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The role of AHR-inducible cytochrome P450s in metabolism of polyunsaturated fatty acids

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Abstract

The environmental pollutant 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD) is the prototype of a large number of non-genotoxic carcinogens, dietary phytochemicals and endogenous metabolites that act via binding the aryl hydrocarbon receptor (AHR). The TCDD-liganded AHR massively upregulates CYP1A1, CYP1A2 and CYP1B1 in many mammalian organs. We demonstrated that TCDD treatment markedly increases the levels of several epoxides and diol metabolites of the epoxides of both ω-6 and ω-3 polyunsaturated fatty acids (PUFA) in the liver and lungs of mice, in an aryl hydrocarbon receptor-dependent fashion, and most likely via the activities of the CYP1 family members. ω-6 epoxides are known to stimulate tumor growth, angiogenesis, and metastasis in mice. Interestingly, ω-3 epoxides have the opposite effect on these parameters. TCDD and other AHR agonists may, therefore, impact angiogenesis, growth and metastasis of tumors in either a positive or negative way, depending on the relative levels of ω-6 epoxides and ω-3 epoxides generated in the host and/or tumor cells. This is of potential relevance to carcinogenesis by AHR agonists in the human, since the human population is exposed to widely varying ω6:ω3 PUFA ratios in the diet.

Key Terms

2,3,7,8-Tetrachlorodibenzo-ρ-dioxin (TCDD); Aryl Hydrocarbon Receptor (AHR); eicosanoid; oxylipin; polyunsaturated fatty acids (PUFA); CYP1; omega-3 (ω-3) PUFA; omega-6 (ω-6) PUFA; tumor growth; metastasis

The Aryl Hydrocarbon Receptor mediates the toxic effects of a wide variety of environmental pollutants, certain dietary components and endogenous compounds

The Aryl Hydrocarbon Receptor (AHR) was originally identified as a protein that binds 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD). TCDD is a persistent environmental pollutant

Declaration of Interest Section

None

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that has a large number of toxic effects, including carcinogenicity in all species tested, including humans. All the toxic effects of TCDD are mediated by the AHR. TCDD is not mutagenic, but acts as a non-genotoxic carcinogen (tumor promoter). A large number of other polychlorinated dibenzodioxins, dibenzofurans, and polychlorinated biphenyls that are important environmental contaminants bind the AHR, and have similar toxic effects as TCDD, although at higher doses. Polycyclic aromatic hydrocarbons (PAH), which are important carcinogens found in tobacco smoke, smog and overcooked meats, also bind and activate the AHR (Gasiewicz 2011 and Denison 2011). Several PAHs and polychlorinated compounds (including TCDD) are included in the 2013 list of 126 “Priority Pollutants” by the US Environmental Protection Agency.

PAH, such as benzo(a)pyrene, have a different mechanism of carcinogenic action from TCDD. Unlike TCDD, benzo(a)pyrene is rapidly metabolized by CYP1A1, CYP1A2 and CYP1B1 and thereby quickly eliminated from the body. One of its metabolites, benzo(a)pyrene 7,8-diol, 9,10-epoxide is highly mutagenic (genotoxic) and represents its principal “ultimate carcinogenic” derivative. Heterocyclic amines represent important carcinogens in overcooked fish and meats. Their carcinogenicity largely depends upon their binding to the AHR in gut epithelial cells resulting in the induction of CYP1A2, which then metabolizes them to mutagenic derivatives (Fontana 1999). Recently, several compounds have been proposed to be endogenous ligands for AHR, including the endogenous tryptophan catabolite kynurenine, which is overproduced in several human tumors, and enhances tumor progression (Opitz 2011). The AHR also impacts several facets of the immune system. Thus several bacterial pigments can activate AHR, and thereby initiate an antibacterial response (Moura-Alves *et al* 2014). TCDD can induce Treg development and suppress T_H17 differentiation. Kynurenine probably plays a role in the regulation of these T cell subsets *in vivo* by activating the AHR. Certain vegetable constituents exhibit AHR agonist activities and can regulate intestinal immunity via AHR. The AHR also controls endotoxin tolerance via its activation by kynurenine (Bessede *et al* 2014). Humans are therefore exposed to a variety of AHR ligands.

