Acta Pharmaceutica Turcica
XXXX (4) 169-173 (1998)

OMEGA-OXIDATION OF ARACHIDONIC ACID

ARAKİDONİK ASİT'İN OMEGA-OKSİDASYONU

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In the present study, the importance of omega-oxidantion metabolism of arachidonic acid by animal tissues is summarized. Tissue and species differences are explained by cytochrome P450 isoforms catalyzing the reactions. Attention is drawn to the biological significance of arachidonic acid omega-oxidation metabolites.

Bu çalışmada, arakidonik asitin hayvan dokularındaki omega oksidasyonu metabolizmasının önemi özetlenmektedir. Doku ve tür farklılıkları, reaksiyonları katalizleyen sitokrom P450 izoformları ile açıklanmaktadır. Arakidonik asitin omega-oksidasyonu sonucu oluşan metabolitlerin önemine dikkat çekilmektedir.

Keywords: *Omega-oxidation; Omega-hydroxylation; Arachidonic acid; Microsomal metabolism*

Anahtar kelimeler: *Omega-oksidasyonu; Omega-hidroksilasyonu; Arakidonik asit; Mikrozomal metabolizma*

Introduction

Ordinary straight-chain fatty acids can be omega-oxidized (ω-oxidized) to dicarboxylic acids in the animal body. For normal straight-chain fatty acids, the ω-oxidation is quantitatively a minor pathway. The pathway acquires more significance for branched-chain or substituted fatty acids which are poorly, or not at all oxidized by normal β-oxidation (1). Arachidonic acid (AA), the key eicosanoid, plays a functionally significant role in physiology. It is the precursor of prostanoids and leukotrienes (Fig.1).

The membrane-bound endogenous fatty acid AA can be released from membranes by phospholipases and then metabolized to biologically active compounds by different enzyme systems. AA contributes a number of oxidative pathways during metabolism which are collectively termed *"The Arachidonate Cascade"* (2). In addition to being a substrate for various oxidative enzymes such as cyclooxygenases and lipoxygenases, AA is also a substrate for cytochrome P450 (P450) catalyzed oxidations such as omega-hydroxylation. Recent research in this field aims at elucidating and defining the cytochrome P450-generated metabolites for a better and comprehensive understanding of AA's central role in physiology and pharmacology in wich lipid-derived mediators play a role in cell and organ function.

The ω-hydroxylation of arachidonic acid, as well as that of other fatty acids, has been demonstrated as an activity associated with several forms of cytochrome P450 4A gene family, mainly 4A1, 4A2 and 4A3 in the rat kidney. Rat liver and kidney P450 4A1, although very active as lauric acid ω-hydroxylase, carries out relatively well the ω-hydroxylation of arachidonic acid and is susceptible to induction by clofibrate (3,4).

Outline of ω-hydroxy metabolites formed from AA by the cytochrome P450 monooxygenase system

Cytochrome P450 monooxgenases metabolize polyunsaturated fatty acids (PUFA) by various routes: (i) omega/omega-1 hydroxylation (ii) epoxidation

