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Exposure to endocrine disruptors 17alpha-ethinylestradiol and estradiol influences cytochrome P450 1A1-mediated genotoxicity of benzo[*a*]pyrene and expression of this enzyme in rats

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ABSTRACT

Endocrine disruptors (EDs) are compounds that interfere with the balance of the endocrine system by mimicking or antagonising the effects of endogenous hormones, by altering the synthesis and metabolism of natural hormones, or by modifying hormone receptor levels. The synthetic estrogen 17α-ethinylestradiol (EE2) and the environmental carcinogen benzo[*a*]pyrene (BaP) are exogenous EDs whereas the estrogenic hormone 17β-estradiol is a natural endogenous ED. Although the biological effects of these individual EDs have partially been studied previously, their toxicity when acting in combination has not yet been investigated. Here we treated Wistar rats with BaP, EE2 and estradiol alone or in combination and studied the influence of EE2 and estradiol on: (*i*) the expression of cytochrome P450 (CYP) 1A1 and 1B1 in rat liver on the transcriptional and translational levels; (*ii*) the inducibility of these CYP enzymes by BaP in this rat organ; (*iii*) the formation of BaP-DNA adducts in rat liver *in vivo*; and (*iv*) the generation of BaP-induced DNA adducts after activation of BaP with hepatic microsomes of rats exposed to BaP, EE2 and estradiol and with recombinant rat CYP1A1 *in vitro*. BaP acted as a strong and moderate inducer of CYP1A1 and 1B1 in rat liver, respectively, whereas EE2 or estradiol alone had no effect on the expression of these enzymes. However, when EE2 was administered to rats together with BaP, it significantly decreased the potency of BaP to induce *CYP1A1* and *1B1* gene expression. For EE2, but not estradiol, this also correlated with a reduction of BaP-induced CYP1A1 enzyme activity in rat hepatic microsomes. Further, while EE2 and estradiol did not form covalent adducts with DNA, they affected BaP-derived DNA adduct formations *in vivo* and *in vitro*. The observed decrease in BaP-DNA adduct levels in rat liver *in vivo* resulted from the inhibition of CYP1A1-mediated BaP bioactivation by EE2 and estradiol. Our results indicate that BaP genotoxicity mediated through its activation by CYP1A1 in rats *in vivo* is modulated by estradiol and its synthetic derivative EE2.

1. Introduction

The term "endocrine disruptor" (ED) is used for compounds that mimic or antagonise the effects of endogenous hormones, alter the synthesis and metabolism of natural hormones or modify hormone receptor levels. The synthetic estrogen 17α-ethinylestradiol (EE2) and the carcinogenic environmental pollutant benzo[*a*]pyrene (BaP) belong to a group of chemicals assigned as exogenous endocrine disruptive compounds while the estrogenic hormone estradiol, or more precisely, 17β-estradiol, is a natural endogenous ED. The biological effects of these EDs depend on their metabolism. Although the toxic effects of these EDs are partially known, apart from BaP, information on their genotoxic and carcinogenic properties mediated during metabolism is scarce.

Abbreviations: AhR, aryl hydrocarbon receptor; BaP, benzo[*a*]pyrene; BPDE, benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide; COMT, catechol-*O*-methyltransferase; CYP, cytochrome P450; EE2, 17α-ethinylestradiol; DMSO, dimethylsulfoxide; ED, endocrine disruptor; dG-N^2-BPDE, 10-(deoxyguanosin-N^2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; EROD, 7-ethoxyresorufin *O*-deethylation; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NADPH, nicotinamide adenine dinucleotide reduced; *m*EH, microsomal epoxide hydrolase; NQO1, NAD(P)H:quinone oxidoreductase 1; PAH, polycyclic aromatic hydrocarbon; POR, NADPH:CYP reductase; TLC, thin layer chromatography; RAL, relative adduct labelling