Mechanism of action of the AHR

The unliganded AHR is located in the cytosol complexed with two molecules of heat shock protein 90 (HSP90), p23, and also the immunophilin-like protein, XAP2. After binding agonist, the AHR translocates to the nucleus, releases its chaperone proteins, and dimerizes with the Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT). The liganded AHR acts as a transcription factor. Much of our knowledge of the further steps of AHR action come from studies on the *CYP1A1* gene. In the nucleus, AHR/ARNT dimers bind to specific short DNA sequences, termed Xenobiotic Responsive Elements (XREs) in the enhancer region of the *CYP1A1* gene (and other responsive genes), and recruits a number of coactivator proteins, which remodel, relocate or dissociate nucleosomes in chromatin at the enhancer and promoter, leading to recruitment of RNA polymerase II and transcriptional initiation (Hankinson 2012). The liganded AHR turns on the transcription of a large number of genes. Surprisingly, the spectra of genes modulated by TCDD treatment differs greatly between different mammalian species (Forgacs *et al* 2012). However CYP1A1, CYP1A2 and CYP1B1 are induced in many tissues in all mammals, and are also routinely the most, or

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among the most massively induced genes. The levels of AHR and the maximal level that each CYP1 family members can be induced by TCDD varies greatly between different tissues/organs (Harper, Riddick and Oakey 2006). In addition, different CYP1 family members are inducible in different tissues. The AHR is overexpressed and constitutively active in a proportion of many types of cancer, and CYP1A1 and CYP1B1 are overexpressed in many cancers, further reinforcing the notion that these proteins are intimately involved in cancer progression (Androutsopoulos, Tsatsakis and Spandidos 2009).

Epoxides of omega-6 (ω-6) and omega-3 (ω-3) polyunsaturated fatty acids (PUFA) exhibit potent biological activities

Excessive intake of ω-6 PUFA, such as arachidonic acid (C20:4, ω6) and linoleic acid (C18:2, ω6), characteristic of the typical western diet, is associated with a number of detrimental health effects, including cancer, which appear to result mainly from the formation of their eicosanoid metabolites (i.e. oxidation products of the ω6 PUFAs). In contrast, the ω-3 PUFAs, α-linolenic acid (C18:3, ω3), eicosapentaenoic acid (EPA, C20:5, ω3), and docosahexaenoic acid (DHA, C22:6, ω3) are associated with beneficial health effects. Epidemiological and preclinical evidence supports the notion that a diet rich in omega-3 dietary fatty acids is associated with reduced risks of several diseases, including heart diseases and cancer.

Arachidonic acid is metabolized via three pathways: the “cyclooxygenase”, “lipoxygenase” and “cytochrome P450 epoxidation/hydroxylation” pathways (Figure 1). Mammalian cytochromes P450 from many different subfamilies, including the CYP1A, CYP1B, CYP2B, CYP2C, CYP2D, CYP2E, CYP2F, CYP2G, CYP2J, CYP2P, CYP2U, CYP3A, CYP4A, CYP4B and CYP4F subfamilies exhibit arachidonic acid epoxidation and/or hydroxylation activities. The immediate products of the epoxidation/hydroxylation pathway of arachidonic acid metabolism include four *cis*-epoxyeicosatrienoic acids (EETs), 5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET, the “terminal” hydroxides, 16-HETE, 17-HETE, 18-HETE, 19-HETE and 20-HETE, and certain “midchain” hydroxides, including 5-HETE, 8-HETE, 12-HETE and 15-HETE. The midchain hydroxides are also products of the lipoxygenase catalyzed metabolism of arachidonic acid. Several of the *EETs* and hydroxides, including some of the terminal hydroxides (particularly 20-HETE), exhibit potent biological activities. For example, EETs are anti-hypertensive, anti-inflammatory, analgesic and cardio-protective. The epoxides can be further metabolized, particularly by soluble epoxide hydrolase, which converts them to the dihydroxyeicosatrienoic acid diol derivatives (DHETs). The DHET metabolites are generally considered biologically inactive (Norwood *et al* 2010). The other PUFAs are metabolized in a similar fashion to arachidonic acid (Fer *et al* 2008). Of interest, the epoxides of omega-3 PUFA have the opposite effects from the epoxides of arachidonic acid on tumor growth, angiogenesis and metastasis (see later).

