STRUCTURE-DEPENDENT HORMETIC CYTOTOXICITY INDUCED BY PCB CONGENERS

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Abstract

The effects of exposure of Vero cells to polychlorinated biphenyl (PCB) congeners were examined. An *ortho*-substituted PCB congener (PCB 52) and a coplanar congener (PCB 77) were assessed for their cytotoxicity on Vero cell, with the attempt to assess the possible hormetic biphasic dose-response curve and to compare their structure-activity relationship. Flow cytometry was used to monitor the changes of biochemical endpoints caused by those two PCB congeners. Both *ortho*-substituted and non *ortho*-substituted coplanar PCB congeners showed the hormetic cytotoxicity: cell proliferation stimulation at low doses, while loss of viability with high dose. Their cytotoxic profile was different along the incubation prolongation. These two different trends might be associated with their genotoxicity and Ah agonist characteristics respectively, which was related with their chemical structures.

Introduction

Polychlorinated biphenyls (PCBs) are widespread persistent environmental contaminants, which have been extensively used for a variety of industrial applications. Their high lipophilicity has resulted in bioaccumulation in various organisms through the food chain. Many PCBs have been detected in human blood, milk, and other tissues [1, 2]. PCB mixtures are considered to be a present risk to both wild life and humans for their characteristics of immunotoxic, carcinogenic, neurotoxic and cause birth defects. PCBs consist of up to 209 different congeners; the toxicity of individual PCBs is structure-dependent. The mean intake of PCBs is estimated to be about four times lower than the estimated tolerable dose. Low doses of toxic substances may induce a range of stimulatory responses has long been known. Various investigators have observed the biphasic dose-response curve through cytotoxicity tests [3,4].The history of such observations extends well over a century, and has been concluded as hormesis. Hormesis is an adaptive response characterized by biphasic dose responses with similar stimulatory response. Hormesis rejects the standard toxicological assumption that effects at low doses can be extrapolated from data obtained from high doses. This phenomenon has important implications for the hazard assessment process and challenges the current risk assessment practices as well [5].

Cellular membranes are composed of lipid bilayers, which mediate various cellular functions. As highly lipophilic substances, PCBs are stored in body fats, including cellular membranes. It is reported that PCBs can disrupt cellular membrane integrity and alter the physiological function of cells [6]. Cell cycle progression,

through the controlled process of DNA replication and cell division, is initiated in quiescent cells by mitogen stimulation. Perturbations of tissue homeostasis due to disruption of cell-to-cell communication have been linked to growth and developmental diseases, such as cancer. Some PCB congeners have been implicated as potential tumor-initiating compounds. Their tumor-promoting activity has been associated with cell proliferation, which involved in cell cycle regulation [7]. PCBs exposure has been involved in renal toxicity from both in vitro and in vivo experiments [8]. This study investigates the cytotoxicity of PCBs congeners in Vero cells, which from African green monkey kidney. The objectives of this study were: (1) to evaluate the potential of hormesis caused by testing PCB congeners, (2) to investigate whether the toxicity of PCBs depends on the particular structure it has formed and finally (3) to assess the effects on cellular cycle caused by PCBs.

Materials and methods

MTT assay was employed to measure cell proliferation. Cells at exponential proliferation phase were harvested, seeded in 96-well plates at 1×10^4 cell/well in 100 l of cell medium, and left to adhere to the plastic plates overnight before being exposed to different concentrations of PCB congeners. Prior to PCB exposure, the growth medium was discarded and replaced with the medium containing varied concentrations of PCB congeners, and incubated for 24, 48 and 72 h at 37 ℃. After incubation period, 20 µl of 5 mg/ml MTT solution was added to each well, and the cells were continued incubated in the dark at 37 ℃ for 4 h. Thereafter, 150 µl DMSO was added to solubilise the converted purple dye in culture plates. The absorbance was measured on a spectrophotometer microplate reader (SpectraMax Plus 384) at a wavelength of 570 nm.

Following the exposure, the medium was removed; cells were harvested with trypsin. For cell cycle analysis, harvested cells were washed with phosphate-buffered saline (PBS), and fixed in 70% ethanol at 4℃ overnight. Fixed cells were washed once with PBS and resuspended in 0.5 ml PI/RNase staining buffer and incubated for 15 minutes at room temperature before analysis. Cells were analyzed on FACSCalibur, and CELLQuest software for data acquisition. A minimum of 15,000 events was collected per sample. Data were analyzed using ModFit LT version 3.0 software. PI dye was also used to monitor changes in membrane injury, where some increase in labelling indicates sublethal changes in membrane permeability [9].

