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Review

Lipoxygenase – a versatile biocatalyst for biotransformation of endobiotics and xenobiotics

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Abstract. Lipoxygenase, a member of the arachidonate cascade enzymes, dioxygenates polyenoic fatty acids to finally yield products with profound and distinct biological activity. This review summarizes the available evidence for another role played by lipoxygenases in the metabolism of endobiotics and xenobiotics. Although other mechanisms exist, a direct hydrogen abstraction by the enzyme and the peroxyl radical-dependent chemical oxidation appear to be central to the co-oxidase activity of lipoxygenases. Besides polyunsaturated fatty acids, H_2O_2, fatty acid hydroperoxides, and synthetic organic hydroperoxides support the lipoxygenase-catalyzed xenobiotic oxidation. The major reactions documented thus far include oxidation, epoxidation, hydroxylation, sulfoxidation, desulfuration, dearylation, and N-dealkylation. It is noteworthy that lipoxygenases are also capable of glutathione conjugation of certain xenobiotics. The enzyme system appears to be inducible following exposure to chemicals. Lipoxygenases are inhibited by a large number of chemicals, some of which also serve as co-substrates. Available data suggest that lipoxygenases contribute to in vivo metabolism of xenobiotics in mammals.

Key words. Xenobiotic oxidation; glutathione conjugation; lipoxygenase induction and inhibition; free radical; reactive metabolite; co-oxidation; teratogen; carcinogen bioactivation.

Introduction

The perpetuation of human and other animal species depends, among other factors, on successful defense against the deleterious effects of naturally occurring toxic chemicals in plants and synthetic toxicants. A spectrum of enzymes that have evolved with time make up the defense system that endows survival against toxicants. The system aids the body in the conversion of lipophilic chemicals into water-soluble products that are eliminated via urine or feces. Most metabolic reactions decrease while some increase in the toxicity of chemicals. Since a balance between these opposing reactions determines the magnitude of the biological response, biotransformation represents a key element in understanding the pharmacological efficacy of drugs and the toxicity pesticides, industrial/environmental chemicals, and naturally occurring toxicants. Ample documentation exists for the role played by microsomal cytochrome P450 (P450), flavin-containing mono-oxygenase (MFMO) and prostaglandin H synthase (PGS) in the oxidation of endobiotics and xenobiotics. Recent publications from different laboratories have established lipoxygenase (LO) as another major enzyme responsible for xenobiotic oxidation [1]. This review is primarily intended to summarize the available literature on LO-mediated metabolism of chemicals. The discussion is intended to be provocative to promote future research rather than definitive, because this area of science is just beginning to evolve.

LOs are ubiquitous in aerobic organisms. They occur in many species of algae, plants, aquatic invertebrates, and vertebrates. In mammals, a significant amount of LO activity occurs in blood cells and many organs in the body [1]. Several excellent reviews on LO summarize the cur-

rent information on general aspects [2–7], molecular biology, catalytic properties [8–15], structure and function [16, 17], regio- and stereo-chemistry [18], and their role in health and disease [e.g., refs 19–21]. Specific information pertaining to 5-LO [22–26], 12-LO [27, 28], and 15-LO [29, 30] is also available. For more complete understanding of the LO system, the reader is encouraged to consult these reviews.

Biochemistry and properties of LOs

Dioxygenase activity

Arachidonic acid, an essential polyunsaturated fatty acid (PUFA), is oxidized in the body by P450, PGS and LO. LOs incorporate O_2 into the PUFA-containing 1-*cis*,4-*cis*-pentadiene system. The dioxygenation (fig.1) involves hydrogen abstraction, the first rate-limiting step, to produce a fatty acid radical ($L^{\bullet}$), radical rearrangement to form a conjugated diene, and oxygen insertion to produce a fatty acid peroxyl radical intermediate ($LOO^{\bullet}$) before the release of lipid hydroperoxide (LOOH). Current nomenclature of LO classification is based on the positional specificity of arachidonate oxygenation. Of the different forms known, 5-, 8-, 12-, 15-, and 12/15-(S)LO have received the most attention. The dioxygenation of arachidonate, for example, by 5-LO yields 5(S)-hydroperoxy-6,8,11,14-(*E,Z,Z,Z*)-eicosatetraenoic acid (5-HPETE), while 12-LO and 15-LO produce 12-HPETE and 15-HPETE, respectively. Inside the cell, HPETEs are either reduced to the corresponding hydroxy derivatives, viz. 5-HETE, 12-HETE, and 15-HETE, or serve as intermediates in the genesis of a wide array of bioactive molecules such as leukotrienes (LTs), lipoxins, and hepoxilins. Although current nomenclature implies a strict positional specificity of fatty acid oxygenation, the data gathered with different substrates and from site-directed mutagenesis studies have revealed that an alteration in this parameter can be observed with mammalian LOs [31–33]. More than half a dozen genes regulate LO in mice and human and their expression varies with tissue [e.g. ref. 34]. Except for red blood cells and possibly lymphocytes, multiplicity exists in the occurrence of LO in mammalian cells. At present, the exact number of LOs in mammals is not known. Leaping advances in genetic and molecular biology techniques have unveiled the existence of multiple forms of 12-LO and 15-LO [10, 27, 29, 35]. Additional forms and subspecies will certainly be discovered in the future. Human LOs have been purified and characterized in detail from different blood cells [27, 29]. High-performance liquid chromatography (HPLC) analyses of arachidonate metabolites suggested the predominance of 15-LO in human lung [36]. Some data on the properties of LOs from human term placenta and intrauterine conceptual tissues [37, 38] and other tissues are also available. Among plants, the most studied LO is from soybean (SLO). Of the known multiple forms of SLO, L-1 is routinely used by many investigators as a model LO in various xenobiotic oxidation studies. It is referred to hereafter simply as SLO.

LOs are non-heme iron proteins. Although the major portion of LO activity resides in tissue cytosol, some activity can be observed associated with the nuclear envelope, mitochondria, and microsomes. Under certain experimental conditions, LOs translocate from the cytosol to intracellular membranes [39]. Typically, PUFAs containing two or more double bonds serve as the best substrates for the dioxygenase activity of LOs. However, some LOs can also oxygenate complex lipids such as esters of polyenoic

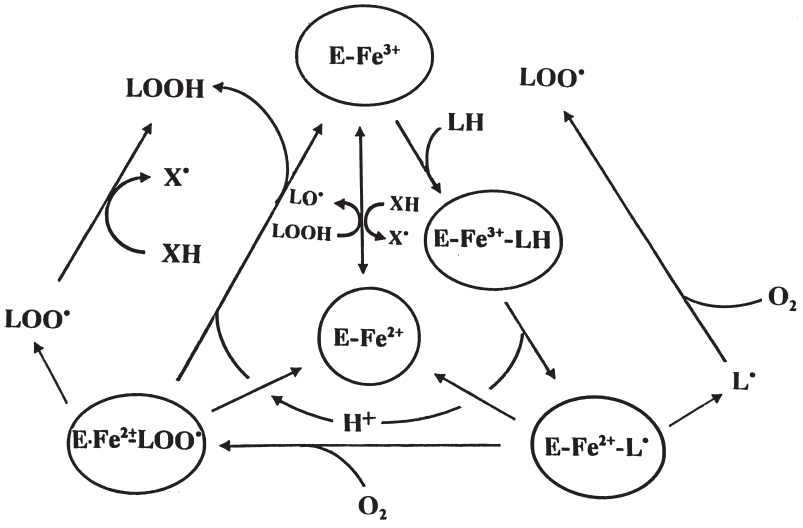

Figure 1. Schematic representation of dioxygenation of fatty acid (LH) and co-oxidation of xenobiotic (XH) by lipoxygenase (E). See text for further details.

fatty acids. Even biomembranes are attacked and certain LOs are known to oxygenate mitochondria and erythrocyte ghosts. Lipid peroxidation catalyzed by 15-LO provides a precise mechanism by which membrane-enclosed organelles are digested during the maturation and differentiation of such cells such as reticulocytes, central fiber cells of the eye lens, and keratinocytes. Atherosclerosis involves oxidative modification of low-density lipoprotein by 15-LO [1, 40, 41].

The commonly used assays for the dioxygenase activity of LO include an estimation of oxygen uptake by means of a Clark electrode, spectrophotometric recording of conjugated diene formation absorbing at 234 nm and HPLC (plus radiometry) of (^{14}C or ^{3}H)-arachidonic acid metabolites [42]. Hemoglobin, a common contaminant in preparations of mammalian tissue LOs, poses a serious problem, since it exhibits both pseudo-dioxygenase and pseudo-peroxidase activities. A recently developed method using $ZnSO_4$ [38] was found to alleviate this problem without significantly affecting either the dioxygenase or co-oxidase activities of human term placental LO (HTPLO). The K_m values for dioxygenation noted with different LOs range between 6 and 28 μM for arachidonate and between 3.5 and 26 μM for oxygen [43]. An initial lag period of several seconds is commonly noted while assaying the dioxygenase activity of most LOs. This is related to the fact that LOs are catalytically inactive in the ferrous form and require activation by peroxide. LOOH addition (usually $\leq$10 μM) shortens this lag period. Higher LOOH concentrations, however, inactivate most LOs. Nanomolar H_2O_2 can also effect a spontaneous activation of SLO [44, 45]. Ca^{2+}, Mg^{2+}, or ATP stimulate dioxygenase and co-oxidase activities of LOs in vitro.

