PCB 126 INDUCES APOPTOSIS OF CHONDROCYTES IN CULTURE

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Introduction

Recently, strong positive association of dioxin-like PCBs and arthritis in women, based on the NHANES study, was reported from the background exposure levels[1] Arthritis, in particular, osteoarthritis (OA), involves the progressive destruction of cartilage matrix by a pathological imbalance of the normal chondrocyte function[2]. Chondrocytes, the only cell type in the articular cartilage, maintain the equilibrium between synthesis and degradation of cartilage matrix and play an essential role in keeping cartilage integrity. Impairment of the normal chondrocyte function is responsible for the cartilage damage, which is the most prominent feature of the arthritis[3]. Chondrocyte cell death has long been assumed as a central feature in osteoarthritis cartilage degradation. More recently, apoptotic cell death has become a focus of interest in the pathogenesis of the cartilage disease and its process has been suggested as a target for the therapeutic intervention[4]. Regulators of chondrocyte cell death include a variety of extracellular matrix (ECM) components, mechanical injury, nitric oxide, death receptor, and mitochondrial functionality, etc[5]. However, it remains unclear what initiates apoptotic processes of chondrocytes at the early stage of the arthritis and what is the underlying mechanism. Thus, the present study attempted to examine the effects of PCB126, the most potent congener of its class with a high TEF value, on chondrocyte apoptosis, which is a critical event in the cartilage disease.

Materials and Methods

Culture of Rabbit Articular Chondrocytes. Articular chondrocytes were isolated from cartilage slices of 2-week-old New Zealand White rabbits by enzymatic digestion as described previously[6].

Exposure. Cells grown on 96-well plates were exposed to 0, 10,100 and 1000 nM PCB 126 for 24 hrs for apoptosis analysis. For the immunoblot analysis of PKC-delta, cells were exposed for 15 min.

Western Blot Analysis. 10 μg of whole cell lysates were immunoblotted as described previously[6]. PKC-delta was detected using the respective monoclonal antibodies (BD Transduction Laboratories, Lexington, KY).

ROS measurement. Formation of ROS was measured with use of the fluorescent probe DCFH-DA (50μM), as described previously[7]

Analysis of apoptosis. Fragmented nucleosomal DNA was quantified by ELISA kit (Cell Death Detection ELISA Plus; Roche, Mannheim, Germany) and Caspase-3 activity was determined with ApoAlert caspase colorimetric assay kit (Clontech Laboratories, Palo Alto, CA).

TUNEL assay. DNA fragmentation was detected with terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL) assay kit (fluorescein in situ cell death detection kit; Roche, Mannheim, Germany) according to the manufacturer's protocol.

Results and Discussion

We used the primary rabbit chondrocytes in culture to analyze the effects of PCB 126 on chondrocytes apoptosis and suggest the possible mechanism of PCB-induced cartilage disease.

The metabolism of chondrocytes in articular cartilage is subject to a complex environmental control. ROS emerges as a major factor in the regulation of metabolic processes in chondrocytes[8]. It mediates damage of the extracellular matrix by induction of apoptosis or metalloproteinase. Patients with joint diseases such as OA and RA showed an increase of ROS production[9]. This study demonstrated that PCB126 is an effective inducer of ROS generation in chondrocytes (Fig. 1). It is noteworthy that generation of ROS by PCB 126, based on TEF value calculation, was higher than TCDD, the most potent agent of HAH compounds. This result suggests that PCB may be more potent on chondrocyte than TCDD with respect to ROS generation.

Activation of PKC-δ in chondrocytes was observed upon dioxin exposure (Fig. 2). Because activation of PKC-δ may contribute to the induction of cartilage disease through the induction of MMP-13, a cartilage degrading enzyme [10], altered activation of this isozyme may adversely affect the normal function of chondrocytes via aberrant PKC-mediated phosphorylation, which may initiate a cascade of cartilage damage.

Survival and death of chondrocytes are closely linked to cartilage matrix integrity. Thus, apoptotic cell death is considered as an important factor contributing to the breakdown of the extracellular matrix in joint diseases[5]. While numerous methods are available to quantitate apoptosis for *in vitro* analysis, each method has its own drawbacks. This study used a strategy to combine a panel of different technical approaches for the optimal detection of chondrocyte apoptosis. Apoptotic effects measured by caspase-3 activity and nucleosomal DNA fragmentation clearly demonstrated that PCB 126 is a potent inducer of chondrocyte apoptosis (Figs. 3A and 3 B). These apoptotic measures were further confirmed by analyzing microscopic images of TUNEL staining (Fig. 4). The effects were dose-dependent and more severe at higher doses. Inhibition of apoptotic effects by antioxidants suggests that PCB 126-induced apoptosis of chondrocyte may be ROS-mediated. ROS-mediated damage is known as a major signal in precipitating apoptosis and a total action of dioxin-responsive genes represents a pivotal upstream event in the apoptosis cascade[11]. Therefore, it is suggested that PCB 126 induces ROS production which may lead to the subsequent apoptosis of chondrocytes.

