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**COMMENTARY**

# Impact of Dioxin-Type Induction of Drug-Metabolizing Enzymes on the Metabolism of Endo- and Xenobiotics

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**ABSTRACT.** The induction of a number of drug-metabolizing enzymes is among the best-understood biochemical effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related agonists of the aryl hydrocarbon receptor (AhR). Among the cytochrome P450s (CYPs), the genes encoding CYP1A1, 1A2, and 1B1 are responsive to AhR agonists, i.e. their expression is inducible in various mammalian tissues and organs as well as in many types of cell lines and primary cells in culture. In addition, an aldehyde dehydrogenase, an NADPH-quinone-oxidoreductase, and the phase II conjugating enzymes glutathione-*S*-transferase (GST) Ya and UDP-glucuronosyltransferase 1A1 have been identified as responsive to AhR agonists. Induced expression of these members of the AhR gene battery is thought to be aimed at an improved elimination of the inducing agent and its metabolites. However, the identity of the physiological ligand(s) of the AhR is still obscure. The consequences of induced expression of AhR-regulated genes encoding drug-metabolizing enzymes have been investigated in human populations, e.g. in smokers, and in various experimental models. A prominent example of increased adverse effects due to the induction of CYP1A isozymes is the metabolic activation of carcinogenic aromatic amines and polycyclic aromatic hydrocarbons. An increasing amount of data is also available on the impact of dioxin-type induction on the metabolism of drugs, food constituents, and endogenous substrates. For example, the hepatic clearance of the drug theophylline, which is widely used in asthma therapy, is enhanced significantly in smokers. Increased glucuronidation of thyroxine in rats treated with TCDD or other potent AhR agonists is thought to result in hypothyroxinemia and its biological consequences, such as sustained hyperplasia of the thyroid, bearing a higher risk of thyroid cancer. The relevance of these observations for humans exposed to dioxin-type inducers is discussed. BIOCHEM PHARMACOL **55**;8:1155–1162, 1998. © 1998 Elsevier Science Inc.

**KEY WORDS.** aryl hydrocarbon receptor; drug metabolism; drug-metabolizing enzymes; induction; TCDD

## DRUG-METABOLIZING ENZYMES AS MEMBERS OF THE AH RECEPTOR GENE BATTERY

The AhR† has been found in most, if not all, investigated mammalian and a number of nonmammalian vertebrate species. It is a member of the PAS (per-arnt-sim) family of basic helix-loop-helix proteins [1, 2]. Upon binding of an agonist, it dissociates from the chaperone molecule hsp90, and associates with a structurally related protein, the ARNT. The whole complex acts as a nuclear transcription factor that binds to consensus sequences, DREs, in the 5′-flanking region of responsive genes, thereby enhancing their transcription [3–8]. Among these genes of the so-called AhR gene battery are a number of genes encoding drug-metabolizing enzymes [9, 10] such as CYP1A1 and 1A2, a GST of the alpha class, an NADPH-quinone-oxidoreductase (DT diaphorase), a UDP-glucuronosyltransferase (UGT1A1), and an aldehyde dehydrogenase. The TCDD-inducible isozyme CYP1B1 is not discussed here in more detail. It should be kept in mind that in many cases, e.g. the human GSTA1/A2 [11], the role of DRE-dependent transcriptional activation in induction is by far less clear than in the case of CYP1A1.

Interestingly, most of the isozymes of the AhR gene battery exhibit a clear preference for substrates that either fulfill the structural requirements of an AhR agonist or, in the case of phase II substrates, are derived from such a structure. This finding indicates that the drug-metabolizing enzymes of the AhR gene battery are part of a defense system aimed at the elimination of the inducer and its metabolites.

