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Review

Significance of aldehyde oxidase during drug development: Effects on drug metabolism, pharmacokinetics, toxicity, and efficacy

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ABSTRACT

Aldehyde oxidase contributes to drug metabolism and pharmacokinetics (PK), and a few clinical studies were discontinued because of aldehyde oxidase metabolism. Its AOX1, AOX3, AOX3L1, and AOX4 isoforms are expressed in mammals, and species differences in expression profiles reflect differences in drug metabolism and PK between animals and humans. Individual differences in aldehyde oxidase activity also influence drug metabolism in humans. Moreover, the reduced solubility of the aldehyde oxidase metabolites may induce drug toxicity. Because various drugs inhibit aldehyde oxidase, assessments of ensuing drug–drug interactions (DDI) are critical for drug optimization. Although drug metabolism, PK, safety, and DDI are important, drugs such as famciclovir and O6-benzylguanine that affect aldehyde oxidase activity in humans have been reported. Recently, various *in vitro* approaches have been developed to predict PK in humans. However, *in vitro* studies on aldehyde oxidase may be hampered because of its instability. In contrast, *in vivo* studies on chimeric mice with humanized livers have also been focused on to predict aldehyde oxidase-mediated metabolism. Additionally, the ratios of N1-methylnicotinamide to metabolites in urinary excretions may represent useful biomarkers of aldehyde oxidase activity in humans. Thus, assessing the contributions of aldehyde oxidase to drug metabolism in humans is necessary.

1. Introduction

During drug discovery and development, assessments of drug metabolism and pharmacokinetics (PK) are essential to achieve efficacy and safety. Kinetic and metabolic examinations of novel pharmaceuticals involving cytochrome P450 (CYP) have been widely performed using human liver microsomes. With the development of screening systems for drug metabolism, discontinuation rates of clinical studies on drug candidates with unexpected drug metabolism and PK have decreased by 10% in 2000 [1]. Drug-metabolizing enzymes that contribute to the metabolism of the top 200 marketed drugs in the US in 2002 include CYP, UDP-glucuronosyltransferase (UGT), esterase, and flavin monooxygenase [2]. Although many drugs are metabolized by CYPs, the contribution of non-CYP drug metabolism may be significant [3]. In particular, studies on drug metabolism by aldehyde oxidase, and the resulting PK, have become increasingly important, and clinical studies on various drug candidates which are susceptible to aldehyde oxidase metabolism have been discontinued in clinical studies [4]. Specifically, BIBX1382 (pyrimido-pyrimidine) N8-(3-chloro-4-fluorophenyl)-N2-(1-methyl-4-piperidinyl)-pyrimido[5,4-d]pyrimidine-2,8-diamine, pyrimido-pyrimidine), carbazeran {6,7-dimethoxy-1-[4-(ethylcarbamoyloxy)-piperidino] phthalazine}, FK3453 {[6-(2-amino-4-phenylpyrimidin-5-yl)-2-isopropylpyridazin-3(2H)-one]}, and RO-1 {6-[2,4-difluoro-phenoxy]-2-[(R)-2-hydroxy-1-methyl-ethylamino]-8-[(S)-2-hydroxy-propyl]-8H-pyrido(2,3-d)pyrimidin-7-one} were discontinued because of poor bioavailability and rapid elimination in humans [4–8]. High activity of aldehyde oxidase in humans has led to low systemic exposures. On the other hand, SGX-523 {6-[6-(1-Methyl-1H-pyrazol-4-yl)-(1,2,4)triazolo(4,3-b)pyridazin-3-ylthio]

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quinolone} was discontinued because of suspected involvement in kidney failure following metabolism by induced aldehyde oxidase in humans [9]. In addition to aldehyde oxidase activity, this enzyme reportedly hydroxylates carbon–hydrogen bonds adjacent to nitrogen atoms in aromatic heterocyclic rings of drug compounds (Fig. 1).

2. Enzymology of aldehyde oxidase

Aldehyde oxidase as well as xanthine oxidoreductase which converts to two forms, xanthine dehydrogenase and xanthine oxidase belong to molybdo-flavoenzyme. Similar to CYPs, aldehyde oxidase also contributes to oxidation, but it does not require NADPH as a co-factor. Moreover, oxidation by CYP follows electrophilic attack of substrates, whereas that by aldehyde oxidase follows nucleophilic attack using oxygen derived from water [10].

Aldehyde oxidase has two subunits of about 150 kDa, and these comprise 20-, 40-, and 85-kDa domains containing an Fe–S cluster, an FAD-binding site, and a substrate pocket near the molybdenum co-factor (MoCo), respectively [11]. After binding to the pocket, substrates are oxidized, and molybdenum in the active site is reduced from hexavalent to tetravalent, and it returns to the initial valence following the supply of electrons to FAD and Fe–S clusters. Subsequently, reduced $FADH_2$ transmits electrons to oxygen, which is then converted to hydrogen peroxide. This redox cycle is also identical to that shown for xanthine oxidase [11].

Aldehyde oxidase is widely expressed in various species, including protists, plants, algae, insects, and vertebrates, but it has not been identified in fungi [12]. Kurosaki et al. proposed a phylogenetic tree of aldehyde oxidase evolution in prokaryotic and eukaryotic organisms. Originally, prokaryotic- and eukaryotic aldehyde oxidase evolved separately from xanthine dehydrogenase in bacteria, protists, plants, algae, and insects, but it evolved in vertebrates following gene duplication from xanthine dehydrogenase [12]. Aldehyde oxidase has been identified in various species, and its four isoforms AOX1, AOX3, AOX3L1, and AOX4 have been identified in vertebrates, with wide expressions in mammalian organs [12].

