

## Short Communication

# *N*-Isopropyl-*p*-iodoamphetamine Hydrochloride Is Predominantly Metabolized by CYP2C19<sup>[S]</sup>

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### ABSTRACT:

[<sup>123</sup>I]*N*-Isopropyl-*p*-iodoamphetamine hydrochloride ([<sup>123</sup>I]IMP) is clinically used to evaluate blood flow in the brain on single photon emission-computed tomography. This is a rare radiopharmaceutical that undergoes metabolism. The first step is reported to be [<sup>123</sup>I]*p*-iodoamphetamine formation. The drug-metabolizing enzyme(s) involved remain(s) unclear. This study examined the roles of human cytochrome P450 (P450) in the metabolism of nonradiolabeled IMP with the use of human liver microsomes (HLM) and recombinant human CYP1A1, -1A2, -1B1, -2A6, -2B6, -2C8, -2C9, -2C19, -2D6, -2E1, -3A4, and -3A5. Disappearance of IMP was examined because *p*-iodoamphetamine was not available. IMP (0.5 μM) time-dependently disappeared when HLM and NADPH-generating system were added to the reaction mixture. (S)-Mephenytoin (1 mM) inhibited the IMP disappearance by approximately 90%. The disappearance

of IMP was predominantly catalyzed by recombinant CYP2C19, with  $K_m$  and  $V_{max}$  of 8.6 μM and 9.7 nmol · min<sup>-1</sup> · nmol P450<sup>-1</sup>, respectively. IMP disappearance in CYP2C19-deficient HLM (CYP2C19\*2/\*2) was approximately 30% of that in the presence of HLM harboring wild-type CYP2C19, indicating that IMP is polymorphically metabolized by CYP2C19. High-performance liquid chromatography of the incubation mixture of IMP and CYP2C19 revealed an unidentified peak. As the area of the IMP peak decreased, the area of this unidentified peak increased in a time-dependent fashion. The peak was also detectable on incubation of IMP with HLM. Mass spectrometry revealed that the molecular weight of a compound in this unidentified peak was the same as that of *p*-iodoamphetamine. Thus, we demonstrated that IMP was predominantly metabolized by CYP2C19 to form *p*-iodoamphetamine.

### Introduction

[<sup>123</sup>I]*N*-Isopropyl-*p*-iodoamphetamine hydrochloride ([<sup>123</sup>I]IMP) is a radiopharmaceutical used clinically to evaluate blood flow in the brain on single photon emission-computed tomography (see chemical structure in Baldwin and Wu, 1988). This radiopharmaceutical has been amply demonstrated to be distributed in brain tissue in a clinically useful fashion. With the application of nuclear medicine techniques in the 1980s, pharmacokinetic analysis revealed uptake of [<sup>123</sup>I]IMP by the liver after a high uptake by lung during the first pass (Moretti et al., 1983). Subsequently, [<sup>123</sup>I]IMP was found to undergo biotransformation in the body (Baldwin and Wu, 1988), although few imaging diagnostics were thought to be metabolized. Analyses of human or rat plasma and urine samples obtained after [<sup>123</sup>I]IMP administration have revealed that the first step in metabolism was the formation of [<sup>123</sup>I]*p*-iodoamphetamine followed

by sequential generation of [<sup>123</sup>I]*p*-iodobenzoic acid and [<sup>123</sup>I]*p*-iodohippuric acid (Baldwin and Wu, 1988). The biotransformation of [<sup>123</sup>I]IMP to metabolite(s) with lower lipid solubility could practically impair penetration of <sup>123</sup>I to the brain. Therefore, to accurately evaluate blood flow in the brain, radioactivity derived from the water-soluble metabolite(s), which cannot reach the brain, has to be subtracted from the total radioactivity of all components with <sup>123</sup>I. To date, drug-metabolizing enzyme(s) involved in the metabolism of nonradiolabeled IMP remain(s) unclear, although one study has demonstrated binding of IMP and its initial metabolite to rat cytochrome P450 (P450), suggesting that P450 plays a role in the metabolism of IMP (Joulin et al., 1992).

This study first examined whether or not nonradiolabeled IMP was metabolized by drug-metabolizing enzyme(s) in human liver microsomes (HLM) and then attempted to elucidate human P450 isoform(s) responsible for the biotransformation of IMP. Because the first metabolite *p*-iodoamphetamine was not available, IMP disappearance was examined.

### Materials and Methods

#### Human Liver Microsomes and Recombinant Human P450 Isoforms.

Pooled HLM (wild-type CYP2C19) and HLM harboring CYP2C19\*2/\*2 (CYP2C19\*2/\*2 HLM) were obtained from BD Biosciences (Woburn, MA). Twelve recombinant P450 isoforms (i.e., CYP1A1, -1A2, -1B1, -2A6, -2B6, -2C8, -2C9.1, -2C19, -2D6.1, -2E1, -3A4, and -3A5) were purchased from BD Biosciences.

