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Oxidation of *R*- and *S*-omeprazole stereoselectively mediated by liver microsomal cytochrome P450 2C19 enzymes from cynomolgus monkeys and common marmosets

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ABSTRACT

Racemic omeprazole has been used for clinically treating gastric acid-related diseases and also as a typical human cytochrome P450 (P450) 2C19 probe substrate in preclinical studies. *S*-Omeprazole has been developed as a single enantiomer medicine, which has been reported not to be associated with polymorphic human P450 2C19 phenotypes. In this study, 5-hydroxylation and sulfoxidation activities, with respect to stereoselective *R*- and *S*-omeprazole oxidations by liver microsomes from experimental animals including non-human primates and humans, were investigated *in vitro*. Liver microsomes from humans, cynomolgus monkeys, and mice preferentially mediated *R*-omeprazole 5-hydroxylations, however those from marmosets, minipigs, dogs, and rats preferentially mediated *S*-omeprazole 5-hydroxylations. High catalytic activities were observed for recombinant human P450 2C19 in *R*-omeprazole 5-hydroxlations, cynomolgus monkey P450 2C19 in both *R*- and *S*-omeprazole 5-hydroxlations, and marmoset P450 2C19 in *S*-omeprazole 5-hydroxlations. On the other hand, human, cynomolgus monkey, and marmoset P450 3A enzymes preferentially mediated *S*-omeprazole sulfoxidations. Correlation and kinetic analyses revealed a high affinity of polymorphic cynomolgus monkey and marmoset liver microsomal P450 2C19 enzymes with respect to *R*- and *S*-omeprazole 5-hydroxylations, respectively, and a high capacity of cynomolgus monkey and marmoset liver microsomal P450 3A4 for omeprazole 5-hydroxylations and sulfoxidations. *R*-and *S*-omeprazole 5-hydroxylation activities in cynomolgus monkey and marmoset liver microsomes were significantly different among wild-type, heterozygous, and homozygous animals genotyped for cynomolgus monkey *P450 2C19* p.[(Phe100Asn; Ala103Val; Ile112Leu)] and for marmoset *P450 2C19* p.[(Phe7Leu; Ser254Leu; Ile469Thr)], respectively. The results of this study demonstrate polymorphic cynomolgus monkey and marmoset P450 2C19-dependent omeprazole oxidation activities with individual variations similar to humans.

1. Introduction

Species differences for metabolic clearances and drug disposition of candidate chemicals are important issues for new drug development. In order to predict pharmacokinetics and toxicokinetics of drug candidates, non-human primates have recently become the focus due to their physiological and genetic similarity to humans [1]. Generally, cynomolgus monkeys (*Macaca fascicularis*), an "Old world primate" [2], and common marmosets (*Callithrix jacchus*), a "New world primate" [3], are used for preclinical drug metabolism studies. Drug metabolizing forms of polymorphic cytochromes P450 (P450) mediate oxidative clearances of a variety of compounds in humans [4]. Individual differences in humans in terms of drug metabolism may be caused predominantly by whole gene deletion or impaired variants of P450s, as reported [5]. In general, cynomolgus monkey or marmoset P450 enzymes show high sequence homology to their

Abbreviation: P450, general term for cytochrome P450 (EC 1.14.14.1).

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human counterparts [6] and genetic polymorphisms have recently been found in cynomolgus monkey and marmoset *P450 2C19* gene [7–10], similar to humans [11].

The pharmacokinetics of omeprazole, a typical human P450 probe substrate [12], has been reported to be associated with polymorphic human P450 2C19 phenotypes [13,14]. Racemic mixtures of omeprazole have been used as marketed proton pump inhibitors for treating gastric acid-related diseases, but the *S*-enantiomer of omeprazole, esomeprazole, has been developed as a single enantiomer drug [15]. In our recent studies, genetic variant forms of cynomolgus monkey and marmoset P450 2C19 enzymes showed substantially reduced activities compared to wild types in stereoselective *R*- and *S*-warfarin 7-hydroxylations [7–10], respectively. The roles of non-human primate *P450* genes, especially polymorphic *P450 2C19*, in drug metabolism need to be further elucidated for preclinical studies.

In realization that the stereoselectivity is generally different between marmoset and cynomolgus enzymes, the conformation of conserved affinity and capacity of corresponding P450 enzymes are equally important to explore among non-human primates and humans in *in vivo* preclinical studies. In this study, omeprazole oxidation activities were evaluated with respect to stereoselective *R*- and *S*-omeprazole oxidation in a variety of experimental animals including non-human primates, and humans. We report herein the high affinity of polymorphic cynomolgus monkey and marmoset P450 2C19 enzymes with respect to *R*- and *S*-omeprazole 5-hydroxylations, respectively, and the high capacity of cynomolgus monkey and marmoset P450 3A4 for omeprazole 5-hydroxylations and sulfoxidations.

2. Materials and methods

2.1. Chemicals

R- and *S*-omeprazole were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and 5-hydroxyomeprazole from Toronto Research Chemicals (Toronto, ON, Canada). Omeprazole sulfone, ticlopidine, and ketoconazole were obtained from Sigma-Aldrich (St. Louis, MO). The other chemicals and reagents used were obtained in the highest grade commercially available [7,8,16].

