The Virgin Olive Oil Model of Essential Fatty Acid Metabolism.

New Aspects on Inflammation and Carcinogenesis via the LOX Pathway of Biodegradation of Lipids

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SUMMARY

Virgin Olive Oil is a unique divine mixture of extreme biological value. The percentage of essential fatty acids, phenolic, sterolic and stanolic composition reflect its nutritional and pharmaceutical characteristics. Recent data of progress on Lipoxygenase metabolic pathway of arachidonic acid has proven molecules like 5-OXO- HETE responsible for inflammation and metastatic carcinogenesis. Special attention should be drawn for suitability of the chronic use of Virgin Olive Oil and other oils as in mixtures like cosmetic devices, instead of consuming as a food.

Keywords : Virgin Olive Oil, arachidonic acid, LOX pathway, 5-OXO-ETE, HETE, inflammation, metastatic carcinogenesis, cosmetic use of oils.

INTRODUCTION

Among vegetable oils, virgin olive oil has naturally derived chemicals that correspond to nutritional and pharmaceutical characteristics that make it unique. In real sense it constitutes a miracle mixture of omega polyunsaturated fatty acids (PUFA's), phenolic, and stanolic anti-oxidant molecules of ever increasing medicinal properties nowadays. This biochemical composition reflects complex metabolic pathways that are carried on in olive fruit cells (1).

The biodegradation of polyunsaturated fatty acids as well as that of the other biologically significant metabolites depends primarily among others on the degree of fruit ripeness which is also influenced by genetic and environmental factors (2). The stability of virgin olive oil change as the fruit ripens, and the concentrations of PUFA's such as linoleic, linolenic arachidonic and linolic acids change accordingly (3). Equally important to this ripening are the technological treatments of the process of olive oil extraction (4).

During ripening of olive fruit metabolic processes of auto-oxidation are carried out where protagonist role have the Lipoxygenases (LOX) and Cycloxygenases (COX) metabolizing enzymes (5, 6).

In particular LOX proteins constitute an important class of monomeric, nonheme iron containing dioxygenases commonly found in animals, plants and fungi (7) that catalyze the oxygenation of polyunsaturated fatty acids such as linolenic, linoleic and arachidonic acids Plant and animal LOX in particular have been extensively identified for their action (8,9). Specifically this enzyme catalyzes the insertion of molecular oxygen into PUFA containing a Z,Z,1,4-pentadiene moiety yielding the corresponding hydroperoxides (10). These in respect are subsequently metabolized into secondary metabolites that possess important physiological properties. These properties range from the initiation of biosynthesis of leucotriens and lipoxins which are important potent mediators of inflammatory responses (10, 11) to the biosynthesis of signaling compounds that exert significant mitotic activity stimulation (12). In plants, linolenic and linoleic acid are the primary substrates of LOX due to their abundance, and LOX are classified as 9 and 13-LOXs with respect to their positional specificity of linoleic acid oxygenation (13, 14).

THE LOX BIOCHEMICAL MECHANISM

LOX are becoming of most interest in food science because their activity results in free radical formation that can exert deleterious actions from nutritionally important compounds such as essential polyunsatutared fatty acids which are no other than linolenic, linolic and arachidonic acids (15,20). On the other hand what is more interesting is that the action of LOX and COX enzymes is implicated in the degree of inflammatory initiative response, the development and metastasis of cancer via the metabolism of arachidonic acid and prostaglandin biosynthesis (16).

During tissue disruption of olive fruit cells, lipid degradation is carried instantaneously by auto-oxidative enzymes such as LOX (sheme 1). Hexanal (E)-2-hexenal, (E)-2-hexen-ol, 1-hexanol and (Z)-3-hexen-1-yl acetate are five biomarkers produced as a consequence of lipid degradation following tissue disruption, and they are among the most important volatile compounds in olive oil aroma (17-20).

Arachidonic acid is converted to a large number of biologically active products (eicosanoids) that are important in a variety of pathological conditions including inflammatory and allergic diseases (21) especially on the skin. The biochemical synthesis of eicosanoids involves a complex array of compounds with different chiral centers and double bond configurations, on this respect, LOX enzymes catalyze the stereospecific oxidation of arachidonic acid to form hydroperoxyeicosatetraenoic acids (HPETEs) (22). This in turn is catalyzed by a specific peroxidase to yield a specific hydroeicosatetranoic acid (HETE) which in is further transformed to –OXO-

ETE_2 by the $NAPD^+$-dependent enzyme–hydroxy eicosanoid dehydrogenase. Although there is a lack of stereospecificity between mammalian and plant LOX enzymes, in biotechnology the soybean lipoxygenase which is widely used as a source of 15 LOX produces also the 5-HPETE and thus is called as 5/15LOX (23). 5-HPETE is also converted by peroxidase to 5-HETE which is further transformed to 5-OXO-ETE by $NADPH^+$ -dependent enzyme 5-hydroxy eicosanoid dehydrogenase (5-HEDH) (Scheme 2) (24).

