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Induction of phase-1 metabolizing enzymes by oltipraz, flavone and indole-3-carbinol enhance the formation and transport of benzo[*a*]pyrene sulfate conjugates in intestinal Caco-2 cells

Bettina Ebert [a], Albrecht Seidel [b], Alfonso Lampen [a,*]

[a] *Institut für Lebensmitteltoxikologie, Stiftung Tierärztliche Hochschule Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany*
[b] *Biochemisches Institut für Umweltcarcinogene, Prof. Dr. Gernot Grimmer Stiftung, Lurup 4, 22927 Großhansdorf, Germany*

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Abstract

The small intestine is well equipped with various phase-1 and phase-2 xenobiotic metabolizing enzymes (XME), which contribute to the detoxification process of the body. Many XME are regulated via aryl hydrocarbon receptor (AhR)-dependent pathways, and numerous naturally occurring AhR agonists (e.g. flavonoids, dietary indoles) have been identified to date. In the present study we show that pretreatment of Caco-2 cells with food-associated compounds (flavone and indole-3-carbinol) and with the anticancer chemopreventive agent oltipraz enhances the formation of the major metabolites of the procarcinogen benzo[*a*]pyrene (BP) formed by intestinal Caco-2 cells, namely BP-1-sulfate and BP-3-sulfate, and their transport to the apical compartment of a Transwell™ chamber. Oltipraz treatment was most effective in this regard followed by flavone and indole-3-carbinol. The effect observed here after pretreatment with oltipraz, flavone and I3C was the result of the induction of both CYP1A1 and CYP1B1, as was confirmed by analysis of CYP1A1 (protein and mRNA) and CYP1B1 (mRNA) expression. In summary, our study shows that the induction of both CYP1A1 and CYP1B1 resulted in an accelerated metabolism and an enhanced clearance of the potent procarcinogen BP, indicating that flavone, indole-3-carbinol and oltipraz have an impact on the biochemical barrier against BP in intestinal cells.

Keywords: Benzo[*a*]pyrene; CYP1A1; CYP1B1; Oltipraz; Flavone; Caco-2 cells

Abbreviations: ABC, ATP-binding cassette; AhR, aryl hydrocarbon receptor; BP, benzo[*a*]pyrene; 3-OH-BP, 3-hydroxybenzo[*a*]pyrene; INF, indeno[1,2,3-cd]fluoranthene; 3-MC, 3-methylcholanthrene; β-NF, β-naphthoflavone; CYP, cytochrome P450; DMSO, dimethylsulfoxide; GST, glutathione S-transferases; I3C, indole-3-carbinol; MRP, multidrug resistance-associated proteins; PAH, polycyclic aromatic hydrocarbons; PBS, phosphate buffered saline; SULT, cytosolic sulfotransferases; TBS, tris-buffered saline; Tris, tris-(hydroxymethyl)-aminomethane; UGT, UDP-glucuronosyltransferase; XME, xenobiotic metabolizing enzymes

* Corresponding author. Tel.: +49 511 856 7602; fax: +49 511 856 7680.
E-mail address: alfonso.lampen@tiho-hannover.de (A. Lampen).

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1. Introduction

The small intestine plays an important role in the detoxification of ingested xenobiotics, since it is equipped with several phase-1 (e.g. CYP1A1, CYP1B1 and CYP3A4 [Lin et al., 1999; Ding and Kaminsky, 2003]) and phase-2 (e.g. sulfotransferases [SULT], UDP-glucuronosltransferases [UGT] and glutathione *S*-transferases [GST]) xenobiotics metabolizing enzymes (XME) (Lin et al., 1999) as well as with transport proteins of the ABC (ATP-binding cassette) superfamily (e.g. multidrug resistance associated proteins [MRP], *p*-glycoprotein [P-gp] (Taipalensuu et al., 2001; Haimeur et al., 2004)

Benzo[*a*]pyrene (BP), the model xenobiotic used in this study, is a prominent member of the group of polycyclic aromatic hydrocarbons (PAH) and it is a well-known food contaminant with high carcinogenic potential. Tobacco smoke and polluted air at specific work places are well-known sources of human exposure to BP. However, the main route for exposure of the general population to BP seems to be the food chain, which reportedly accounts for 97% of the total daily BP intake (Hattemer-Frey and Travis, 1991).

