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Impact of *Alternaria* toxins on CYP1A1 expression in different human tumor cells and relevance for genotoxicity

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HIGHLIGHTS

- CYP1A induction by alternariol is AhR-dependent.
- CYP1A induction by alternariol has no substantial impact on genotoxicity.
- ATX-II acts as an inducer of CYP1A expression.

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ABSTRACT

The *Alternaria* toxins alternariol (AOH) and alternariol monomethyl ether (AME) have been reported previously to act as activators of the aryl hydrocarbon receptor (AhR) in murine hepatoma cells, thus enhancing the expression of cytochrome P450 (CYP) 1A monooxygenases. Concomitantly, both benzopyrones represent substrates of CYP1A, giving rise to catecholic metabolites. The impact of AOH and AME on CYP1A expression in human cells of different tissue origin colon (HT29), esophagus (KYSE510), liver (HepG2) and their effects on cell viability, generation of reactive oxygen species (ROS) and DNA integrity were investigated. ROS production was induced by both mycotoxins in all cell lines with AOH exhibiting the highest potency in esophageal cells concomitant with the most prominent CYP1A induction level. Of note, altertoxin-II (ATX-II), the more potent DNA-damaging mutagen formed by *Alternaria alternata*, induces CYP1A even at significant lower concentrations. AhR-siRNA knockdown in human esophageal cells supported the hypothesis of AhR-mediated CYP1A1 induction by AOH. However, DNA damage was minor at CYP1A1-inducing AOH concentrations. AhR-depletion did not affect the DNA-damaging properties of AOH indicating no substantial impact of AhR in this regard. However, in combination with xenobiotics prone to metabolic activation by CYP1A the induction of CYP1A by *Alternaria* toxins deserves further attention.

1. Introduction

Moulds of the genus *Alternaria* are ubiquitous pests known to infest various foods and feed (Ostry, 2008; Solfrizzo et al., 2004). *Alternaria spp.* are able to produce diverse secondary metabolites under a wide range of environmental conditions (Magan, 1984). Their chemical structures span the group of benzopyrones, tetramic acid derivatives and perylene quinones. Two major metabolites of *Alternaria*, the benzopyrones alternariol (AOH) and its 9-*O*-monomethyl ether (AME) are detected in a variety of different food products, bearing a potential risk for health of man and animals (Fig. 1). The consumption of *Alternaria* contaminated grain has been associated with an increased incidence of

Abbreviations: AhR, aryl hydrocarbon receptor; AME, alternariol monomethyl ether; AOH, alternariol; ATX-II, altertoxin II; B[*a*]P, benzo[*a*]pyrene; cRNA, control siRNA; CYP1A, cytochrome P450 1A; DCF, dichlorofluorescein; DCFH-DA, dichloro-dihydro-fluorescein diacetate; DMSO, dimethlysulfoxid; EROD, ethoxyresorufin *O*-deethylase; MEN, menadione; ROS, reactive oxygen species; siRNA, small interfering ribonucleotide acid; SRB, sulforhodamine B; WST-1, stable tetrazolium salt.

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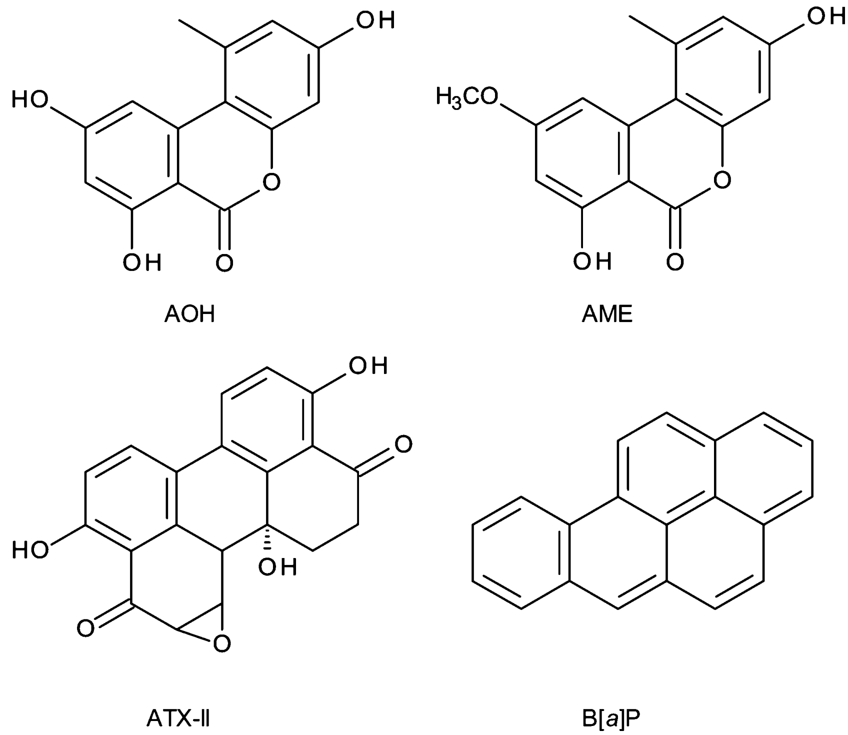

