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Inhibition of organic anion transporter (OAT) activity by cigarette smoke condensate

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ABSTRACT

Cigarette smoke condensate (CSC) has previously been shown to impair activity and expression of hepatic drug transporters. In the present study, we provided evidence that CSC also hinders activity of organic anion transporters (OATs), notably expressed at the kidney level. CSC thus *cis*-inhibited OAT substrate uptake in OAT1- and OAT3-transfected HEK293 cells, in a concentration-dependent manner (IC_{50} = 72.1 µg/mL for OAT1 inhibition and IC_{50} = 27.3 µg/mL for OAT3 inhibition). By contrast, OAT4 as well as the renal organic cation transporter (OCT) 2 were less sensitive to the inhibitory effect of CSC (IC_{50} = 351.5 µg/mL and IC_{50} = 226.2 µg/mL, for inhibition of OAT4 and OCT2, respectively). OAT3 activity was further demonstrated to be blocked by some single chemicals present in cigarette smoke such as the heterocyclic amines AαC (IC_{50} = 11.3 µM) and PhIP (IC_{50} = 1.9 µM), whereas other major cigarette smoke components used at 100 µM, like nicotine, the nitrosamine NNK and the polycyclic aromatic hydrocarbons benzo(a)pyrene and phenanthrene, were without effect. AαC and PhIP however failed to *trans*-stimulate activity of OAT3, suggesting that they were not substrates for this transporter. Taken together, these data establish OAT1 and OAT3 transporters as targets of cigarette smoke chemicals, which may contribute to smoking-associated pharmacokinetics alterations.

1. Introduction

Cigarette smoke is a complex mixture of > 5000 chemicals, including nicotine, polycyclic aromatic hydrocarbons, heterocyclic amines, nitrosamines, aminobiphenyls and metals like cadmium, lead and arsenic (Talhout et al., 2011). It is highly toxic for human health, notably causing cancers, cardiovascular diseases, endocrine disruption and stroke (Ezzati et al., 2005; Sasco et al., 2004; Windham et al., 2005). These well-established and pleomorphic deleterious effects of smoking have been linked to the activation of various cellular and molecular pathways (Sobus and Warren, 2014), including ones related to drug detoxifying proteins. Indeed, cigarette smoke-contained chemicals like polycyclic aromatic hydrocarbons induce expression of the drug metabolizing enzymes cytochrome P-450 (CYP) 1A1 and CYP1B1 in various tissues, through mobilizing the aryl hydrocarbon receptor signaling cascade (Kitamura and Kasai, 2007). CYP1A1/1B1 can in turn bio-activate polycyclic aromatic hydrocarbons into reactive metabolites, that cause DNA adducts and mutation, which ultimately may result in cancers, notably lung cancers (Moorthy et al., 2015; Nebert et al., 2004). Up-regulation of hepatic CYPs, but also of other drug metabolizing enzymes like glutathione S-transferases, by cigarette smoke additionally results in increased metabolism of various drugs, and thereby contributes to impaired pharmacokinetics in smokers (Miller, 1989; Sohn et al., 2015).

In addition to drug metabolizing enzymes, drug transporters, which belong to either the solute carrier (SLC) or the ATP-binding cassette (ABC) transporter superfamilies, are now recognized as major contributors to drug absorption, disposition and elimination (Giacomini et al., 2010). These transporters, mainly expressed in organs/tissues implicated in drug disposition and elimination such as gut, blood-brain barrier, liver and kidney, also constitute targets for various cigarette smoke chemicals. Indeed, some polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbon metabolites have been shown to be handled, and/or to regulate, the ABC transporters breast cancer resistance protein (BCRP/*ABCG2*) and multidrug resistance protein (MRP) 4 (*ABCC4*) (Ebert et al., 2005; Gelhaus et al., 2012). The heterocyclic amine 2-amino-1-methyl-6-phenylimidazol[4,5-*b*]pyridine (PhIP) as well as the heavy metal cadmium interact with BCRP activity (Kummu et al., 2012; van Herwaarden et al., 2003). Cadmium is moreover transported by the SLC transporters organic cation

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transporter (OCT) 1 (*SLC22A1*), OCT2 (*SLC22A2*), multidrug and toxin extrusion protein (MATE) 1 (*SLC47A1*) and MATE2-K (*SLC47A2*) (Soodvilai et al., 2011; Yang et al., 2017). Another heavy metal present in cigarette smoke, *i.e.*, arsenic, is handled by the ABC transporters MRP1 (*ABCC1*) and MRP2 (*ABCC2*) (Leslie, 2012) and regulates their expression (Takeshita et al., 2003; Vernhet et al., 2001).