The capability of phytochemicals occurring in plant foods to modulate XME activities is believed to contribute to reduction of cancer risk and therefore is under intense investigation (Singletary et al., 1998; Lin and Lin-Shiau, 2001; Conney, 2003) The majority of these studies have focused on the modulation of phase-2 enzymes (e.g. GST and UGT [Guyonnet et al., 1999; Van der Logt et al., 2003]). However, although the activation of phase-1 enzymes (e.g. CYP1A1 and CYP1B1) is commonly associated with adverse effects, such as pro-carcinogen activation, only little is known about the effect of an increased induction of phase-1 enzymes on the detoxification process of potential carcinogens, such as PAH.

It has been shown that PAH are able to induce their own metabolism by upregulating XME in Caco-2 cells (e.g. CYP1A1, CYP1B1 and UGT) via aryl hydrocarbon receptor (AhR)-dependent pathways (Lampen et al., 2004). This has raised the question of whether non-toxic compounds that are constituents of our daily diet could act in a similar way, since recently many food-associated compounds (e.g. flavonoids, dietary indoles, polyphenolic compounds) with AhR-agonistic activities have been discovered (Denison and Nagy, 2003).

Although derived from a colon tumor, the human colon adenocarcinoma cell line Caco-2 is an established model cell line for the human small intestine, since the cells differentiate into polarized epithelial cell monolayers and show all biochemical (e.g. expression of brush border membrane enzymes, such as sucrase–isomaltase) and morphological characteristics of human small intestinal enterocytes (e.g. tight junctions, microvilli [Hidalgo et al., 1989]). Furthermore, they have been shown to be a suitable model system to study biotransformation processes [Meunier et al., 1995] since they express various phase-1 and phase-2 enzymes (e.g. CYP1A1 and CYP 1B1 [Boulenc et al., 1992; Lampen et al., 1998; Buesen et al., 2002] SULT1A1 and 1A3 [Tamura et al., 2001]), and transport proteins of the ABC superfamily, e.g. *p*-glycoprotein [Hunter et al., 1993; Taipalensuu et al., 2001], and MRP [Hirohashi et al., 2000].

In previous investigations we found that the major phase-2 metabolites of BP formed by Caco-2 cells were BP-1-sulfate and BP-3-sulfate and were transported actively to the apical (luminal) compartment of the Transwell^{TM} chamber (Buesen et al., 2002, 2003). Furthermore, the PAH did induce CYP1A1, CYP1B1, and UGT, however, it had no effect on the expression of sulfotransferases at all (Lampen et al., 2004). Therefore, we hypothesize that prior exposure of Caco-2 cells to naturally occurring weak AhR-agonists and the chemopreventive agent oltipraz may lead to a faster clearance of BP.

Oltipraz (see Fig. 1C) was originally developed as an antischistosomal agent (Gentilini et al., 1980) and is now considered one of the most promising cancer chemopreventive agents. As a substituted 1,2-dithiole-3-thione, oltipraz resembles naturally occurring compounds found in cruciferous vegetables. Oltipraz has been reported to inhibit chemically induced carcinogenesis in various organs of experimental animals (Wattenberg and Bueding, 1986) and it has been shown to be an inducer of GST (Davidson et al., 1990) and CYP1A1 (Le Ferrec et al., 2002). Flavonoids are polyphenolic compounds that are known to have beneficial health effects by mediating a broad spectrum of biological responses. Flavone, the non-substituted parent compound of the subclass of flavones has been shown to be an AhR-agonist (Ashida et al., 2000).