Ethylphenidate: From biomarker to designer drug

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KEYWORDS

ethylphenidate, stimulant medication, substance abuse

The following is a brief background and account of the psychostimulant "designer drug" and curious methylphenidate-ethanol transesterification metabolite, ethylphenidate (EPH; **Fig 1**). That is ***ethyl***phenidate with an "E", not it's well known homolog, ***methyl***phenidate (MPH; Ritalin®, Concerta®, others) – the first-line agent for the treatment of attention-deficit/hyperactivity disorder (ADHD). Note that the only structural difference resides in the ester where EPH has an ethyl group rather than the methyl group in MPH.

dl-EPH is described chemically as (RR/SS)-ethyl phenyl(piperidin-2-yl)acetate or ritalinic acid ethyl ester. It has been characterized in the literature as the racemic crystalline hydrochloride salt and as its separate enantiomers.[1,2,3] Interestingly, EPH holds unique pharmacological significance in both legal and illicit arenas which will be touched upon in this paper.

The earliest investigation of EPH in the biomedical literature was reported over 50 years ago by Portoghese and Malspeis, who found EPH to be 80% as potent as MPH in antagonizing sedation in mice. Beyond this report, little to any information was published until Schweri and associates reported that EPH was ~50% as potent as MPH in inhibiting dopamine uptake in rat striatal synaptosomes.[1,4] However, due to the structural similarity of EPH to MPH, it was frequently used as an internal standard for MPH pharmacokinetic studies from the 1970s-1990s. Analytical methods incorporating EPH as an internal standard became problematic once it became known that EPH was also a MPH metabolite. Contemporary analytical methods generally incorporate deuterated MPH as an internal standard.[5]

In the late 1970s, the cocaine-ethanol transesterification pathway, which yields the active metabolite cocaethylene (ethyl cocaine or benzoylecgonine ethyl ester), emerged as a precedent for a methyl ester containing drug to be metabolically transformed into an ethyl ester.[6] This unique drug interaction eventually became the focus of numerous investigations. This peculiar bioconversion requires a catalytic enzyme to extract two separate drugs from the bloodstream, and covalently bond them. In the context of cocaine-ethanol co-ingestion and potential toxicity, plasma cocaethylene concentrations can exceed those of the parent drug cocaine and this metabolite appears to be cardiotoxic.[7,8]

We hypothesized the potential for EPH formation in individuals who co-abused MPH and alcohol, or even in adult ADHD patients who consume modest amounts of alcohol while being treated with MPH. There were, however, some practical impediments to testing this in humans. It was appealing to speculate that EPH would be formed in humans in view of both MPH and cocaine sharing a common binding site of stimulant action, i.e., the dopamine transporter (DAT).[9] In 1997, utilizing a rat liver preparation, Bourland and co-workers reported that MPH may serve as a substrate for ethanol transesterification, as could the ester-containing drug meperidine which, like MPH, is otherwise primarily metabolized by hydrolysis.[10] In this approximate time frame (i.e. late 1990's) we received two sets of postmortem blood and tissue samples recovered from unrelated overdose victims whose case histories had documented multi-drug ingestion, including large quantities of MPH along with evidence of ethanol consumption. These samples held the potential to demonstrate that humans metabolically generate EPH from MPH and ethanol. Following the in-house synthesis of an authentic reference standard of EPH, we confirmed by liquid chromatography–mass spectrometry (LC-MS) the novel detection of EPH formed in vivo in these human samples.[11] It was not suggested that EPH factored into the toxicology of either fatality, as it was not part of the initial toxicological report and its presence would not

have been suspected or assayed for. These were multi-drug ingestion cases and other medications and ethanol explained the fatalities.

Although the above findings confirmed that EPH could be formed in humans, questions remained. Due to the history of these biological samples, the circumstances surrounding their collection (i.e., autopsy) and the unknown MPH-ethanol doses, it remained to be established whether EPH formation occurs using typical therapeutic MPH doses (10-20mg) and moderate ethanol consumption. Subsequently, our group conducted a normal volunteer study in which 6 healthy volunteer subjects were administered a single fixed 20 mg dose of MPH followed 30 min later by a weight-based dose (0.6 g/kg) of ethanol.[12] The 20mg dose of MPH was chosen as it most closely approximated a commonly studied 0.3mg/kg weight based dose which would be taken by a 70kg individual. The dose of ethanol chosen in the study was within the range of dosing parameters being utilized in analogous cocaethyelene studies, and approximated a double vodka (80 proof) and orange juice in a 70 Kg subject. Using a non-enantiospecific LC-MS/MS assay, EPH was detected in both blood and urine samples in every subject. Although concentrations were low relative to MPH, this provided a proof-of-concept regarding EPH formation under simulated clinical drug utilization.

To advance our investigations, the National Institutes of Health supported our more comprehensive and enantiospecific study of the pharmacokinetics and pharmacodynamics of MPH-ethanol interactions.[13] ***dl***-MPH (0.3 mg/kg) was administered orally 30 min ***before*** ethanol (0.6gm/kg), 30 min ***after*** ethanol (0.6gm/kg), or ***without*** ethanol, in a randomized, normal subject three-way crossover study of 10 men and 10 women. Ethanol (1) elevated the C_{max} and AUC of ***d***-MPH 40% and 25%, respectively; (2) the transesterification pathway yielded approximately 10 times more ***l***-EPH than ***d***-EPH; and (3) ethanol significantly increased positive subjective effects of MPH. Further, a novel MPH poor metabolizer was discovered. Plasma concentrations of ***l***-MPH reached ~70 times that found in normal metabolizers and, distinct in this individual, no EPH was formed.[14] Although it had long been recognized that MPH underwent substantial stereoselective metabolism favoring the de-esterification (and deactivation) of ***l***-MPH over ***d***-MPH, Sun and associates[15] identified the specific carboxylesterase CES1A1 as the enzyme responsible for the first-pass, stereoselective metabolism of MPH. CES1A1 is also now known to catalyze the transesterification reaction. In concordance with this enantioselective hydrolysis of ***l***-MPH to the metabolite ritalinic acid (**Fig 1**), it follows that transesterification of MPH also favors ***l***-MPH as the substrate, and indeed the vast majority of EPH detected as the ***l***-EPH isomer.[13]

