NITRIC OXIDE MEDIATES DIOXIN-INDUCED APOPTOSIS OF CHONDROCYTES IN CULTURE

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Abstract

Positive associations between halogenated aromatic hydrocarbons and arthritis have been reported in human populations. 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. NO was produced in a dose-dependent manner with a maximum of 2.2-fold increase at 100 nM TCDD. Levels of matrix metalloproteinase (MMP)-13 were increased. ELISA analysis showed increase of apoptosis, which was further supported by TUNEL staining. The dioxin-induced increases of MMP-13 and apoptosis were effectively blocked by iNOS inhibitor, L-NMMA, indicating that these events are mediated via NO-dependent pathway. The present study suggests that dioxin induces apoptosis of chondrocyte via NO-dependent pathway, which also mediates an increase of cartilage degradation enzyme, MMP-13. Since this is the first study to analyze the mechanism of dioxin-induced apoptosis, our results may contribute to understand the initial roles of the environmental pollutants in the etiology of arthritis.

Introduction

Recently, strong positive association between dioxin-like PCBs and arthritis in women was reported from the background exposure levels[1]. Chondrocyte apoptosis is responsible for the cartilage damage, which is the most prominent feature of the arthritis. Clinical specimens from rheumatoid arthritis (RA) and osteoarthritis (OA) cartilages showed apoptotic chondrocyte death. Since dioxin-like compounds are associated with the outcome of arthritis and chondrocytes have clinical importance in the pathogenesis of the disease, we hypothesized that TCDD, the most toxic compound of HAH, may play important roles in the disease outcome by impairing the normal function or survival of chondrocytes. Thus, the present study attempted to examine the effects of dioxin on chondrocyte apoptosis and its mechanism of action.

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[2].

Exposure. Cells grown on 96-well plates were exposed to 0, 0.1, 1, 10 and 100 nM 2,3,7,8-tetrachlorodibenzo-*p*-

dioxin (TCDD, or dioxin; >99% purity; CIL, Andover) for 24 hrs for NO analysis. For the immunoblot analysis of MMP-13, cells were exposed to 0, 1, 10 and 100 nM TCDD for 24 hrs.

Western Blot Analysis. 10 μg of whole cell lysates were immunoblotted as described previously[2]. MMP-13 was detected using the monoclonal antibodies (BD Transduction Laboratories, Lexington, KY).

NO measurement. NO production was measured by estimating the stable NO metabolite, nitrite, in conditioned medium using a spectrophotometric method based on the Griess reaction[3].

Analysis of apoptosis by internucleosomal DNA fragmentation. Fragmented nucleosomal DNA was quantified by ELISA kit (Cell Death Detection ELISA Plus; Roche, Mannheim, Germany) as described in the manufacturer's manual.

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

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. The metabolism of chondrocytes in articular cartilage is subject to a complex environmental control. NO plays a catabolic role in OA disease pathology and mediates apoptosis and inflammatory responses[4]. It is also involved in regulation of matrix metalloproteinases and inhibits the synthesis of collagen and proteoglycans[5]. While the effects of NO depend on a variety of surrounding conditions including the amount of production, its diffusion and the concentrations of reactant species[6], it is a prevailing hypothesis that NO presence in excess contributes to detrimental outcome of joint diseases. NO was produced in a dose-dependent manner with a maximum of 2.2-fold increase at 100 nM TCDD (Fig. 1A). The dioxin-induced NO production was effectively blocked by iNOS inhibitor, L-NMMA (Fig. 1B). As demonstrated in this study, it is suggested that dioxin-induced overproduction of NO may lead to detrimental effects via an induction of apoptosis and matrix metalloproteinases (MMP).

Multiple family members of MMP are expressed in articular cartilage and play an important role in cartilage destruction. In particular, MMP-13 preferentially digests type II collagen among interstitial collagens and is expressed by chondrocytes and synovial cells in human OA and RA[7]. MMP-13 causes loss of cartilage, which prompts apoptosis of chondrocyte[8]. In our study, dioxin increased the amount of metalloproteinase-13, a key enzyme for the cartilage damage in dose- dependent manner (Fig. 2). This increase was blocked by L-NMMA, α-NF, or Trolox. The results indicate that MMP-13 increase may be associated with NO and ROS productions, which are mediated via the AhR-dependent pathway. Since MMP-13 is correlated with loss of ECM[7], which

initiates apoptosis of chondrocytes, dioxin-induced MMP-13 may also play a certain role in accelerating the apoptosis process. Since survival and death of chondrocytes are closely linked to the cartilage matrix integrity, apoptotic cell death is considered as an important factor contributing to the breakdown of extracellular matrix in joint diseases[9]. Damaged chondrocyte viability may accelerate the progression of the lesion[10]. The present study clearly demonstrated that dioxin is an inducer of chondrocyte apoptosis (Fig. 3A). Inhibitory effects of apoptosis by the NO inhibitor in our study (Fig. 3B) suggest that NO mediates the dioxin-induced apoptotic process.