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Treatment with TCDD markedly increases the levels of a number of epoxides and diols of omega-6 and omega-3 PUFAs in several organs/ tissues of mice

To directly address the potential role of eicosanoids in TCDD toxicity, it is important to ascertain the levels of these compounds in the relevant organs and tissues. Recent advances in liquid chromatography-tandem mass spectrometry (LC-MS/MS) have allowed for the identification and quantitation of a large number of metabolites of PUFA in tissue extracts. Of particular relevance for the current studies are the cytochrome P450 catalyzed products of PUFA (both ω3 and ω6), collectively referred to as oxylipins.

We analyzed the levels of up to twenty-five eicosanoid metabolites of the ω6 PUFA, arachidonic acid and linoleic acid in the liver, lung, heart, spleen and serum of male and female adult C57BL/6 wild-type or AHR knockout mice injected intraperitoneally with 50 μg/kg TCDD or with vehicle (corn oil). Three days later, the organs/tissues were harvested and subsequently analyzed for the levels of certain free (i.e. non-esterified) eicosanoids. This dose and duration of exposure were used as they are known to maximally induce most TCDD-responsive genes (Hayes *et al* 2007 and Forgacs *et al* 2012). In addition, the levels of total eicosanoids (i.e. free and esterified) were measured in the livers of wild-type male mice. To obtain total eicosanoids, liver extracts were treated with KOH in order to hydrolyze membrane phospholipids (Bui *et al* 2012). In collaboration with Dr. Bruce Hammock at the University of California, Davis, we then measured 67 oxylipins, including 42 that we had not measured previously, in the liver, lung and heart of TCDD-treated and untreated male wild-type C57BL/6 mice, including metabolites of the ω3 PUFA, linolenic acid, EPA and DHA (Yang *et al* 2013). Our results are summarized in Figure 2. The major findings are as follows:

1. TCDD had little effect on the levels of any of the twelve prostanoids measured in any of the organs/tissues examined, except for the heart. This suggests that the increases in the levels of the other metabolites in these organs and tissues that are discussed below, are not ascribable to TCDD induction of phospholipase A2. It is also consistent with our observation that under our experimental conditions, TCDD did not increase the levels of cyclooxygenase 2 in any organ or tissue examined (Bui *et al* 2012).
2. In the serum, liver and spleen, TCDD treatment increased the levels of several metabolites of arachadonic acid (AA) or linoleic acid that are generally categorized as lipoxygenase products, but which can also be generated by particular cytochromes P450.
3. Where analyzed, the levels of the total metabolites in the liver were in all cases greater than the levels of the corresponding free metabolites, generally exceeding them by 2- to 10-fold (Bui *et al* 2012). The levels of total prostanoids could not be measured as they are degraded by KOH.
4. For those metabolites in the liver affected by TCDD treatment, total levels generally increased in parallel with the free levels of the same metabolite.

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This was particularly striking for the cytochrome P450-derived DHETs and terminal hydroxides of arachidonic acid.

5. TCDD did not increase eicosanoid levels in *Ahr*$^{-/-}$ mice. Thus the observed increases in the eicosanoids induced by TCDD are dependent on AHR.

6. In the absence of TCDD, the wild type and *Ahr*$^{-/-}$ mice exhibited similar levels of eicosanoids.

7. There were markedly different levels of the prostanoids and putative lipoxygenase products between different (TCDD-untreated) organs; with the levels generally decreasing in the following order: spleen, lung, heart, liver, serum; such that the spleen contained up to 200-fold higher levels of some of these metabolites than the liver. Such differences were not observed for the metabolites derived from the cytochrome P450 epoxidation/hydroxylation pathway.

8. We observed differences in the levels of certain eicosanoids between (TCDD-untreated) male and female mice. However, the differences that were observed generally did not exceed three-fold.

9. In the liver, TCDD increased the levels of several epoxides of arachidonic acid (EETs), α-linolenic acid (EpoDEs), EPA (EpETEs) and DHA (EpDPEs), in several cases more than ten-fold. TCDD increased the levels in the liver of an even greater number of the diols of arachidonic acid, EPA and DHA (DHETs, DiHETEs and DiHDPEs, respectively) and also increased the levels of the diols of linoleic acid (DiHOMES). These metabolites are generated from the epoxides by epoxide hydrolase. The presence of diols in organs *in vivo* is therefore a reflection of the generation of the corresponding epoxides.

