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Genotoxic effects of PCB 52 and PCB 77 on cultured human peripheral lymphocytes

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ABSTRACT

Polychlorinated biphenyls (PCBs) are known to be carcinogenic, but the mechanisms of this action are uncertain. Most, but not all, studies have concluded that PCBs are not directly mutagenic, and that much if not all of the carcinogenic activity resides in the fraction of the PCB mixture that contains congeners with dioxin-like activity. The present study was designed to determine genotoxic effects of an *ortho*-substituted, non-coplanar congener, 2,2′,5,5′-tetrachlorobiphenyl (PCB 52), and a non-*ortho*-substituted coplanar congener with dioxin-like activity, 3,3′,4,4′-tetrachlorobiphenyl (PCB 77) on cultured human peripheral lymphocytes. DNA damage was assessed by use of the comet assay (alkaline single-cell gel electrophoresis). After cell cultures were prepared, test groups were treated with different concentrations of PCB 52 (0.2 and 1 μM) and PCB 77 (1 and 10 μM) for 1 h at 37 °C in a humidified carbon dioxide incubator, and compared to a DMSO vehicle control group. The cells were visually classified into four categories on the basis of extent of migration such as undamaged (UD), low damage (LD), moderate damage (MD) and high damage (HD). The highest concentration of PCBs 52 and 77 significantly increased DNA breakage in human lymphocytes ($p < 0.001$). Our results indicate that both the non-coplanar PCB 52 and coplanar PCB 77 cause DNA damage, and that the *ortho*-substituted congener was significantly more potent than the dioxin-like coplanar congener.

1. Introduction

Polychlorinated biphenyls (PCBs) were widely used as industrial fluids, flame-retardants, diluents and fluids for capacitors and transformers because of their lipophilic and insulating features [1]. Although their production was banned in most countries in the late 1970s [2,3], industrial and household products containing PCB material are still in use. In addition PCBs are very persistent, both in the environment and in the human body.

PCBs are a mixture of up to 209 different congeners having various numbers (1–10) and positions (*ortho*, *meta* and *para*) of chlorine atoms on the biphenyl rings [2,4]. The toxicity of individual PCB congeners varies with their structure [5]. Congeners with no chlorines and a few with one chlorine in the *ortho* position (non-*ortho* and mono-*ortho*, respectively) are able to assume a planar configuration and bind to the aryl hydrocarbon (Ah) receptor. These congeners act like the highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [6]. The Ah receptor is a member of the steroid family of receptors, which binds DNA and acts via alteration of gene expression [7]. However, there is also abundant evidence from *in vivo* and *in vitro* studies that the *ortho*-substituted, non-coplanar, non-dioxin-like congeners have toxic effects on various organ systems [8]. At least some *ortho*-substituted congeners are metabolized by other cytochrome P450s, but not via binding to the Ah receptor. Both coplanar and many non-coplanar PCB congeners cause gene induction, with some genes up regulated and others down regulated [9–11].

PCB mixtures can be immunotoxic [12,13], carcinogenic [14], clastogenic [15,16] and cause birth defects [6] in both animals and humans. While they, like dioxin, are usually considered to be non-genotoxic carcinogens [17] there remains some uncertainty in this regard. The mechanisms by which PCBs exert their adverse effects are not fully understood and data on their genotoxic properties are still controversial [18].

Environmental contaminants may cause DNA damage directly and/or by generation of reactive species such as electrophilic metabolites or free radicals. Silberhorn et al. [19] reported that Aroclor 1254, a commercial mixture of PCB congeners, gave negative results in the majority of genotoxicity assays. However, Aroclor 1254 was reported to be clastogenic in dove embryos by Peakall et

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al. [15]. Sargent et al. [16] reported that PCB 77 exerted genotoxic effects in human lymphocyte cultures. Aroclor 1254 also produced DNA single-strand breaks in an alkaline elution/rat hepatocyte assay [20], but did not induce unscheduled DNA synthesis in primary rat hepatocytes [21]. Belpaeme et al. [22,23] suggested that the planar PCB 77 did not induce DNA damage in human lymphocytes or in brown trout erythrocytes in the comet assay. None of the individual PCB congeners and PCB mixtures tested induced a significant increase of DNA damage in nuclei of human sperm in the neutral comet assay [24].

In the present study, two structurally different congeners of the same molecular weight, 3,3′,4,4′-tetrachlorobiphenyl (PCB 77, a dioxin-like coplanar congener) and 2,2′,5,5′-tetrachlorobiphenyl (PCB 52, an *ortho*-substituted, non-coplanar congener) were selected to examine and compare their effects on DNA damage in acutely cultured human lymphocytes by the comet assay.

2. Materials and methods

2.1. Chemicals and reagents

PCBs 52 and 77 were purchased from Ultra Scientific (North Kingstown, RI). Lymphorep were purchased from Asis-Shield (Oslo, Norway). Newborn calf serum (FCS) was obtained from Biological Industries (Israel). NaCl, NaOH, dimethyl sulfoxide (DMSO) and HCl were provided from Merck (Dormstadt, Germany). The other chemicals and reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA). Bi-distilled water was used in all the experiments. PCBs were dissolved in DMSO so that the final concentration of DMSO was never greater than 0.1% (v/v).

