Toxicology in Vitro 26 (2012) 841–848

Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

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

Polychlorinated biphenyl quinone metabolites lead to oxidative stress in HepG2 cells and the protective role of dihydrolipoic acid

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ARTICLE INFO

Article history:
Received 7 November 2011
Accepted 30 April 2012
Available online 8 May 2012

Keywords:
PCB
Oxidative stress
ROS
Antioxidant
α-Lipoic acid

ABSTRACT

Parent polychlorinated biphenyls (PCBs) have been shown to induce cellular oxidative stress. However, the effects of PCB active metabolites have not been extensively investigated. Parent PCBs are first converted to hydroquinone metabolites *via* cytochrome P-450-catalyzed hydroxylation, and the hydroquinone metabolites are then further oxidized into the corresponding quinone metabolites. Quinones are responsible for a wide range of toxic effects because of their high reactivity. Previous studies have suggested that reactive oxygen species (ROS) play important roles in multiple toxic mechanisms. In this context, the present study was undertaken to investigate oxidative stress resulting from treatment with PCB quinones in HepG2 cells. The protective effects resulting from co-administration of dihydrolipoic acid (DH-LA) were also investigated. We have found that exposure to PCB quinones leads to: (1) a decrease in cell viability; (2) an increase in both the total ROS production and superoxide production; (3) only 3Cl-PCBQ caused significant increase in the thiobarbituric acid reactive substances (TBARS) level; (4) an increase in SOD activity and a decrease in catalase activity; and (5) a decrease in GST activity and GSH level. We have also found that quinones possessing a higher number of chlorine atoms on the quinone ring display a greater activity and that DH-LA is an effective protective agent as it diminishes PCB quinone-induced cellular oxidative stress.

1. Introduction

Polychlorinated biphenyls (PCBs) are a family of persistent organic pollutants with 209 possible congeners. Due to their great heat transfer characteristics, commercial PCB congeners had been widely used industrially as coolants and lubricants (Safe, 1993, 1994). However, many countries have banned their production and usage since the 1970s because of their chronic and acute toxic effect. Despite this ban and because they are resistant to decomposition, PCBs are still largely present in the biota (Robertson and Hansen, 2001). Researchers have recently claimed that 3,3′-dichlorobiphenyl (PCB11) is produced as a by-product during the manufacturing process of azo and phthalocyanine pigments (Hu and Hornbuckle, 2010).

Exposure to PCBs is believed to be associated with various diseases, such as carcinogenesis, atherosclerosis and neurodegenerative diseases (Carpenter, 2006; Lehmler et al., 2005). Despite the number of studies published on this topic, the mechanisms underlying the toxicity of PCBs are not yet fully understood due to the complexity of PCB isomers, especially downstream metabolites. Studies have shown that ROS generation is involved in the metabolic pathway of PCBs in cells (Srinivasan et al., 2002). It has also been shown that the toxicity of PCBs is associated with an elevated ROS production (Tharappel et al., 2002).

ROS play critical roles in a number of biological functions, including cell growth, proliferation and death (Foreman et al., 2003; Ishikawa et al., 2008; Tsukagoshi et al., 2010; Xia et al., 2007). At the same time, several antioxidant enzyme defense systems, such as superoxide dismutase (SOD), catalase and glutathione peroxidase, also play important roles as they scavenge the excess of ROS and keep the number of ROS at a certain level. Despite these defense systems, exposure to PCBs leads to uncontrolled ROS accumulation and cellular oxidative stress. Oxidative stress weakens the defense systems in cells by inhibiting antioxidant enzyme activities, which results in adverse health effects and diseases (Lee et al., 2005). Hence, ROS levels and cellular oxidative stress in biological systems are usually used to assess toxic

Abbreviations: AhR, aryl hydrocarbon receptor; CAT, catalase; DCFH-DA, 2′,7′-dichlorodihydrofluorescein diacetate; DH-LA, dihydrolipoic acid; GSH, glutathione; GST, glutathione S-transferase; α-LA, α-lipoic acid; PCB, polychlorinated biphenyl; MDA, malondialdehyde; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

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http://dx.doi.org/10.1016/j.tiv.2012.04.028

effects resulting from exposure to chemical toxins such as PCBs (Valavanidis et al., 2006). The relevancy of both the toxicity of PCBs and an irregular ROS generation has been well documented (Hennig et al., 2002; Spencer et al., 2009; Srinivasan et al., 2001; Twaroski et al., 2001; Zhu et al., 2009).

Excessive ROS accumulation restrains the proper function of intracellular antioxidant enzymes. However, oxidative stress can be counteracted by non-enzymatic small molecule antioxidants. α-Lipoic acid (α-LA) is a sulfur-containing fatty acid that is believed to function as an antioxidant in therapies for chronic diseases associated with oxidative stress (Smith et al., 2004). α-LA can be found in a reduced form (dihydrolipoic acid, DH-LA) and in an oxidized form (lipoic acid, LA) that seem to recycle. DH-LA is a potent reducing agent that is able to regenerate vitamin C and glutathione from their oxidized forms (Jones et al., 2002; Kagan et al., 1992). A recent publication has suggested that α-LA inhibits rat liver fibrosis through the attenuation of ROS signaling in hepatic stellate cells (Foo et al., 2011). Although both the reduced and oxidized forms of α-LA are considered to be antioxidants, the reduced form is believed to possess higher antioxidant effectiveness (Moini et al., 2002; Zhao and Liu, 2010). DH-LA was therefore chosen over other antioxidants to demonstrate its protective role towards oxidative stress.