Interestingly, cigarette smoke condensate (CSC) and cigarette smoke extract as whole mixtures impair activity and/or expression of various ABC and/or SLC transporters (Pan et al., 2009; Sayyed et al., 2016; Takano et al., 2016; van der Deen et al., 2007). CSC notably blocks activity of hepatic transporters handling anionic drugs such as the canalicular ABC transporter MRP2 and the sinusoidal SLC transporters organic anion transporting polypeptide (OATP) 1B1 (*SLCO1B1*) and OATP1B3 (*SLCO1B3*) (Sayyed et al., 2016). Functional activity of MRP1, which handles drugs conjugated to anionic ligands (Cole and Deeley, 2006), has also been shown to be altered in lung epithelial cells exposed to cigarette smoke extract (van der Deen et al., 2007). Whether CSC may additionally block other SLC drug transporters handling anionic drugs, such as organic anion transporter (OAT) 1 (*SLC22A6*) and OAT3 (*SLC22A8*), remains however unknown. This issue is likely important to consider because OAT1 and OAT3, notably located at the basolateral pole of proximal tubule cells and acting as dicarboxylate exchangers, play a major role in tubular secretion of drugs, and by this way, in their renal elimination and pharmacokinetics (Burckhardt, 2012). OAT1 and OAT3 are consequently identified as drug transporters that have to be studied during the pharmaceutical development of new molecular entities according to a regulatory point of view, notably because inhibition of these transporters can be the cause of drug-drug interactions (DDI) (Giacomini et al., 2010; Maeda and Sugiyama, 2013). The present study was therefore designed to analyze the effects of CSC and various cigarette smoke-contained chemicals on activity of these OATs.

2. Materials and methods

2.1. Chemicals

CSC was supplied by Murty Pharmaceuticals (Lexington, KY, USA). It was prepared by smoking University of Kentucky's 3R4F standard research cigarettes on a Federal Trade Commission smoke machine (Nagaraj et al., 2006). The amount of smoke particulates, collected on a glass fiber filter, was determined by weight increase of the filter. It corresponds to a mean of 9.5 mg total particular matter/cigarette (Eldridge et al., 2015). CSC was finally prepared by dissolving the collected smoke particulates in dimethyl sulfoxide (DMSO) to yield a 40 mg/mL solution. Probenecid, amitriptyline, verapamil, 6-carboxyfluorescein, nicotine, benzo(a)pyrene, phenanthrene, 4-aminobiphenyl, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), glutarate, sodium arsenate and lead chloride were provided by Sigma-Aldrich (Saint-Quentin Fallavier, France), whereas cadmium chloride was from Merck Millipore (Fontenay-sous-Bois, France). The heterocyclic amines PhIP, 2-amino-9H-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9H-pyrido[2,3-*b*]indole (MeAαC) and 3-amino-1-methyl-[5H]-pyrido[4.3-b]indole (Trp-P-2) were from Santa Cruz Biotechnology (Dallas, TX, USA). [6,7-^{3}H(N)]-estrone-3-sulfate (E3S) (specific activity 51.8 Ci/mmol), p-[glycyl-2-^{3}H]-aminohippuric acid (PAH) (specific activity 3.0 Ci/mmol), and [1-^{14}C] tetra-ethylammonium (TEA) (specific activity 3.5 mCi/mmol) were from Perkin-Elmer (Courtaboeuf, France).