2.2. Subjects

Six healthy male non-smoking donors (mean age, 29.33 ± 2.33 years) provided blood samples. Subjects had not been exposed to radiation or drugs 6 months prior to the study. Each person was interviewed about the possible influence of confounding factors. This study was approved by the Ethics Committee of Firat University Medical School (Elazig, Turkey).

2.3. Blood sampling and cell preparation

Whole peripheral blood samples were obtained by venous puncture. The isolation of lymphocytes was performed by centrifugation in a density gradient of lymphorep (15 min, 280 × *g*) and the cells washed with RPMI-1640 medium supplemented with 10% FCS. In order to determine cell viability 0.4% tryphan blue was used and the cells were counted with a hemocytometer. After cell cultures were prepared, test groups were treated with different concentrations of PCB 52 (0.2 and 1 μM) and PCB 77 (1 and 10 μM), and the vehicle control group was treated with DMSO for 1 h at 37 °C in a Nuaire humidified carbon dioxide incubator (Playmouth, MN, USA). As a positive control, lymphocytes were treated with 50 μM H_2O_2 and incubated for 5 min on ice. After incubation, cell viability was again examined using tryphan blue.

2.4. Comet assay

The alkaline comet assay technique was performed as described in Singh et al. [25] with minor modifications. Briefly, microscope slides were precoated with 0.75% (w/v) high melting agarose at PBS. The lymphocytes were mixed with 100 μl of 0.5% (w/v) low melting agarose (LMA) at 37 °C and the cell suspension was pipetted and layered onto precoated slides. The slides were immediately covered with a large cover slip and kept at 4 °C for 5 min to allow the agarose to solidify. After the cover slips were removed, the slides were immersed in ice cold freshly prepared lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium sacosinate, pH 10) with 1% Triton X-100 and 10% DMSO for 1 h. Each experiment was repeated eight times.

2.5. Electrophoresis

After lysis, the slides were placed in a horizontal gel electrophoresis tank (Bio-Rad, USA) positioned close to the anode and covered with fresh alkaline electrophoresis buffer solution containing 1 mM EDTA, 300 mM NaOH (pH > 13) for 20 min at 4 °C. Electrophoresis was conducted at 25 V (0.83 V/cm) and 300 mA for 20 min. After electrophoresis, the slides were washed three times with neutralizing buffer (0.4 M Tris, pH 7.5) at 4 °C for 5 min each. Then the slides were dried and stained with 20 μg/ml ethidium bromide and covered with a cover slip. The slides were incubated at 4 °C in a refrigerator for 20 min before scoring. All of the steps were conducted in the dark to prevent additional DNA damage.

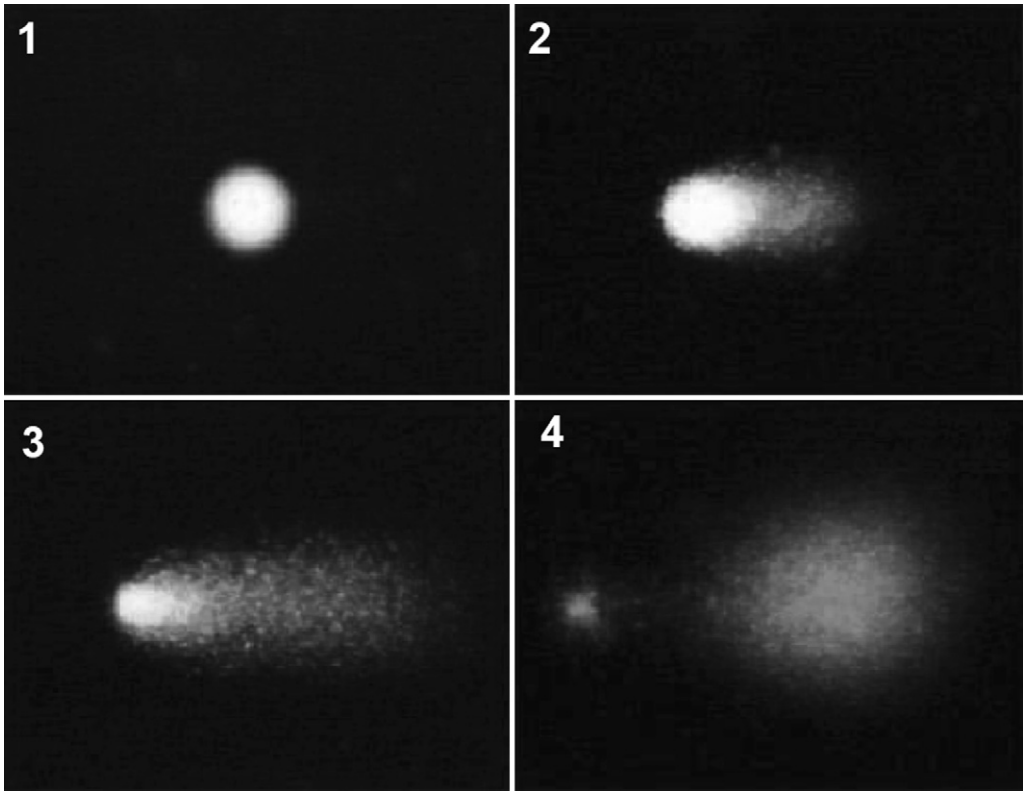

Fig. 1. Undamaged (1) low damaged (2), moderate damaged (3) and high damaged (4).