10. In the lung, TCDD increased the level of the most abundant epoxide of DHA (19,20-EpDPE), and increased the levels of nearly all the diols of arachidonic acid, linoleic acid, α-linolenic acid, EPA and DHA. This indicates that TCDD does generate the corresponding epoxides in the lung but these are rapidly metabolized to diols by epoxide hydrolase.

11. TCDD increased the levels of some of the hydroxyl metabolites of arachidonic acid (HETEs), EPA (HEPEs) and DHA (HDoHE) in both the liver and lung. However, the levels of these metabolites were generally much lower than the levels of the corresponding epoxides (and many were undetectable).

12. TCDD decreased the levels of several oxylipins in the heart.

Enzymes responsible for the TCDD-mediated increases in oxylipin levels

It is important to consider which TCDD-inducible enzymes are likely to be responsible for the increased oxylipin levels we observed. A number of microarray studies have been

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performed to identify TCDD-inducible genes in mouse liver. Most of the 88 mouse cytochromes P450 and virtually all the eicosanoids-metabolizing enzymes (including the seven known lipoxygenases) were analyzed in one study, and of these only CYP1A1, CYP1A2 and CYP1B1 were upregulated more than two-fold by TCDD (Forgacs *et al* 2012), which is in agreement with another less comprehensive study (Boutros 2008). However, in a different study, the eicosanoid-generating enzymes CYP2C29, CYP2C37 and CYP2C50 were reported to be upregulated by TCDD, although few details were given (Buczynski, Dumlao, and Dennis 2009, 2012). We measured the levels of the mRNAs for CYP1A1, CYP1A2, CYP1B1, cytosolic phospholipase A2 (PLA2G4A and PLA2G12A), soluble epoxide hydrolase (EPHX2) and prostaglandin endoperoxide synthase 2 (cyclooxygenase 2 {PTGS 2}) in the male mice used for the oxylipin analyses (Bui *et al* 2012). The levels of the CYP1A1 and CYP1B1 mRNAs were increased by TCDD treatment in the wild-type male mouse in the liver, lung and heart. CYP1A2 increased only in the liver. None of the mRNA levels were increased by TCDD in the spleen. These observations are in general agreement with those obtained by other investigations (Stejskalova and Pavek 2011). It should be noted that TCDD characteristically affects the levels of these enzymes exclusively at the transcriptional level, and increases in their mRNAs are reflected in increases in the corresponding proteins (Hankinson 1995). We found that the mRNA for the phosopholipase A2 form PLAG2G12A was inducible in the male liver about 2.5-fold, consistent with previous microarray studies (Boutros *et al* 2008 and Kopec *et al* 2010). PLA2G4A has been reported to represent a major form of phosopholipase A2 with regard to the release of arachidonic acid (Kita *et al* 2006). However, although it was reported to be increased by TCDD treatment in the Hepa-1 mouse hepatoma cell line (Kinehara *et al* 2009), this enzyme was not induced by TCDD in our studies. Although TCDD was reported to transiently increase the levels of the mRNA for prostaglandin endoperoxide synthase 2 (cyclooxygenase) in the lung and spleen (but not in the liver) of female C57BL/6 mice (Vogel *et al* 1998), we did not observe an increase in this mRNA in any organ in our study. If any increase in cyclooxygenase occurred at earlier time points, this had minimal effects on the levels of prostanoids in our experiments. Recent studies have demonstrated that ω3 and ω6 PUFA, as well as total saturated and monounsaturated fatty acids, were elevated in the livers of mice chronically exposed to TCDD (Lin *et al* 2011, Kopec *et al* 2011 and Forgacs *et al* 2012). Although such changes may impact the generation of oxylipins after longer term exposure to TCDD, they are unlikely to have contributed significantly to the TCDD-induced increases in these metabolites in our short term exposure experiment, since the levels of the prostanoids were refractory to TCDD treatment in our studies. In conclusion, TCDD induction of CYP1A1, CYP1A2, and CYP1B1 correlated with TCDD-induced increases in oxylipin levels in the lung and liver, and are most likely to be mainly responsible for the TCDD-mediated increases in the oxylipins in these organs. Although CYP1A1 and CYP1B1 were induced in the heart, they were induced to considerably lower levels than in the liver or lung, and this may explain why TCDD did not increase the levels of the arachidonic acid or linoleic acid metabolites in the heart, although we have no explanation for the decreased levels of certain metabolites in this organ. An increase in some metabolites occurred after TCDD treatment in the spleen, despite the lack of TCDD induction of the CYP1 enzymes in this organ, indicating that other TCDD-inducible enzymes may be induced. Alternatively, this could conceivably be due to transport of these metabolites from other organs in the

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blood. Transport of such metabolites in the circulation is known to occur (Diani-Moore et al 2014).