3. Physiological significance of aldehyde oxidase

The intrinsic physiological substrates of aldehyde oxidase include retinaldehyde, indol, pyridoxal, nicotinamide, (*S*)-methylmalonate, semialdehyde, gentisate aldehyde, 5-hydroxyindole acetaldehyde, and phytochrome indole-3-acetaldehyde [13].

In plants, endogenous indole-3-acetaldehyde and abscisic aldehyde are catalyzed by aldehyde oxidase to indole-3-acetic acid and abscisic acid (ABA), respectively, during plant hormone biosynthesis [14,15]. ABA has important roles in seed development and germination and in stress response [15]. Zdunek-Zastocka et al. reported that in pea plants, changes in the expression and activity of aldehyde oxidase were observed during plant development and under stress conditions [16].

However, physiological roles of endogenous substrates catalyzed by aldehyde oxidase in mammalians remain poorly understood.

Metabolism of benzaldehyde by aldehyde oxidase was demonstrated in male mice that lacked circulating growth hormones in comparison with female mice and was increased after treatment with growth hormones, and the subsequent administration of testosterone to female mice increased aldehyde oxidase activity [17]. These data were supported by increased mRNA expressions of AOX1 and AOX3 following treatment with testosterone [18].

Sugihara et al. reported that in mice, the activity of aldehyde oxidase increased by approximately 1.5-fold after the administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [19]. Furthermore, the induction of aldehyde oxidase through the aryl hydrocarbon receptor (AhR) and its receptor nuclear translocator (ARNT) was demonstrated after the dosing of TCDD in mice, and the mRNA inductions of AOX1 and AOX3 mRNA inhibited in AhR- and ARNT-deficient cells [20]. However, because 3-methylcholanthrene, an AhR ligand, has no inductive effects on aldehyde oxidase in mice [19], multiple factors may also contribute to the induction of aldehyde oxidase expression by AhR ligands.

Maeda et al. revealed that antioxidant responsive elements (AREs), to which NF-E2-related factor (Nrf2) binds, are located in 5′ upstream region of rat *AOX1*. Dissociation of Kelch-like ECH-associated protein (Keap-1) from Nrf2 by treatment with the electrophilic regent diethylmalate in hepatoma cells can contribute to the induction of AOX1 mRNA expression [21]. The results suggest that the expression of AOX1 is regulated by the Nrf2-Keap1 pathway [21].

Knockdown of AOX1 in 3T3-L1 adipocytes impaired adipogenesis and inhibited the release of adiponectin, indicating a physiological function of AOX1 [22]. In AOX4 knockout mice, direct downregulation of retinoid-dependent genes and alteration of lipid homeostasis and secretion were observed [23].

It is important to investigate endogenous substrates and the mechanism of induction to elucidate the physiological significance of aldehyde oxidase.

4. Aldehyde oxidase-targeting clinical drugs and drug candidates

The functions of aldehyde oxidase as a drug-metabolizing enzyme have recently been proposed, and the drugs methotrexate, famciclovir, and zaleplon are reportedly substrates of aldehyde oxidase (Fig. 1) [24]. The indoloquinoline alkaloid cryptolepine is isolated from the West African climbing shrub *Cryptolepis sanguinolenta* as a traditional treatment for malaria and is metabolized by aldehyde oxidase (Fig. 1) [25]. Importantly, nitrogen-containing aromatic heterocyclic rings are common to all of these aldehyde oxidase substrates, and the metabolism of the aldehyde groups of the alkylating agent cyclophosphamide has been shown (Fig. 1) [10]. The ring of cyclophosphamide is cleaved after microsomal oxidation, and the resulting open-ring aldehyde aldophosphamide is metabolized to carboxyphosphamide by aldehyde oxidase (Fig. 2) [10]. The S-enantiomer of RS-8359 [(±)-4-(4-cyanoanilino)-5,6-dihydro-7-hydroxy-7H-cyclopenta(d)pyrimidine] selectively and reversibly inhibits monoamine oxidase A (Fig. 1) and is eliminated more rapidly than the R-enantiomer. Because this compound is metabolized by aldehyde oxidase to the 2-keto form, chiral selectivity is an important predictor of drug metabolism [26].

Numerous nitrogen-containing aromatic heterocyclic compounds are present in chemical libraries of drug candidates with kinase inhibitory activities [27]. Thus, aldehyde oxidase may be an important drug-metabolizing enzyme.

5. Substrate specificity of aldehyde oxidase

Structural specificities and structure–substrate activity relationships of aldehyde oxidase metabolism were discovered more than 40 years ago, and by monitoring the rates of ferricyanide reduction, differences between rabbit liver aldehyde oxidase and bovine milk xanthine oxidase were examined [28]. High metabolic activities of aldehyde oxidase and xanthine oxidase were observed in the cases of 2-hydroxypurine, 4-hydroxypteridine, 4-hydroxypyrido[2,3-d]pyrimidine, 4-hydroxypyrido[3,2-d]pyrimidine, 4-hydroxypyrimidine, 2-mercaptopurine, pteridine, purine, and 6-purinecarboxamide. Whereas aldehyde oxidase mainly