THE ADVERSE EFFECTS OF PFOS AND PCB 153 ON NEONATAL TESTICULAR CELLS IN VITRO

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Introduction

Perfluorooctane sulfonate (PFOS) is widely used in industry; it is non-biodegradable and persistent in the human body and in the environment. Although reports have indicated that young people might have higher PFOS levels in serum or blood than do older people, its adverse effects on neonatal testicular cells had not been investigated previously. PCB 153 is one of the most prevalent polychlorinated biphenyl (PCB) congeners in biological tissues, but the direct adverse effect of PCB 153 on neonatal testis remains unclear. In this study, we exposed a neonatal Sertoli cell/germ cell co-culture system to PFOS and PCB 153 to determine and compare the toxic features of PFOS and PCB 153 on the co-culture system.

Materials and methods

Animals, Sertoli cell/gonocyte co-cultures, and treatment

Animal experiments were performed according to the guidelines for animal welfare at Fudan University. Primary Sertoli cells and gonocyte were obtained from testes of five-day-old pups (day of delivery: Day 1). The co-cultures were prepared as follows: briefly, testes were aseptically removed from pups, decapsulated, and subjected to sequential digestion with collagenase/hyaluronidase and DNase I. Cells were re-suspended in hormone- and serum-free DMEM on ice. Cells were plated in gelatin-coated dishes. All cultures were maintained in hormone- and serum-free DMEM containing 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 3 mM sodium lactate, 5 mg/mL transferrin, and 50 ng/mL retinol in a water-saturated environment of 95% air and 5% CO_2. Solutions of PCB 153 and PFOS in DMSO were added directly to the culture medium (DMSO 0.01%) 48 h after cells had been plated in the dishes. The final concentrations tested in the culture medium, for both PCB 153 and PFOS, were 0, 1, 10, 50, and 100 μM. The determination was performed 24 h after exposure.

Cellular viability assay

Cell viability was determined through a 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-8)–based colorimetric assay (CCK-8, Dojindo, Gaithersburg, MD) according to the manufacturer's instructions.

Apoptosis by flow cytometric analysis

Apoptosis was tested using an apoptosis detection kit (Major Biotech, Shanghai), according to the manufacturer's instructions. Briefly, co-culture cells were collected 24 h after treatment with PCB 153 or PFOS; they were then incubated in buffers (400 μl) containing Annexin V (5 μl) in the dark at room temperature for 15

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min and PI (10 μl) for 5 min. The stained cells were analyzed using a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA, USA). The data were analyzed using Cellquest software (Becton Dickinson, San Jose, CA, USA).

Measurement of ROS formation

ROS generation was determined by adding 2´,7´-dichlorofluorescin diacetate (DCFH-DA). The fluorescence intensity of the ROS product was measured using Image Pro-plus 6.0. The percentage increase in the ROS formed in the exposed cell dish was determined relative to control fluorescence intensity values.

Morphologic structure of actin filaments and vimentin immunostaining

The distribution of filamentous actin within the cultured Sertoli cells was examined after incubating with rhodamine-conjugated phalloidin (Molecular Probes, Eugene, OR; 0.25 ug/mL) for 30 min and then viewing under a (Leica TCS SP5) confocal argon ion laser scanning microscope. The images were captured and digitized using a Spot Camera equipped with LEICA software. Vimentin immunostaining are as following: After 24 h of treatment with PCB 153 or PFOS, co-culture cells were fixed in ice cold acetone for 30 min. The cells were then incubated with the first vimentin antibodies (Abcam) overnight at 4 °C. Secondary antibodies marked with FITC were added and then the sample was left at room temperature for 1 h. Vimentin was viewed under a Leica TCS SP5 confocal argon ion laser scanning microscope. The images were captured and digitized using a Spot Camera equipped with LEICA software. Ten photographs (about 300 cells) for each group sample with stained vimentin filaments were captured and quantified.

Statistical Analyses

SPSS (version 11.0) software for Windows (SPSS, Chicago, IL, USA) was used for analyses. All values are presented as means ± SD. The significance of the differences between mean values was evaluated through one-way ANOVA or chi-square analysis.