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**ABBREVIATIONS:** [<sup>123</sup>I]IMP, [<sup>123</sup>I]*N*-isopropyl-*p*-iodoamphetamine hydrochloride; P450, cytochrome P450; HLM, human liver microsomes; HPLC, high-performance liquid chromatography.

**IMP Metabolism by Pooled Human Liver Microsomes.** NADPH-dependent IMP metabolism by pooled HLM was examined in the presence or absence of an NADPH-generating system. The mixture without the addition of the pooled HLM and NADPH-generating system served as the control. IMP metabolism was monitored based on the disappearance of IMP. When the inhibition of IMP disappearance by (*S*)-mephenytoin was examined, the inhibitor was added 5 min before the initiation of metabolism. IMP was analyzed with a high-performance liquid chromatography (HPLC) system.

**IMP Metabolism by P450 Isoforms.** IMP metabolism by 12 recombinant P450 isoforms was examined based on the disappearance of IMP. IMP disappearance in the absence of the NADPH-generating system for each P450 served as the control.

**Enzyme Kinetics of IMP Metabolism.** Substrate disappearance velocity ( $v$ ) was calculated as  $[(C_{s,initial} - C_{s,t})/incubation\ time/P450\ concentration]$ , where  $C_{s,initial}$  was the substrate concentration at time 0 and  $C_{s,t}$  was the substrate concentration after  $t$  min incubation with each P450 (Li et al., 2007). Kinetic constants ( $K_m$  and  $V_{max}$ ) are reported as the means  $\pm$  S.E.

**IMP Metabolism by Human Liver Microsomes Harboring CYP2C19\*2/\*2.** IMP metabolism was monitored based on the disappearance of IMP. The incubation period of 0.5  $\mu$ M IMP was 60 min. IMP disappearance velocity ( $v$ ) was calculated as  $[(C_{s,initial} - C_{s,60}) \times volume\ of\ reaction\ mixture/60\ min]$ .

**Analysis of IMP Metabolite by Liquid Chromatography-Electrospray Ionization Mass Spectrometry.** Liquid chromatography-electrospray ionization mass spectrometry was performed with a micrOTOF-Q II mass spectrometer (Bruker Daltonics, Billerica, MA) to identify the IMP metabolite produced by CYP2C19. Detailed descriptions of the methods are given in the supplemental data.

## Results and Discussion

IMP time-dependently disappeared when pooled HLM and an NADPH-generating system were contained in the reaction mixture (Fig. 1A). Among 12 recombinant P450 forms, CYP2C19 was demonstrated to predominantly catalyze the reaction (Fig. 1B). The effects of substrate concentrations on IMP disappearance in the presence of

CYP2C19 or CYP1A1 are shown in Supplemental Fig. 1. The apparent  $K_m$  and  $V_{max}$  were calculated to be  $8.6 \pm 0.8\ \mu$ M and  $9.7 \pm 0.4\ \text{nmol} \cdot \text{min}^{-1} \cdot \text{nmol P450}^{-1}$  for CYP2C19 and  $18 \pm 5.0\ \mu$ M and  $7.3 \pm 1.0\ \text{nmol} \cdot \text{min}^{-1} \cdot \text{nmol P450}^{-1}$  for CYP1A1. The intrinsic metabolic clearance calculated with the  $K_m$  and  $V_{max}$  values obtained for CYP2C19 was approximately 2.5-fold higher than that for CYP1A1. We could not determine these apparent enzyme kinetic parameters for CYP1A2 because of a slight decrease in IMP at concentrations higher than 8.0  $\mu$ M where IMP disappearance was not saturated. (*S*)-Mephenytoin, a specific CYP2C19 substrate (Wrighton et al., 1993), inhibited IMP disappearance catalyzed by pooled HLM in a concentration-dependent manner (Fig. 1C). IMP disappearance was inhibited by 1 mM (*S*)-mephenytoin by approximately 90%. IMP disappearance activities seen in CYP2C19\*2/\*2 HLM for respective concentrations of microsomal protein were approximately 30% compared with those seen in the pooled HLM (wild-type CYP2C19) (Fig. 1D). These results indicate that IMP is predominantly metabolized polymorphically by CYP2C19.