Co-oxidase activity

Besides dioxygenation of PUFAs, LOs are also capable of oxidizing many xenobiotics. The ability of LO to couple peroxidation of PUFAs with xenobiotic oxidation is termed co-oxidase activity (see fig.1). This aerobic reaction is called 'co-oxidation' and the chemical oxidized is referred to as the co-substrate. LOs are unique in that they themselves synthesize the oxidants (LOO$^{\bullet}$ and LOOH) needed for xenobiotic co-oxidation. The relationship between dioxygenase and co-oxidase activities of SLO has been examined using SLO preparations varying in specific activity obtained from different commercial sources [46]. Linoleic acid and dianisidine were used as substrates for dioxygenase and co-oxidase activity, respectively. If one presumes that the co-oxidase activity is due to protein(s) other than LO, then large variation among values for the dioxygenase/co-oxidase activity ratio is expected. Despite a 15- to 73-fold variation in the dioxygenase activity, an activity ratio of 4.75 was noted for each LO preparation. This correlation between the dioxygenase and co-oxidase activities of SLO suggests their inter-dependence and reveals that LO catalyzes not only dioxygenation but also the co-oxidation reaction. Other terms such as 'peroxidase-like activity, pseudo-peroxidase, or hydroperoxidase activity of LO' have been used by some investigators to signify xenobiotic oxidation reactions mediated by LOs in the presence of various LOOHs or H_2O_2.

Importance of LO-mediated xenobiotic metabolism

LO is a versatile biocatalyst capable of many reactions involved in xenobiotic metabolism. The following discussion illustrates clearly how LO serves as the sole pathway for the activation of certain toxicants and operates as an alternate or additional pathway for oxidation of other chemicals.

Benzidine is a known bladder carcinogen. However, a few reports have also implicated human exposure to benzidine and other arylamines in cancers of the pancreas [47], kidneys and liver [48]. In rats, mice, and hamsters, benzidine causes hepatocarcinogenicity [49]. Benzidine undergoes bioconversion to electrophiles that covalently bind to DNA, induce mutations, and initiate carcinogenesis. Even though liver is rich in P450, benzidine activation may not be mediated by P450 since it is a very poor substrate for the NADPH-dependent oxidation by rat hepatic P450 [50]. PGS involvement can be ruled out because it occurs in negligible amounts in liver [51]. LO appears to be the sole catalyst in light of the fact that benzidine activation occurs readily with the rat and human liver LO [1].

Enzymatic oxidation of guaiacol offers another example. SLO can rapidly convert guaiacol into tetraguaiacol in the presence of linoleic acid or H_2O_2 [46, 52]. Apparently, P450 is unable to mediate this reaction in the presence of organic hydroperoxide [53]. Acrylonitrile (ACN), a high-volume industrial chemical, is an animal lung carcinogen that requires bioactivation to 2-cyanoethylene oxide (CEO) and cyanide to exert toxicity. Although ACN bioactivation by P450 is debatable (discussed later), human lung LO and SLO can extensively oxidize ACN to these products [36]. In extrahepatic tissues, which are often targets for chemical toxicity and contain only moderate to low levels of P450, PGS is suggested to contribute to the process of xenobiotic bioactivation [51, 54]. In these tissues, LO can serve as an efficient alternate or additional pathway of xenobiotic oxidation. The reported desulfuration of parathion by the rat brain LO [55], oxidation of benzo(a)pyrene (BP) [56], and epoxidation of aflatoxin B_1 [57] by the pulmonary LO lend strong support for this contention. The bioactivation of p-aminophenol, a potent nephrotoxicant, represents another notable example, since neither P-450 [58] nor PGS [59] is appar-

ently responsible for its toxicity. The results of our study [60] suggest that the LO pathway may be the only route that generates GSH-adducts, the presumed ultimate nephrotoxic species from p-aminophenol. Human term placentas from non-smoker donors exhibit a marked deficiency in P450, MFMO, and PGS activities. However, LO is plentiful in human placenta [37, 38]. It assumes a unique role in the activation of chemical teratogens and developmental toxicants [1]. The same can be argued, at least to some extent, for human intrauterine tissues at early or mid-gestation [37], and other target organs.

The subcellular location of the enzymes may be a critical issue in the oxidation of certain hydrophilic xenobiotics. P450, MFMO, and PGS are microsomal enzymes while LO is mainly cytosolic. For example, imipramine, a widely used tricyclic antidepressant, is freely soluble in water and sparingly soluble in organic solvents such as acetone. Thus, in theory, the intracellular accessibility of imipramine is expected to be much higher to LO than to P450, MFMO, or PGS. The reported LO-mediated extensive N-demethylation of imipramine [61] strongly supports this view.

Although a demonstration of the presence of P450 in trace amounts, for example, may be of some academic interest, the efficiency of the enzyme, in a practical sense is far more important and should not be ignored. In this regard, P450, although it can oxidize many chemicals, is widely accepted to be a relatively weak biocatalyst of xenobiotic oxidation when the turnover numbers for different reactions are considered. In contrast, the reported catalytic potential of the LO estimated under optimal conditions for the same oxidative reactions appears quite impressive (table 1). This, in theory, also translates into a much lower tissue requirement for LO to generate the same amount of metabolite(s) from different xenobiotics.

Another noteworthy point relates to the dependency of P450 and MFMO on pentose phosphate shunt enzymes for NADPH supply. This cellular pool of NADPH is relatively small (e. g., ~100 µM in hepatocytes) and both P450 and MFMO must compete with many other biochemical pathways for the available NADPH. In contrast, the cellular supply of free (plus esterified) PUFAs is plentiful (e. g. free arachidonate alone occurs at 210 ng/mg in human term placenta [1]) to support co-oxidase activity of LO.

Proposed mechanisms of xenobiotic co-oxidation

At least four mechanisms have been identified in LO-mediated xenobiotic oxidation.

LO-mediated, hydroperoxide-dependent reactions

This mechanism is directly linked with the redox cycling of iron at the catalytic center of LO. A large number of reducing co-substrates such as phenols and aromatic amines can donate electrons and reduce the Fe^{3+} in LO to the Fe^{2+} form (fig.1) and concurrently undergo one-electron oxidation to free radical species. The process requires active participation of LO in the hydrogen abstraction from the co-substrate. LOOH simply oxidizes Fe^{2+} back to Fe^{3+} in the enzyme to regenerate the active LO for the next enzyme cycle, as shown:

$$E(Fe^{3+}) + XH \rightarrow E(Fe^{2+}) + X^{\bullet} \quad (1)$$

$$E(Fe^{2+}) + LOOH \rightarrow E(Fe^{3+}) + LO^{\bullet} + OH^{-} \quad (2)$$

The reduction of hydroperoxide during LO-mediated xenobiotic co-oxidation does not yield the corresponding hydroxy derivative of the fatty acid, as noted with PGS. Instead, LOOH undergoes a homolytic cleavage by LO to generate an alkoxy radical ($LO^{\bullet}$) and hydroxide ion [65, 66]. LOs do not display a strict specificity toward peroxide, as 5-HPETE [66], 15-HPETE [67], and 13-HPOD [68–70] have been shown to support xenobiotic oxidation. Kulkarni and Cook [52] were the first to demonstrate that H_2O_2 can be substituted for LOOH to observe the co-oxidase activity of SLO toward different xenobiotics. Subsequent reports have confirmed these observations for SLO [71–75] as well as human tissue LOs [75]. A recent study from our laboratory [76] has revealed that synthetic organic peroxides such as tert-butyl hydroperoxide or cumene hydroperoxide can also support xenobiotic oxidation by SLO or HTPLO with notable efficiency.

Table 1. Selective data on the comparative ability of lipoxygenase and cytochrome P450 to mediate xenobiotic oxidation in vitro.