The most potent AhR agonists, however, including 2,3,7,8-substituted PCDD, PCDF, and non-ortho-substituted PCB are anthropogenic contaminants found in the environment and in food and tissue samples. Their occurrence is linked historically to industrial development, making it unlikely that the AhR defense program is

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† *Abbreviations:* AhR, aryl hydrocarbon receptor; ARNT, AhR nuclear translocator; CYP, cytochrome P450; DRE, dioxin-responsive element; GST, glutathione-*S*-transferase; 3-MC, 3-methylcholanthrene; PAH, polycyclic aromatic hydrocarbons; PCB, polychlorinated biphenyls; PCDD, polychlorinated dibenzo-*p*-dioxins; PCDF, polychlorinated dibenzofurans; and TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

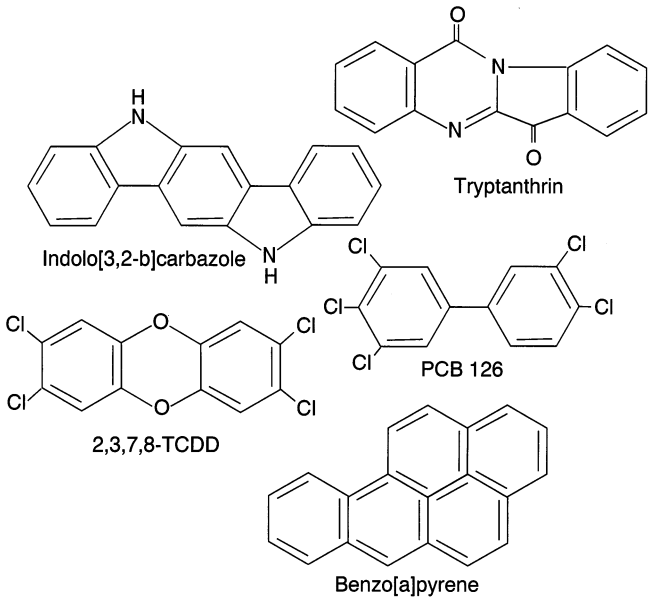


**FIG. 1. Chemical structures of synthetic and natural AhR agonists.**

directed against these compounds. The same may be true for another group of agonists, the PAH. In this case, preindustrial exposure may have resulted from wood fires, but it appears unlikely that the AhR gene battery, more or less common among higher vertebrates, developed to protect the organism from the chemical consequences of wood fire.

Heterocyclic aromatic amines formed during high-temperature cooking of meat or present in tobacco smoke represent another group of AhR agonists [12] relevant to humans.

The search for natural/physiological AhR ligands has focused on various other classes of compounds (Fig. 1). These include rutaecarpine alkaloids [13], indole derivatives such as indole-3-carbinol [13], or certain oxocarotenoids [14]. Another possibility is that intestinal microflora plays a role in generating AhR agonists such as indolo[3,2*b*]carbazole formed from dietary indolo-3-carbinol [15], or tryptanthrins from anthranilic acid derivatives and tryptophan [16]. Moreover, tryptophan was found to be converted into AhR agonists upon incubation with rat feces [17] or upon UV irradiation [18].

In humans, these sources of AhR agonists may contribute to the background expression of AhR-regulated genes. In addition, exposure to PCDD, PCDF, or PCB above background level, or to PAH particularly in smokers, was shown to result in induction of CYP1A1 and 1A2 [19, 20]. The important question of a possible polymorphism of responsiveness of human populations has been addressed in a number of studies [20–22]. A recent sequence analysis of the AhR exons 1–10 in a Japanese population, however, failed to link a diversity in responsiveness to AhR agonists to a variation in the amino acid sequence [23] found in 43% of the population.

## CONSEQUENCES OF INDUCTION FOR THE METABOLISM OF XENOBIOTICS

An induction of AhR-regulated genes encoding drug-metabolizing enzymes in humans was studied primarily in smokers. Most investigators suggested that PAH are the most important AhR agonists in tobacco smoke; the contribution of aromatic and heterocyclic amines and other AhR agonists to the overall inducing effect of tobacco smoke is less clear. The relatively specific functional CYP1A parameters 7-ethoxyresorufin *O*-deethylase and phenacetin *O*-deethylase were shown to be induced in the liver of smokers [24, 25].