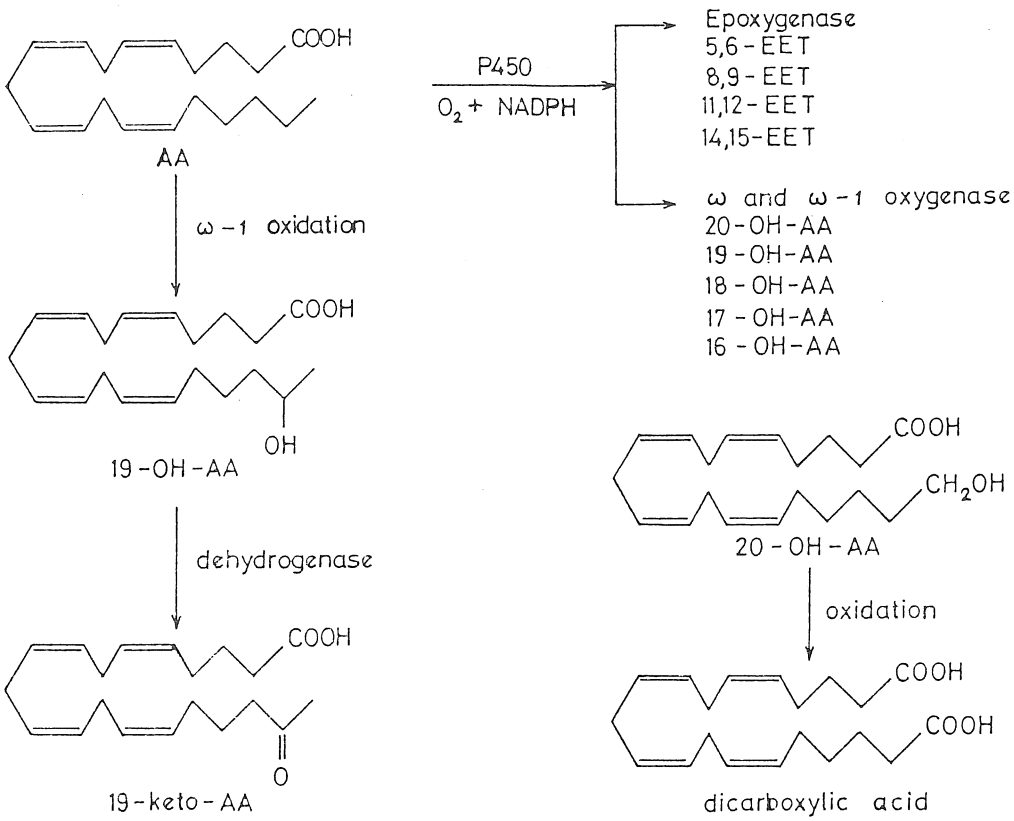

Fig.1. Arachidonic acid metabolites formed by the cytochrome P450 monooxygenase system

(iii) allylic and *bis*-allylic hydroxylation and (iv) hydroxylation with double bond migration. Although the liver and kidney are the major sites for these P450 catalyzed oxidations, a wide range of other organs and tissues also show P450 activity towards AA and other PUFA. Interestingly enough, many of the P450 AA metabolites have been shown to carry biological activites.

In general, fatty acid ω-oxidation is regulated by factors such as animal age, diet, starvation, administration of fatty acids and by agents such as hypolipidemic drugs, steroids and aspirin (2).

Hydroxylation of the ω-side chain of arachidonic acid

Earlier studies have shown ω 1-and ω 2-hydroxy metabolites of AA to be the most abundant hydroxy metabolites formed by liver and renal cortical microsomes of many species(3) including the human (5). Recent studies have shown that C_{16}-C_{18} of AA also can be hydroxylated by hepatic microsomes. These hydroxylation metabolites are also found in microsomal incubations of β-naphthoflavone, 3, 4, 5, 3', 4'-pentachlorobiphenyl or 3-methylcholanthrene treated rats.

Ciprofibrate and clofibrate-induced rat liver microsomes form 20-HETE as the main metabolite(3). Incubation of rat anterior pituitary microsomes with AA results in the formation of 20-HETE and at least three epoxides. Rabbit olfactory epithelium and intestine have been reported to produce 20-HETE and 20-HETE plus 19-HETE as major *in vitro* metabolites, respectively.

The entire ω-side chain of AA can be hydroxylated by NADPH-dependent hepatic P450 to yield 16-HETE, 17-HETE, 18-HETE, 19-HETE and 20-HETE. 20-HETE and its glucuronide conjugate have been identified in human urine leading to the conclusion that AA is metabolized *in vivo* by ω-hydroxlation. Enzymes of the P450 4A gene family preferantially oxidize the terminal carbon atom of AA, as well as of a variety of fatty acids, prostaglandins and eicosanoids, in hepatic and renal microsomal incubations(3,6,7).