Substrate	Reaction	Enzyme Activity (nmol/min per nmole enzyme)		Relative activity (A/B)	Reference
		soybean lipoxygenase (A)	mouse liver P450 (B)		
Aldrin	Epoxidation	4.0	0.15–0.31	13–26	62
Parathion	Desulfuration	3.0	0.15	20	55
Aminopyrine	N-demethylation	214	4–8	27–54	63
Thiobenzamide	Sulfoxidation	241	2.9	83	64

Peroxyl radical-mediated reactions

The LO-generated $LOO^{\bullet}$ can serve as a potent, direct-acting oxidizing agent. Hydrogen abstraction from a donor molecule represents one of the prominent reactions displayed by $LOO^{\bullet}$ (fig. 1). As illustrated in Eq. 3, this process yields a free radical as the product of one-electron oxidation of xenobiotic and LOOH.

$$LOO^{\bullet} + XH \rightarrow X^{\bullet} + LOOH \quad (3)$$

Oxygenation of acceptor molecules is another important attribute noted with peroxyl radicals. The peroxyl radical serves as an efficient epoxidizing agent and can donate its terminal oxygen atom to an aliphatic double bond to give rise to an epoxide from the co-substrate (4).

$$LOO^{\bullet} + RC{=}CR \rightarrow LOOH + RC(O)CR \quad (4)$$

This has been noted in the LO-catalyzed epoxidation of aldrin [62], BP-diol [77], and other chemicals. In the oxygenation of sulfur-containing compounds, oxygen transfer from the LO-generated $LOO^{\bullet}$ to a co-substrate results in the sulfoxidation as observed with thiobenzamide [64] or desulfuration (displacement of sulfur atom by oxygen atom), as in the case of parathion [55]. Some of the sources for the supply of $LOO^{\bullet}$ to the LO system proposed by many are shown in figure 1. The primary source is the generation of $LOO^{\bullet}$ as intermediates during dioxygenation of PUFAs by LO before the LOOH release. LO is a leaky system. A portion of the $L^{\bullet}$ pool resulting from hydrogen abstraction of PUFA by LO escapes into the medium and after reaction with O_2 yields $LOO^{\bullet}$. Futile catalysis of the E-Fe^{2+}-$LOO^{\bullet}$ complex can also yield $LOO^{\bullet}$. Another pathway originates from the decomposition of LOOH. This involves an intermolecular rearrangement of $LO^{\bullet}$ to form an epoxy allylic radical which reacts with O_2 to form the epoxy peroxyl radical that triggers xenobiotic oxidation [67].

Electron transfer-dependent reactions

In certain cases, a 'shuttle oxidant' is generated by LO from a good substrate in a primary reaction that oxidizes another chemical non-enzymatically in the secondary reaction. This is exemplified by the reported hyperoxidation of benzidine (BZ) and several other xenobiotics [78] by the primary cation radicals of phenothiazines [e.g., chlorpromazine (CPZ)] produced by SLO in the presence of H_2O_2 as shown below:

$$CPZ + H_2O_2 \rightarrow CPZ^{+\bullet} \text{ (shuttle oxidant)} \quad (5)$$
$$CPZ^{+\bullet} + BZ \rightarrow CPZ + BZ^{+\bullet} \quad (6)$$

Other mechanisms

Some xenobiotics are directly oxidized by LO in the absence of exogenous PUFAs or LOOH. LO inhibitors, 5,8,11-eicosatriynoic acid (ETI) and 5,8,11,14-eicosatetraynoic acid (ETYA) serve as the suicide substrates and are directly oxidized by different LOs. Other examples include the SLO-mediated oxidation of hexanal phenylhydrazone to α-azo hydroperoxide [79], 2-[(4′-hydroxy-3′-methoxy)-phenoxy]-4-(4″-hydroxy-3″-methoxyphenyl)-8-hydroxy-6-oxo-3-oxabicyclo[3.3.0]-7-octene formation from curcumin [80], hydroperoxide generation from alkenes [81], and a few others. Additionally, SLO-generated peroxyl radicals of low-density lipoprotein can co-oxidize α-tocopherol and probucol to their respective phenoxyl radicals [82]. Similar oxidant production by SLO from human [83] or mouse [84] lung microsomes was shown to couple oxidation of a tobacco-specific animal lung carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl-1-butanone (NNK) to its keto aldehyde and keto alcohol derivatives, and the process can be blocked significantly by nordihydroguaiaretic acid (NDGA), a classical LO inhibitor [83].

Fate of xenobiotic free radicals

The fate of primary free radicals generated during one-electron oxidation of reducing co-substrates by LOs depends on the nature of the radicals and the surrounding environment. They may (i) undergo coupling reactions to yield dimers or polymers as revealed by the conversion of guaiacol to tetraguaiacol, (ii) undergo further oxidation to yield two-electron oxidation products as noted with aminopyrine or benzidine [44, 63]; (iii) serve as a shuttle oxidant and react with another chemical as shown for the phenothiazine-benzidine combination [78], (iv) react with thiol and trigger thiol pumping (reduction of a free radical back to the parent chemical and the generation of $GS^{\bullet}$), or (v) react with macromolecules to produce protein, DNA, or RNA adducts.

Reactions catalyzed

Oxidation

Endobiotics and relevant chemicals

Several endogenous chemicals are known to be oxidized by different LOs [1]. Thus SLO easily oxidizes both NADH and NADPH in the presence of linoleic acid. A blockade of ferricytochrome-C or nitroblue tetrazolium reduction by superoxide dismutase suggests the generation of superoxide anions ($O_2^{\bullet -}$) during NAD(P)H oxidation by SLO. 15-LO, purified from rabbit reticulocytes, also co-oxidizes NAD(P)H in the presence of linoleic acid [85]. This reaction is not observed with 13-HPOD suggesting that $LOO^{\bullet}$ may be the oxidant involved. Reduced glutathione (GSH) is the most abundant non-pro-

tein intracellular thiol. Of note is that GSH is an extremely poor substrate for horseradish peroxidase (HRP) [86]. It is neither oxidized by P450 nor by purified preparations of PGS in the presence of arachidonate [87]. In fact, GSH inhibits the cyclooxygenase activity of PGS [80]. LO is unique in this regard since GSH serves as an excellent substrate. SLO in the presence of PUFAs invokes an extensive oxidation of GSH to GS$^{\bullet}$ [88, 89]. The process is accompanied by a low rate of $O_2^{\bullet-}$ generation [88]. The reaction seems to be linked with the LOO$^{\bullet}$ generation since negligible GSH oxidation by SLO occurs in the presence of 13-HPOD [88] or H_2O_2 [60]. HTPLO also displays similar ability to oxidize GSH [90]. Considering the data on NAD(P)H and GSH oxidation and concurrent reactive oxygen species production, it is tempting to speculate that the LO pathway plays a significant contributory role in the genesis of cellular oxidative stress.

Ascorbic acid, an essential dietary vitamin, and a readily available reductant in mammalian cells, is avidly oxidized by SLO in reaction media supplemented with either linoleic or arachidonic acid but not with 13-HPOD or H_2O_2 [1]. Although a similar lack of 13-HPOD-dependent ascorbate oxidation has also been reported for potato tuber 5-LO [66], the authors found the palmitate ester of ascorbate to serve as a substrate for the hydroperoxidase activity of SLO as well as potato tuber 5-LO. The absence of $O_2^{\bullet-}$ generation suggests that LOO$^{\bullet}$ oxidizes ascorbate to free radicals which disproportionate to yield dehydro-L-ascorbate [1]. As expected, ascorbate addition to incubates markedly decreases the rate of LO-mediated oxidation of several xenobiotics due to a competitive inhibition and/or reduction of xenobiotic free radical back to the parent compound. As a consequence, several phenols and arylamines enhance ascorbate oxidation in the reaction media containing linoleic acid and SLO [1]. Vitamin E (α-tocopherol) and its seven analogues are oxidized at different rates by SLO and potato tuber 5-LO in the presence of 13-HPOD [66]. The oxidation of other endobiotics via the LO pathway includes the conversion of noradrenaline, N-acetyldopamine, epinephrine, and L-dopa to their corresponding melanin pigments [74] and the genesis of pheomelanins from 5-S-cysteinyl-dopa and 5-S-cysteinyl dopamine [91]. Catecholic tetrahydroisoquinolines such as salsolinol, tetrahydro-papaveroline, laudanosoline, apomorphine [92], and dihydroxyindoles [93] are also oxidatively converted by SLO in the presence of H_2O_2 into their respective melanins.

Xenobiotics

Occupational exposure to 4-aminobiphenyl (4-ABP) elevates the risk of bladder cancer. 4-ABP also occurs in tobacco smoke. Several reports have implicated an increased risk of hematopoietic malignancies and lung cancers in adults to cigarette smoking during pregnancy. Prior oxidative metabolism of 4-ABP is essential for it to exert its carcinogenic effect. Datta et al. [75] demonstrated that both SLO as well as HTPLO can oxidize 4-ABP. The major metabolite, 4,4-azobis(biphenyl), was reported to arise via a mechanism involving an initial one-electron oxidation of 4-ABP to a free-radical species. These results explain, at least partly, the reported detection of 4-ABP-adducts in the placentas of non-smokers which are deficient in both P450 and PGS activities.

Although the use of benzidine, another arylamine, is currently banned in the USA, benzidine-based dyes are still employed in many other countries in textile and other industries. Thus, cancer and mortality due to exposure to aromatic amines are still important public health issues on a global scale. Many reports have documented benzidine as serving as an excellent substrate for co-oxidation by SLO in the presence of linoleic acid or H_2O_2 [44, 46, 52, 78]. Initial one-electron oxidation of benzidine yields a nitrogen-centered cation radical which may either undergo a second one-electron oxidation to produce benzidine di-imine or disproportionates to produce the parent diamine and benzidine di-imine. Both the cation radical and di-imine are electrophilic derivatives capable of covalent binding to macromolecules. Like SLO, extensive benzidine oxidation to reactive benzidine di-imine can be observed with animal LOs isolated from lung, brain, liver, and embryo as well as with purified LOs from human tissues such as lung, liver, term placenta, and intrauterine conceptual tissues [1].