As a non-invasive method for the assessment of CYP1A2 activity in humans, molar ratios of urinary caffeine metabolites are in use. The various ratios used differ, however, in their distribution patterns, i.e. monophasic as well as biphasic distributions of CYP1A2-linked ratios have been reported in both smokers and nonsmokers [19–22]. Furthermore, the relationship between individual ratios from the same cohort was reported to be weak [26]. These findings indicate the need for biochemical and molecular approaches to clarify whether a genetic polymorphism of inducibility and/or expression of CYP1A2 exists among humans.

PAH such as benzo[*a*]pyrene or benz[*a*]anthracene not only are AhR agonists, but the parent compounds and many of their metabolites also are substrates for AhR-regulated phase I and phase II isozymes [27–30]. In V79 cells expressing transfected human CYP1A1, almost exclusive oxidation of benzo[*a*]pyrene at the 7,8,9,10-position yielding the ultimate carcinogen 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene was reported [31].

Another prominent group of carcinogens that are activated metabolically by CYP1A1/1A2 are carcinogenic aromatic and heterocyclic amines. Human CYP1A2 was shown to be primarily responsible for the hepatic *N*-oxidation of carcinogenic aromatic amines [32], a crucial step in their conversion into ultimate carcinogenic metabolites. In fact, pretreatment of rats with 3-MC resulted in a marked increase in *N*-hydroxylation and DNA binding when hepatocytes isolated from these animals were incubated with 2-naphthylamine [33] or 4-aminobiphenyl [34]. The metabolic activation of a number of procarcinogenic heterocyclic amines is also dioxin inducible [35, 36].

Metabolic activation of aflatoxin $B_1$ was stimulated in cultured human epidermal cells treated with 5 nM of TCDD [37], and antibodies against CYP1A2 effectively inhibited the metabolism of aflatoxin $B_1$ in human liver microsomes [38]. In contrast, the oxidative metabolism of benzene leading to myelotoxic metabolites was reduced in hepatocytes isolated from 3-MC-treated rats [39]. This effect was due to both an induction of UGT1A1 conjugating phenolic benzene metabolites and a decrease in hepatic CYP2E1, the high-affinity benzene/hydroquinone monooxygenase [40]. These findings are in agreement with the

**TABLE 1. Substrates* of drug-metabolizing enzymes inducible by dioxin-type inducers**

| | Xenobiotics | |
|---|---|---|
| Endobiotics | Contaminants | Drugs |
| 17β-Estradiol | Aromatic amines | Bufuralol |
| Retinoids | PAH | Chlorzoxazone |
| Thyroxine (T4) | Heterocyclic amines | Clozapine |
| Uroporphyrinogen | | Cyclobenzaprine |
| | | Imipramine |
| | | Propranolol |
| | | Theophylline |
| | | Zoxazolamine |

*In many cases, phase I metabolites of the substrates listed are substrates for AhR-regulated conjugating enzymes.

reported attenuation of benzene-induced lymphocytopenia by pretreatment of rats with dioxin-type inducers [41].

A number of clinically important drugs are metabolized by isozymes of the AhR gene battery. The high-affinity demethylation of caffeine and its primary dimethylxanthine metabolites, paraxanthine and theophylline, is metabolized preferentially by CYP1A2 in human liver [42]. For theophylline, a drug widely used in asthma therapy, systemic clearance was predicted to be approximately 50% greater in smokers than in nonsmokers [43]. The analgesic drug phenacetin was used as a high-affinity substrate for CYP1A2, e.g. in rat liver microsomes [44]. Other drugs that have been shown or suggested to be substrates of CYP1A1 or 1A2 [45–51] are listed in Table 1.

