective presystemic metabolism within the context of the existing biomedical literature. The following discussion is intended to better define the pharmacokinetics (PK) of oral IR-dl-MPH. In addition, in this letter we offer a rationale for considering selection of less-complex, cost-curtailing bioanalytical methods that obviate chromatographic chiral discriminators when monitoring the psychoactive d-methylphenidate (d-MPH) isomer component of this racemate (Patrick et al., 1987; Patrick and Straughn, 2016). Understanding the PK of IR-dl-MPH underpins rational study designs of new modified-release (MR) dl-MPH formulation technologies (at least six unique branded MR-dl-MPH products have been approved in the last 5 years, with a seventh tentatively approved (Drugs@FDA: FDA Approved Drug Products; https://www.accessdata.fda.gov/scripts/cder/daf/); others are in the regulatory pipeline (Patrick et al., 2019). Furthermore, understanding dl-MPH absorption and disposition provides guidance for bioanalytical methods used in ongoing gene-/dose-effect studies, which are now showing increasing promise in the advancement of personalized attention-deficit/hyperactivity disorder (ADHD) pharmacotherapy (Zhu et al., 2008; Lyauk et al., 2016; Stage et al., 2017, 2019).

IR-dl-MPH typically provides 4 hours of efficacy in the treatment of ADHD. This psychostimulant undergoes extensive oral first-pass metabolism in humans (Chan et al., 1983) (Table 1), monkeys, and rats (Wargin et al., 1983), whereby the absolute bioavailability of the combined methylphenidate (MPH) enantiomers has been reported to be approximately 30%, 22%, and 19% in these species, respectively. These early 1980s determinations used achiral (nonenantiospecific) gas chromatography (GC) methods. Subsequently, an enantiospecific GC approach using chiral MPH derivatization, chlorphentermine as an internal standard, and electron capture detection found that the isomeric composition of the absolute bioavailable fraction following an oral racemic MPH dose exists as 23% d-MPH (the *R*,*R*-isomer) and 5% l-methylphenidate (l-MPH) (the *S*,*S*-isomer) in humans (Srinivas et al., 1993); equating to 82% of total plasma MPH exposure representing the d-MPH enantiomer (Table 1). Similar percentage differences between d-MPH and l-MPH plasma exposure have persisted in more contemporary secondary (Srinivas, 2004) and tertiary biomedical literature studies (Thummel et al., 2018).

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ABBREVIATIONS: ADHD, attention-deficit/hyperactivity disorder; CES1, carboxylesterase 1; d-MPH, d-methylphenidate; dl-MPH, dl-methylphenidate; GC, gas chromatography; HFP, heptafluorobutyryl-*S*-prolyl chloride; IR, immediate release; LC, liquid chromatography; l-MPH, l-methylphenidate; MPH, methylphenidate; MR, modified release; MS, mass spectrometry; PK, pharmacokinetics.