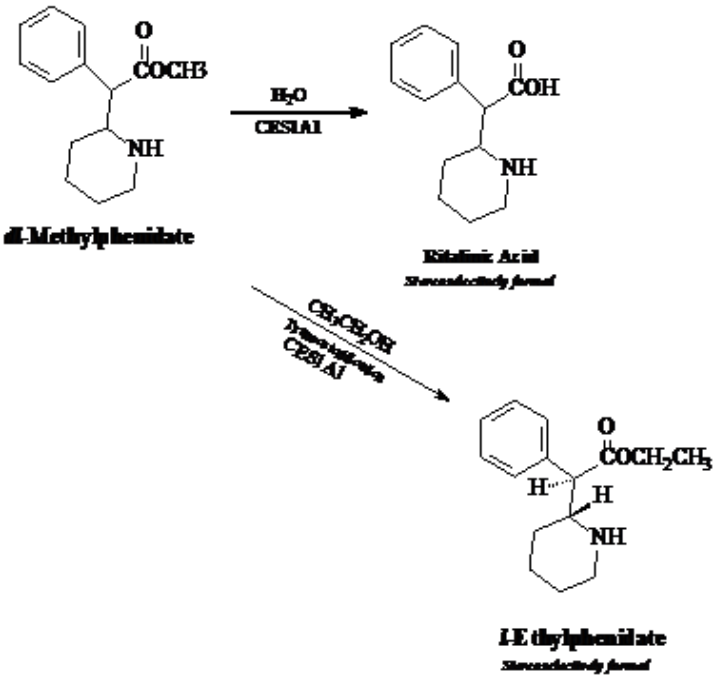
We are also interested in developing novel therapeutic agents. Though Schweri and coworkers reported evidence of lower potency for EPH compared to MPH in inhibiting DA uptake in synaptosomes from a dopaminergic region of rat brain, ***d***-EPH exhibits approximately the same low nanomolar DAT inhibition as ***d***-MPH when tested in a more substrate-specific assay using DAT-transfected human embryonic kidney cells.[4] Perhaps importantly, ***d***-EPH was found to be only 10% as potent as ***d***-MPH at inhibiting the norepinephrine transporter.[3] This dopaminergic selectivity of EPH relative to MPH offers potential for exploitation in drug discovery. Consistent with EPH DAT selectivity relative to MPH, we have now shown that isopropylphenidate (the isopropyl ester homolog of MPH) is DAT selective and offers potential as a novel therapeutic agent.[16]

Recognizing the structural similarity of EPH to MPH (**Fig 1**), and its aforementioned effects on monoamines, the issue is raised – "Is EPH subject to abuse?" Although EPH is not explicitly controlled in the US presently, it could ***possibly*** be considered an analog of MPH, a Schedule II substance covered under the Federal Analog Act, in that it is "substantially the same structure as a Schedule II drug, i.e., MPH".[2] Two recent reviews of so-called "Legal Highs" and designer drugs have indicated that EPH is being sold online as an illicit stimulant or cognitive enhancer which is sometimes called "nopaine".[17,18] Indeed, discussion rooms and accounts of EPH use as a "legal high" abound on the internet- as do vendors selling EPH in gram quantities. As evidence of a wider concern of EPH use and abuse, EPH is now being included in MS assays designed to screen for and detect many other designer drugs which are of growing concern.[19] Although pure EPH can be purchased for research use by legitimate vendors which supply analytical standards and research biochemicals, our laboratory recently received a sample of EPH purchased from an internet vendor, and purported to be EPH hydrochloride. Using a validated LC-MS/MS assay established in our laboratory we were able to determine that the substance was indeed racemic (***dl***-EPH), and of high purity.[5] This finding suggests that EPH purchased in this way, could be readily ingested by almost any individual such that they were effectively "dosed" far in excess of any amount metabolically formed during the ethanol-MPH transesterification pathway. The toxicological implications are unknown.

However, there are further concerns. For those individual users not finding the EPH "experience" to be to their liking, they could easily convert EPH to MPH using readily available methanol and other items stocked in their local hardware store- at far less effort than routinely employed by clandestine "chemists" manufacturing methamphetamine for instance. Alternatively, criminally enterprising non-users may engage in MPH manufacture using the EPH precursor, one synthetic step removed from MPH, to supply crystalline MPH hydrochloride for intranasal, intravenous or oral abuse.

We have chronicled the evolution of EPH over the past several decades. First as a member of a structure-activity series, then as an internal standard, followed by it serving as a MPH-ethanol biomarker, and now as a potential therapeutic agent with a burgeoning designer drug of abuse presence.

Figure 1: Formation of ***l***-Ethylphenidate



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How to cite:
Markowitz JS, Patrick KS. Ethylphenidate: From biomarker to designer drug. Ment Health Clin [Internet]. 2013;3(6):318-20. Available from: http://dx.doi.org/10.9740/mhc.n183949