## CONSEQUENCES OF INDUCTION FOR THE METABOLISM OF ENDOBIOTICS

A well-described effect of TCDD and other potent AhR agonists is the perturbation of retinoid homeostasis. Treatment of rats with a high dose of 10 μg of TCDD/kg body weight led to a 95–97% reduction of hepatic retinol and to a marked reduction of retinol in the lung, the intestine, and the adrenals [52], while the retinol-derived radioactivity in the kidney was strongly enhanced. The possible contribution of enhanced metabolism of retinoids was investigated by a number of authors. In fact, the dose–response relationship in rats treated with TCDD was similar for effects on hepatic retinoids and for induction of CYP1A1/1A2 [53]. Studies with purified rabbit CYP isozymes showed that CYP1A2 has high activity in both the 4-hydroxylation of retinoids and the oxidation of retinal to retinoic acid [54]. Furthermore, it was found that glucuronidation of 13-*cis*-retinoic acid and all-*trans*-retinoic acid was enhanced by a factor of 4 to 7 in liver microsomes from TCDD- or 3-MC-treated rats [55, 56]. A recent study, however, suggests that suppressed retinol esterification in retinoid-storing hepatic stellate cells plays an important role in the loss of hepatic retinoids in TCDD-treated rats [57]. The relevance of the reported suppression by TCDD of the retinoic acid-mediated induction of type II of the cellular

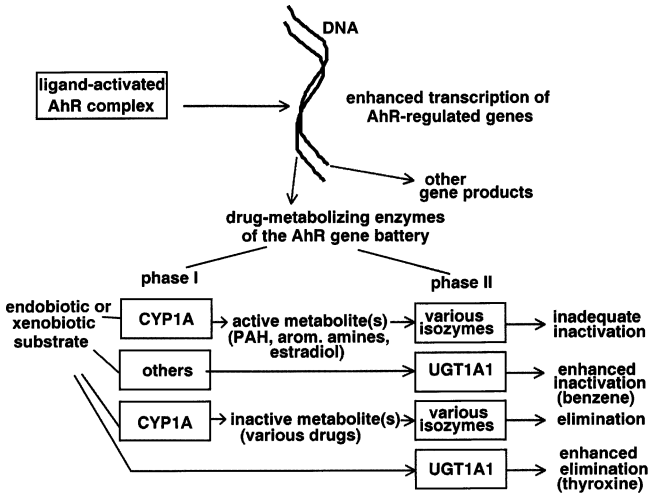


**FIG. 2. Possible consequences of dioxin-type induction of drug-metabolizing enzymes.**

retinoic acid binding protein (CRABP-II) and of the retinoic acid receptor β in murine palate mesenchymal cells [58] is currently unknown.

Acute and subchronic administration of TCDD to rats induced the 2-hydroxylation of 17β-estradiol [59], and MCF-7 breast cancer cells treated with indolo[3,2-*b*]carbazole showed increased rates of 2-, 4-, 6α-, and 15α-hydroxylation of 17β-estradiol [60]. Induction of CYP1A1/1A2 in the liver of TCDD-treated female rats was suggested to contribute to the genotoxic and/or tumor-promoting effect of TCDD by enhancing the formation of reactive estrogen metabolites [61]. In laboratory animals, TCDD led to a decline in serum thyroxine [62]. Because both the induction of a hepatic UDP-glucuronosyltransferase and a decrease in serum thyroxine occurred in the same dose range in rats treated with TCDD [63], it was suggested that thyroxine is eliminated to a higher extent as a glucuronide in treated animals.

Porphyria is produced by many chlorinated organic compounds in experimental animals. Recently, it was found that induction of hepatic CYP1A2 activities significantly correlated with hepatic porphyrin levels [64]. In fact, CYP1A2 was shown to catalyze the oxidation of uroporphyrinogen [65], suggesting that porphyria may result, at least in part, from CYP1A2 induction.

## DIOXIN-TYPE INDUCTION AND RISK ASSESSMENT

Two major types of adverse effects can be linked to dioxin-type induction of drug-metabolizing enzymes (Fig. 2). First, the underlying activation of the AhR not only leads to an induction of drug-metabolizing enzymes, but also affects the expression of other genes that influence basic cellular processes, such as growth, differentiation, and programmed cell death [66]. Examples for this type of effects of TCDD in tissue culture are inhibited or accelerated differentiation of epidermal cell lines [67, 68], inhibi-