Results and Discussion

PCB congeners have been classified as possible human carcinogens. The tumor-promoting activity of PCBs has been suggested to be associated with their capacity to activate signal transduction pathways, leading to cell proliferation promotion. Several studies have demonstrated the capability of PCB congeners to stimulate cell proliferation at low doses [10]. The hormetic cytotoxicity induced by PCB congeners was hypothesized to be a common phenomenon. This study employed Vero cells as the test model, cytotoxicity as the endpoint to evaluate potential hormesis induced by PCB congeners. A notable observation was the significant stimulatory effect detected in Vero cells, which was exposed to the lower doses of PCB congeners. This phenomenon can be incorporated into hormesis. Clear examples were evident from our results where increase in MTT uptake over that of controls occurred at low concentrations, while pronounced cytotoxic effect manifested at higher doses of PCB 52 (as shown in Fig. 1.). Figure 1 shows the effects of PCB congeners on cell viability measured at 24, 48 and 72h after exposure. Both PCB congeners were found to stimulate proliferation of Vero cells in a biphasic

manner. PCB 77 was found to be a more efficient inducer of proliferation on Vero cells. Meanwhile, the coplanar PCB 77 did cause an uptake proliferation trend as exposure time expansion, while different trend was observed in PCB 52 treatment.

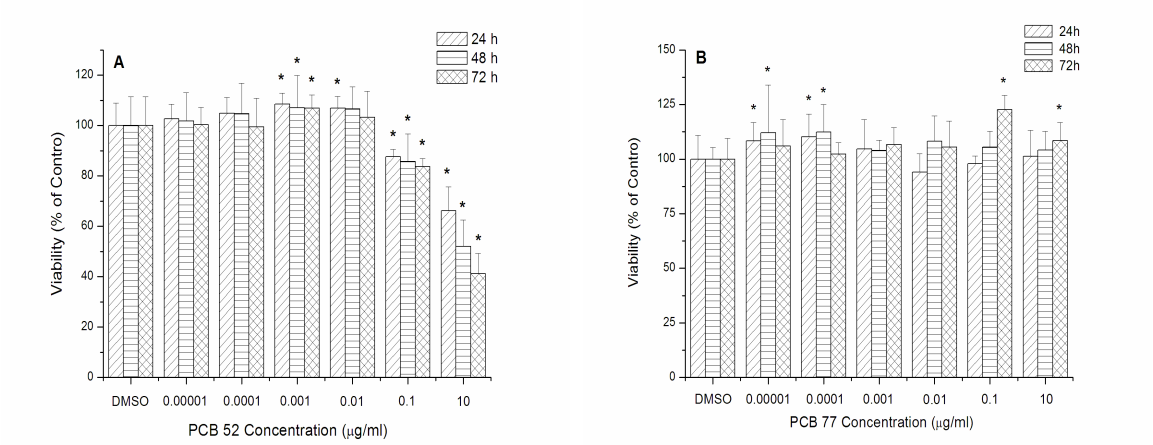

Fig. 1. Dose-dependence of effects of PCB 52(A) and 77(B) on Vero cell proliferation; measurement made at 24, 48 and 72h after addition of PCB congeners. Data expressed as mean± S.D. * denotes significant difference from control ($p<0.05$).

The different trend between those two PCBs might be associated with their structure, as the properties of individual congeners depend upon both the number of chlorines and their positions around the biphenyl rings. Our studies have chosen two PCB congeners of the same molecular weight and chlorine atoms, with the different at chlorines positions around the biphenyl ring. The presence of chlorine molecule(s) in the *ortho* position causes the increased angle between the biphenyl rings, leading to the three-dimensional structure of PCB molecule. Whereas with the chlorines in the *meta* and *para* positions, PCB molecule assumes a planar configuration. The *ortho*-substituted and coplanar congeners differ greatly in their ability to dissolve in biological membranes. The bulky structure of the *ortho*-substituted congeners can cause a greater perturbation of the lipid bilayer and further change membrane physiological functions, such as ion permeabilities through voltage- or ligand-gated channels, as well as changes in activity of membrane-bound enzymes [11]. Many previous studies have demonstrated that *ortho*-substituted PCBs disrupt the structure of cell membrane in a relatively nonspecific fashion, independent of cell types or membrane types [9, 12]. Tan et al. have compared *ortho*-substituted and coplanar PCB congeners with their ability on altering membrane structure [9]. Their observations showed that that *ortho*-substituted congeners cause a greater disruption of membrane integrity than do coplanar congeners. Our observations with Vero cells are consistent with their results; *ortho*-substituted PCB 52 disrupts the structure of plasma membrane in a dose- and time- dependent fashion, whereas there is little or no apparent effect of the coplanar PCB 77(as shown in Fig. 2.).

