Toxicology Letters 206 (2011) 204–209

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Gene expression and inducibility of the aryl hydrocarbon receptor-dependent pathway in cultured bovine blood lymphocytes

Flavia Girolami[a,1], Veronica Spalenza[b,1], Monica Carletti[a], Giovanni Perona[c], Paola Sacchi[b], Roberto Rasero[b], Carlo Nebbia[a,*]

[a] Department of Animal Pathology, University of Turin, Via Leonardo da Vinci 44, 10095 Grugliasco, Italy
[b] Department of Animal Production, Epidemiology and Ecology, University of Turin, Via Leonardo da Vinci 44, 10095 Grugliasco, Italy
[c] Centro Interdipartimentale Servizio Ricoveri Animali (C.I.S.R.A.), University of Turin, Via Leonardo da Vinci 44, 10095 Grugliasco, Italy

ARTICLE INFO

Article history:
Received 10 June 2011
Received in revised form 12 July 2011
Accepted 14 July 2011
Available online 23 July 2011

Keywords:
Aryl hydrocarbon receptor
Dioxin-like compounds
Lymphocytes
Cattle
Real-time PCR

ABSTRACT

The exposure to dioxin-like (DL) compounds, an important class of persistent environmental pollutants, results in the altered expression of target genes. This occurs through the binding to the aryl hydrocarbon receptor (AhR), the subsequent dimerization with the AhR nuclear translocator (ARNT), and the binding of the complex to DNA responsive elements. A number of genes are up-regulated, including, among others, the AhR repressor (AHRR) and several biotransformation enzymes, such as the members of CYP1 family and NAD(P)H-quinone oxidoreductase (NOQ1). The expression and the inducibility of the above genes were investigated in mitogen-stimulated cultured blood lymphocytes from cattle, which represent a notable source of DL-compound human exposure through dairy products and meat. As assessed by real-time PCR, all the examined genes except CYP1A2 and NQO1 were detected under basal conditions. Cell exposure to the DL-compounds PCB126 or PCB77 in the 10^{-6}–10^{-9} M concentration range resulted in a 2–4-fold induction of CYPIA1 and CYP1B1, which was antagonized by α-naphthoflavone or PCB153. This study demonstrates for the first time the presence and inducibility of the AhR pathway in easily accessible cells like bovine peripheral lymphocytes and prompts further investigations to verify whether similar changes could occur under *in vivo* conditions.

1. Introduction

Polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzofurans (PCDFs) and polychlorobiphenyls (PCBs) are chlorinated aromatic hydrocarbons long recognized as persistent and widespread environmental pollutants of great concern to human health. The prolonged exposure to such contaminants causes a broad spectrum of adverse effects, including tumor promotion and teratogenesis, as well as perturbation of the immune, endocrine, and nervous systems (Mandal, 2005). Among hundreds of congeners, only a few, collectively identified as dioxin-like (DL) compounds, are capable of binding, albeit with different affinities, to a specific cytosolic receptor, the aryl hydrocarbon receptor (AhR), which is responsible for most of their toxic effects. The accumulation of DL-compounds at the top soil level, following combustion fallout or organic fertilization with sewage sludges, represents an important source of contamination for food producing animals at the farm level (Brambilla et al., 2004). Due to the high degree of lipophilicity, these substances tend to build up to a significant extent in animal productions (eggs, fat, meat, milk and dairy products), which represent the main source of exposure for humans (Donato et al., 2006; Hirako, 2008), and are therefore included in the monitoring procedures for risk assessment and management.