2.2. Analysis of R- and S-omeprazole oxidation

Pooled liver microsomes from humans, cynomolgus monkeys, marmosets, minipigs, dogs, rats, and mice and recombinant human P450 enzymes expressed in insect cells were purchased from Corning Life Sciences (Woburn, MA). Individual cynomolgus monkey and marmoset liver microsomes were prepared as described previously [8,17]. Liver microsomes from nine wild-type, eight heterozygous, and four homozygous cynomolgus monkeys genotyped previously [7,8] for *P450 2C19* p.[(Phe100Asn; Ala103Val; Ile112Leu)], and from four wild-type, ten heterozygous, and nine homozygous marmosets genotyped previously [7,8] for *P450 2C19* p.[(Phe7Leu; Ser254Leu; Ile469Thr)] were used. This study was approved by its Institutional Animal Care and Use Committee. Recombinant cynomolgus monkey and marmoset P450 2C19, P450 3A4, and other isoforms co-expressed with NADPH-P450 reductase in bacterial membranes were prepared as described [8,14,18]. Omeprazole oxidation activities were determined as described previously [8,14]. Briefly, *R*- and *S*-omeprazole (1–500 μM) were incubated with liver microsomes (1.0 mg/mL) or recombinant P450 (40 pmol/mL) at 37 °C for 15 min in the presence of an NADPH-generating system. To elucidate the effects of a human P450 2C19 inhibitor, ticlopidine, the omeprazole substrate concentrations were set up at 20 μM. The product formation rates were determined using a reverse phase high-performance liquid chromatography with UV detection [8,14].

Kinetic analysis for the substrate concentrations [S]-dependent omeprazole 5-hydroxylation activities (v) were carried out with one or two K_m values in combined Michaelis–Menten equations by non-linear regression: $v = V_{max1}\ [S]/(K_{m1} + [S]) + V_{max2}\ [S]/(K_{m2} + [S])$; and parameters were calculated from a fitted curve by non-linear regression (mean ± S.E.). One-way analysis of variance (ANOVA) with Dunnett's post test was carried out using Prism (Graphpad Software, La Jolla, CA) to compare the liver microsomal omeprazole oxidation activities from 21 cynomolgus monkeys and 23 marmosets genotyped for *P450 2C19*.

2.3. Docking simulations into P450 enzymes

Marmoset P450 2C19 primary sequence was aligned with a crystal structure of human P450 2C19 (Protein Data Bank code 4GQS) for modeling of three dimensional structures in a similar manner to the cynomolgus monkey P450 2C19 model [9,19] using MOE software (ver. 2015.10, Computing Group, Montreal, Canada). Prior to docking simulation, the energy of the P450 structures was minimized using the CHARM22 force field. Docking simulation was carried out for *R*- or *S*-omeprazole enantiomer binding to P450 enzymes using the MMFF94x force field distributed in the ASEDock software [9,19] and ranked according to the ligand-interaction energies (U values, kcal/mol).

3. Results

3.1. Stereoselective R- and S-omeprazole oxidation in liver microsomes from humans, non-human primates, and other experimental animals

R- and *S*-omeprazole oxidation activities by liver microsomes from humans, cynomolgus monkeys, marmosets, minipigs, dogs, rats, and mice were determined at substrate concentrations of 10 and 100 μM (Table 1). Some of the *R*-omeprazole sulfoxidation activities were under the detection limits; however, liver microsomes from humans and animal species tested efficiently ($\geqslant$2) catalyzed *S*-omeprazole sulfoxidation over *R*-omeprazole sulfoxidation. Liver microsomes from humans, cynomolgus monkeys, and mice preferentially mediated *R*-omeprazole 5-hydroxylations. In contrast, liver microsomes from other animal species, including marmosets, catalyzed *S*-omeprazole 5-hydroxylations preferentially under the present conditions (Table 1).

R- and *S*-omeprazole oxidation activities by human, cynomolgus monkey, and marmoset recombinant P450 enzymes were determined at the substrate concentration of 100 μM (Fig. 1). High catalytic activities of *S*-omeprazole sulfoxidation in human, cynomolgus monkey, and marmoset recombinant P450 3A4 enzymes were observed. High catalytic activities of recombinant human P450 2C19 in *R*-omeprazole 5-hydroxlations (Fig. 1A), cynomolgus monkey P450 2C19 in both *R*- and *S*-omeprazole 5-hydroxlations (Fig. 1B), and marmoset P450 2C19 in *S*-omeprazole 5-hydroxlations (Fig. 1C) were observed. Human, cynomolgus monkey, and marmoset P450 3A enzymes mediated *S*-omeprazole 5-hydroxlations under these conditions.

Because cynomolgus monkeys and marmoset liver microsomes showed stereoselective *R*- and *S*-omeprazole 5-hydroxylations, respectively, roles of cynomolgus monkey and marmoset P450 enzymes in the omeprazole oxidation were determined. Correlations between *R*- and *S*-omeprazole 5-hydroxylation activities and P450 probe oxidation activities in individual cynomolgus monkey and marmoset liver microsomes were determined (Fig. 2). *R*-Omeprazole 5-hydroxlation activities in cynomolgus monkey liver