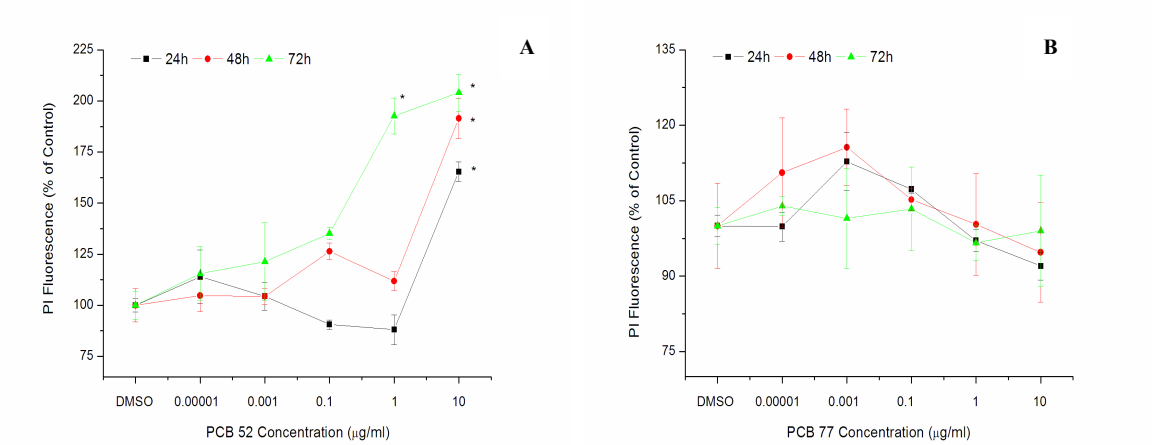

Fig.2 Time-dependent accumulation of PI in Vero cells exposed to various concentrations of PCB 52 (A) and 77(B) (which we assume correlates with cell membrane integrity). PI fluorescence was normalized to 100% of control in each group. Data expressed as mean± S.D. of three independent experiments run in duplicate. * Significantly difference between control and treated samples ($p<0.05$).

Table 1 shows a time-dependent modulation of cell cycle by PCB 52 in Vero cells. PCB 52 significantly increased the percentage of cells in the G0/G1 phase with exposure time increased from 24h to 72h exposition. Simultaneously a loss of cells in G2/M was observed. Disruption of normal control of the cell cycle is one of the important steps in carcinogenesis. The balance between genotoxic and proliferative effects of PCBs might determine the fate of cells. Cells have DNA surveillance mechanisms, and if excessive DNA damage is detected, cell cycle arrest occurs, which allows more time for DNA repair to rectify this DNA damage before entering mitosis. Several studies have implicated some PCB congeners as potential tumor-initiating compounds that may either induce oxidative DNA damage or formation of DNA adducts. The formation of DNA adducts by genotoxic compounds may lead to activation of DNA damage checkpoints associated with G1 cell cycle arrest and activation of DNA repair mechanisms [13, 14]. The decrease trends of cell viability incubated with *ortho*-substituted PCB 52 as exposure expansion might be a presence of genotoxicity caused by PCBs. As shown in Table 1, our observation also concluded the massive accumulation of cell in G1 phase as incubation prolongation.

Table 1 Time-dependent modulation of cell cycle by PCB 52 in Vero cells.

Treatments	G1			G2		
	24h	48h	72h	24h	48h	72h
DMSO	65.78±4.30	74.73±4.39	87.37±3.40	17.05±1.31	13.38±3.66	8.67±2.80
T0.00001	67.67±3.45	76.06±0.41	82.81±3.40	17.01±0.88	14.26±2.45	12.84±2.16
T0.001	64.95±5.39	77.08±2.84	82.16±0.98	18.22±1.07	13.25±2.54	12.22±2.18
T0.1	66.45±3.28	74.29±4.31	85.02±3.19	17.15±2.21	13.18±5.04	10.88±3.24

T1	69.76±4.75	77.82±2.99	83.23±3.19	18.44±4.26	12.27±4.88	11.15±1.80
T10	58.57±1.80	52.62±13.35*	66.39±5.91*	12.03±5.06	11.09±2.69	9.68±3.41

* indicates a significant difference ($p<0.05$) compared with control.

Coplanar PCB congeners that act as AhR ligands might alter the normal control of cell proliferation through 1qthe mechanism of contact inhibition disruption. The elevated trends of cell proliferation accompanied by PCB 77 incubation prolongation might be involved in this mechanism. Contact inhibition is a mechanism through which non-transformed adherent cells decreases with increased cell density, as they enter a reversible in G1 phase of the cell cycle, when receiving anti-proliferative signals mediated through cell-cell contacts. The loss of contact inhibition can lead to deregulated growth and is often associated with malignant transformation. Several studies have demonstrated the capability of AhR ligands to stimulate cell proliferation through disruption of contact inhibition [3, 15]. Vondracek et al. have reported the different effect on cell proliferation caused by *ortho*-/non *ortho*-PCB congeners [7]. The 'dioxin-like' PCB congeners were found to induce cell proliferation in a concentration-dependent manner; whereas the 'non-dioxin-like' compounds, which were not aryl hydrocarbon receptor (AhR) agonists, had no effect on cell proliferation. The cell proliferation promotion with PCB 77 exposure might be the result of release from contact inhibition caused by coplanar AhR agonist PCB congener. In conclusion, our results of the presented data confirm that both *ortho*-substituted PCB 52 and coplanar PCB 77 presented hormetic cytotoxicity on Vero cells. Our observations also shown that the bulky structure of the *ortho*-substituted congeners results in a greater disruption of membrane function than the planar, dioxin-like congeners, which also suggest a structure-dependent mechanism of PCB congeners. The different trends induced by the two PCB congeners might be link with its own genotoxicity and the release of contact inhibition as well.

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