TABLE 1

d- and l-MPH exposure, time course parameters, and methods

Reference	Dose	Early Exposure $pAUC_{0-3\ h}$ (Plasma % d-MPH)[a]		Total Exposure $AUC_{0-\infty}$ (% d-MPH)		*F*		T_{max}		$T_{1/2}$		Analytical Method
		d-MPH	l-MPH	d-MPH	l-MPH	d-MPH	l-MPH	d-MPH	l-MPH	d-MPH	l-MPH	
		ng·h/ml	*ng·h/ml*	*ng·h/ml*	*ng·h/ml*	%	%	*h*	*h*	*h*	*h*	
Chan et al. (1983)												
IR-MPH (*N* = 5)	10 or 15 mg (oral)	N/A	N/A	N/A	N/A	27.86 ± 11.48 Total d- + l-MPH (fasted)[b]	27.86 ± 11.48 Total d- + l-MPH (fasted)[b]	1.6 ± 0.4	1.6 ± 0.4	2.1 ± 0.4	2.1 ± 0.4	Achiral TFA GC-MS
IR-MPH (*N* = 5)	10 or 15 mg (oral)	N/A	N/A	N/A	N/A	31.40 ± 15.87 Total d- + l-MPH (fed)	31.40 ± 15.87 Total d- + l-MPH (fed)	1.0 ± 0.4	1.0 ± 0.4	2.1 ± 0.3	2.1 ± 0.3	Achiral TFA GC-MS
IR-MPH (*N* = 5)	10 or 15 mg (i.v.)	N/A	N/A	N/A	N/A	100	100	0.5	0.5	2.0 ± 0.4	2.0 ± 0.4	Achiral TFA GC-MS
Srinivas et al. (1987)												
IR-MPH (*N* = 5)	10 mg (oral)	9.00 (89% d-MPH)	1.13 (89% d-MPH)	30.46 ± 9.57 (82% d-MPH)	6.66 ± 1.38 (82% d-MPH)	N/A	N/A	2.2 ± 0.5	2.01 ± 1.16	3.1 ± 1.1	5.59 ± 1.07	Chiral HFB-GC-ECD
Srinivas et al. (1992)												
IR-MPH (*N* = 9)	10 mg (oral)	7.43 (83% d-MPH)	1.50 (83% d-MPH)	27.71 ± 9.53 (86% d-MPH)	4.61 ± 1.77 (86% d-MPH)	N/A	N/A	2.3 ± 0.5	2.4 ± 0.5	1.87 ± 0.65	1.43 ± 0.76	Chiral HFB-GC-ECD
Srinivas et al. (1993)												
IR-MPH (*N* = 13)	40 mg (oral)	19.50 (84% d-MPH)	3.75 (84% d-MPH)	120.21 ± 30.68 (89% d-MPH)	14.79 ± 4.14 (89% d-MPH)	23 (82%)	5 (18%)	2.4 ± 0.8	2.14 ± 0.64	5.7 ± 1.2	3.93 ± 0.76	Chiral HFB-GC-ECD
IR-MPH (*N* = 11)	10 mg (i.v.)	15.00 (56% d-MPH)	12.00 (56% d-MPH)	147.74 ± 47.91 (63% d-MPH)	88.64 ± 43.13 (63% d-MPH)	100	100	0.25	0.25	6.0 ± 1.7	3.61 ± 1.12	Chiral HFB-GC-ECD
Modi et al. (2000b)												
OROS-MPH (*N* = 33)	18 mg (oral)	2.25 (99% d-MPH)	0.03 (99% d-MPH)	42.2 ± 16 (99% d-MPH)	0.43 ± 0.7 (99% d-MPH)	N/A	N/A	7.9 ± 2.0	7.1 ± 2.0	3.8 ± 0.8	N/A	Chiral LC-MS
Patrick et al. (2007)												
IR-MPH (*N* = 19)	0.3 mg/kg (oral)	20.25 (98% d-MPH)	0.38 (98% d-MPH)	82.9 ± 26.2 (N/A)	N/A	N/A	N/A	2.3 ± 32.7	3	2.8 ± 14.3	N/A	Chiral LC-MS
Patrick et al. (2013)												
IR-MPH (*N* = 24)	0.15 mg/kg (oral)	25.10 (99% d-MPH	0.27 (99% d-MPH)	52.1 ± 29 (99% d-MPH)	0.77 ± 73 (99% d-MPH)	N/A	N/A	2.4 ± 47	1.8 ± 49	2.9 ± 19	2.1 ± 41	Chiral LC-MS

$AUC_{0-\infty}$, area under the curve from time zero to infinity; ECD, electron capture detection; *F*, absolute bioavailability; HFB, *N*-heptafluorobutyryl-*S*-prolyl chiral derivative; N/A, not available; OROS, osmotic-controlled release oral-delivery system; $pAUC_{0-3\ h}$, partial area under the curve at 0–3 hours; TFA, *N*-trifluoroacetyl; $T_{1/2}$, half-life; T_{max}, time to maximum plasma concentration.