During the HPLC analyses of the IMP disappearance by CYP2C19, an unidentified peak was found on HPLC chromatograms (Fig. 2, A–C). As the area of the IMP peak decreased, the area of this unidentified peak increased in a time-dependent fashion (Fig. 2D). The unidentified peak was also detectable when IMP was incubated with pooled HLM (wild-type CYP2C19) (Supplemental Fig. 2A). Ultraviolet spectra of both peaks seen with pooled HLM and recombinant CYP2C19 were nearly the same (Supplemental Fig. 2, B and C). The unidentified peak was fractionated and analyzed with liquid chromatography-electrospray ionization mass spectrometry (Fig. 2E and Supplemental Fig. 3). A major peak at 232 nm was detected at 11.2 min (Supplemental Fig. 3), and a parent mass  $[M + H]^+$  was observed at  $m/z$  262.0085 (Fig. 2E). High-resolution mass spectrometry (electrospray ionization) calculated for  $C_9H_{12}IN [M + H]^+$  (262.0087) was found at

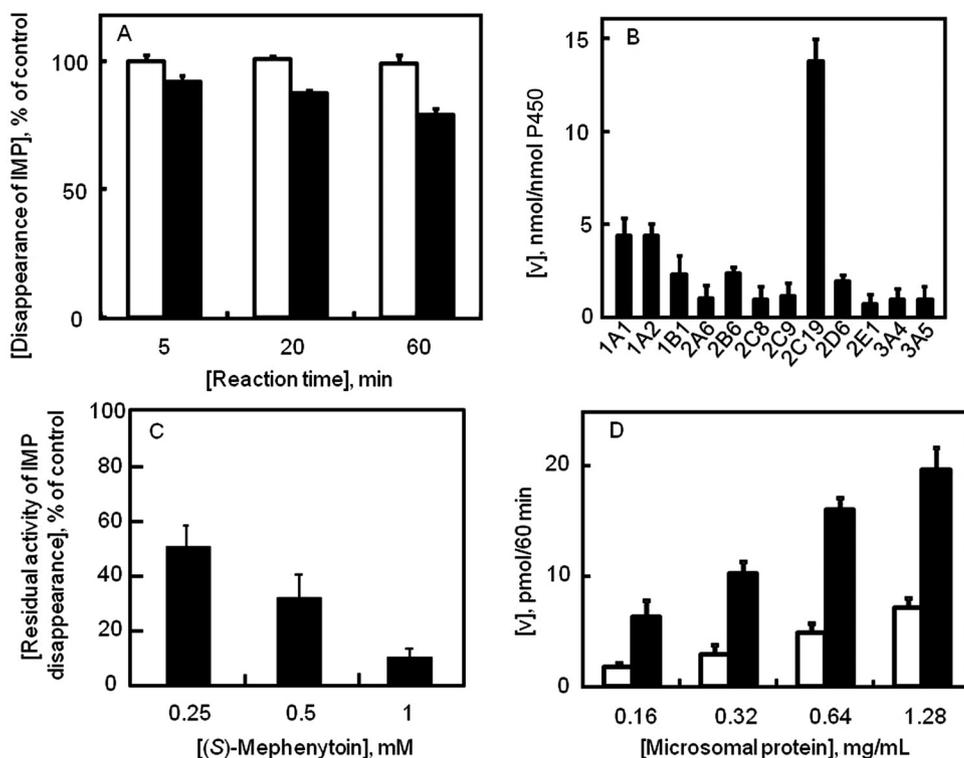


FIG. 1. Disappearance of IMP in the presence of both pooled HLM and NADPH-generating system (A), catalyzed predominantly by recombinant CYP2C19 (B), showing inhibition of HLM-catalyzed IMP disappearance by (*S*)-mephenytoin (C), and demonstrating polymorphic metabolism of IMP by CYP2C19 (D). The IMP concentration used was 0.5  $\mu$ M. A, open bars, the presence of pooled HLM and absence of NADPH-generating system; closed bars, presence of both pooled HLM and NADPH-generating system; D, open bars, CYP2C19\*2/\*2 HLM; closed bars, pooled HLM (wild-type CYP2C19). Each point shows the mean of three independent analyses with S.D. bars.

