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Review

α-Methylacyl-CoA racemase (AMACR): Metabolic enzyme, drug metabolizer and cancer marker P504S

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ABSTRACT

α-Methylacyl-CoA racemase (AMACR; P504S) catalyzes a key chiral inversion step in the metabolism of branched-chain fatty acids, ibuprofen and related drugs. Protein levels are increased in all prostate and some other cancer cells and it is used as a marker (P504S). The enzyme requires no cofactors and catalyzes its reaction by a stepwise 1,1-proton transfer *via* an enolate intermediate. The biological role of AMACR in cancer is complex, linking lipid metabolism with nuclear receptor (*e.g.* FXR and PPAR) activity and expression of enzymes such as cyclooxygenase-2 (COX-2). The roles of the various splice variants and the effects of single-nucleotide polymorphisms (SNPs) in cancers are discussed. A number of rationally designed AMACR inhibitors have been reported in the literature as potential cancer treatments. The opportunities and challenges for development of acyl-CoA esters as inhibitors are discussed from a medicinal chemical viewpoint. Other challenges for drug development include the problems in assaying enzymatic activity and the prediction of structure–activity relationships (SAR). Inhibitors of AMACR have potential to provide a novel treatment for castrate-resistant prostate cancers but this potential can only be realized once the biology is well understood. Recent work on the role of AMACR in parasitic diseases is also reviewed.

Contents

1. Introduction

Branched-chain fatty acids and related compounds are important components of cells and are abundant in the human diet. Human cells produce unsaturated fatty acids branched with methyl groups (*e.g.* geranylgeranoic acid) from isoprenoid lipids, which are intermediates in the biosynthesis of cholesterol [1–3]. Saturated 2-methyl fatty acids including pristanic acid (2*R*,*S*,6*R*,10*R*,14-tetramethylpentadecanoic acid) are present in the diet. The major source of pristanic acid is its dietary precursor, phytanic acid (3*R*,*S*,7*R*,11*R*,15-tetramethylhexadecanoic acid), which is abundant in foods such as red meat, dairy products and

Abbreviations: AMACR, α-methylacyl-CoA racemase (also known as P504S); 2-APA, 2-arylpropanoic acid ('profen'); CAR, constitutive androstane receptor; CoA, coenzyme A; FXR, farnesoid X receptor; MCR, AMACR homolog from M. tuberculosis; NMR, nuclear magnetic resonance; NRs, nuclear receptors; NSAID, non-steroidal anti-inflammatory drug; PPAR, peroxisome proliferation activation receptor; PSA, prostate-specific antigen; PXR, pregnane X receptor; RXR, retinoid X receptor; siRNA, small interfering RNA; SNP, single-nucleotide polymorphism.

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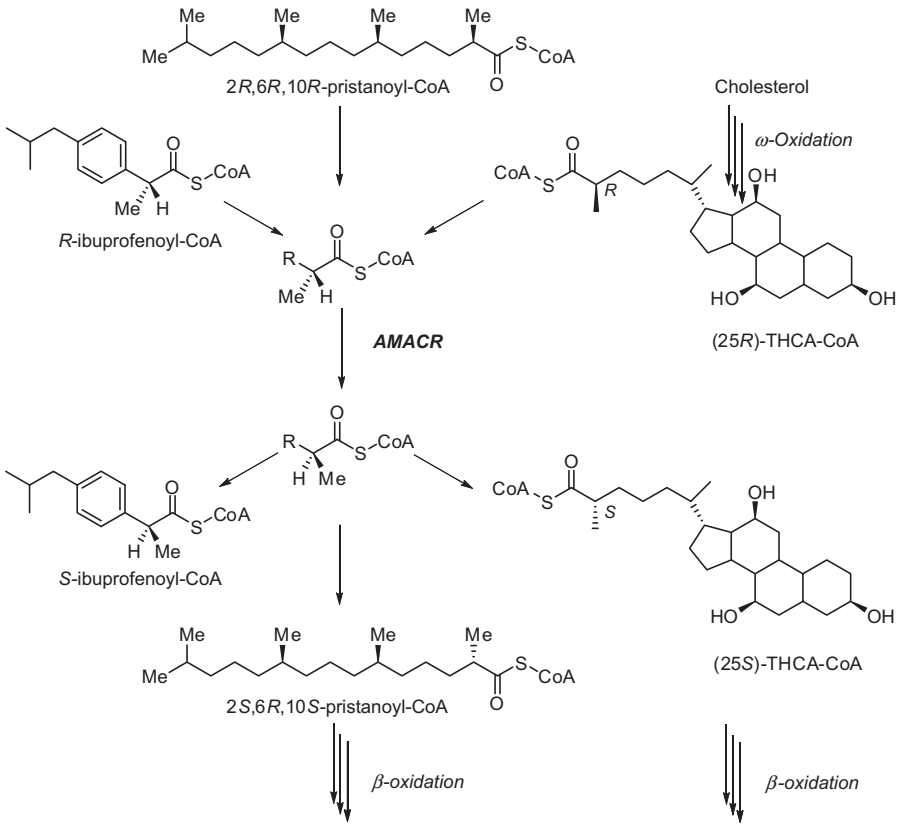
some other foods. Bile acids, with a steroid skeleton, are produced endogenously from cholesterol by ω-oxidation and these have α-methyl groups to the carboxylic acid moiety. Defects in the metabolism of these branched-chain fatty acids result in a number of diseases with mainly neurological symptoms [4–11].