2-Aminofluorene (2-AF) is a potent carcinogen and teratogen to which humans may be exposed from environmental, industrial, and dietary sources. Metabolic activation is obligatory for 2-AF toxicity. 2-AF activation by P450 and MFMO plays some role in hepatocarcinogenesis. However, mammary gland and Zymbal gland, which are deficient in these enzymes, are also targets for 2-AF-induced cancer. 2-AF is reported to be a poor substrate for PGS. In contrast, SLO has been found to catalyze a rapid oxidation of 2-AF in the presence of linoleic acid while arachidonate, linolenic acid, and cis-11,14-eicosadienoic acid were less than half as effective [94]. Radiometry revealed the generation of electrophilic 2-AF intermediates by SLO which bind covalently to bovine serum albumin and calf thymus DNA. As expected, 2-AF bioactivation is blocked by ETI, ETYA, NDGA, gossypol, and esculetin, the classical LO inhibitors. These results clearly suggest that LO may serve as an alternate pathway to P450 in 2-AF bioactivation in mammalian extra-hepatic tissues.

The colon carcinogen 1,2-dimethylhydrazine is activated by P450 to the proximate carcinogen methylazoxymethanol. Further metabolism by PUFA-supported co-oxidation or NAD-dependent oxidation releases the final methylating agent and formaldehyde. Apparently, rat colonic mucosal LO, but not P450, mediates this reaction in the presence of arachidonate, linoleate, or 15-HPETE [95].

Phenytoin, a known human teratogen, is used during pregnancy as an efficacious anticonvulsant. The relevance of maternal and embryonic P450 in its bioactivation has been questioned [96, 97]. SLO-mediated PUFA-dependent oxidation of phenytoin to free-radical species is accompanied by covalent binding to proteins which can be suppressed by the classical LO inhibitors [96, 97]. Cyclophosphamide is a prodrug whose toxicity and therapeutic efficacy depends on its metabolic activation. It undergoes linoleate-supported co-oxidation by SLO and 15-LO from rabbit reticulocytes to yield a free-radical species [98]. The reaction generates the unstable tautomer of a 4-hydroxy intermediate, an active metabolite, which breaks down spontaneously to release acrolein and phosphoramide mustard. Diethylstilbestrol (DES) is a human transplacental carcinogen. DES-quinone, one of the metabolites of DES, binds to DNA and is presumed to be the ultimate toxicant. Although DES-quinone formation by human tissue LO has yet to be examined, SLO has been shown to initiate one-electron oxidation to DES semiquinone in the presence of H_2O_2 [99]. Subsequent dismutation of two molecules of DES semiquinone yields one molecule each of DES-quinone and DES. Isoproterenol, a β-adrenoreceptor agonist, is used as a bronchodilator in the treatment of asthma. This two-electron donor chemical serves as a co-substrate for the peroxidase-like activity of SLO in the presence of H_2O_2 and is easily oxidized to aminochrome [74, 100]. Sequential generation of o-quinone and leukaminochrome as intermediates was postulated in aminochrome production [100]. A number of other arylamines and phenols are routinely used as model substrates to assay the co-oxidase activity of various LOs in the presence of either PUFA or H_2O_2. Many workers have studied oxidation of 3,3′-dimethoxybenzidine (dianisidine) and 3,3′,5,5′-tetramethyl-benzidine to their respective di-imines, *p*-phenylenediamine to its free radical, or the formation of Wurster's Blue free radical from N,N,N′,N′-tetramethyl phenylenediamine [44, 46, 52]. Although phenol is oxidized slowly by SLO [66, 68], substituted phenols and catechols undergo extensive one-electron oxidation and yield the corresponding reactive phenoxyl radicals or semiquinones. These free radicals polymerize to yield a mixture of complex metabolites. Examples include the PUFA-, H_2O_2-, or LOOH-supported LO-mediated oxidation of *p*-aminophenol [65, 66, 68], *o*-methoxyphenol (guaiacol) [46, 52, 68], catechol(s), hydroquinone [65, 66, 68, 101, 102], resorcinol, pyrocatechol [66, 68], and pyrogallol [46, 52].

Hydroxylation

Although the information is relatively limited, at least three examples of LO-mediated hydroxylation of chemicals are known. The amino acid proline undergoes oxidative conversion by SLO to yield hydroxyproline in the presence of linoleic acid [1]. SLO generates a 4-hydroxy derivative from the clinically used antiinflammatory agent oxyphenbutazone in the presence of linoleic acid [103]. In the case of NNK, initial activation by α-carbon hydroxylation yields several metabolites. P450 is only partially responsible for this process in human lung microsomes [83]. Increased formation of keto aldehyde and keto alcohol by the added arachidonate and an inhibition of their formation by NDGA led the authors [83] to conclude that LO in human lung microsomes plays a role in NNK oxidation. A similar NDGA-sensitive decline noted in the rate of keto alcohol and keto aldehyde production with patas monkey lung microsomes is in harmony with this postulate [104]. Although the inclusion of NADPH in the reaction media containing SLO complicates interpretation, the data, in general, tend to support a role for LO in NNK oxidation [83, 84, 105].

Epoxidation

Endobiotics and relevant chemicals

Hypervitaminosis-A has been linked with birth defects in humans and animals. The parent retinoid per se may not be teratogenic, and bioactivation seems to be a prerequisite. The oxidation of β-carotene by SLO and pea LO yields apocarotenal, epoxycarotenal, apocarotenone, and epoxycarotenone [106]. All-trans retinol acetate is an excellent substrate for co-oxidation for SLO and HTPLO in the presence of linoleic acid [107]. The peroxyl radicals of linoleic acid generated by the LOs were proposed to attack the π-electrons of the C=C bond to produce 5,6-epoxide of all-trans-retinol acetate. All-trans-retinol acetate is also co-oxidized by the purified LO from chickpeas [108]. Other studies have also observed the formation of 5,6-epoxy metabolites during SLO-catalyzed co-oxidation of all-trans-retinoic acid [109], retinol, and β-ionone [110].

Xenobiotics

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous environmental pollutants, many of which are mutagenic, carcinogenic, and teratogenic. BP, the prototype of this class of compound, requires double epoxidation to produce the ultimate toxicant, BP-diolepoxide. Although P450 is required for the initial epoxidation of BP, the LO pathway may be more important in some tissues for the final epoxidation step. Arachidonate- or 13-HPOD-dependent metabolism of BP triggers cytotoxicity and mutagenesis in Chinese hamster lung fibroblasts (V79 cells) [111]. Although V79 cells possess both PGS and LO activities, NDGA but not indomethacin, the inhibitor of PGS, exhibited an inhibitory response, suggesting an involvement of the LO pathway in the induc-

tion of BP toxicity. Possibly neither P450 no-PGS is involved in BP activation by the human or rat colonic mucosal microsomes since addition of linoleic acid, arachidonic acid, or their hydroperoxides enhances the process four- to fivefold whereas NADPH has no effect [112, 113]. Studies on the covalent binding of BP to proteins in rat liver and lung revealed that linoleate is more effective than arachidonate for the activation by microsomal enzymes, whereas in the cytosol, different PUFAs but not oleic acid supported the reaction, arachidonate being the best cofactor [56]. Indomethacin did not inhibit BP activation, however, both NDGA and quercetin effectively reduced the binding. The linoleate-dependent binding was > 3 and > 16-fold greater with liver and lung microsomes, respectively, than that after incubation with NADPH. The authors [56] suggested that LO plays a dominant role in BP activation in rat liver and lung. In line with this postulate, Adriaenssens et al. [114] did not observe the expected decline in the covalent binding of BP to DNA by indomethacin in mice. Aspirin, another specific inhibitor of PGS, did not alter the number of pulmonary adenomas in mice treated with BP suggesting that PGS does not activate this carcinogen in vivo. The linoleic acid-supported co-oxidation of [^{14}C]BP in rat lung cytosol yields 1,6-dione, 3,6-dione, and 6,12-dione of BP, with the predominant production of BP-6,12-dione [1]. The quinones derived from BP are reactive enough to bind covalently to the critical tissue macromolecules or they can undergo further redox reactions in cells.

Byczkowski and Kulkarni [77] were the first to demonstrate that BP-diol can be activated by SLO in the presence of linoleic acid. BP-trans-anti-7,8,9,10-tetrahydrotetrol, the product of the hydrolytic breakdown of the ultimately mutagenic BP-anti-7,8-dihydrodiol-9,10-epoxide, was detected as the major metabolite by radiometry combined with HPLC. These results provide the most crucial direct evidence that purified LO can mediate the final step in BP bioactivation. The peroxyl radical derived from linoleic acid during dioxygenation by SLO was postulated to be responsible for the epoxidation of BP-diol. Subsequently, Hughes et al. [67] confirmed these findings and noted that the SLO-catalyzed BP-diol epoxidation can also occur in the presence of arachidonate, γ-linolenic acid, and 15-HPETE. Based on the oxygen consumption studies, the authors [67] proposed that 15-HPETE is reduced to an alkoxyl radical and hydroxyl anion. The radical rearranges to an allylic epoxyl radical which reacts with molecular oxygen to form a peroxyl radical that serves as an oxidant for the epoxidation. However, attempts to detect 15-HPETE-derived peroxyl radical by electron spin resonance (ESR) spectroscopy were unsuccessful. Although the fatty acid metabolites were not analyzed, simultaneous formation of epoxy-hydroxy, dihydroxy, and trihydroxy fatty acids was proposed [67]. These observations [67, 77] with SLO raise the question whether LO-mediated BP-diol bioactivation occurs in human tissues. Scharping et al. [115] observed NDGA-sensitive epoxidation of BP-diol in human liver microsomes and by SLO in the presence and absence of arachidonate. The SLO-mediated reaction was augmented by the addition of phenylbutazone. Apparently, phenylbutazone is oxidized by LO to a carbon-centered free radical which traps molecular oxygen to form a peroxyl radical that enhances epoxidation of BP-diol. A possibility that pulmonary LO may also mediate this reaction is strongly suspected, since LO capable of xenobiotic oxidation occurs in human [36] and animal lungs [56]. The inability of indomethacin to inhibit the arachidonate-dependent BP-diol epoxidation by either hamster trachea or human bronchus explant in culture strongly implies that the activation proceeds via the LO pathway and not by PGS [116]. Joseph et al. [37] have shown that purified LO from human intrauterine conceptual tissues and HTPLO can easily epoxidize BP-diol to several metabolites. The production of BP-trans-anti-tetrol by LO was about eight times above the control value noted with boiled enzyme preparations. These results explain, in part, the report [117] on the detection of DNA adducts of BP in the placentas of non-smokers which lack both P450 and PGS activities.