The AhR is an intracellular transcription factor, which, in the absence of a ligand, is stabilized in a cytosolic protein complex with heat shock protein 90 kD (Hsp90), hepatitis B virus X-associated protein (XAP2) and p23. Ligand binding leads to nuclear translocation, release of the chaperon proteins, and formation of a heterodimer with its partner molecule, the AhR nuclear translocator (ARNT). The AhR/ARNT complex interacts with xeno-

Abbreviations: AhR, aryl hydrocarbon receptor; AhRR, aryl hydrocarbon receptor repressor; ARNT, AhR nuclear translocator; CYP, cythochrome P450; cDNA, complimentary DNA; DL, dioxin-like; DMSO, dimethylsulfoxide; Hsp90, heat shock protein 90 kD; α-NAF, α-naphthoflavone; β-NAF, β-naphthoflavone; NQO1, NAD(P)H-quinone oxidoreductase; PBMC, peripheral blood mononuclear cell; PCB, polychlorobiphenyl; PCDD, polychlorodibenzo-p-dioxin; PCDF, polychlorodibenzofuran; PHA, phytohemagglutinin; q-PCR, real-time PCR; TCDD, 2,3,7,8 tetrachloro-dibenzo-*p*-dioxin; TEF, toxic equivalency factor; XAP2, hepatitis B virus X-associated protein; XME, xenobiotic metabolizing enzyme; XRE, xenobiotic responsive element.

* Corresponding author. Tel.: +39 011 670 9015; fax: +39 011 670 9016.
E-mail address: carlo.nebbia@unito.it (C. Nebbia).

[1] These authors contributed equally to this work.

0378-4274/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2011.07.014

biotic responsive elements (XREs) located in the regulatory region of a number of target genes encoding for phase I and phase II biotransformation enzymes, including cythochrome P450 (CYP) 1A1, 1A2, and 1B1, and NAD(P)H-quinone oxidoreductase (NQO1), as well as proteins involved in the regulation of development, proliferation and differentiation (Abel and Haarmann-Stemmann, 2010). It is worth noting that CYP1A1 (Olson et al., 1994) and, to a lesser extent, CYP1B1 (Santostefano et al., 1997) are likely involved in the oxidative biotransformation of DL-compounds yielding –OH derivatives, which may be subsequently glucuronidated or sulphated and then excreted via the biliary and urinary routes. In rodents and in humans CYP1A1 and CYP1B1 are predominantly expressed in extra-hepatic tissues, while CYP1A2 is constitutively expressed to a notable extent in the liver (Ioannides, 2006). Finally, the activation of the AhR-dependent pathway induces the up-regulation of the AhR repressor (AhRR), which inhibits the AhR transcriptional activity in a negative feedback loop manner (Hahn et al., 2009).

Although the liver plays a capital role in the biotransformation of foreign compounds, many xenobiotic metabolizing enzymes (XMEs) are also expressed in extrahepatic organs or tissues (Ding and Kaminsky, 2003; Pavek and Dvorak, 2008), including circulating lymphocytes, where they may be involved in the bioactivation of a number of pro-carcinogens, like polycyclic aromatic hydrocarbons (Fung et al., 1999) and aflatoxin B1 (Wilson et al., 1995). As blood cells are readily accessible from living animals, they have been proposed as surrogate for XME expression in liver or other target organs (Furukawa et al., 2004). In particular, several reports have addressed the expression of genes involved in the AhR signaling pathway in leukocytes from laboratory species and humans (Saurabh et al., 2010; Shah et al., 2009; Siest et al., 2008), and changes in their pattern of expression and/or inducibility have been found in circulating lymphocytes from 2,3,7,8 tetrachloro-dibenzo-*p*-dioxin (TCDD)-exposed individuals approximately 20 years after Seveso's (Italy) accident (Landi et al., 2003).

The modulation of AhR-dependent gene transcription caused by the exposure to DL-compounds has been mainly characterized in liver cell lines (Beedanagari et al., 2010; Kim et al., 2006) and in hepatocyte primary cultures from laboratory animals and humans (Budinsky et al., 2010; Le Vee et al., 2010); by contrast, only scant information is available for food producing species (Guruge et al., 2009; Kennedy et al., 1996; Landi et al., 2003). As regards cattle, which have been implicated in several outbreaks of environmental contamination from DL-compounds (Brambilla et al., 2004), both CYP1A1 and CYP1B1 transcripts have been detected in calf liver and found to be induced upon the exposure to TCCD and other PCDDs and PCDFs (Guruge et al., 2009). However, there are no data about bovine blood cells. Here we report for the first time the expression of a number of AhR-dependent genes and their *in vitro* inducibility in cattle peripheral blood lymphocytes. It is anticipated that data generated from this study will be applied to monitor the changes in target gene expression in circulating mononuclear cells from cattle reared in dioxin-contaminated areas.