Role of different cytochrome P450 isoforms

Mammalian P450 enzymes are formed from a gene superfamily comprising at least 12 gene families and 22 subfamilies (6). These hemoproteins are widely distributed in nature. Most P450s are very limited in their tissue distribution. However, they catalyze reactions of a significant number of diverse substrates. The 4A family includes genes for rat liver and kidney fatty acid ω-hydroxylases (4).

There are definite species differences: the major P450 arachidonate product in uninduced chick embryo and adult chicken liver is ω-OH AA, whereas *cis*-epoxyeicosatrienoic acids (EETs) predominate in untreated rat liver microsomes(8). In human liver microsomes, AA metabolism is principally catalyzed by 2C enzymes. The contribution of 1A2, 2E1 and 2B6 may be enhanced by exposure to the inducers of the enzymes. The main microsomal metabolite is ω-OH AA (5). Expression of 4A forms is sex dependent, and male rats exhibit greater induction of 4A1 and 4A3 in liver than female rats. In the male rat kidney, 4A1 is not expressed and 4A2 is the predominant form. In the female, 4A2 is not expressed in livers or kidneys(9).

In the liver, one of the major hepatic responses to peroxisome proliferators (classified as nongenotoxic carcinogens) is the induction of drug metabolizing enzymes such as fatty acid ω-hydroxlases (10,11). More specifically, increased transcription of lauric acid ω-hydroxylase has been demonstrated (11). Ethanol, acetone, imidazole and isopropanol can induce 2E1: the isoform catalyzing ω-1, ω-2 and ω-3 hydroxylation reactions of AA (12).

A recent study on the effects of prolonged administration of the hypolipidemic saturated 3-thio fatty acid analogue tetradecylthioacetic acid on lipid metabolism in rats has revealed that AA levels are decreased in heart tissue with possible consequences for cardiac membrane phospholipid dynamics and function(13). A stereospecific AA ω-2 hydroxylase is present in the genital glands(12).

Yamane *et al.* have compared AA ω-hydroxylation activity in the brain, liver and colonic adenocarcinoma, with special emphasis on subcellular localization of the activity and have found that there is a difference regarding subcelluar localization depending on the type of organs(14).

19-OH-PGEs and 19-OH-PGFs in semen are not formed by hydroxylation of PGs but are formed by the prior 19-hydroxylation of eicosatrienoic and arachidonic acid with the subsequent action of the cyclooxgenase to form the corresponding 19-OH-PGs(15).

The kidney monooxygenase

It is proposed that the renal AA monooxygenase may participate in the control of body fluid volume and/or composition, and thus contribute to the pathophysiology of hypertension (16,17). The oxidation of AA and lauric acid by kidney microsomes is greater than oxidation of steroid hormones(4). The major kidney

isoforms in the human kidney cortex is 4A2(12).

The major kidney P450 isoforms catalysing ω/ω-1 oxidation are predominantly members of the P450 4A gene family; several rat, rabbit and human P450 4A isoforms have been purified and/or cloned, expressed and after reconstitution in the presence of P450 reductase, shown to catalyze AA ω- and, to a lesser extent, ω-1 oxidation. The rat P450 4A1 and rabbit 4A4 produce only 20-OH-AA. Purified P450 2E1 metabolized AA to 19-(S)- and 18(R)-OH-AA, along with other metabolites.

Recent studies have shown several 4A isoforms to be localized at S1, S2 or S3 segments of the proximal tubule, brush border or the renal medulla(16).

Significance of the kidney metabolites

A wide variety of biological activities have been attributed to the AA ω/ω-1 oxygenase products, mainly vasoactive properties. Many of these are specific for species and vessels and even dependent on systemic pressure. In some cases, biological activity requires their further oxidative metabolism and/or transformation by prostaglandin H_2 synthase. These effects include vasoactivity and the modulation of cellular ion fluxes. Different vascular tissues have different capacities to metabolize exogenous AA to 20-OH-AA Prostaglandin H_2 synthase-dependent and -independent vasoconstrictor activities for 20-OH-AA have been reported. In contrast, 19-OH-AA is a stereospecific prostaglandin H_2 synthase-independent renal vasodilator.