Aflatoxin B_1 (AFB), a substituted coumarin produced as a secondary metabolite by *Aspergillus flavus* and *A. parasiticus,* is a known contaminant in various food products. AFB is a potent hepatocarcinogen and teratogen in humans. The requisite event in AFB toxicity is its prior oxidation at the 8,9-vinyl ether bond to form the AFB-8,9-epoxide, an electrophilic species which is the ultimate carcinogenic metabolite. Linoleic acid, linolenic acid, and arachidonic acid strongly inhibit NADPH-dependent hepatic microsomal AFB activation [118, 119]. This leads to a logical question as to whether P450 serves as the sole catalyst of this reaction. Although PGS can epoxidize AFB, this ability cannot explain hepatocarcinogenicity since liver contains biologically non-significant levels of this enzyme [51]. An earlier study noted that the binding of AFB metabolites to DNA in mouse embryo fibroblasts is inhibited by LO inhibitors [120], and SLO can mediate AFB activation [121]. A recent study by Roy and Kulkarni [122] has provided the necessary evidence for the ability of adult human liver LO to epoxidize AFB in the presence of PUFAs. Thus, LO clearly represents an additional or alternate pathway of AFB bioactivation in human liver. AFB contamination occurs in grain dust, and handling of grains in large quantity can result in significant pulmonary exposure to AFB. Available data indicate that AFB epoxidation can be effected by human lung cytosolic LO [57] as well as by the cytosolic LO from guinea pig lungs and kidney [121]. Similar experimental data on AFB bioactivation collected for the LO purified from human intrauterine conceptual tissues and HTPLO

[123] explain, at least partly, the developmental toxicity associated with exposure to this fungal alkaloid during pregnancy. A recent study has examined the role of PGS and LO in ochratoxin-A genotoxicity in human epithelial lung cells. NDGA blocked while indomethacin was actually found to enhance the formation of DNA adducts tenfold [124].

Epoxidation of aldrin to dieldrin is a model reaction noted with P450 and PGS. The demonstration that SLO [62] can easily oxidize aldrin to dieldrin clearly suggests LO as an additional pathway for this epoxidation. The rate of the SLO-mediated reaction was 8- to 20-fold greater than that of different isozymes of P450. Styrene is a high-volume industrial chemical used in the manufacture of plastics, resins, synthetic rubber, and insulators. Workers' exposure to styrene seems unavoidable. Metabolic studies have established that prior styrene oxidation to styrene-7,8-oxide is essential to observe toxicity, since it binds covalently to cellular macromolecules. P450, hemoglobin, myoglobin, and HRP have been shown to mediate this epoxidation reaction. For reasons unclear at present, PGS does not catalyze styrene epoxidation [125]. The results reported by Belvedere et al. [126] suggest that the LO pathway can serve as an additional efficient route for styrene epoxidation. The rate of the arachidonate-supported SLO-mediated reaction was about four-fold greater than that noted with the NADPH-supported P450-mediated hepatic microsomal reaction. The peroxyl radicals of arachidonate and/or those derived from hydroperoxide degradation by LO are expected to mediate this reaction.

ACN is another important industrial chemical widely used in the manufacture of plastics, rubber, and acrylic fibers. Residues of ACN are found in water, food, and cigarette smoke. ACN is carcinogenic in the rat but its potential carcinogenic risk to humans is uncertain. The mechanism by which ACN initiates tumor formation is not established; however, prior bioactivation appears to be prerequisite. ACN undergoes epoxidation to produce reactive CEO and, ultimately, cytotoxic cyanide. The proposal that P450 is responsible for the bioactivation of ACN seems inconsistent in view of the following: (i) the covalent binding of ACN to brain, lung, and liver microsomes from untreated rats is not significantly increased by the addition of NADPH [127]; (ii) in a physiologically based dosimetry study, Gargas et al. [128] concluded that an extrapolation of in vitro data to the whole animal is not valid since the V_{max} for ACN epoxidation by rat liver P450 accounts for <8% of the in vivo estimate for CEO formation, and (iii) in animals, ACN carcinogenicity is also observed in Zymbal's gland, stomach, brain, and uterus that contain very low or undetectable levels of P450. A detailed study conducted by Roy and Kulkarni [36] revealed that the LO pathway is involved. SLO as well as the partially purified human lung LO preparations containing 15-LO, and smaller amounts of 5-LO and 12-LO, were found to trigger extensive metabolism of ACN to CEO and cyanide in the presence of PUFAs. Among the fatty acids tested, linoleate was most effective in supporting epoxidation of ACN to CEO by the human lung LO. Interestingly, the human lung enzyme was about six-fold better as a catalyst than SLO in converting ACN to cyanide. Significant covalent binding of the radioactivity derived from [^{14}C]-ACN to bovine serum albumin and calf thymus DNA occurred when the reaction media contained either active SLO or human lung LO. These reactions were strongly inhibited by NDGA and the antioxidant butylated hydroxytoluene (BHT). Available evidence clearly suggests the potential toxicological relevance and importance of the LO pathway in ACN bioactivation in humans in vivo.

Sulfoxidation

Functional groups bearing a sulfur atom are frequently present in many drugs, pesticides, and naturally occurring compounds. The ability of P450, MFMO, PGS and other peroxidases to invoke an initial oxidative attack on the sulfur atom in the substrate molecule is amply documented. Naidu and Kulkarni [64] explored the possibility that the LO pathway may effect oxidation of thiobenzamide, a hepatotoxicant in laboratory animals. The evidence gathered suggested that SLO rapidly converts thiobenzamide to its toxic metabolite sulfoxide in the presence of PUFAs. Linoleic acid and linolenic acid were most effective in supporting the reaction while cis-11,14-eicosadienoic acid and arachidonic acid were about 76% and 40% as effective, respectively. Negligible thiobenzamide sulfoxidation occurred in the presence of 13-HPOD. The rate of SLO-mediated sulfoxidation was about 45- and 83-fold greater than the values reported for MFMO and P450 (table 1), respectively. Since significant amounts of LO occur in human liver [122], examining whether hepatic LO can mediate sulfoxidation of thiobenzamide is essential. Currently, phenothiazines enjoy wide acceptance as relatively safe and efficacious drugs for the treatment of psychotic illnesses. Other therapeutic applications include their use as, e.g., tranquilizers, sedatives, antiemetics, and antimicrobials. Disruption of endocrine function, and untoward effects on the cardiovascular and reproductive systems are among the side effects noted after acute exposures. Phenothiazines undergo extensive oxidative metabolism in the body. The process involves the formation of free radicals, the first essential step responsible for their biological activity. SLO has been shown to oxidize several phenothiazines into free radicals [71, 72]. CPZ, the prototype of this class of drugs, undergoes SLO-mediated sulfoxidation in the presence of H_2O_2 [71]. The postulated biochemical mechanism involves an initial formation of

CPZ cation radical ($CPZ^{+\bullet}$), the one-electron oxidation product of CPZ, which subsequently degrades non-enzymatically to CPZ sulfoxide. $CPZ^{+\bullet}$ is relatively stable and its accumulation in reaction medium can be monitored spectrophotometrically. HTPLO can also produce $CPZ^{+\bullet}$ from CPZ in the presence of H_2O_2 [129]. Recent studies indicate that besides H_2O_2, the SLO- and HTPLO-catalyzed $CPZ^{+\bullet}$ genesis from CPZ is also efficiently supported by PUFAs [130], cumene hydroperoxide, and tert-butyl hydroperoxide [76].