[a]The partial area under the curve was calculated using the arithmetic trapezoidal rule.

[b]Fasted; all other studies included breakfast, generally light.

This reported PK relationship between the d:l percentages of plasma MPH isomers notwithstanding, more recent relative bioavailability studies (i.e., where no comparative intravenous dl-MPH PK studies were conducted) have consistently shown that approximately 99% of total dl-MPH exposure is attributable to the d-MPH isomer. This was revealed by Ramos et al. (1999) in the course of validating a vancomycin-based chiral liquid chromatography (LC)/mass spectrometry (MS) method using IR-dl-MPH in a test subject prior to conducting bioavailability studies of a new MR-dl-MPH product (Lee et al., 2003). This determination of approximate 99% of oral dl-MPH reaching the systemic circulation has since been replicated in multiple studies of both IR-dl-MPH (Modi et al., 2000a; Patrick et al., 2007, 2013) (Table 1) and MR-dl-MPH formulations (e.g., Modi et al., 2000b). We note that 99%:1% d-MPH:l-MPH systemic exposure following oral dl-MPH could be viewed in practical terms as representing presystemic biocatalytic resolution of racemic dl-MPH to the racemic switch drug: pure d-isomer dexmethylphenidate. Indeed, the package labeling for dexmethylphenidate recommends using one-half the milligram dose when converting a maintenance dose of dl-MPH in an ADHD patient to the enantiopure d-MPH product (Drugs@FDA: FDA Approved Drug Products; https://www.accessdata.fda.gov/scripts/cder/daf/).

More recent bioanalytical studies characterizing enantiospecific plasma MPH concentrations have used chiral LC stationary phases rather than the GC method for MPH enantiomeric separations. These LC methods avoid problematic chiral derivatization (as discussed subsequently), provide the molecular specificity of tandem MS to suppress chemical noise, and incorporate deuterated internal standards that offer optimal analytical control of potential: 1) variability in extraction recovery, 2) extent of postsampling hydrolysis (Ramos et al., 1999), and 3) changes in intraday instrumental performance. Advantages of such an LC-MS analytical approach are evident from published representative chromatograms from LC-MS (Zhu et al., 2011) compared with chiral derivatization GC studies where chemical interferences and loss of baseline resolution are clearly evident (Srinivas et al., 1987).

Chiral derivatization GC methods for PK studies of dl-MPH have most often used heptafluorobutyryl-*S*-prolyl chloride (HFP) to form the corresponding (*S*,*R*,*R*)- and (*S*,*S*,*S*)-HFP-MPH diastereomers from d-(*R*,*R*)-MPH and l-(*S*,*S*)-MPH. The commercial availability of HFP, as used by Lin et al. (1999) for the chiral derivatization of dl-MPH extracted from human plasma, ended many years ago due to the unacceptable degree of in situ racemization during chemical synthesis (enantiomeric excess $<80\%$; technical support personal communication, January 2019; Campbell Supply Co., Rockton, IL).

The strong electron-withdrawing inductive effects of the seven fluorine bonds on the acyl group of HFP, taken together with the prolyl moiety existing as its acid chloride, can be expected to render the α-hydrogen atom especially acidic (Sykes, 1986), and accordingly predispose the stereogenic center to inversion of configuration to the unnatural amino acid *R*-proline antipode. Serving as a precedent, even when using the less fluorinated homolog *N*-trifluoroacetyl-*S*-prolyl chloride as a chiral GC derivatization reagent required corrections to the relative GC peak areas to establish the enantiomeric excess in the course of preparative scale resolution of dl-MPH isomers. In this example, a reference standard of pure (*S*)-methamphetamine was derivatized with *N*-trifluoroacetyl-*S*-prolyl chloride in parallel with resolution product characterization to adjust for the extent of actual enantiopurity (Patrick et al., 1987). Furthermore, Lui and Ku (1981) found that commercially available *N*-trifluoroacetyl-*S*-prolyl chloride contains 5% to 6% of the opposite *R*-enantiomer, thus again requiring analogous corrections for enantiospecific GC analysis of a range of amphetamine enantiomeric mixtures.