Fig. 1. Structures of the benzopyrones alternariol (AOH) and alternariol monomethyl ether (AME) the perylene quinone altertoxin-II (ATX-II) and the polycyclic aromatic hydrocarbon benzo[*a*]pyrene (B[*a*]P).

esophageal cancer in Linxian, China (Liu et al., 1992). Furthermore *Alternaria* extracts as well as the single toxins AOH and AME have been described to possess genotoxic and mutagenic properties (Brugger et al., 2006; Dong et al., 1987; Fehr et al., 2010, 2009; Lehmann et al., 2006; Liu et al., 1992, 1991; Schrader et al., 2006). The perylene quinone altertoxin II (ATX-II, Fig. 1) has been reported being even more potent than the two benzopyrones regarding genotoxic and mutagenic effects potentially due to a different mechanism of action (Fleck et al., 2012; Schwarz et al., 2012; Stack and Mazzola, 1989; Tiessen et al., 2013b). Nevertheless, concerning the underlying modes of action data are still limited.

Previously we demonstrated that AOH and AME interfere with the activity of human topoisomerases (Fehr et al., 2009). Thereby the mode of action is based on the stabilization of topoisomerase II-DNA-intermediates, leading to the conclusion that AOH and AME act as topoisomerase II poisons. In contrast, ATX-II in genotoxic concentrations did not show any impact on the stability of these intermediates (Tiessen et al., 2013b). Recently we showed that AOH and AME modulate the redox balance in human colon carcinoma cells yet without apparent negative impact on DNA integrity (Tiessen et al., 2013a). Both mycotoxins represent substrates of cytochrome P450 monooxygenases, thus resulting in hydroxylated metabolites, yielding either catechols or hydroquinones (Pfeiffer et al., 2007b). Pfeiffer et al. (2008) reported the highest activity of the aryl hydrocarbon receptor (AhR)-regulated enzyme CYP1A1 for AOH and AME. The hydroxylation of AOH and AME was studied under *in vivo*-like conditions in precision-cut rat liver slices of male Sprague Dawley rats. The pattern of *in vivo* metabolites was comparable to the one of *in vitro* metabolites of AOH, clearly supporting the relevance of oxidative metabolism *in vivo* (Burkhardt et al., 2011). Fleck et al. (2014) investigated the metabolism of ATX-II in the intestinal Caco-2 transwell system and observed a very low absorption of the mycotoxin and partly metabolism to ATX-I.

The transcription factor aryl hydrocarbon receptor (AhR) is known to mediate the biochemical and toxic effects of dioxins, polyaromatic hydrocarbons (PAHs) and related compounds (Abel and Haarmann-Stemmann, 2010). It controls the regulation of drug metabolizing enzymes, predominantly the expression of the cytochrome P450 family genes and some phase II enzymes. Schreck et al. (2012) investigated the impact of AME and AOH on CYP1A1 expression in murine hepatoma cells differing in AhR-signalling. Dependency of CYP1A1 induction on AhR and aryl hydrocarbon receptor nuclear translocator (Arnt) was shown by comparison of wild type Hepa-1c1c7, Arnt-deficient Hepa-1c1c4 and AhR-deficient Hepa-1c1c12 cells. In AhR- and Arnt-expressing Hepa-1c1c7 cells, CYP1A1 protein-levels were already induced after 5 h of incubation with 40 μM AME, whereas 40 μM AOH induced CYP1A1 expression only after 24 h. As AME is a better substrate for CYP1A1 than AOH (Pfeiffer et al., 2008), it might also be a better AhR ligand. Since no induction of CYP1A1 expression by AOH or AME was detected in AhR- and Arnt-deficient cells, the hypothesis is supported that the AhR-pathway is involved in AOH-/AME-induced CYP1A1 expression in murine hepatoma cells. The AhR pathway is not only a regulator of drug metabolism, it can also be activated by endogenous ligands and influence cell cycle control, immune response and cell differentiation (Stejskalova et al., 2011).

In the present study the question was addressed, whether and to what extent AME, AOH and ATX-II impact CYP1A1 expression in human tumor cells. Whether the *Alternaria* toxins AOH and AME show selectivity towards an organ, especially regarding the AhR-mediated induction of CYP1A1, was investigated by comparing their effect on cell growth, generation of reactive oxygen species (ROS) and induction of CYP1A1 in human colon carcinoma cells HT29, in HepG2 hepatocellular carcinoma cells, which are expected to possess potent detoxification properties, and in esophageal carcinoma cells KYSE510, which are of special interest concerning the association between an increased incidence of esophageal cancer and the contamination of food with *Alternaria* toxins (Liu et al., 1992, 1991). Furthermore the question was addressed, whether in human tumor cells AOH-mediated CYP1A1 induction is dependent on AhR expression and whether