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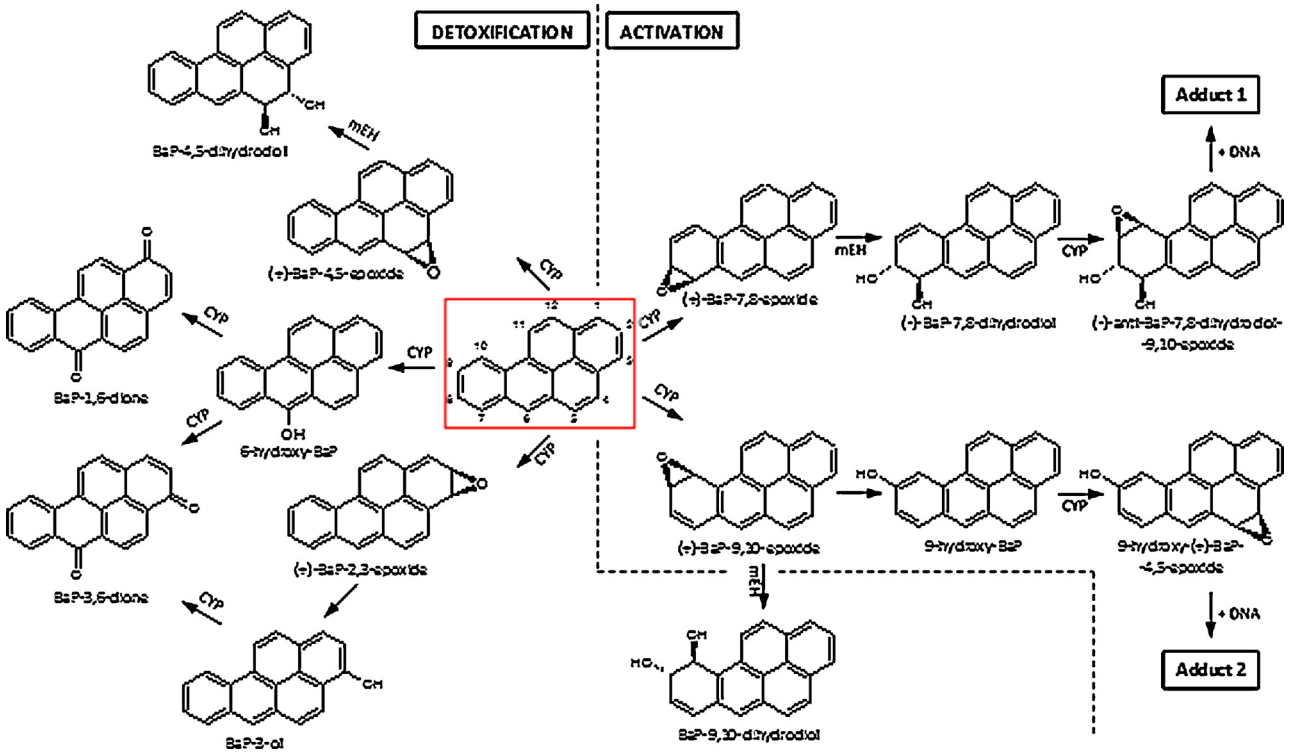

Fig. 1. Proposed pathways of biotransformation and DNA adduct formation of BaP catalysed by CYP enzymes and *m*EH. The typical three-step activation process by CYPs followed by hydrolysis by *m*EH leads to BPDE which forms dG-N^2-BPDE (adduct 1) and the two-step activation process by CYP leads to the formation of 9-hydroxy-BaP-4,5-epoxide that can react with deoxyguanosine in DNA (adduct 2). Formation of BaP detoxification metabolites are shown in the left part of the figure.