Purified human CYP1A1, CYP1A2 and CYP1B1 generate different spectra of eicosanoids from arachidonic acid *in vitro*. CYP1A1 favors terminal HETEs, CYP1A2 favors ETEs and CYP1B1 favors mid-chain HETEs. The only mouse form to be studied, CYP1B1, favors EETs. Human CYP1A1 and CYP1A2 epoxidize EPA and DHA even more efficiently than they do arachidonic acid (Fer *et al* 2008, Choudhary *et al* 2004, Schwarz *et al* 2004, Rifkind 2006, and Buczynski, Dumlao, and Dennis 2009). The wide spectrum of oxylipins that we observed to be increased by TCDD suggests that two or even three of the CYP1 family members may be involved in particular organs. It should also be noted that although analysis of PUFA metabolism by purified cytochromes P450 is useful, the metabolic profile of arachidonic acid in a particular organ is difficult to predict from its content of cytochromes P450, for several reasons, including the effects of regulatory interactions between metabolic products of some cytochromes P450, and became, as noted above, oxylipins in one organ can be transmitted to other organs via transport in the blood. Schlezinger and coworkers found, for example, that TCDD had a stronger effect on the *in vivo* metabolism of arachidonic acid than on *in vitro* metabolism by liver microsomes (Bui *et al* 2012). Furthermore, it should be noted that the changes in levels in particular regions or cell types of particular organs may be much greater than appears from the levels measured in the whole organ. This is likely to be particularly true in the case of the lung, which contains dozens of different cell types.

Our studies provided a comprehensive determination of the changes in oxylipin levels in various organs of mice/tissues after TCDD treatment. Previously, Dalton and coworkers showed that TCDD exposure increased the levels of three cyclooxygenase-derived arachidonic metabolites in the urine of mice (Dalton *et al* 2001). More recently, Diani-Moore and coworkers have reported that TCDD increased the levels of EETs and DHETs in the heart and liver of chick embryos (Diani-Moore et al 2014).

Potential effects of AHR-dependent changes in ω-3 and ω-6 PUFAs on tumor growth and metastasis

A large number of studies have been performed on the effects of TCDD and other AHR agonists on the tumor-associated properties of cancer cells in culture. These studies have demonstrated that the effects of TCDD on tumor growth, angiogenesis and metastasis are highly variable. For example, AHR agonists stimulate or inhibit these parameters in a cell-specific manner. Furthermore, as described earlier, the AHR is found to be overexpressed in several types of human cancer, as is also the case for CYP1A1 and CYP1B1. These studies relate to the potential role for the activated AHR within the tumor cells themselves on the development of cancer. Few studies have addressed the potential role of the host in mediating the effects of TCDD or tumor progression, and those that have been performed have not allowed for consistent conclusions (Feng, Cao, and Wang 2013). The variation in results obtained may partly be due to the fact that under standard laboratory conditions, as we have shown, mouse tissues contain approximately equal levels of ω-6 and ω-3 epoxides