Bioaccumulative and ubiquitous characteristics of PCBs and its related compounds in our environment may further increase the possibility of chondrocyte apoptosis and subsequent cartilage diseases. Since humans are continuously exposed with the persistent environmental pollutants and some of these pollutants are associated with the skeletal dysfunction, this finding may shed a new light in studying roles of the environmental pollutants

in the etiology of arthritis

Acknowledgment

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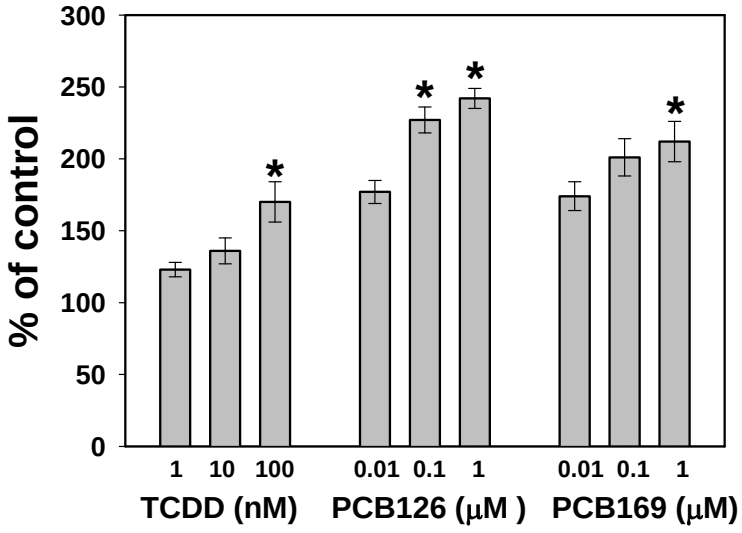

Fig. 1. ROS generation on chondrocytes with exposure to TCDD, PCB126 and PCB169.

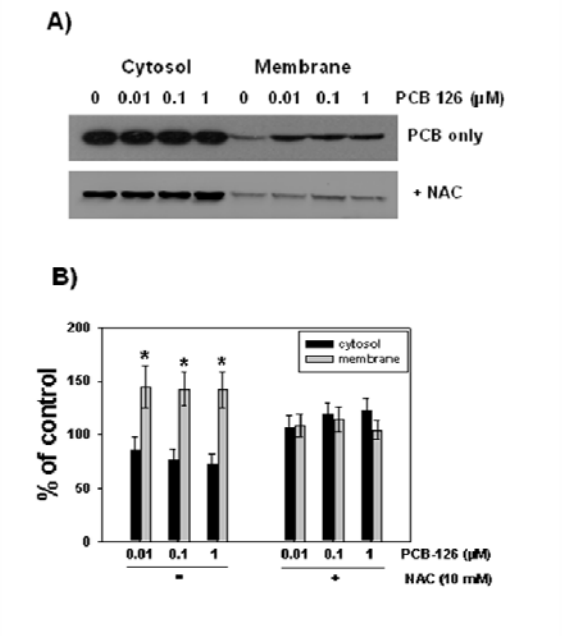

Fig. 2. Western blot analysis of PKC-δ with representative bands (A) and histogram (B).

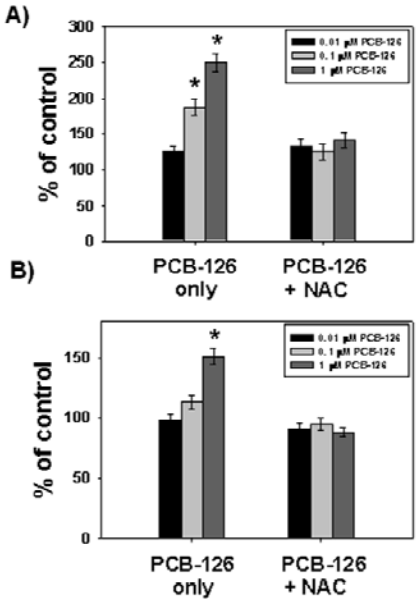

Fig. 3. Analysis of apoptosis with Caspase-3 assay (A) and ELISA (B).

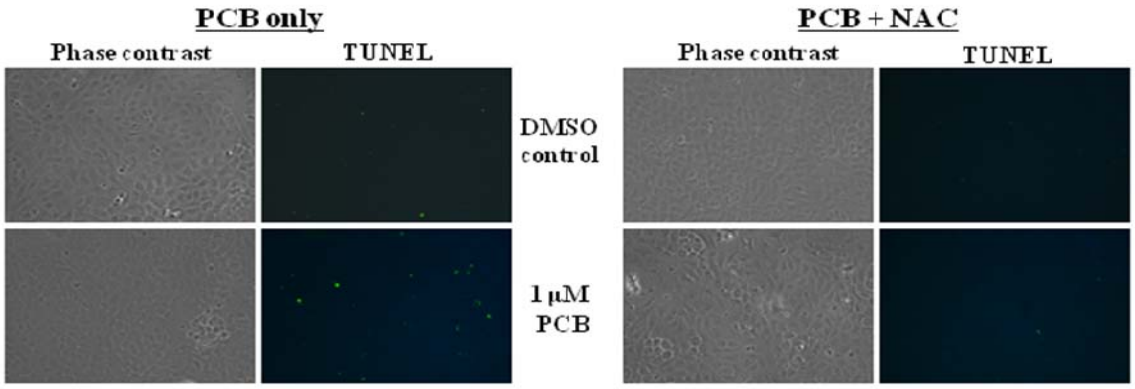

Fig. 4. TUNEL assay for apoptotic effects. Representative image from three independent experiments are presented. Magnification × 400.