tion of adipose differentiation [69], enhancement of growth factor-stimulated DNA synthesis [70, 71], suppression of apoptosis in hepatocytes [72], and stimulation of apoptosis in thymocytes [73]. It remains to be elucidated if and how these responses are mediated by AhR-regulated genes such as those encoding the plasminogen activator inhibitor-2 or interleukin-1β [74], and which role the direct activation of kinases linked to the AhR [75] plays in this scenario. Furthermore, the functional relationships between these basic observations in tissue culture and the complex *in vivo* effects of TCDD and related potent AhR agonists including thymic atrophy and immune suppression, tumor promotion, developmental and reproductive toxicity, and perturbation of steroid hormone action [76] need further investigation. Irrespective of the knowledge of the detailed mechanistic links, however, a reasonable correlation was found for the relative potencies of AhR agonists and/or effects of various doses of a certain AhR agonist comparing CYP1A induction and various toxic endpoints such as thymus atrophy [77] or promotion of preneoplastic liver foci [78]. Furthermore, animals that bear an AhR with lower affinity [79] or that are AhR-deficient [80] were significantly less sensitive to different toxic effects of TCDD.

The other type of adverse effect more closely linked to the induction of drug-metabolizing enzymes results from an enhanced metabolic activation of endogenous or exogenous substrates. The CYP1A2-catalyzed metabolic activation of estrogens was suggested to lead to oxidative DNA damage [61]. This assumption was based on the findings of a lower formation of 8-oxo-deoxyguanosine in TCDD-treated rats after ovariectomy. Likewise, the promoting effect of TCDD on the development of preneoplastic liver foci was found to depend on ovarian hormones [81]. Increased estradiol 2-hydroxylase activity in liver microsomes was observed when female rats were treated for over 30 weeks with 0.01 μg of TCDD/kg per day.

In many instances in humans, the outcome of an enhanced metabolic activation of xenobiotics by drug-metabolizing enzymes induced by AhR agonists is difficult to analyze. This is particularly true in smokers, probably the most important cohort of "induced" persons. Since smokers are exposed to a variety of tobacco smoke constituents that act both as AhR agonists and as CYP1A substrates being activated into ultimate carcinogens, it is difficult to separate both effects. Experiments in animals, in tissue culture, and in subcellular cell fractions indicate, however, that an enhanced metabolic activation and/or DNA binding of polycyclic aromatic hydrocarbons [82, 83] or aromatic amines [33, 34] occur(s) in Ahr agonist-inducible/induced models.

A number of conjugating phase II enzymes that can detoxify intermediate metabolites of PAH or aromatic amines are also inducible, and thus may counterbalance the enhanced activation by phase I enzymes. However, in animal experiments, some phase II enzymes such as UDP-glucuronosyltransferase were less inducible by TCDD than CYP1A1 or CYP1A2 [84]. In human hepatocytes treated with TCDD in primary culture, induction of GST activity [85] or GSTA1 (A2) gene expression [86] was observed only in a minority of the samples, suggesting that this mechanism of inducible detoxification may be polymorphic among humans. A similar combination has been described in human lymphoblastoid B-cell lines [87]. In moderate but not in heavy smokers, the "rapid oxidizer" phenotype exhibiting a polymorphic, high CYP1A2 activity showed higher amounts of 4-aminobiphenyl-derived hemoglobin adducts than the "slow oxidizer" phenotype [88]. These findings argue for an increased risk of adverse effects including cancer when exposure to persistent AhR agonists is followed by exposure to chemical carcinogens of the classes of PAH or aromatic/heterocyclic amines. This is particularly true in cases where the expression of AhR-regulated or other phase II isozymes is low due to a polymorphic distribution in the population.

Heterocyclic aromatic amines formed in high-temperature-cooked meat are another relevant source of dioxin-type inducers. In a study in nonsmokers, Sinha *et al.* [89] reported an induction of CYP1A2, measured as increased oxidative caffeine metabolism, after 7 days of consumption of meat pan-fried at high temperature. Also, in this case, the coincidence of CYP1A2 induction and exposure to procarcinogenic heterocyclic aromatic amines known to be activated by this isozyme may pose an increased cancer risk.