Any *R*-prolyl impurity in the (*S*)-HFP derivatization reagent will yield the (*R*,*R*,*R*)-MPH-prolyl diastereomer, which is the opposite enantiomer of (*S*,*S*,*S*)-HFP-MPH and thus coelutes with l-(*S*,*S*)-MPH to pose a likely potential for overestimating the percent of l-MPH at the expense of underestimating d-MPH. Were this to have been the case, the variance in the late 1980s and early 1990s reports regarding a lesser degree of enantioselective metabolism than now appears to occur finds a compelling scientific basis to explain these conflicting reports of differential isomeric extents of absorption. Thus, it would even appear rational to use less-expensive and less-complex achiral GC or LC methods for plasma d-MPH determinations in view of the vast preponderance of circulating MPH ($\sim$99%) existing as the active d-MPH enantiomer.

However, simultaneous quantitation of d- and l-MPH is warranted: 1) for transdermal dl-MPH delivery PK, where circumvention of the oral hepatic first-pass effect—the organ that expresses carboxylesterase 1 (CES1), which catalyzes dl-MPH hydrolysis—results in substantial l-MPH exposure (Patrick et al., 2009); 2) in cases of concomitant dl-MPH and CES1 inhibitor ethanol, where l-MPH concentrations rise (Patrick at al., 2007, 2013; Zhu et al., 2017); 3) for dl-MPH in combination with other CES1 inhibitors used in clinical practice, e.g., aripiprazole and fluoxetine (Zhu et al., 2010); or 4) in forensic studies of intravenous or intranasal dl-MPH abuse, which again circumvents the hepatic first-pass effect. (Use of intravenous dl-MPH for barbiturate overdose, anesthesia recovery, or intractable hiccups was discontinued in the 1960s.) Were dl-MPH to be formulated as a free base sublingual product, direct oral membrane solubility/transport would again bypass the early hydrolytic effects of hepatic CES1 on dl-MPH (Patrick et al., 2010). It is noted that an orally disintegration tablet (Cotempla XR-ODT) was approved in 2017, which contains dl-MPH not as the hydrochloride salt but rather bound to a nonabsorbable exchange resin. In this formulation, the potential for appreciable oral cavity l-MPH absorption may warrant investigation.

Also included in Table 1 are the analyses for partial area under the curve at 0–3 hours. Not only does this time interval cover the primary duration of therapeutic action of IR-dl-MPH, it extends a comprehensive study of partial area under the curve at 0–3 hours values for a broad range of MR-MPH formulations (Patrick et al., 2019) and carries relevance to this specific partial area under the curve metric in recently instituted bioequivalence parameters now required by the Food and Drug Administration for select dl-MPH products (Jackson, 2014). Taken together with the time to maximum plasma concentration and half-life parameters, Table 1 provides key aspects of both the rate and extent of dl-MPH oral bioavailability.

In summary, a very tenable hypothesis is presented that explains the disparities between older dl-MPH PK data compared with more recent findings, owing to evolving advances in the quality of chiral bioanalytical methodology. As described previously, perfluoroacylated prolyl derivatizing agents have a history of imprecision in enantiospecifically quantifying drugs, including dl-MPH and amphetamines. Currently, dl-MPH serves as a first-line pharmacotherapeutic agent for the treatment of the most commonly diagnosed childhood and adolescent central disorder: ADHD. As empirical approaches to drug individualization give way to pharmacogenomics and precision medicine, fundamental knowledge of well-validated pharmacology is critically important in achieving desired outcomes. This letter serves to correct specific misunderstandings regarding dl-MPH absorption and disposition, which have persisted in mainstream secondary and tertiary biomedical literature (Thummel et al., 2018).

Authorship Contributions

Performed data analysis: Patrick, Rodriquez.

Wrote or contributed to the writing of the manuscript: Patrick, Rodriquez.

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