2.2. Cell culture

HEK293 cells overexpressing OAT1 (HEK-OAT1 cells), OAT3 (HEK-OAT3 cells), OCT2 (HEK-OCT2 cells) or MATE2-K (HEK-MATE2-K cells), kindly provided by Technologie Servier (Orléans, France) and characterized in previous studies (Bruyere et al., 2017; Chedik et al., 2017), were cultured in Dulbecco's modified Eagle medium (DMEM) (Life Technologies, Saint Aubin, France) supplemented with 10% (vol/vol) fetal calf serum, 10 IU/mL penicillin, 10 µg/mL streptomycin, 1% nonessential amino acids, and 1 µg/mL insulin. HEK293 cells overexpressing OAT4 (HEK-OAT4 cells) were prepared by transduction of HEK293 cells by lentiviral pLV-EF1-hOAT4-hPGK-GFP vector, as previously described (Jouan et al., 2014). Construction of the lentiviral vector, production of lentivirus supernatants, transduction of HEK293 cells and cloning and initial characterization of HEK-OAT4 cells were performed by Vectalys (Labège, France). HEK-OAT4 cells were next routinely cultured in DMEM medium as described above.

2.3. Transporter activity assays

The effects of CSC and cigarette smoke-contained chemicals on SLC transporter activities were analyzed through determining intracellular accumulation of reference substrates using a well-defined transport medium, as previously reported (Chedik et al., 2017; Sayyed et al., 2016). The transport assay medium consisted of 5.3 mM KCl, 1.1 mM KH_2PO_4, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 11 mM D-glucose, 10 mM HEPES, and 136 mM NaCl; pH was adjusted to 7.4 value, except for the pH-sensitive MATE2-K transport assay for which pH was set at 8.4 (Chedik et al., 2017). Cells were first washed with phosphate-buffered saline and then incubated at 37 °C for 5 min with transport assay buffer containing reference substrates, in the presence or absence of reference transporter inhibitors, CSC or cigarette smoke chemicals. The used substrates were: 33.3 nM [^{3}H]-PAH (for OAT1 activity), 3.86 nM [^{3}H]-E3S (for OAT3 or OAT4 activities), 10 µM 6-carboxyfluorescein (for OAT1 or OAT3 activities) and 28.6 nM [^{14}C]-TEA (for OCT2 or MATE2-K activities). The used reference inhibitors were: 10 mM probenecid (for OAT1 and OAT3 activities), 2 mM probenecid (for OAT4 activity), 200 µM amitriptyline (for OCT2 activity) and 200 µM verapamil (for MATE2-K activity). Cells were next washed twice with ice-cold phosphate-buffered saline (PBS) and finally lysed in distilled water. Intracellular accumulation of radiolabeled substrates was next measured by scintillation counting of cell lysates with a Beckman LS6500 (Beckman Coulter Inc., Fullerton, CA, USA). Intracellular accumulation of 6-carboxyfluorescein was determined by spectrofluorimetry using a SpectraMax Gemini SX spectrofluorometer (Molecular Devices, Sunnyvale, CA, USA); excitation and emission wavelengths were 492 and 517 nm, respectively. Values of substrate accumulation were then normalized to total protein content, determined by the Bradford method (Bradford, 1976). Data were finally expressed as percentage of substrate accumulation found in control cells not exposed to reference inhibitor, CSC or cigarette smoke chemicals. Percentage of reduction of substrate accumulation was defined as 100% (accumulation in control cells) minus percentage of substrate accumulation in the presence of CSC or cigarette smoke chemical, whereas percentage of stimulation of substrate accumulation corresponded to percentage of substrate accumulation in the presence of CSC or cigarette smoke chemical minus 100% (accumulation in control cells). Data were also alternatively expressed as percentage of transporter activity found in control cells, arbitrarily set at 100%, according to the following equation:

$$\%\text{SLC transporter activity} = \frac{(\text{Accumulation}_{\text{CSC/chemical}} - \text{Accumulation}_{\text{Reference inhibitor}}) \times 100}{\text{Accumulation}_{\text{Control}} - \text{Accumulation}_{\text{Reference inhibitor}}} \quad \text{(A)}$$

where $\text{Accumulation}_{\text{CSC/chemical}}$ corresponds to substrate accumulation in the presence of CSC or cigarette smoke chemical, $\text{Accumulation}_{\text{Control}}$ corresponds to substrate accumulation in control cells and $\text{Accumulation}_{\text{Reference inhibitor}}$ corresponds to substrate accumulation in the presence of a reference transporter inhibitor.

Half maximal inhibitory concentrations (IC_{50}) of CSC or some cigarette smoke-contained chemicals towards transporter activities were determined from nonlinear regression of concentration-response data