FIGURE 1. THE BIOCHEMICAL PATHWAY BY WHICH LOX ENZYMES CONVERT LINOLIC AND LINOLEIC ACIDS TO TOXIC BY PRODUCTS

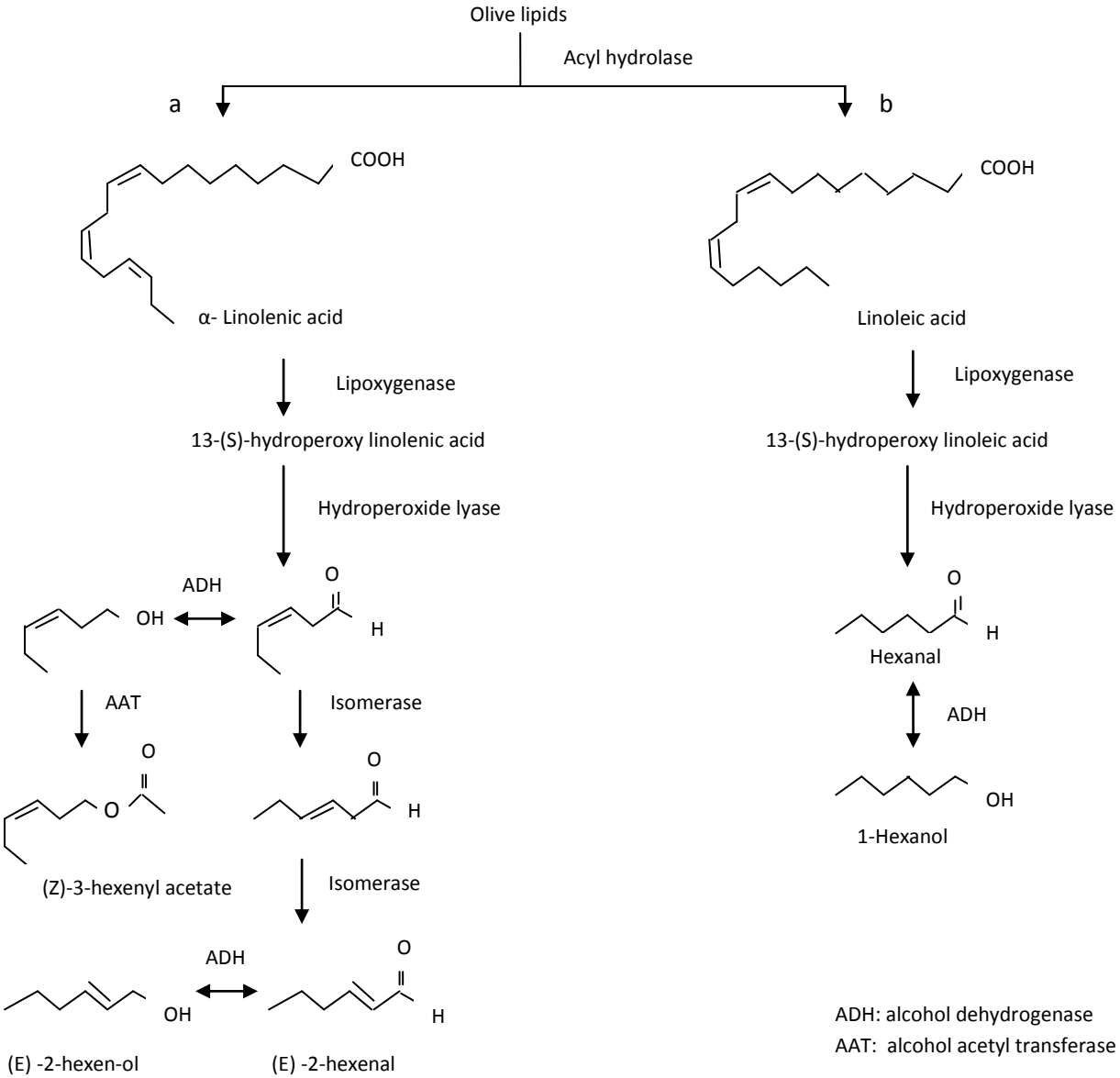
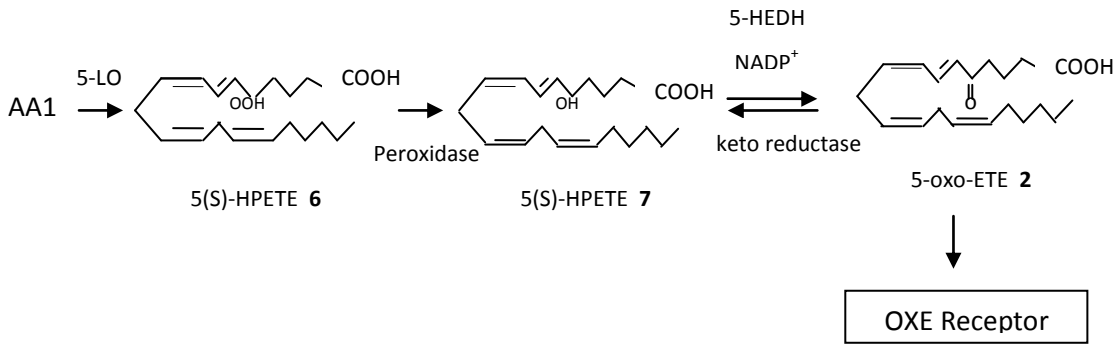