Owing to their branched-chain structure, these fatty acids are initially metabolized in peroxisomes and a number of pathways exist, including β-oxidation which is the central pathway. Branched-chain fatty acids possessing 3-methyl groups, such as phytanic acid, cannot be immediately degraded by β-oxidation because the 3-methyl group blocks conversion of the 3-hydroxylacyl-CoA to the 3-ketoacyl-CoA [7]. Instead, these fatty acids undergo preliminary α-oxidation within peroxisomes to produce a 2-methyl fatty acid (pristanic acid) which, upon conversion to the corresponding acyl-CoA ester, is β-oxidized in peroxisomes and mitochondria for chain-shortened derivatives. Some chain-shortened derivatives also possess 2*R*-methyl groups. The ω-oxidation pathway converts terminal methyl groups to carboxylic acids and is important for initial production of bile acids from cholesterol [12–14]. The pathway also plays an important role in the detoxification of phytanic acid [15,16] in patients with adult Refsum's disease (who are deficient in the α-oxidation pathway), by converting it into its corresponding dicarboxylic acid. The ω-oxidation pathway produces α-methyl fatty acids which can be metabolized by β-oxidation thereby overcoming the metabolic block. This pathway is thought to have limited capacity [15] and so is probably not very significant when a functioning α-oxidation pathway is present. The peroxisomal α- and β-oxidation pathways are well defined and most of the enzymes involved have been well characterized [4–10]. More recent efforts have concentrated on studying the enzymes involved in the ω-oxidation pathway (reviewed in [8,17]).

An important aspect of the branched-chain β-oxidation pathway is that only *S*-2-methylacyl-CoA esters can be metabolized (Scheme 1). This is because very-long-chain, long-chain, and medium-chain acyl-CoA oxidases (ACOXs) have an absolute requirement for the 2*S*-stereoisomer of the substrate [18–20]. However, bile acids are produced exclusively with *R*-configuration at the α-carbon (carbon 25 in the standard numbering system for steroids) by mitochondrial ω-oxidation and a mixture of *R*- and *S*- configurations by microsomal ω-oxidation [12–14]. The pristanic acid derived from α-oxidation of phytanic acid is a mixture of 2*R*- and 2*S*-epimers [7,21]. Chain-shortened derivatives of pristanic acid with 2*R*-methyl groups are also produced by the β-oxidation pathway. The metabolism of *R*-2-methyl fatty acids is accomplished by conversion to their corresponding acyl-CoA esters, followed by chiral inversion to the *S*-2-methylacyl-CoA ester by α-methylacyl-CoA racemase (AMACR; EC 5.1.99.4) [22–24]. The CoA moiety of the substrate contains multiple chiral centers, so the chiral inversion reaction is formally an epimerization reaction (the configuration of only one of the several chiral centers is changed). Recent studies on the human enzyme [25,26] have shown that the reaction occurs by deprotonation of the substrate followed by a non-stereospecific reprotonation event, yielding a near 1:1 mixture of acyl-CoA esters with the original and epimeric configurations. Thus, the net effect of the enzyme is to racemize the configuration of the substrate at the 2-position.

The importance of AMACR in metabolism of branched-chain fatty acids is underlined by the observation that severe reduction of AMACR activity in humans results in a neurological disorder due to the accumulation of *R*-2-methyl fatty acids [27–29]. A mouse knock-out model of AMACR deficiency has been reported [30]. These mice showed the expected increase in levels of bile acid



Scheme 1. Role of AMACR in the metabolism of 2-methylacyl-CoA esters. Phytanic acid (3*R*,7*R*,11*R*,15-tetramethylhexadecanoic acid) can be ω-oxidized to 3*R*,7*R*,11*R*-trimethylhexadecan-1, 16 dioic acid [15]. The configuration of the new chiral center at carbon-15 is unknown, but AMACR is likely to be involved in the metabolism of acyl-CoA esters with 15*R*-configuration. AMACR, α-methylacyl-CoA racemase; CoA, coenzyme A; THCA, trihydroxycholestanic acid.