BIOACTIVATION AND EFFECTS OF ENVIRONMENTAL POLLUTANTS IN HUMAN AND RODENT BLOOD VESSEL ENDOTHELIAL CELLS

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Introduction

Recent epidemiological studies reveal associations between exposure to environmental pollutants and cardiovascular disorders in humans. Elevated serum concentrations of polychlorinated biphenyls (PCBs) have for instance been associated with cardiovascular risk factors such as hypertension (1-3). Exposure to the carbonate plastic monomer bisphenol A (BPA) has been associated with an increased incidence of cardiovascular disease and atherogenic changes in the vascular wall (4-6). The contention that the human cardiovascular system is a sensitive target for toxic chemicals gain support from our earlier and recent experimental studies in rodents, birds and fish, as well as in cultured human primary endothelial cells. It is also compatible with earlier observations that certain polycyclic aromatic hydrocarbons (PAHs) are environmental carcinogens that may also contribute to atherosclerosis in mice and birds (7,8).

In this presentation we will briefly discuss effects of Ah receptor (AhR) agonists (e.g. the coplanar PCB126 or BNF, ß-naphthoflavone) on the expression of cytochrome P450 (CYP)1 enzymes in various endothelia in rodents *in vivo* or *ex vivo*, as well as in cultured human umbilical vein endothelial cells (HUVEC). The CYP1-dependent bioactivation and irreversible binding of prototype polyaromatic hydrocarbons (PAH) and heterocyclic amines such as benzo(a)pyrene (BaP), 7,12-dimethyl-benz(a)anthracene (DMBA) and 3-amino-1,4-dimethyl-5*H*-pyrido- [4,3-*b*]indole (Trp-P1) in these endothelia will be reviewed. We will also report how PCB126 affects vasoactive factors in HUVEC, and how these effects are modulated by physiological 17ß-oestradiol concentrations. Some effects of PCB126, 1-nitropyrene (1-NP) and bisphenol A (BPA) on biomarkers for endothelial dysfunction, cell stress and DNA damage in HUVEC will finally be presented.

Material and methods

Human umbilical vein endothelial cells (HUVEC) were purchased from Science Cell Research laboratories, Carlsbad, CA. C57Bl mice and Wistar or Sprague Dawley rats were purchased from various suppliers. All animal experiments were approved by the Local Ethical Committee for Research on Animals in Uppsala and the studies followed the guidelines laid down by the Swedish and European Union legislation on animal experimentation. Rodents, tissue-slices and cultured cells were treated with model chemicals as previously described. Tape section and light microscopy autoradiographic imaging using ^{3}H-labelled BaP, DMBA and Trp-P-1 and immunohistochemistry was performed as previously described (9-19). Precision-cut tissue slices for in vitro autoradiography were prepared as described in (14) and the slices were incubated with various ^{3}H-labelled chemicals. HUVEC were exposed to

various compounds and the detection of biomarkers of endothelial dysfunction, DNA damage were performed as described (20-22). Finally, female Fischer rats were exposed to BPA (0.025, 0.25 and 2.5 mg/l) and fructose (50 g/l) in the drinking water from 5 to 15 weeks of age to mimic human exposure (unpublished data).

Results and discussion

Co-localization of CYP1A1 expression and BaP, DMBA and Trp-P-1 adduct formation in endothelial linings

As demonstrated by immunohistochemistry, a high CYP1A immunoreactivity occurred in capillaries of the heart, skeletal muscle, uterus and in blood-brain interfaces such as the leptomeninges and *plexus choroideus*, whereas no expression was observed for instance in cerebral capillary endothelial cells of mice treated with AhR agonists (9-11). No, or very low constitutive immunoreactivities were observed in these endothelia in vehicle-treated animals. No basal or induced CYP1B1 expression was observed in endothelial cells, while a weak CYP1B1 immunostaining was detected in the muscle layer of small arteries. It should be noted that in subcellular preparations of whole organs, e.g. heart and brain, the CYP1A1 in endothelial cells is diluted due to cells that do not express high levels of CYP1A1, for examples myocytes or neurons, in excess. A cell-specific metabolism in endothelial cells may therefore remain undetected due to the presence of metabolically inactive cells. In order to detect minor sites of bioactivation such as endothelial linings we employed light microscopic autoradiographic imaging to examine the bioactivation and subsequent irreversible binding of the radiolabelled prototype toxicants in tissues of animals pretreated with AhR-agonists. As determined by light microscopic autoradiography of AhR-agonist-treated mice exposed to ^{3}H-labelled BaP, DMBA or Trp-P-1 and birds exposed to ^{3}H-Trp-P-1 a significant accumulation of non-extractable radioactivity occurred in endothelial linings (9-18). The bound radioactivity occurred in the nuclei and the perinuclear cytoplasm, suggesting that the autoradiograms depict both DNA- and protein-bound adducts. Since the binding sites of ^{3}H-labelled BaP, DMBA or Trp-P-1 corresponded with the sites of CYP1A1 induction, we concluded that rodents express a constitutively low but highly inducible and functional CYP1A1 in endothelial cells. The binding of reactive metabolites in endothelial cells exceeded the binding in all other cell types in AhR-agonist treated mice and was abolished by pretreatment with the CYP1A1 inhibitor ellipticine, supporting a CYP1A1-catalysed metabolic activation *in situ* to a reactive species (9, 10,12). These findings imply that there is a preferential CYP1A1-catalysed formation of reactive metabolites from all three carcinogens in endothelial cells expressing high CYP1A1 levels. Interestingly, however, carcinogenesis in endothelial cells is a relative rare finding, suggesting that degenerative lesions and cell death may be more prevalent responses to metabolism-activated carcinogens/mutagens in these cells. Experiments with ^{3}H-DMBA and ^{3}H-Trp-P-1 in HUVEC confirmed that AhR-agonists induced an increased bioactivation, suggesting that also human endothelial cells should be targets for toxicity of reactive intermediates formed from CYP1A1-activated carcinogens/mutagens (17-18). This conclusion is supported by immunohistochemical studies on the heavily vascularized human endometrium demonstrating an expression of CYP1A1 and CYP1B1 protein in and around human endometrial blood vessels, although a large interindividual