Dose–response analyses in rat liver using polymerase chain reaction (PCR) techniques showed that TCDD-mediated increases in CYP1A1 mRNA were detectable following a single dose of 0.1 ng/kg, which led to a TCDD liver concentration equivalent to a chronic dose of 2–5 pg/kg per day [90]. In a chronic study in female rats, Sewall *et al.* [63] found a significant increase in hepatic CYP1A1 mRNA with a dose between 0.1 and 1.0 ng of TCDD/kg per day given over 30 weeks. In another study, 95% confidence limits for the no-effect level of 0.7 to 4 ng of TCDD/kg for the daily intake and of 0.06 to 0.4 ng of TCDD/g wet weight for the liver level were calculated, using increases in CYP1A1 and CYP1A2 activities as parameters [53]. However, the correlation between CYP1A induction and tumor promotion in the liver of TCDD-treated rats is very limited since, for example, in Sprague–Dawley rats the hepatocarcinogenic but not the CYP1A-inducing effect of TCDD is restricted to females [90]. Furthermore, the dose–response relationships for both endpoints are not necessarily identical [91, 92].

Significant effects on serum thyroxine were observed in female rats treated with 10 ng of TCDD/kg per day [64]. In the same range, a significant increase in hepatic UGT1A1 mRNA was obtained. The maximal increase of UGT1A1 mRNA was approximately 3-fold, CYP1A1 mRNA was induced about 260-fold. The average minimal TCDD concentration in the liver leading to a decrease in thyroxine was 1.7 μg/kg. Parallelism between the increase in specific thyroxine glucuronidation and the decline in plasma thyroxine in TCDD- or PCB-treated rats was shown by Van Birgelen *et al.* [93]. Kohn *et al.* [94] presented a

mechanistic model including enhanced glucuronidation of thyroxine by a TCDD-inducible hepatic UDP-glucuronosyltransferase, and a resulting increase in thyrotropin (TSH) release regulated by the hypothalamic factors thyrotropin-releasing hormone and somatostatin. The calculated increases in blood TSH are consistent with a sustained stimulation of the thyroid and may represent a critical event in the promotion of thyroid cancer.

A strong reduction in liver retinoid levels was observed in female Sprague–Dawley rats after 13 weeks of feeding of a diet containing 0.2 μg of TCDD/kg (corresponding to 14 ng of TCDD/kg per day) [95]. The authors conclude that the sensitivity of hepatic retinoid depletion indicates the need for further investigation of the effects of low-dose exposure with AhR agonists.

## CONCLUSIONS

The induction of drug-metabolizing enzymes has two major facets. The first one is that induction is part of a pleiotropic response of the cell towards an endogenous or exogenous challenge. Induction is frequently, but not necessarily, linked to other responses, including alterations in growth, differentiation, rate of apoptosis, and intracellular communication. Since these changes play a major role in many adverse effects of chemicals such as immune response, teratogenicity, and tumor promotion, induction of drug-metabolizing enzymes is used frequently as a surrogate parameter for these more complex effects. In the case of TCDD and related AhR agonists, this concept has been applied successfully. It has to be kept in mind, however, that induction *per se* is not a toxic endpoint, i.e. there are a number of examples, such as tumor promotion in rat liver, where the correlation between induction of CYP1A isozymes and the toxic endpoint is insufficient.

The other facet of induction of drug-metabolizing enzymes is the possible direct effect on the activation/inactivation of endobiotic or xenobiotic substrates. AhR agonists have been shown in various experimental models and in a number of studies in humans to enhance the metabolic activation of carcinogenic PAH and aromatic and heterocyclic amines, thereby increasing the risk of cancer. In the case of noncarcinogenic substrates such as drugs, induction may lead to an increased metabolic clearance. The most thoroughly investigated example for this type of interaction is the drug theophylline. The outcome of a dioxin-type induction on plasma thyroid levels is an example of an adverse effect on the metabolism of an endogenous compound. A prenatal lack of thyroxine may lead to developmental defects of the central nervous system. Furthermore, it may cause thyroid hyperplasia resulting in an increased risk of thyroid cancer. In this field, more research is necessary to assess the effects of background exposure to persistent AhR agonists and to identify human subpopulations that may be at higher risk due to higher exposure and/or polymorphic responses to AhR agonists.

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