Our previous studies have suggested that ROS generate *in vitro via* redox cycling of the hydroquinone–semiquinone–quinone triad (Song et al., 2008a,b). It was shown that highly chlorinated PCB quinones lead to the highest steady-state level of semiquinones and to the highest level of ROS such as H_2O_2 and hydroxyl radicals. However, to the best of our knowledge, the relationship between the degree of chlorination of PCB quinones and oxidative stress has not been studied. We have therefore investigated ROS formation and oxidative stress in HepG2 cells using PCB quinones with different degree of chlorination (0–3 chlorine atoms) on the quinone ring. The protective role of DH-LA towards oxidative stress induced by PCB quinones was also studied.

2. Materials and methods

2.1. Chemicals and cell culture

The PCB quinones used in this study were 2-(4-chloro-phenyl)-[1,4]benzoquinone (0Cl-PCBQ), 2-chloro-5-(4-chloro-phenyl)-[1,4]benzoquinone (1Cl-PCBQ), 2,5-dichloro-3-(4-chloro-phenyl)-[1,4]benzoquinone (2Cl-PCBQ) and 2,3,5-trichloro-6-phenyl-[1,4]benzoquinone (3Cl-PCBQ). The corresponding structures are shown in Fig. 1. In this study, the nomenclature of PCB quinones is based on the number of chlorine atoms on the quinone ring.

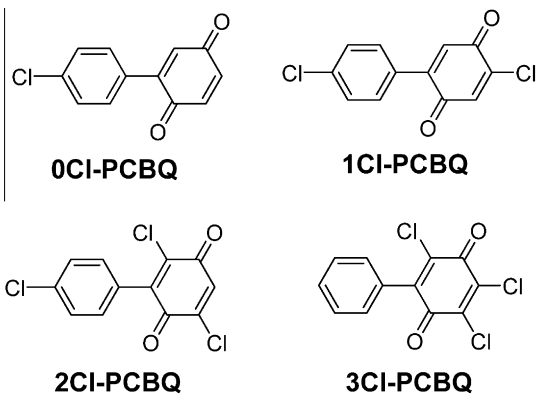

Fig. 1. Structures and nomenclature of the tested PCB quinones. *Note*: only the quinone ring chlorine atoms were counted in their nomenclature.

The PCB quinones were synthesized and characterized as previously described (Song et al., 2008a). Stock solutions (10 mM each) of PCB quinones were prepared in DMSO; the final concentration of DMSO was below 0.5% in each assay. DMSO alone had no significant effect in any of the experiments. Glutathione (GSH), GSSG, thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) detection kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). DH-LA was purchased from Aladdin Reagent Database Inc. DCFH-DA and MTT were purchased from Sigma–Aldrich Inc., Shanghai, PRC. Triton X-100 was purchased from Dingguo Biotechnology Co., Beijing, China, and DHE was purchased from Beyotime Institute of Biotechnology, Haimen, China. All other chemicals were of the highest grade commercially available and were used without further purification.

A human hepatocellular carcinoma (HepG2) cell line was kindly provided by Prof. Xuegang Li, Southwest University, PR China. Cells were cultured in RPMI 1640 medium (Gibco Invitrogen Co., USA) with 10% fetal bovine serum (HyClone, USA) and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin) at 37 °C and 5% CO_2. Cells were exposed to the PCB quinone compounds for a typical time of 3 h. DH-LA was added 1 h before the addition of the PCB quinones in the given experiments.

2.2. Cell viability

Cell viability was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983; Shi et al., 2011). Cells were transferred onto 96-well culture plates at 1×10^4 cells/well and permitted to adhere overnight at 37 °C. After incubation with a PCB quinone for 3 h, the cells were treated with MTT (final concentration of 5 mg/mL) for 4 h at 37 °C. The MTT-containing medium was removed, and 100 μL of DMSO were added to all wells and mixed thoroughly using a 10 min shake. The optical density (OD) of each well was measured at 490 nm using a microplate reader (BioTek ELX800).

2.3. Measurement of ROS levels

The amount of ROS was determined following a previously described procedure with minor modifications (Xue et al., 2011). In brief, cells in 24-well culture plates were loaded with 10 μM DCFH-DA directly in the culture medium for 20 min at 37 °C and 5% CO_2. The cells were then washed twice with PBS (pH 7.4), and the PCB quinone tested was added and incubated in RPMI 1640 medium for 3 h. The intracellular oxidants oxidized DCFH to fluorescent DCF. Subsequently, the cells were harvested and lysed in 300 μL of 0.1% Triton X-100 on ice for 10 min and sonicated for 10 s. The homogenates were centrifuged at 12,000 rpm, 4 °C for 20 min and the supernatant was collected. The fluorescence was recorded with an excitation wavelength of 488 nm and an emission wavelength of 526 nm (F7000, HITACHI). The fluorescence of the control group was normalized to 100%.

2.4. Measurement of intracellular superoxide ($O_2^{\cdot-}$)

Cells were plated in 24-well culture plates and incubated with 10 μM DHE (stock solution: 10 mM in DMSO stored at −20 °C) for 40 min. The culture medium was then removed and the cells were washed twice with PBS (pH 7.4). The PCB quinone tested was added to the plates and incubated in RPMI 1640 medium for 3 h. The cells were then lysed with 300 μL of 0.1% Triton X-100 on ice for 10 min and sonicated for 10 s. The homogenates were centrifuged at 12,000 rpm, 4 °C for 20 min, and the supernatant was collected. The fluorescence was recorded with an excitation wavelength of 488 nm and an emission wavelength of 575 nm.