Desulfuration and dearylation

The presence of a phosphothionate group is a common feature of many currently used pesticides. Their commercial importance has garnished considerable interest in their metabolic fate in mammals. Extensive studies in the past with P450 and MFMO established that organophosphothionate insecticides undergo desulfuration. A single report [55] documents parathion desulfuration and dearylation by SLO in the presence of linoleic acid. Paraoxon formation by SLO proceeds at a rate which is 20 times greater than the reaction mediated by purified mammalian P450 (table 1). 13-HPOD alone was about 30% as effective as linoleic acid in supporting the reaction. An initial oxidative attack on sulfur in the parathion molecule by the LO-generated fatty acid peroxyl radical is proposed to yield an unstable oxthirane-type intermediate. Rearrangement and decomposition of the intermediate give rise to paraoxon and elemental sulfur. Concurrently, the relatively non-toxic dearylation products, p-nitrophenol and diethyl phosphate are also formed. Thus, the displacement of the sulfur atom by oxygen (desulfuration) results in the bioactivation of parathion since paraoxon is a very potent anticholinesterase, while elemental sulfur, which covalently binds to macromolecules, is regarded as a potential tissue necrogen. Rat brain LO also yields qualitatively similar results [55].

Dealkylation

Besides model compounds [131], a large number of xenobiotics are known to undergo oxidative N-dealkylation via the LO pathway (table 2). Aminopyrine is extensively used by many investigators as a prototype in mechanistic studies on N-demethylation of xenobiotics catalyzed by P450, PGS and HRP. In vivo metabolism of aminopyrine in humans exhibits ~200-fold variation and this is not related to gender, intake of caffeine or alcohol, or P450 polymorphism [132]. Since LO activity occurs in the liver and many other tissues, a hypothesis that this pathway may be involved in aminopyrine metabolism was tested using SLO in the presence of H_2O_2 [63]. The results (table 1) indicate that aminopyrine N-demethylation by SLO occurs at a rate which is several-fold greater than that reported for the P450- and PGS-mediated reaction [63]. The accumulation of formaldehyde, the end product of N-demethylation, in the reaction media is strongly inhibited by NDGA and gossypol. The generation of a nitrogen-centered free-radical cation, the expected initial one-electron oxidation product of aminopyrine, can be studied by spectrophotometry [63, 73]. The rate of accumulation of this radical species and formaldehyde depends on pH, the amount of the enzyme, the concentration of aminopyrine and H_2O_2. Ascorbate, GSH, and dithiothreitol (DTT) markedly suppress radical formation, supporting the contention that a free-radical mechanism is involved in the N-dealkylation of aminopyrine via the LO pathway. The cation radical of aminopyrine is postulated to be further converted to an iminium cation either by deprotonation or hydrogen atom abstraction. Subsequent hydrolysis of the iminium cation yields the expected monomethylamine and formaldehyde [63, 73].

Tricyclic antidepressants such as imipramine and closely related compounds represent a group of drugs used today worldwide for the treatment of major depression. Their remarkable efficacy in alleviating depression is well established. However, a few side effects such as cardiovascular toxicity and species-specific teratogenicity in animals have been noted. Imipramine, a prototype of this class, undergoes extensive metabolism in the body. However, the enzyme(s) responsible for its oxidation has not been clearly determined. The facts that (i) pretreatment of rats with phenobarbital and β-naphthoflavone does not cause any major change in the rate of its oxidation by brain and liver microsomes, (ii) metabolic reactive intermediates of imipramine form stable complexes, accumulate and inactivate P450, (iii) imipramine being highly water soluble, its intracellular translocation into the microsomal lipid phase is likely to be difficult, and (iv) a >16-fold inter-individual variation occurs in the blood levels of imipramine in patients suggest that enzymes other than P450 may be involved. The role of MFMO in imipramine oxidation is debatable since purified enzyme does not catalyze this reaction unless detergent is added. Since LO activity occurs in the liver and brain [55, 122], its involvement in imipramine oxidation can be suspected. Exploration of this hypothesis revealed that imipramine is an excellent substrate for N-dealkylation by SLO in the presence of H_2O_2 [61]. Desipramine, the expected product of imipramine mono N-demethylation, was identified by HPLC. When tested separately, desipramine also yielded formaldehyde suggesting double dealkylation of imipramine by SLO. Among the compounds tested, trimipramine was oxidized most rapidly while desipramine, clomipramine, diltiazem, amitriptyline, and doxepin exhibited a low oxidation rate.

Besides sulfoxidation, several phenothiazines are easily demethylated, releasing formaldehyde in the incubation media containing H_2O_2 and either SLO or HTPLO (table

Table 2. Lipoxygenase-mediated N-dealkylation of xenobiotics.

Chemical	Enzyme	Co-factor	Reference
Model compounds			
N-methylaniline	SLO, HTPLO	H_2O_2	131
N,N-dimethylaniline	SLO, HTPLO	H_2O_2	131
N,N,N′,N′-tetramethylbenzidine	SLO, HTPLO	H_2O_2	131
N,N-dimethyl-p-phenylenediamine	SLO, HTPLO	H_2O_2	131
N,N-dimethyl-3-nitroaniline	SLO, HTPLO	H_2O_2	131
N,N-dimethyl-p-toluidine	SLO, HTPLO	H_2O_2	131
Pesticides			
Aminocarb	SLO	H_2O_2	133
	SLO, HTPLO	linoleic acid	130
Zectran	SLO	H_2O_2	133
	SLO, HTPLO	linoleic acid	130
Dicrotophos	SLO	H_2O_2	133
	SLO, HTPLO	linoleic acid	130
Chlordimeform	SLO	H_2O_2	133
	SLO, HTPLO	linoleic acid	130
Famphur	SLO	H_2O_2	133
Formetanate	SLO	H_2O_2	133
Pirimicarb	SLO	H_2O_2	133
Tetramethiuram	SLO	H_2O_2	133
Drugs			
Aminopyrine	SLO	H_2O_2	63, 73
Chlorpromazine	SLO	H_2O_2	129
	SLO	CHP, TBHP	76
	SLO, HTPLO	linoleic acid	130
Promazine	SLO	H_2O_2	129
	SLO	CHP, TBHP	76
	SLO, HTPLO	linoleic acid	130
Promethazine	SLO	H_2O_2	129
	SLO	CHP, TBHP	76
	SLO, HTPLO	linoleic acid	130
Triflupromazine	SLO	H_2O_2	129
Trimeprazine	SLO	H_2O_2	129
	SLO	CHP, TBHP	76
	SLO, HTPLO	linoleic acid	130
Trifluoperazine	SLO	H_2O_2	129
Imipramine	SLO	H_2O_2	61
Desipramine	SLO	H_2O_2	61
Trimipramine	SLO	H_2O_2	61
Clomipramine	SLO	H_2O_2	61
Amitriptyline	SLO	H_2O_2	61
Diltiazem	SLO	H_2O_2	61
Doxepin	SLO	H_2O_2	61

SLO, soybean lipoxygenase; HTPLO, human term placental lipoxygenase; CHP, cumene hydroperoxide; TBHP, tert-butyl hydroperoxide.

2). Among the phenothiazines tested [129], promazine was oxidized by SLO at the highest rate while triflupromazine exhibited the slowest oxidation rate. A similar structure-activity response was displayed by HTPLO for N-demethylation of phenothiazines. Cumene hydroperoxide and tert-butyl hydroperoxide also support the reaction (table 3) [76]. Additional experimental evidence [130] revealed that PUFAs also support N-dealkylation of phenothiazines by SLO and HTPLO. Linoleic acid was up to eight times more efficient in supporting N-dealkylation of CPZ than either γ-linolenic acid or arachidonic acid.

Recently, Hu and Kulkarni [133] documented N-demethylation of several pesticides in reaction media containing H_2O_2 and SLO (table 2). Among the pesticides tested, the formaldehyde production rate was highest with aminocarb. Significant suppression of the reaction by GSH, DTT, BHT, and butylated hydroxyanisole (BHA) suggest a free-radical nature of the aminocarb N-demethylation. Besides SLO, HTPLO can also efficiently catalyze this reaction in the presence of linoleic acid [130]. The experimental data gathered in our laboratory on the structure-activity relationship of xenobiotics undergoing dealkylation suggest that the accessibility of the N atom-bearing methyl groups is critical for N-demethylation. Thus, compounds such as antipyrine, theophylline, caffeine, nicotine, and cocaine in which the N atom bearing the alkyl groups is endocyclic and which are known

Table 3. Peroxide specificity of lipoxygenase-mediated N-demethylation of phenothiazines.

Substrate	Specific activity (nmol HCHO/min per milligram protein)					
	SLO			HTPLO		
	H_2O_2	CHP	TBHP	H_2O_2	CHP	TBHP
Chlorpromazine	132	106	117	6.0	3.2	3.9
Promazine	66	57	68	N.D.	2.0	2.3
Promethazine	828	294	356	N.D.	11.3	15.8
Trimeprazine	205	151	157	N.D.	5.0	7.9

SLO, soybean lipoxygenase; HTPLO, human term placental lipoxygenase; CHP, cumene hydroperoxide, TBHP, tert-butyl hydroperoxide, N.D., not determined [from refs. 76, 129].

to undergo N-demethylation by P450 do not serve as co-substrates for N-demethylation by SLO and HTPLO in the presence of H_2O_2 [63] or synthetic organic hydroperoxides [76]. The absence of detectable O-dealkylation of 4-nitroanisole or S-dealkylation of methiocarb clearly marks a unique substrate specificity exhibited by the LO pathway (SLO) for dealkylation of xenobiotics [131].