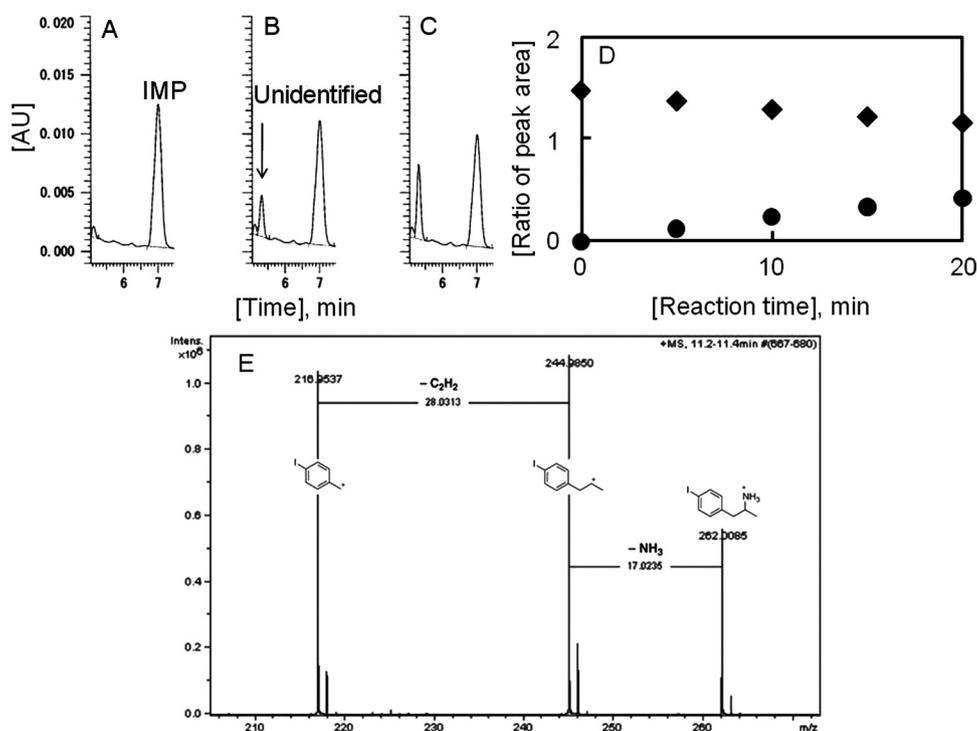


Fig. 2. HPLC chromatograms of IMP and unidentified peak obtained by the incubation of IMP with CYP2C19 for reaction times of 0 (A), 10 (B), and 20 min (C), showing a reaction-time-dependent decrease in IMP (◆) and an increase in the unidentified peak (●) (D) and demonstrating total mass spectra between 11.1 and 11.5 min and assignment of fragmentations (E). D, ratio of peak area was calculated by dividing the peak area of IMP or unidentified peak by that of internal standard. Each data point in D represents the mean of triplicate determinations and is shown with S.D. bars.

262.0085. The fragments at  $m/z$  244.99 and 216.95 can be assigned as successive neutral losses of  $\text{NH}_3$  ( $\Delta m = 17.0265$ ) and  $\text{C}_2\text{H}_2$  ( $\Delta m = 28.0313$ ), both determined with mass accuracies below 3 mDa (Fig. 2E). These results suggest that the unknown metabolite is *p*-iodoamphetamine [1-(4-iodophenyl)-propan-2-amine].

In clinical practice, the metabolism of lipid soluble [ $^{123}\text{I}$ ]IMP to water-soluble metabolites, most likely [ $^{123}\text{I}$ ] *p*-iodobenzoic acid and [ $^{123}\text{I}$ ] *p*-iodohippuric acid, can impair the penetration of  $^{123}\text{I}$  to the brain. Therefore, to accurately evaluate blood flow in the brain, radioactivity derived from water-soluble metabolites must be subtracted from the total radioactivity derived from all components labeled with  $^{123}\text{I}$ . The water-soluble metabolites are usually separated by octanol/water extraction of blood samples collected after [ $^{123}\text{I}$ ]IMP injection, and the radioactivity in the water phase is subsequently quantified. If the formation of water-soluble metabolites *in vivo* is predominantly determined by CYP2C19 activity, radioactivity in the water phase might parallel CYP2C19 activity. The quantification of water-soluble metabolites of [ $^{123}\text{I}$ ]IMP might be applied to CYP2C19 phenotyping to identify poor metabolizers. Our CYP2C19-phenotyping method might facilitate patient selection or dose adjustment for medicines that are substrates of polymorphic CYP2C19 (<http://www.cypalleles.ki.se/cyp2c19.htm>). However, future studies are necessary to put our idea into clinical practice. First, the role(s) of CYP2C19 in the subsequent steps of IMP metabolism should be comprehensively clarified. We are synthesizing *p*-iodoamphetamine to examine secondary metabolism in more detail. Second, whether [ $^{123}\text{I}$ ]IMP is predominantly metabolized by CYP2C19 should be confirmed with a variety of recombinant human P450s and pooled HLM at concentrations similar to those in human plasma, which are much lower than the concentrations used in this study. Our preliminary study using radiolabeled IMP and HPLC equipped with a radioactivity detector indicated that recombinant CYP2C19 and pooled HLM-mediated metabolism of labeled IMP. Third, after comprehensive *in vitro* studies of metabolism, *in vivo* correlations between CYP2C19 phenotyping as measured by the proposed methods and CYP2C19

genotyping should be examined. In conclusion, our results showed that IMP metabolism was mainly catalyzed polymorphically by CYP2C19.

#### Authorship Contributions

*Participated in research design:* Fujita, Kunishima, and Kawai.

*Conducted experiments:* Sugiyama, Akiyama, Hioki, and Nishi.

*Performed data analysis:* Sugiyama, Fujita, Hioki, and Kunishima.

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*Other:* Fujita and Sasaki acquired funding for the research.

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