BaP is a polycyclic aromatic hydrocarbon (PAH) that has been classified as human carcinogen (Group 1) by the International Agency for Research on Cancer (IARC) (IARC, 2010). BaP and other PAHs are produced mainly by incomplete combustion of organic matter. Their ubiquitous presence in the environment leads to measurable background levels of exposure in the general population (IARC, 2010). Beside the inhalation of polluted air, the main sources of exposure are tobacco smoke and diet (Baird et al., 2005). BaP has been shown to cause cytotoxic, genotoxic, neurotoxic, mutagenic and carcinogenic effects in various tissues and cell types (Siddens et al., 2012; Wohak et al., 2016; Krais et al., 2016; Long et al., 2016, 2017; Chepelev et al., 2016). BaP requires metabolic activation prior to reaction with DNA (Reed et al., 2018). Cytochrome P450 (CYP) enzymes, mainly CYP1A1 and 1B1, are the most important enzymes involved in this process, in combination with microsomal epoxide hydrolase (*m*EH) (Fig. 1) (Nebert et al., 2013; Arlt et al., 2015; Stiborová et al., 2014, 2016a,b). First, CYP1A1 enzyme oxidises BaP to an epoxide that is then converted to a dihydrodiol by *m*EH (*i.e.* BaP-7,8-dihydrodiol). Further bioactivation by CYP1A1 leads to the ultimately reactive species, BaP-7,8-dihydrodiol-9,10-epoxide (BPDE) that can react with DNA, forming adducts preferentially at guanine residues (Fig. 1). The 10-(deoxyguanosin-N^2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (dG-N^2-BPDE) adduct is the major product of the reaction of BPDE with DNA *in vivo* (Arlt et al., 2008, 2012) and preferentially leads to the induction of G to T transversion mutations (Alexandrov et al., 2016; Kucab et al., 2015; Nik-Zainal et al., 2015). Alternatively, BaP-7,8-dihydrodiol can be activated by aldo-keto reductases leading to BaP-7,8-dione which is also capable of forming DNA adducts and generating oxidative damage to DNA (Penning, 2014). However, BaP is also oxidised to other metabolites such as other dihydrodiols, BaP-diones and further hydroxylated metabolites (Indra et al., 2013, 2014; Stiborová et al., 2014, 2016a,b; Sulc et al., 2016). Although most of these metabolites are detoxification products, BaP-9-ol (9-hydroxy-BaP) is the precursor of 9-hydroxy-BaP-4,5-epoxide that can form another adduct with deoxyguanosine in DNA (Fig. 1). Expression of CYP enzymes of the family 1 (CYP1A1 and 1B1), which predominantly metabolise BaP, are known to be up-regulated by the aryl hydrocarbon receptor (AhR); BaP itself can bind to and activate AhR thereby enhancing its own metabolic

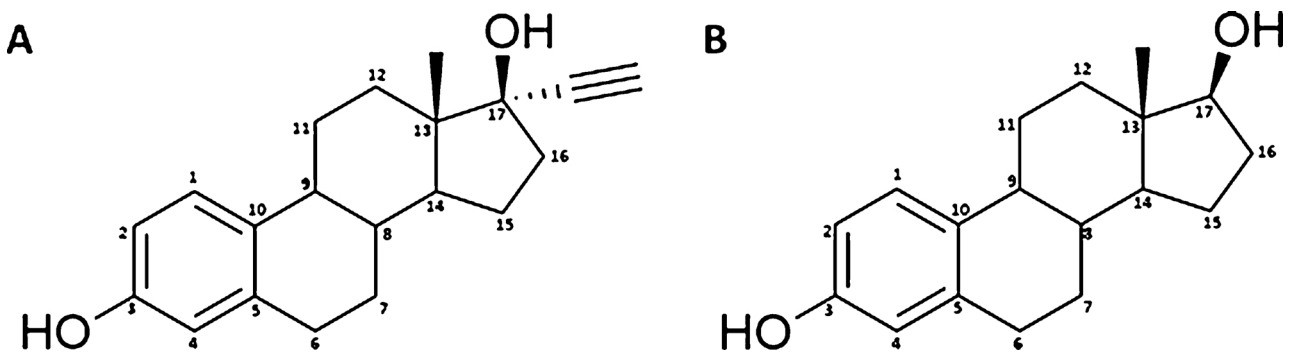

Fig. 2. Structures of EE2 and estradiol.