Drug-chemical interaction

Hu and Kulkarni [78] hypothesized that under certain conditions, the LO-generated primary free radical metabolites of efficient substrates can stimulate oxidation of other chemicals in the secondary reaction. In experiments designed to test the hypothesis, the SLO-generated $CPZ^{+\bullet}$ from CPZ was found to serve as a shuttle oxidant capable of simultaneous oxidation of several hydrogen donors. Thus the metabolic interaction resulted in marked stimulation of benzidine di-imine formation from benzidine by the SLO-generated $CPZ^{+\bullet}$ in the H_2O_2-supplemented reaction media. The combinations containing seven other phenothiazines also displayed a similar phenomenon and stimulated benzidine oxidation albeit to a lesser extent than CPZ. Besides benzidine, $CPZ^{+\bullet}$ also stimulated the oxidation of tetramethyl benzidine, o-dianisidine, guaiacol, pyrogallol, phenylenediamine, and tetramethyl phenylenediamine. The highest degree of $CPZ^{+\bullet}$-caused stimulation (94-fold) was noted in the Wursters Blue radical formation from tetramethyl phenylenediamine [78]. About 25-fold hyperoxidation of L-dopa to dopachrome by $CPZ^{+\bullet}$ generated by SLO in the presence of H_2O_2 offers another example of chemical-chemical interaction affecting the rate of oxidation [1]. An enhancement of H_2O_2-dependent SLO-catalyzed oxidation of L-dopa, 5-S-cysteinyl-dopa, and 5-S-cysteinyl dopamine by catechols such as tetrahydropapaveroline, dopa methyl ester, hydrocaffeic acid, NDGA, salsolinol, and caffeic acid has also been reported in the literature [91].

Lipoxygenase inhibition

To establish the role of a specific enzyme in xenobiotic oxidation in vivo, most investigators use 'so called selective inhibitors.' However, a serious problem of non-specificity, often ignored by many, exists. Thus, for example, SKF-525 and metyrapone, the presumed selective inhibitors of P450, also inhibit SLO [134]. Indomethacin and non-steroidal anti-inflammatory drugs not only inhibit PGS but can also denature rat hepatic microsomal P450 [135]. Aspirin, indomethacin, sodium salicylate, phenylbutazone, ibuprofen, naproxen, and sulindac, the presumed PGS inhibitors, can effectively block LO activity in rat neutrophils [136]. Indomethacin has been reported to cause a three-fold increase in LTB_4 formation along with an increase in 5-, 12-, and 15-HETE formation in ionophore-stimulated human neutrophils [137], a blockade of the LO pathway in hamster lungs [138], and inhibition of the PGS and LO pathways in human epithelial lung cells [124]. According to one report [97], indomethacin causes ~50% inhibition of covalent binding of activated phenytoin by SLO while a significant enhancement of protein binding of arachidonate-dependent activated BP in the cytosol of rat liver and lung was noted in another study [56].

In the past, a large number of chemicals have been evaluated as selective inhibitors for 5-, 12-, or 15-LO. Several reviews covering this subject are available [e.g., 139–143]. Unfortunately, lack of a unified approach for the evaluation renders the interpretation of the data on relative inhibitory potency of different chemicals difficult. Despite many problems (see above), continued efforts in this area have led to the discovery of inhibitors which exhibit high potency and desired selectivity. For example, the compound YT-18 [2,3-dihydro-2,4,6,7-tetramethyl-2-[(4-phenyl-1-piperazinyl)-methyl]-5-benzo-furanamine] selectively inhibits 5-LO from human and porcine leukocytes and RBL cells but has almost no effect on 12-LO, 15-LO, and cyclooxygenase-1 and -2 [144]. Gorins et al., [145] tested a series of (car-

boxyalkyl)benzyl propargyl ethers as inhibitors of 12-LO isolated from porcine leukocyte cytosol. The most potent acetylenic (carboxyalkyl)benzyl ethers did not inhibit human platelet 12-LO, human neutrophil 5-LO, rabbit reticulocyte 15-LO or soybean 15-LO [145].

Since a large number of chemicals inhibit LO activity, several mechanisms have been proposed to explain their mode of action. Thus a chemical may inhibit LO activity by reducing iron in LO or by forming a dead-end complex, while others inhibit activity by serving as an antioxidant, iron chelator, substrate analogue, inhibitor of 5-LO-activating protein (FLAP), or a blocker of LO induction. However, the discussion here is limited to some of those chemicals which are also co-oxidized by LO in the inhibition process. The phenolic antioxidants BHT and BHA, which break free radical chain reactions, also block xenobiotic metabolism catalyzed by different LOs [36, 60, 76, 131]. The ESR study [146] revealed that one-electron oxidation of seven BHT homologues by SLO in the presence of linoleic acid generates free-radical species. The metabolism of 2-tert-butyl(1,4)hydroquinone, a demethylated metabolite of the antioxidant BHA, by SLO involves a two-electron oxidation process that yields 2-tert-butyl (1,4)-p-quinone without the semiquinone radical or oxygen radical formation [147]. Trolox C, a phenolic antioxidant undergoes a H_2O_2-supported multistep oxidation process catalyzed by SLO [148]. However, Trolox C oxidation is not supported by 13-HPOD with either SLO or potato tuber 5-LO [66]. Resveratrol, a naturally occurring phytoalexin, is oxidized by LO. Both the parent compound and its oxidation product exhibit a potent, selective inhibitory effect on the dioxygenase activity while the hydroperoxidase activity is spared [149]. Similar to ETI and ETYA, the oxidation of hexanal phenylhydrazone leads to the inactivation of SLO due to the concomitant oxidation of the methionine residue of the protein to corresponding sulfoxide [79]. SLO is rapidly inactivated when incubated with arachidonic acid and either NDGA, the aminopyrazolines BW 755C and BW 540C, or the acetohydroxamic acid derivatives BW A4C and BW A137C [150]. 15-HPETE was as effective as arachidonate in promoting the inactivation, but linoleic acid and 13-HPOD were much less effective. The data link the enzyme inhibition to the pseudoperoxidase activity of SLO [150]. The LO inhibitors, BW 755C and phenidone, serve as co-substrates for SLO and undergo oxidation to the corresponding dehydroderivatives in the presence of 13-HPOD [68]. Several N-alkylhydroxylamines dramatically increase the latency of SLO and are simultaneously oxidized to their nitroxide derivatives [151]. Using a spectrophotometric assay, Riendeau et al. [69] examined the ability of purified 5-LO from porcine leukocytes to degrade 13-HPOD in the presence of derivatives of diphenyl-N-hydroxybenzofurans, 4-hydroxybenzofurans, and 5-hydroxy-dihydrobenzofurans. Strong stimulation of the pseudoperoxidase reaction could be detected only with very effective inhibitors of LTB_4 biosynthesis by human leukocytes. The results indicated that N-hydroxyurea and benzofuranol derivatives can function as reducing agent for the enzyme. N-(4-chlorophenyl)-N-hydroxy-N′-(3-chlorophenyl)urea (CPHU) serves as a reducing agent and stimulates 13-HPOD-supported hydroperoxidase activity of the recombinant human 5-LO, porcine leukocyte 12-LO, and SLO [152, 153]. The nitroxide radical is formed during SLO-mediated metabolism of CPHU in the presence of 13-HPOD. ESR studies [70] have provided the necessary direct evidence that the NOH of the hydroxamate group of CPHU, N–[(E)-3-(3-phenoxyphenyl)prop-2-enyl]acetohydroxamic acid (BW A4C), and N-(1-benzo(b)thien-2-ylethyl)-N-hydroxyurea (Zileuton) is oxidized by SLO to form the corresponding nitroxides when incubated in the presence of linoleic acid. Similar ESR evidence is also available for the formation of the expected nitroxide metabolite as the one-electron oxidation product of desferal, dimethylhydroxylamine, isopropylhydroxylamine, N-hydroxyurea, and cyclohexylhydroxylamine in the incubation media containing SLO and 13-HPOD [70]. N-hydroxyurea is an animal teratogen, while Zileuton, a 5-LO inhibitor, is a drug for the treatment of asthma. As a 5-LO inhibitor, diphenyl disulfide appears to be 1000-fold more potent than diethyldisulfide, and it is noteworthy that glutathione is almost inactive in inhibiting LO even at 80 mM [154]. Other LO inhibitors include n-alcohols and n-alkylthiols [155], flavonoids [156, 157] and many others. Some of these chemicals are expected to be oxidized by LO.