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and may thereby be poised on a knife's edge with regard to a pro- or anti-progression state. Few studies have addressed the role of TCDD on the angiogenesis or metastasis of tumors *in vivo*. Recently, several groups have demonstrated that epoxides of ω-6 PUFA stimulate tumor growth, angiogenesis and metastasis in mouse models. Ligands for proxisomal proliferator activated receptor α (PPARα) repressed the expression of several Cyp2c family members (which are known to exhibit arachidonic acid epoxygenase activity), reduce the levels of EETs in plasma, and decreased the growth and angiogenesis of tumors (Pozzi 2007 and Pozzi 2010). Panigrahy and coworkers showed that systemic administration of EETs enhanced the growth and angiogenesis of a variety of tumors arising from transplanted cancer cells. Furthermore, overexpressing the CYP2J2 or CYP2C8 arachidonic acid epoxygenases in the endothelium enhanced the above parameters as well as enhancing tumor metastasis (Panigrahy *et al* 2012). Recently, the Hammock group demonstrated that in contrast to EETs, systemic administration of 19,20-EDP, an epoxide derivative of DHA, in conjunction with the epoxide hydrolase inhibitor *τ-AUCB*, suppressed the primary growth and angiogenesis of xenografted tumor cells. In addition, coadministration of 19,20-EpDPE or 16,17-EpDPE with *τ-AUCB*, or even treatment with 16,17-EpDPE on its own repressed the metastasis of Lewis lung carcinoma cells (LLC) in a model of spontaneous metastasis (Zhang *et al* 2013). Thus the epoxides of ω-3 PUFA (particularly of DHA) have opposite effects on tumor growth, angiogenesis and metastasis compared with EETs. In the above studies, the tumor cells that were used do not biosynthesize EETs or ω-3 epoxides, and furthermore, the effects of the EETs and EpDPEs on the growth of the primary tumors appeared to be mediated by effects on angiogenesis and not by direct effects on tumor cell proliferation. Interestingly, Jiang and coworkers demonstrated that overexpression of the arachidonic acid epoxygenase, CYP2J2 in breast cancer cells increased angiogenesis in the resulting primary tumors and also enhanced their metastasis to the lung (Jiang *et al* 2007). Thus synthesis of PUFA epoxides within the tumor cells in the absence of such an increase in the host can also impact tumor progression. The mechanism(s) whereby EETs stimulate and EpDPEs inhibit tumor progression have been investigated, and a variety of mechanisms may be involved. These responses have been associated with changes in the expression of Vascular Endothelial Growth Factor (VEGF) or Epidermal Growth Factor or one or more of their receptors, and corresponding alterations in endothelial cell proliferation, changes in the levels of the antiangiogenic protein, thrombospondin in stromal fibroblasts, alterations in the expression of prometastic matrix metalloproteins or of antimestatatic genes, and activation of the MAPK and PI3/AKT pathways (Zhang *et al* 2013). These studies have been handicapped by the fact that the receptor(s) for the PUFA epoxides have not been unequivocally identified (Spector and Kim 2015). Spurprisingly, in a recent study, it has been demonstrated that inhibition of epoxide hydrolase acts synergistically with inhibition of cyclooxygenase-2 activity to inhibit tumor growth and metastasis (Zhang *et al* 2014).

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The changes elicited by TCDD on the levels of ω-6 and ω-3 epoxides in the mouse are in a range likely to impact the growth, angiogenesis and metastasis of tumors

Panigrahy and coworkers reported that tumors generated from xenografted cancer cells (certain of which we subsequently showed to lack expression of AHR) grew considerably more rapidly and metastasized more efficiently when the total plasma levels of 11,12-EET plus 14,15-EET were elevated about two-fold (Panigrahy *et al* 2012). We found that TCDD increased the levels of total EETs and DHETs by this degree or greater in our studies. Thus TCDD increased the levels of total EETs and DHETs 5-fold and 3.6-fold, respectively, in the liver, and increased their levels 2.1-fold and 2.7-fold, respectively, in the lung. The Hammock laboratory found that treatment of mice with 19,20-EpDPE plus the soluble epoxide hydrolase inhibitor *τ-AUCB* increased the plasma and tumor levels of 19,20-EpDPE 2.5-fold and 3-fold, respectively (Table 1), and inhibited metastasis of the above cancer cells (Zhang *et al* 2013). However, we found that TCDD treatment lead to increases in the level of 19,20-EpDPE (the most prevalent ω-3 epoxide), and its diol derivative,19,20-DiHDPE, in the two major sites of metastasis of the above cancer cells (lung and liver) that were equal to or considerably greater than those observed in the Hammock study (Table 1). Furthermore, the levels of 19,20-EpDPE that we measured in the liver and lung were in the same range as those measured by Zhang and coworkers in the tumor tissue (Zhang *et al* 2013). These data suggest that the changes in the levels of PUFA epoxides in the host elicited by TCDD treatment are likely to impact tumor growth, angiogenesis and metastasis.

Future directions and relevance

It will be of interest to ascertain whether AHR agonists, such as TCDD, stimulates tumor growth, and independently, tumor metastasis, when ω-6 PUFA are in abundance, but has the opposite effect when ω-3 PUFA are in abundance. This could be approached by feeding mice with diets with high ratios of ω6:ω3 PUFA or high ratios of ω3:ω6 PUFA, respectively. The data obtained from such a study would be of potentially considerable relevance to the impact of AHR agonists on cancer progression, since the human population is exposed to widely varying ω6:ω3 PUFA ratios in their diet.