2. Materials and methods

2.1. Reagents

Histopaque-1077, dimethylsulfoxide (DMSO), β-naphthoflavone (β-NAF), α-naphthoflavone (α-NAF) and all cell culture reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA). PCB77, PCB126 and PCB153 were supplied by LabService Analytica S.r.l. (Anzola Emilia, BO, Italy). All the materials for the real-time PCR (q-PCR) analysis (including RNA extraction and reverse transcription) were from Qiagen (Valencia, CA, USA).

2.2. Blood lymphocyte isolation and culture

For each experiment, approximately 300 ml of peripheral venous blood was collected from a healthy cow in a transfusion bag containing citrate–phosphate–dextrose–adenine (CPDA-1) as the anticoagulant preservative. Lymphocytes were isolated with the method described by Spalenza et al. (2010) with slight modifications. In brief, all the washes were performed with RPMI-1640 medium, and all the procedures were conducted at room temperature. Although these cell preparations are frequently termed as peripheral blood mononuclear cells (PBMCs) for the presence of monocytes, in our case it has been demonstrated that the majority of them (90%) are lymphocytes (Spalenza et al., 2010). Thus, for ease of nomenclature we will refer to them exclusively as lymphocytes. After isolation, cells were counted with a hemocytometer and viability was assessed with the trypan blue exclusion test (>90%). Cells were seeded at 2×10^6 cells/ml into 10-cm dishes and cultured in RPMI-1640 medium, supplemented with 10% heat-inactivated horse serum, 2 mM L-glutamine and 10 μg/ml phytohemagglutinin (PHA), at 37 °C and 5% CO_2.

2.3. Chemical treatments

All the ligands were dissolved in DMSO, whose final concentration in the growth medium did not exceed 0.1% (v/v). After 2 h of pre-incubation, lymphocytes were treated with the solvent alone (used as control) or with the test compounds under the following conditions. For the time-course experiments, cells were exposed to 100 nM β-NAF or PCB126 and lysed after 2, 4, 16, 24 and 48 h. For the dose–response experiments, lymphocytes were treated with increasing logarithmic concentrations (from 0.1 nM to 1 μM) of PCB126 or PCB77 for 2 h. Finally, α-NAF and PCB153 treatments were performed exposing cells to 100 nM for 1 h before the incubation with 100 nM PCB126 for 2 h. Lymphocyte viability, assessed by the trypan blue exclusion test, was not affected by any of the employed chemical compounds at any examined concentrations.

2.4. RNA extraction and reverse transcription

Total RNA was isolated using RNeasy Mini Kit, according to the manufacturer's protocol. RNA purity and quantity was evaluated by absorbance readings using the NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Illkirch Cedex, France). The ratio of the optical densities measured at 260 and 280 nm were >1.9 for

Table 1
Primers for real-time PCR.

Gene	Accession no.	5′ → 3′ sequence	Amplicon size
AhR	XM_612996	F: GTGCAGAAAACTGTCAAGCC R: GCAACATCAAAGAAGCTCTTG	203
AhRR	NM_001077982	F: TGGAGTCTCTCCACGGCTTC R: GCGTAGAAGATCATCCCTTCC	58
ARNT	NM_173993	F: TTTCCTCACTGATCAGGAAC R: TCCAGGATACGCCCTGTC	183
CYP1A1	XM_588298	F: CGAGAATGCCAATATCCAGC R: TGCCAATCACTGTGTCCAG	173
CYP1A2	NM_001099364	F: CAGTAAGGAGATGCTCAGTC R: CTGTTCTTGTCAAAGTCCTGG	201
CYP1B1	NM_001192294	F: CACCAGGTATTCGGAAGTGC R: AAGAAAGGCCATGACGTAGG	118
NQO1	NM_001034535	F: CGGAATAAGAAGGCAGTGCT R: AGCCACAGAAGTGCAGAGTG	130
GAPDH	NM_001034034	F: GAGAAACCTGCCAAGTATGAT R: GAGTGTCGCTGTTGAAGTCG	125