GSH conjugation of xenobiotics

The formation of GSH conjugate of xenobiotics is an important metabolic reaction. Currently, glutathione transferase (GST) is believed to be responsible for the enzymatic generation of thioethers from xenobiotics in different mammalian tissues. Kulkarni and Sajan [89] were the first to report the GSH conjugation of ethacrynic acid (EA), a diuretic drug, by SLO in the presence of arachidonic acid, linoleic acid, and γ-linolenic acid. The rate of SLO-mediated EA-SG formation was up to 1650-fold greater than that reported for different isozymes of mammalian GSTs. A further study [90] revealed that human tissue LO (HTPLO) is capable of extensive EA-SG formation. A blockade of the reaction by BHT, BHA, and spin traps suggested a free-radical nature of the reaction. Two possible mechanisms of EA-SG formation are proposed. It is envisioned that GSH is first oxidized by LO to GS$^{\bullet}$. In the second step, the GS$^{\bullet}$ directly attacks the C=C bond in the EA molecule to generate a carbon-centered radical which reacts with another molecule of GSH

to finally yield EA-SG. The second mechanism presumes that EA is first oxidized by LO to $EA^{+\bullet}$. Then, in a spontaneous interaction, GSH traps this cation radical leading to EA-SG formation. The LO-mediated reaction occurs at a significant rate under the physiologically relevant concentrations of GSH and fatty acid, and pH. These results suggest a strong possibility of in vivo thioether formation from certain chemicals via the LO pathway. The GSH conjugation of p-aminophenol (PAP) by SLO represents another example. Oxidative metabolism and subsequent conjugation with GSH are believed to be the key steps involved in PAP nephrotoxicity. Apparently, P450 and PGS are not involved in PAP bioactivation [58, 59]. The ESR study conducted with SLO has shown the formation of a short-lived 4-aminophenoxyl radical as the initial one-electron oxidation product of PAP in the reaction media supplemented with linoleic acid [65]. One-electron oxidation of PAP by SLO also occurs in the presence of 13-HPOD [66, 68]. Recently, Yang and Kulkarni [60] noted the SLO-mediated formation of GSH conjugates from PAP in the presence of H_2O_2 at a significant rate. As expected, different LO inhibitors and free-radical scavengers markedly blocked the rate of GS-PAP formation. Since LO is plentiful in human liver [122] and kidney [158, 159], a role for this pathway in the nephrotoxicity of PAP can be suspected.

Modulation of lipoxygenase activity

In vivo and ex vivo exposures to a variety of chemicals can influence cellular and tissue LO activity (table 4). Although systematic studies are few, many anecdotal reports support the notion that LO is an inducible enzyme. Other mechanisms such as the stimulation or activation of latent LO and an inhibition of PGS with a concurrent diversion of arachidonate supply towards the LO pathway can also explain the observed elevation in the production of arachidonate metabolites via LO. Thus phosgene exposure results in a ten-fold increase of in the synthesis of LO-generated arachidonate metabolites in rabbit lungs [160]. Feeding rabbits with the atherogenic diet for 14 weeks leads to an increase up to 100-fold in the 15-LO levels in heart, aortic adventitia, and lung, but not in liver [161]. The treatment of rabbits with the hemolytic agent, phenylhydrazine [162], or hemorrhagic events [163] cause a >1000-fold rise in 15-LO levels in heart, lung, and aorta. An infusion of the oxidant tert-butyl hydroperoxide in isolated rabbit lung elevates LTB_4, C_4, D_4, and E_4 content by two- to three-fold [164]. A significant rise in 15-HETE occurs when human bronchial epithelial cells are exposed in vitro to toluene di-isocyanate [165]. Exposure of cultured bovine tracheal epithelial cells to acrolein leads to an elevation of 12-HETE and 15-HETE production [166]. The administration of NNK to A/J mice for 7 weeks in drinking water results in a more than two-fold increase in the plasma concentration of LTB_4 [84, 105]. Feeding oxidized palm oil to rats for 3 days triggers a more than two-fold increase in liver LO activity [167]. A marked increase in hepatic 15-HETE and LTB_4 production [168] or renal LO activity [169] occurs following streptozotocin-induced experimental diabetes in rats. In mice, the application of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) leads to the induction of epidermal 8-LO activity that peaks at 24 h post-treatment [170]. TPA causes a marked rise in membrane-bound 12-LO activity and protein in human erythroleukemia cells [171]. Phorbol 12-myristate 13-acetate (PMA) exposure invokes a significant rise in the expression of microsomal 12-LO activity and mRNA in human epidermoid carcinoma A431 cells [172]. Isolated perfused rat liver yields higher LT production when exposed to TPA and the Ca^{2+} ionophore A23187 [173] and the same is true for rat hepatocytes in the primary culture exposed to ethanol [174]. Other examples of modulation of LO activity in cells and tissues after in vivo or in vitro exposures to chemicals are given in table 4.

In vivo evidence

Gathering in vivo evidence to document LO-mediated xenobiotic oxidation has been troublesome. Despite the limitations imposed by the debatable selective efficacy of inhibitors, the results of many studies collectively suggest that the LO pathway plays a contributory role in xenobiotic oxidation in vivo. While investigating the preventive efficacy of PGS and 5-LO inhibitors against NNK carcinogenesis in female A/J mice, A-79175, the 5-LO inhibitor, was found to be a stronger inhibitor of lung tumorigenesis than the PGS inhibitor aspirin and reduced both lung tumor multiplicity and incidence [84, 105]. MK-886, which binds to FLAP and inhibits 5-LO, also decreased the mean tumor volume [84]. The authors concluded that besides P450, LO plays a major role in the activation of NNK [84, 105]. The incubation of 82-132 and LM2 murine lung tumor cells with MK-886 or A-79715 was found to decrease NNK-caused cell proliferation in a concentration-dependent manner [84]. The authors opined that an inhibition of NNK activation by LO may be the mechanism responsible for the observed effects. TMK688, another LO inhibitor, not only blocks LO induction but also protects mice from skin carcinogenesis induced by 7,12-dimethylbenz(a)anthracene (DMBA) or TPA plus BP [170]. Other investigators have also reported that an inhibition of the LO pathway prevents DMBA-caused cancer of skin [170, 188] and mammary gland [189, 190] suggesting in vivo DMBA bioactivation by LO. Rodent embryos [191] and human intrauterine conceptual tissues during the early gestation period [37,

Table 4. Activation or induction of lipoxygenase following chemical exposure.

Modulator	Species	Cell/tissue	Parameter measured	Reference
In vivo exposure				
NNK	mouse	lungs	LTB_4	84, 105
Phosgene	rabbit	lung	LTs	160
1% cholesterol diet	rabbit	heart, lung, spleen, kidney, aortic adventia, aorta wall	15-LO	161–163
Phenylhydrazine	rabbit	lung, heart, kidney	15-LO	161–163
tert.-Butyl hydroperoxide	rabbit	lung	LTs	164
Endotoxin	rat	lung	5-HETE, LTC_4	175
Ozone	human	lung lavage fluid	LTC_4, LTB_4	176
Phorbol ester	mouse	skin	8-LO	177
Streptozotocin	rat	liver	5-,15-LO	168
	rat	kidney	15-LO	169
Oxidized palm oil	rat	liver	12-LO	167
Ex vivo exposure				
Toluene diisocyanate	human	bronchial epithelial cells	15-HETE	165
Acrolein	bovine	tracheal epithelial cells	12-, 15-HETE	166
PMA	human	carcinoma A431 cells	12-LO, mRNA	172
TPA	rat	perfused liver	LT	173
N-phenyllinoleamide	mouse	peritoneal macrophages	15-LO	178
1,25-dihydroxy vitamin D_3		HL-60	5-LO	179
1-chloro-2,4-dinitrobenzene		PMN leukocytes	5-LO	180, 181
		BL41-E95A	5-LO	182
Diamide		PMN leukocytes	5-LO	180, 181
		BL41-E95A	5-LO	182
Indomethacin	human	neutrophils	5-,12-,15-HETE, LTB_4	183
Dexamethasone	human	mast cells	5-LO	184
	human	monocytes, THP-1	5-LO	185
Sodium butyrate	rat	intestinal Epithelial cells	12-LO	186
	human	colorectal carcinoma cells	15-LO	187
Ethanol	rat	hepatocytes	LT	174

123] possess a significant level of LO activity capable of xenobiotic oxidation. Yu and Wells [97] observed that pretreatment of pregnant CD-1 mice with ETYA, a dual PGS and LO inhibitor, results in a dose-related decrease in the incidence of phenytoin-induced fetal cleft palate and resorptions. This reduction in phenytoin teratogenicity was considerably greater than that observed with acetylsalicylic acid, which selectively inhibits PGS. Thus, these results provide strong evidence that the LO pathway may be more important than other pathways in the teratogenic bioactivation of phenytoin in vivo.

Conclusions

The literature reviewed here reflects a promising start in understanding the role played by the LO pathway in xenobiotic metabolism; however, much remains unknown. Although documentation is available for oxidation, epoxidation, desulfuration, dearylation, sulfoxidation, and dealkylation of xenobiotics by LO, further studies are needed to reveal fully the spectrum of reactions catalyzed by different LOs. At present, the data are too sparse to establish clearly the in vivo significance of xenobiotic oxidation via the LO pathway in light of other competing pathways. Since the use of inhibitors to selectively block different oxidative pathways may finally turn out to be futile, an exploration of stereochemical differences in the excreted metabolites seems more rewarding. In this regard, exploitation of ex vivo models for organ culture and, especially, the use of knockout mice and/or transgenic animals and LO-transfected cells overexpressing desired LO isoform(s) may prove to be fruitful. The inducibility of LO and its effect on LO-mediated xenobiotic oxidation and GSH conjugation represent some of the important areas which deserve further serious attention, as they may help solve some of the toxicological puzzles. Since the science of toxicology is primarily meant to serve human interests, promotion and execution of future research efforts revolving around data collection using different human tissues seems inevitable.

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