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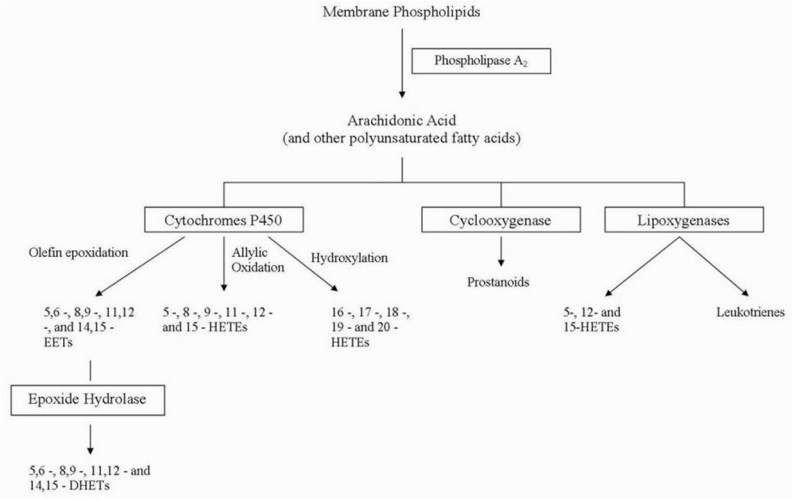

Figure 1.
The metabolism of arachidonic acid.

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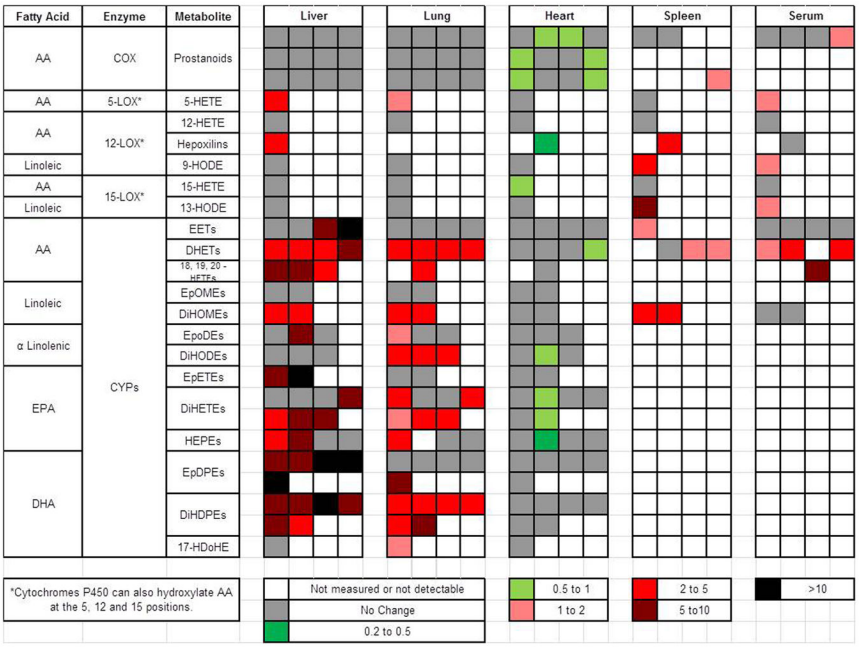

Figure 2.

Heat map summarizing the changes in oxylipin levels elicited by TCDD in mice. Each square represents a different metabolite measured in three or more TCDD-treated or TCDD-untreated male mice.

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Table 1

Comparison of the effects of treatment with 19,20-EDPþs-AUCB with our observations on the effects of TCDD treatment on 19,20- and 19,20-DiHDPE levels.

Zhang *et al.* (2013) [37]	19,20-EpDPE levels in	
	Plasma	Tumor Tissue
No Treatment	10	120
19,20-EpDPE + τ-AUCB	25 (2.5x)	350 (3x)

Yang *et al.* (2013) [28]	19,20-EpDPE levels in		19,20-DiHDPE levels in	
	Liver	Lung	Liver	Lung
No Treatment	14	33	530	610
+TCDD	300 (21x)	100 (3x)	4400 (10x)	1400 (2.3x)

All levels are in picomoles per gram of tissue.