variation was observed (19). None of the endometrial biopsy samples displayed vascular expression of CYP2A6, CYP2B6, CYP2C8/2C9/2C19, CYP2D6, or CYP3A4/5 protein.

Effects of PCB 126, 1-NP, and BPA on biomarkers of endothelial dysfunction and cell stress in endothelial cells

In vitro studies demonstrated that PCB126 increased the levels of vasoconstriction factors and decreased the levels of vasodilating factors in cultured HUVEC in a fashion that is characteristic for endothelial dysfunction related to human hypertension. The study showed that the co-planar PCB126 induced expression of the endothelium-derived vasoconstriction factor COX-2 and stimulated formation of the vasoconstrictor prostaglandin $PGF_{2\alpha}$ via the AhR in HUVEC (20). COX-2 is known to play a role in hypertension by catalysing the formation of vasoconstriction prostaglandins and by stimulating reactive oxygen species (ROS) production. Further studies demonstrated that PCB126 increased the production of the vasoconstriction prostaglandin $PGF_{2\alpha}$ and ROS in HUVEC. The relationship between increased ROS production and human hypertension is well established, ROS promotes vasoconstriction by stimulating the production of vasoconstriction prostaglandins and by reducing bioavailability of the vasorelaxing factor NO. Indeed, exposure to PCB126 slightly reduced the production of NO in HUVEC. Furthermore, the PCB126-induced mRNA expressions of CYP1A1, CYP1B1 and COX-2 in HUVEC were enhanced in the presence of physiological levels of 17β-estradiol. This suggests that increased levels of oestrogen stimulate AhR-dependent transcription of genes previously associated with endothelial dysfunction and hypertension.

In another study we have examined the effects of a nitrated PAH, 1-nitropyrene, that is abundant in diesel exhausts (21). The results revealed that 1-NP induced DNA damage, increased levels of ROS and increased protein expression of the endoplasmic reticulum stress chaperone GRP78 in cultured HUVEC. Induction of CYP1A1 by PCB126 as well as inhibition of nitroreductive metabolism by dicoumarol attenuated the induction of DNA damage, intracellular ROS levels and GRP78 expression. This suggests that the effects of 1-NP on HUVEC were mediated by metabolites mainly formed at nitroreduction and not by CYP1-dependent bioactivation to reactive intermediates.

Recent in vitro studies demonstrated that bisphenol A increased the mRNA expression of genes that regulate vasoconstriction and angiogenesis in HUVEC (eNOS, VEGF, VEGFR2, connexin 43 and ACE1) and in human cardiomyocytes (eNOS and ACE1) (22). The results also showed that BPA increased the expression of P-eNOS(ser1177) and the production of NO in HUVEC. NO is the main effector molecule in angiogenesis downstream of VEGF. Based on the findings that BPA increase the expression of proangiogenic factors we investigated whether BPA could stimulate *in vitro* angiogenesis in HUVEC using the endothelial tube formation assay. The results demonstrated that BPA increased HUVEC tube formation suggesting that BPA can act directly on the endothelium and stimulate angiogenesis. Long-term exposure in rats revealed that environmentally relevant levels of BPA, increased the cardiac mRNA expression of genes that regulate vasoconstriction and angiogenesis. Ten weeks exposure of rats from preadolescence to adulthood to BPA in the drinking water increased the

expression of eNOS, VEGF, VEGFR2 and ACE1 in the heart. Taken together, the genes that were upregulated in rat cardiac tissues *in vivo* were also upregulated in human endothelial cells and cardiomyocytes *in vitro.* The heart is a heavily vascularized tissue that consists mainly of cardiac endothelial cells and cardiomyocytes and although cardiomyocytes dominate the volume of the myocardium the number of endothelial cells exceeds the number of cardiomyocytes by approximately three to one. Thus, the effects of BPA on eNOS VEGF, VEGFR2 and ACE1 mRNA expression in rat cardiac tissues are most likely to be related to an effect of BPA on endothelial cells but may also involve cardiomyocytes.

We conclude that endothelial cells may be targets for bioactivation and toxicity of environmental pollutants. The immunohistochemical and autoradiographic data demonstrated a differential expression of CYP1 enzymes and metabolic activation of pollutants in various endothelial linings suggesting that some but not all endothelial linings may be targets for xenobiotics metabolised by AhR-regulated enzymes. Studies on the effects of PCB126, 1-nitropyrene and BPA in cultured human primary endothelial cells demonstrated up-regulation of various biomarkers for endothelial dysfunction and cell stress suggesting that the human endothelium may be a sensitive target for these pollutants. The bioactivation and effects of environmental pollutants in endothelial cells should be further studied in order to unravel the role of these chemicals in human cardiovascular disease.

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