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Review

Critical overview on the structure and metabolism of human aldehyde oxidase and its role in pharmacokinetics

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ABSTRACT

Aldehyde oxidases are molybdenum and flavin dependent enzymes characterized by a very wide substrate specificity and performing diverse reactions that include oxidations (e.g., aldehydes and aza-heterocycles), hydrolysis of amide bonds, and reductions (e.g., nitro, S-oxides and N-oxides). Oxidation reactions and amide hydrolysis occur at the molybdenum site while the reductions are proposed to occur at the flavin site. AOX activity affects the metabolism of different drugs and xenobiotics, some of which designed to resist other liver metabolizing enzymes (e.g., cytochrome P450 monooxygenase isoenzymes), raising its importance in drug development. This work consists of a comprehensive overview on aldehyde oxidases, concerning the genetic evolution of AOX, its diversity among the human population, the crystal structures available, the known catalytic reactions and the consequences in pre-clinical pharmacokinetic and pharmacodynamic studies.

Analysis of the different animal models generally used for pre-clinical trials and comparison between the human (hAOX1), mouse homologs as well as the related xanthine oxidase (XOR) are extensively considered. The data reviewed also include a systematic analysis of representative classes of molecules that are hAOX1 substrates as well as of typical and well characterized hAOX1 inhibitors. The considerations made on the basis of a structural and functional analysis are correlated with reported kinetic and metabolic data for typical classes of drugs, searching for potential structural determinants that may dictate substrate and/or inhibitor specificities.

Contents

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1. Introduction and scope

Aldehyde oxidases (AOXs, EC 1.2.3.1) are an emerging group of enzymes in the realm of drug and xenobiotic metabolism either in humans or in animal species of veterinarian interest. Along with xanthine oxidoreductase (XOR), the key enzyme in the catabolism of purines [1,2], AOXs are molybdo-flavoproteins, as they require a molybdopterin cofactor (Moco) and FAD for their catalytic activity [3]. Though different in many aspects, AOX and XOR share 50% amino acid identity, a common fold, and are evolutionarily related enzymes. Only a single vertebrate XOR protein is known, while the number of vertebrate AOX proteins and corresponding encoding genes varies among animal species [3–5]. Mammals have up to four AOX coding genes and the two extremes are represented by humans, who synthesize a single AOX enzyme (AOX1) and mice or rats, which are characterized by containing four distinct isoenzymes AOX1, AOX2, AOX3 and AOX4 [6–9]. The physiological function and physiological substrates of XOR are well known. XOR oxidizes hypoxanthine to xanthine and xanthine to uric acid, the final catabolite of purines [10]. On the contrary, very little is known about the endogenous substrates and the tissue-specific or systemic functions of mammalian AOXs [11].

Unlike XOR, which is highly substrate-specific, AOXs have the potential to recognize a broad range of natural and synthetic substrates [12]. As the denomination suggests, AOXs are best known for their ability to oxidize various types of aliphatic and aromatic aldehydes into the corresponding carboxylic acids. However, aldehydes are not the sole substrates of AOXs, as these enzymes metabolize a wide range of substrates, oxidizing various aza-heterocycles. In addition, AOXs are not exclusively oxidases as they hydrolyze amides and reduce nitro compounds as well as nitrogen and sulfo-oxides [13–15]. These diverse reactions occur either at the Mo active site or at the FAD site [16–19]. The broad substrate specificity is the basis for the involvement of human liver AOX1 and other vertebrate AOXs in the metabolism of drugs and compounds of toxicological interest [11].

The vast array of cytochrome P450 monooxygenase isoenzymes (CYP) plays a primary role in drug and xenobiotic metabolism. CYPs are localized in the endoplasmic reticulum of liver hepatocytes and other cell types in different tissues and organs. CYPs metabolize numerous xenobiotics and are key determinants of the pharmacokinetics and pharmacodynamics of many drugs used in the clinical setting. It is calculated that CYPs account for the metabolism of over 75% of the therapeutic agents currently on the market [20]. CYP-dependent metabolism is a major problem in the field of drug development, as it is associated with reduced bioavailability and decreased therapeutic efficacy [21]. This has resulted in strategies aimed to design and synthesize molecules which are not recognized and metabolized/inactivated by CYPs. However, CYP-resistant drugs are often oxidized or reduced by AOXs and a progressive increase in AOX substrates has been observed in the last years [12,22,23]. Concomitantly, the interest in human AOX1, as a drug metabolizing enzyme, has increased, since metabolism by this enzyme is responsible for many clinical trials failures [12,24–28]. Multiple *in vitro* and *in vivo* studies using AOX have been performed including enzymatic activity in fresh human skin [29,30].

As for the involvement of AOXs in drug metabolism, there are several relevant problems, particularly in the field of drug development, which need to be addressed. For instance, there are several *in silico* models for the prediction of human CYP-mediated metabolism which can be used for the design of new and better drugs [31]. Similar models for other non-CYP enzymes, such as AOXs, are either unavailable or largely insufficient [15,32]. In the case of human AOX1, some computational studies have been published [33–35] but, unfortunately, these studies are largely unreliable and outdated, as they are based on the crystal structures of the related bovine XOR [10] and mouse AOX3 [36] proteins. In fact, human AOX1 was crystallized in 2015 [37] and its 3D structure is characterized by significant differences relative to mouse AOX3 and bovine XOR. Only recently was reported a comprehensive study using DFT (Density Functional Theory) to predict human AOX1 activity [38]. In addition, the different complement of AOXs present in human liver and in the hepatic tissue of various animal species used in the pre-clinical phases of *in vivo* drug development is a major obstacle for pharmacokinetic and pharmacodynamics studies involving human AOX1 substrates. Finally, single nucleotide polymorphisms (SNPs) in the coding [39,40] and non-coding regions of human AOX1 have been identified in individuals belonging to different geographical populations. Some of these SNPs are likely to have an impact on the catalytic activity and in the expression levels of the enzyme, which is an important aspect to be considered in terms of personalized treatment with drugs recognized by human AOX1. Clearly, these are just examples that highlight the relevance of AOXs in drug and xenobiotic metabolism, and many other aspects of AOXs biology, enzymatic activity and structure come also into play.

In this review, the current knowledge on the role of human AOX1 in drug metabolism is summarized, with a focus on structure-function relationships. The unique structural features of