Some of the reported effects of the AA ω/ω-1 metabolites on ion transport may underlie their vasoactive properties. Both natriuretic and diuretic effects have been shown for 20-OH-AA. Further work needs to be carried out to elucidate mechanistic aspects. It has been proposed that 20-OH-AA serves an endogenous prohypertensive role in the SHR rats(16).

Conclusion

The role of microsomal P450 is not limited to the metabolism and biodegradation of xenobiotics. Its role in the metabolism of AA assignes important functions to this enzyme system. Further work is needed to understand how P450 inducers and inhibitors alter the spectrum of arachidonate metabolites to which cells are exposed and how the stability of P450 -dependent metabolites effect distant organs from the liver. Work continues on the use of cDNAs to analyze gene expression and to analyze enzymatic mechanisms at the molecular level.

References

1. Bremer, J., Osmundsen, H.: Fatty Acid Oxidation And Its Regulation. In: Numa, S. (Ed) Fatty Acid Metabolism and Its Regulation, pp. 113, Elsevier, Amsterdam 1984
2. Capdevila, J.H., Falck, J.R., Estabrook, R.W.: The FASEB J. 6(Jan.) 731 (1992)
3. Oliw, E.: Prog. Lipid Res. 33(3) 329 (1994)
4. Orellana, M., Fuentes, O., Valdes, E.: FEBS 12398, 322(1) 61 (1993)
5. Rifkind, A.B., Lee, C., Chang, T.H.K., Waxman, D.J.: Arch. Biochem. Biophys. 320(2) 380 (1995)
6. Roman, L.J., Palmer, C.N.A., Clark, J.E., Muerhoff, A.S., Griffin, K.J., Johnson, E.F., Masters, B.S.S.: Arch. Biochem Biophys. 307(1) 57 (1993)
7. Nelson, D.R., Kamataki, T., Waxman, D.J., Guengerich, F.P., Estabrook, R.W., Feyereisen, R., Gonzales, F.J., Coon, M. J., Gunsalus, I.C., Gotoh, O., Okuda, K., Nebert, D.W.: DNA Cell. Biol. 12, 1 (1993)
8. Nakai, K., Ward, A.M., Gannon, M., Rifkind, A.B.: J. Biol. Chem. 267(27) 19503 (1992)
9. Okita, J.R., Castle, P.J., Okita, R.T.: J. Biochem. Toxicol. 8(3) 135 (1993)
10. Gibson, G.G.: Toxicol. Lett. 68, 193 (1993)
11. Moody, D.E., Gibson, G.G., Grant, D.F., Magdalou, J., Rao, M.S.: Drug Metab. Dispos. 20(6) 779 (1992)
12. Hömsten, L., Bylund, J., Oliw, E.H.: Arch. Biochem. Biophys. 332(2) 261 (1996)

13.Asiedu, D.K., FrÌyland, L., Vaagenes, H., Lie, Ø., Demoz, A., Berge, R.K.: Biochem. Biophys. Acta 1300, 86 (1996)
14.Yamane, M., Abe, A., Shimizu, S., Nakajima, M.: J. Chromatogr. A 730, 91 (1996)
15.Kupfer, D.:Pharmacol. Therap. 11, 469 (1981)
16.Makita, K., Falck, J.R., Capdevila, J.H.: The FASEB J. 10(Nov.) 1456 (1996)
17. Lin, F., Abraham, N.G., Schwartzman, M.L.: Cytochrome P450 Arachidonic Acid ω-Hydroxylatino in the Proximal Tubule of the Rat Kidney. (In): Goetzl, E.J., Lewis, R.A., Rola-Pleszczynski, M.(Eds), Cellular Generation, Transport, and Effects of Eicosanoids-Biological Roles and Pharmacological Intervention, pp. 11, Annals of the New York Academy of Sciences, New York, 1994

Accepted: 27.02.1998