FIGURE 2. THE BIOCHEMICAL PATHWAY BY WHICH ARACHIDONIC ACID 1 IS CONVERTED TO 5-OXO-ETE MOLECULES THAT LIGATE TO OXE-RECEPTOR.



IMPLICATIONS OF LOX MECHANISM IN INFLAMMATION AND CANCER

5-OXO-ETE is the extremist of mediators of eosinophil chemotaxis amongst lipid eosinophil activators and has similar effects on neutrophils and monocytes (25). This lipid mediator that derives from peroxidation of arachidonic acid via the 5 LOX pathway that can be energized even from plant derived LOX oxidized lipid particles, has a specific receptor termed OXE (25). In its turn, 5-OXO-ETE is hydrolyzed by both lipoxygenase and cytochrome$_{450}$ enzymes to the respective 5-OXO-HETE derivatives. These are identified in the literature as extreme mediators of inflammation and tumor genesis (26-28). Recently, an inhibitory to fibroblast activation protein, the Niemann-Pick type C2 deficiency, was shown to lead to inflammatory response by upregulation of arachidonic acid bio-degradation via the prostaglandin E2 pathway (29). Prostaglandin E2 is proven to be a key molecule to cellular activation leading to numerous inflammatory diseases and cancer (30).

Effect of 5-OXO-ETE in Inflammation.

The responses elicited by 5-OXO-ETE in eosinophils and neutrophils include actin polymerization, calcium mobilization, integrin expression and degranulation (12). This molecule is considered as an asthma inducing agent as it produces tissue eosinophilia on its presence (31). In human neutrophils 5-HETE that is also a substrate for hydroxylase 20 triggers these cells in a way that resembles polymorphonuclear activation in an alternative route to leucotriene B4 receptor (31). Thus the presence of 5-OXO-ETE may be a major contributor factor to cellular orchestration for the commencement of inflammation. These cells produce a variety of cytotoxic mediators such as reactive oxygen species, and nitrogen species, and among others, enzymes like COX and LOX which activate or are activated by

transcription factors as nuclear factor kB (NF-kB) and signal transducers and activators of transcription-3 (STAT3) (16). Obviously, on a predisposed tissue, the presence of LOX even from plant origin, such as LOX transcripts present in food grade olive oil and other oils (LOX transcript) and of the intermediate metabolites of LOX pathway, such as 5-OXo-ETE may be a contributing factor of an unresolved inflammation. Current epidemiologic data indicate that over 25% of all cancers are related to chronic infections and inflammation of unknown origin that may result from a continuous use of an unidentified agent such as a cosmetic product (32).

Effect of 5-OXO-ETE in Carcinogenesis.

A recent model implicating carcinogenesis via the 5-LOX pathway is in prostate cancer cells where 5-LOX induces cellular growth by 5-OXO-ETE via a receptor called OXER1, which exerts survival signal by generating diacyl-glycerol through phospholipase C-beta (33). Further, 12-LOX metabolite is a critical prostate cancer regulator as it promotes cellular growth by activating the action of a protein called surviving (28). Survivin causes metastasis and promotes angiogenesis in cancer infiltrative tissues.

