A POSSIBLE ROLE OF DIOXIN AS AN INITIATOR OF CARTILAGE DISEASE ANS ITS RELATED MECHANISM OF ACTION

Hyun-Gyo Lee, Hye-Young Kim, and Jae-Ho Yang

Department of Pharmacology/Toxicology, Catholic University of Daegu, School of Medicine, Daegu, Republic of Korea

Introduction

Dioxin and related compounds have been suggested as causes of the musculoskeletal diseases in animals and humans[1]. However, no attempt has been made to investigate the possible role of dioxin, the most potent environmental pollutant, in the etiology of cartilage disease such as arthritis. 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 dioxin on chondrocyte apoptosis, which is a critical event in the cartilage disease and its related mechanism.

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 35mm dishes were exposed to 0, 1, 10 and 100 nM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, or dioxin; >99% purity; CIL, Andover) for 24 hrs for RT-PCR analysis. For the immunoblot analysis of PKC-delta, cells were exposed to 0, 1, and 10 nM TCDD for 15min.

RT-PCR. Total RNA was extracted using TRIzol Reagent (In vitrogen) according to the manufacturer's instructions. 1 μg of total RNA were then reverse-transcribed using the Improm II reverse transcriptase (Promega) according to the manufacturer's instructions supplemented with 500 ng oligo-dT_{15}-primer and 20 U RNasin inhibitor.

Western Blot Analysis. 10 μg of whole cell lysates were immunoblotted as described previously[6]. PKC-δ

and MMP-13 were detected using the respective monoclonal antibodies (BD Transduction Laboratories, Lexington, KY). The blots were reacted with a peroxidase-conjugated anti-mouse IgG and detected by Super Signal (Pierce, Rockford, IL).

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

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. In brief, chondrocytes grown on coverslips were exposed to 0.1% DMSO and 10 nM TCDD for 24 hrs. TUNEL-positive cells were analyzed under a Zeiss Axiophot fluorescence microscope (Zeiss, Oberkochen, Germany).

Results and Discussion

Dioxin is the most potent environmental pollutant and has been suggested as a cause of the musculoskeletal diseases in animals and humans. However, possible role of dioxin in the apoptosis of chondrocytes, a major target cell of the cartilage, has never been elucidated. We used the primary rabbit chondrocytes in culture to analyze the effects of dioxin on chondrocytes apoptosis and suggest the possible mechanism of dioxin-induced cartilage disease.

Dioxin induced mRNA levels of arthritis-associated genes such as IL-1β, iNOS, NF-κB, COX-II and VEGF (Fig. 1). Increase of IL-1β or iNOS leads to induction of COX-II which generates PGE2, implicated in the pain and arthritic disease[8]. Considering roles of these genes in arthritic pathogenesis, our results suggest that dioxin may interfere with the interdependent regulation of arthritis-associated genes in primary condrocytes and lead to the pathogenesis of arthritis.

Activation of PKC-δ in chondrocytes was observed upon dioxin exposure (Fig. 2A). Altered activation of this isozyme may adversely affect the normal function of chondrocytes via aberrant PKC-mediated phosphorylation. Sine the present study showed an increase of MMP-13 protein levels upon dioxin exposure (Fig. 2B), it is suggested that activation of PKC-δ may be associated with the increase of MMP-13, a cartilage degrading enzyme, which may contribute to the induction of cartilage disease[9]. To examine the effect of dioxin on the apoptosis, which is an initiating event of cartilage degradation, TUNEL assay was performed. The apoptotic cell death upon dioxin exposure was detected by the TUNEL staining. Fluorescence microscopy using TUNEL staining identified a higher number of cells with apoptotic bodies after 24 hrs treatment of 10 nM TCDD, as compared to DMSO control (Fig. 3A). The percent of positive cell numbers among the TCDD- treated cells (16.2 ± 1.9) was three times higher than that of the DMSO control cells (5.0 ± 1.2) (Fig. 3B).

There is no answer to what extent apoptosis should be observed in degenerative cartilage in order for it to play a pathogenic role. However, considering the chronic nature of the disease process, the minor but continuous stimulus of apoptosis inducer may initiate and promote the pathogenesis of the cartilage

disease. Bioaccumulative and ubiquitous characteristics of dioxin 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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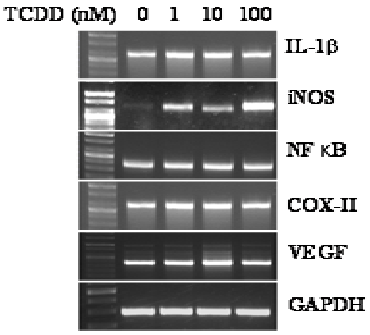

Fig. 1. RT-PCR analysis of arthritis-associated genes in chondrocyte following dioxin exposure

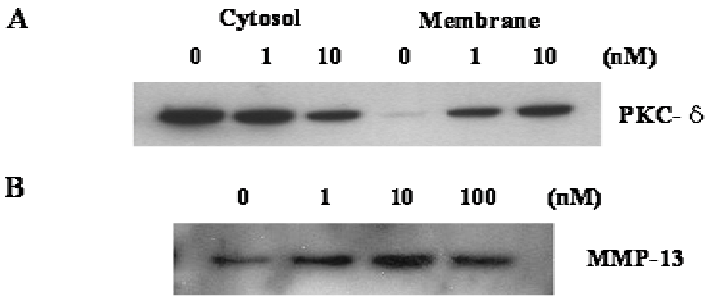

Fig. 2. Western blot analysis of PKC-delta (A) and MMP-13 (B)

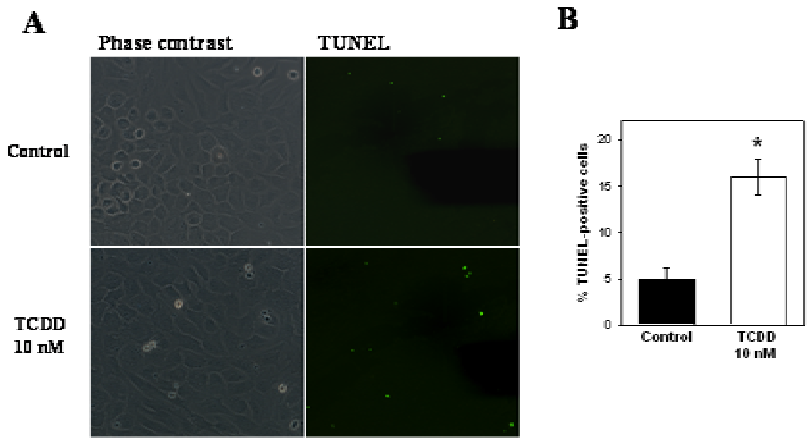

Fig. 3. TUNEL assay for apoptosis induction. Representative image from three independent experiments are presented (A). Magnification × 400. The number of TUNEL-positive cells were presented as a histogram (B).