Taken together, the present study suggests that dioxin induces apoptosis of chondrocyte via NO-dependent pathway, which also mediates an increase of cartilage degradation enzyme, MMP-13.

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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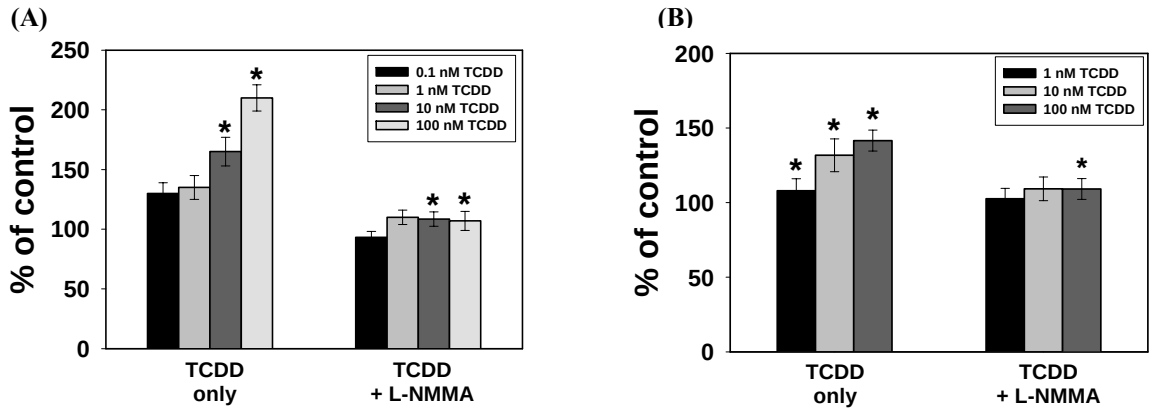

Fig. 1. Effects of NO inhibitor on the NO production (A) and apoptotic effects (B) following TCDD exposure.

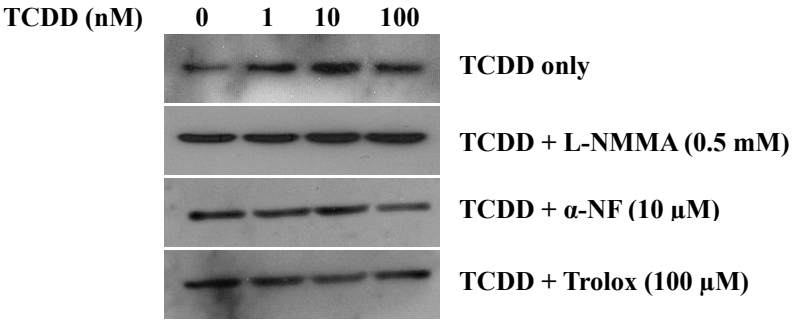

Fig. 2. Western blot analysis of MMP-13 levels with or without respective inhibitors.

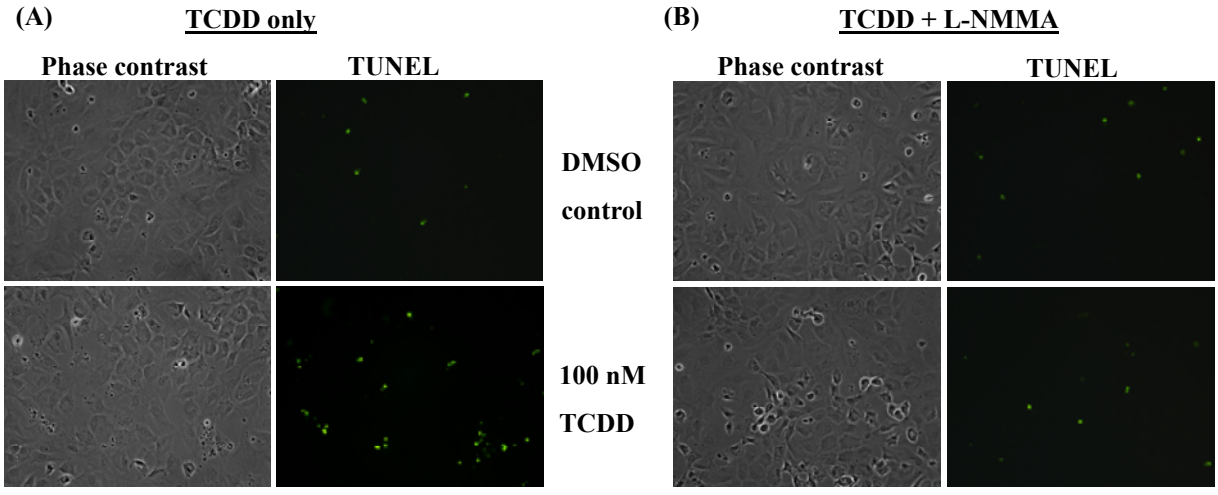

Fig. 3. TUNEL assay for apoptotic effects in presence (B) or absence (A) of NO inhibitor, L-NMMA. Representative image from three independent experiments are presented. Magnification × 400.