Oral cancer is a common neoplasm worldwide, particularly in developed countries (34). In the united States alone oral cancer has been increasing, accounting in more than 7500 deaths in 2005 (32). In oral carcinogenesis, and especially in squamous cell carcinoma (SCC), the 5-LOX pathway is implicated as the metabolites leucotriene B4 was found to be elevated 10 to 30 fold in human squamous cell carcinoma than in normal tissues (26). Arachidonic acid metabolism inhibitors at post-initiation stages of these SCC prevented the further development of disease. Complementary, alcohol drinking is associated for the development of lethal metastatic cancer of the tongue acting synergistically with the 5- LOX pathway metabolites (35).

Effects of LOX pathway on dermal tissue

The wound healing response relies on a mutual response between recruited fibroblasts and the collagenous extracellular matrix (CEM). A key receptor that directs prompt dermal tissue repair is discoidin tissue receptor 2 (DDR2), a tyrosine kinase receptor for fibrillar collagen that is expressed during pathologic scarring, wound healing, arthritis and cancer (36). In DDR2 deficient mice, wound showed decreased tensile strength which correlated with a significant reduction in collagen content and defective collagen crosslinking. This defective response was carried out by the 5-LOX pathway and its intermediate metabolites of arachidonic acid break down. It was restored by inhibition of LOX activity by β-aminopropionitrile (37). In skin cancer and photoaging produced by ultraviolet B irradiation, the 5-LOX protein is translocated from nucleus to cytoplasm in fibroblasts in an elevated manner. When LOX inhibitors were used, such as mizolastine, this traslocation was inhibited

and cellular function returned to normal. Special consideration (36) from these findings arise for cosmetics rich in LOX transcripts (1-3), that are applied regularly on predisposed skin for photoaging and cancer in conjunction to UV exposure (37), such as sunscreens.

DISCUSSION

Clearly the arachidonic acid metabolism of 5-LOX generates the 5-OXO-ETE metabolite that is implicated in an orchestration of eosinophil and neutrophil related inflammatory reactions and in a variety of cancers (5,9,11,16,27). What is of most importance however is that there is no need for specificity for LOX enzymes to produce the same metabolites that contribute to carcinogenesis seen in human as is obvious in the case of soybean oxygenase that produces the same 5-OXO-HETE metabolites both from plant and mammalian origin (5,7) . This is logical as numerous enzymes seen in microbial flora, such as *Thermus aquaticus* polymerase, the basis of polymerase chain reaction technology is specific to polymerize all kinds of DNA (38).

One problem arising is for the industrial use of oil produced by olive fruits, soybean and others as the LOX activity on linolenic, linoleic and arachidonic acids may produce intermediate molecules such as 5-HETE (2,3,5,7) that when not used for eating consumption as in chronic exposure of the dermis layers into cosmetics may provoke the tendency to metastatic malignancies. The data drawn from studies focusing on inflammatory response orchestration and from cases of oral and prostate cancer show that via the 5-LOX pathway of arachidonic acid biodegradation to molecules like 5-OXO-ETE disease status is established (9-12, 26-37). Other important data contribute to this skepticism as the LOX transcript accumulation rise as a results of olive fruit cell senescence (2). This may mean, as it has already been proven (2,3) that when olive oil is extracted from mature fruits and also when cellular decaying debris are left in the olive oil solution, the 5-LOX pathway of linolenic, linoleic and arachidonic degradation continuous in the olive oil solution.

Our preliminary results on a small study underway (data not shown), to detect deleterious effects of virgin olive oil unsuitable use in cosmetic industry show that when a molecular technique of olive oil purification from decaying enzymatic content such as LOX and COX is applied, this attenuates the further biodegradation of in vitro lipid metabolism. In fact in this study, the levels of linolic, linolenic and arachidonic acid show a remarkable increase after 1 and 3 months of incubation ranging from 9 to 33 % as the percentage of whole linoleic (C18 oleic acid) decreases.

Clearly, the food grade mechanical techniques of making oils do not exclude the presence of self decaying and auto-oxidative processes under way in ripening fruits

via LOX pathway metabolites and this may have deleterious effects on human health as is in the case of chronic exposure to cosmetics that contain such oils.

Further research data are needed to provide healthier products, as the application of modern molecular techniques to detect 5-LOX activity in oils and idealize oil purification do become necessary.

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