Results and discussion

Cell viability and cell apoptosis

Significant reductions in the number of viable cells occurred in the PCB 153-exposed group at concentrations of greater than 10 μM and in the PFOS-exposed group at concentrations of greater than 50 μM (Fig.1). On the other hand, marked induction of apoptosis occurred at concentrations greater than 10 μM of PCB 153. However, PFOS did not induce markedly increase of apoptosis. It has been reported that PCB 153 damages MCF10A cells (nonmalignant human mammary epithelial cells) at concentrations from 3 μM [1] and decreases the cell viability on a GT1-7 immortalized hypothalamic cell line at 10 μM after exposure for 24 h[2]; It has also been reported that 10 μM PFOS will inhibit PC12 (a cell model for neuronal development) DNA synthesis and evoke a great degree of lipid peroxidation[3]. The sensitivity of our neonatal Sertoli cell/gonocyte co-culture system is lower than those of these cell lines.

Determination of ROS generation

ROS generation increased in a dose-dependent manner after exposure to either PCB 153 or PFOS. Our primary neonatal Sertoli cell/gonocyte co-culture system is not so sensitive to PFOS for ROS generation as PCB153.

Vimentin and actin filament staining

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The cytoskeleton organization reflects directly on the cell shape and is related to cellular interactions[4-5]. Sertoli cells are primary supporting cells that create the structural and physiological environment for spermatogenic cell development; they also provide a permissive milieu that is necessary for spermatogenesis[6]. In Sertoli cells, actin filaments and vimentin filaments comprise the main cytoskeleton, which may be the target of xenotoxicants. In the present results, 50 μM and 100 μM of PFOS and PCB153 showed an obvious decrease in the fluorescence intensity of vimentin staining as well as exhibited shortened and disorganized actin filaments under the confocal microscope. PFOS induced more obvious morphological changes in the neonatal Sertoli cells than did PCB 153 at the same exposure dose. Sertoli cells, which form the somatic component of the seminiferous tubules, are a major determinant of sperm output by the testis[7]. Thus, the damaged Sertoli cells observed in this present study might eventually affect spermatogenesis.

The above results indicated that both PCB 153 and PFOS directly affect a neonatal Sertoli and gonocyte cell co-culture system, inducing ROS generation in a dose-dependent manner, triggering apoptosis at PCB153 exposed groups, and changing the cytoskeleton of Sertoli cells at PFOS high dose levels. Our findings provide fundamental information regarding the direct effects of PCB 153 and PFOS on neonatal Sertoli cells and gonocytes maintained in a defined co-culture system.
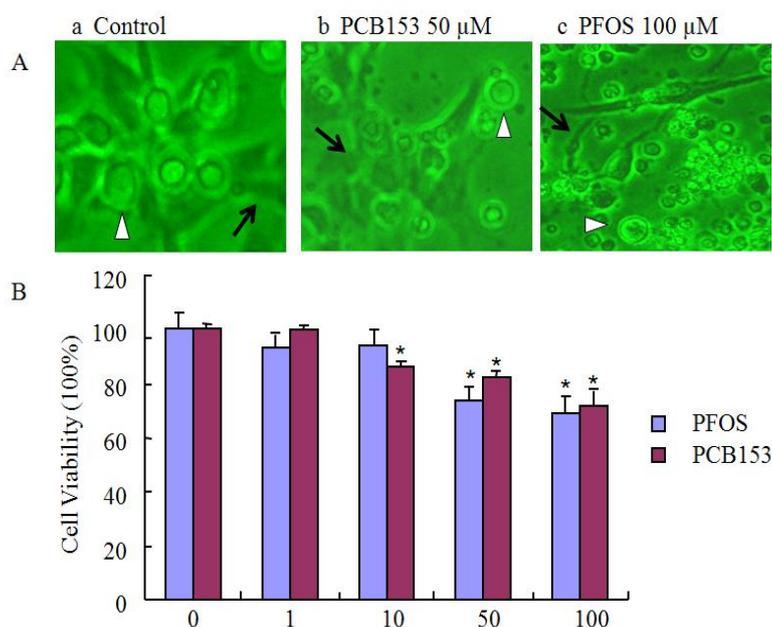
Fig 1: Morphology of co-culture system. A: White triangles indicate gonocytes; black arrowheads, Sertoli cells. Original magnification: ×400. B: Cell viability. Results are expressed as means ± SDs (n=3). *: $P < 0.05$ when compared with corresponding controls (dose: 0 μM).

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