Reactive Oxygen Species Activate Protein Kinase C delta in Chondrocytes Following Dioxin Exposure

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Abstract

Dioxin exposure as low as 0.1 pM induced the mRNA levels of CYP1A1, and CYP1B1, the most prominent marker genes. mRNA levels of iNOS were also induced. The induction of these genes was dose-dependent. Dioxin exposure generated ROS in a dose- and time-dependent manner. Induction of ROS was inhibited by antioxidants such as N-Acetyl Cysteine (NAC) or Trolox and elevated by BSO, GSH depleting agent. Dioxin translocates PKC-delta from the cytosol to the membrane fractions. The translocation was blocked by the antioxidants, suggesting that this translocation was mediated via the ROS generation. The present study demonstrated that the ROS generated by dioxin exposure activates PKC-delta, which stimulates MMP-13, a key enzyme for cartilage degradation. It is suggested that dioxin may be involved in cartilage degradation via generating ROS species, which then activates a matrix degradation enzyme thru PKC signaling.

Introduction

Inflammatory arthropathies such as rheumatoid arthritis (RA) and osteoarthritis (OA) are the major joint diseases which accompany the cartilage degradation. Among the pathogenic factors of joint diseases, ROS generation is closely involved in cartilage damages and joint inflammation.[1] While ROS plays a crucial role in the regulation of normal chondrocyte activities such as cell activation, proliferation and matrix remodeling, excessive ROS is considered to damage all matrix components of the cartilage by a direct attack or by reducing matrix component synthesis[2].

Cigarette smoke contains the components of industrial or environmental pollutants that may produce ROS upon the exposure. It is reported that heavy smoker without a family history of RA showed a stronger association with RA than non-smoker, suggesting the possible link between the smoking and the arthritis[3]. PAHs contained in cigarette smoke are suggested to play a role in the etiology of rheumatoid arthritis[4]. Among the components of cigarette smoke, dioxin is the most potent chemical. However, a possible link between dioxin and joint diseases has never been elucidated.

While effects of dioxin are well documented in a variety of organ and tissue levels, possible effects of dioxin on the cartilage tissues or chondrocytes has been poorly examined. A recent report showed that a battery of

dioxin-responsive genes is induced upon dioxin exposure, suggesting that chondrocyte is a sensitive cell type responding to dioxin[5]. Since ROS generation is associated with cartilage degradation and chondrocyte is a sensitive cell type to dioxin exposure, we attempted to look into possible effects of ROS in chondrocytes upon dioxin exposure to understand the mechanism of environmental toxicant-derived arthritis.

Materials and Methods

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

Exposure. Cells grown on 6-well culture plates were exposed to 0, 1, 10 and 100 nM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; >99% purity; KOR, Boston) 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.

Western Blot Analysis. 10 ug of whole cell lysates were immunoblotted as described previously.[5] PKC-delta were detected using isoform-specific anti- PKC monoclonal antibody (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 (50uM), as described previously[7].

Results and Discussion

Dioxin altered mRNA expression of dioxin-responsive genes in chondrocytes in culture. Dioxin exposure for 24hr revealed mRNA induction of CYP1A1 and CYP1B1, which are known as the most prominent marker genes of dioxin exposure. The induction of these genes was dose-dependent. In particular, induction of marker genes such as CYP1A1 and CYP1B1 was observed at the concentration as low as 10pM (Fig. 1). These results indicate that chondrocyte is a sensitive cellular model to study the mechanistic pathway of dioxin-related joint diseases.

In response to the partial oxygen pressure variation, mechanical stress, and inflammatory mediators, chondrocyte produces abnormally high levels of ROS. The main ROS species produced by chondrocytes in this process is nitric oxide (NO) which is involved in the cartilage destruction.[8] NO stimulates the production of bFGF, a strong angiogenesis factor, and plays an important role in regulating apoptosis of chondrocyte. NO is synthesized by NO synthase (NOS). The present study demonstrated that induction of iNOS mRNA was dramatically induced at 10 nM TCDD, suggesting the important role of this species in dioxin-mediated detrimental effects on cartilage (Fig. 1). Dioxin exposure induced ROS generation in a dose- and time-dependent

manner. Induction of ROS was inhibited by N-Acetyl Cysteine (NAC) or Trolox and elevated in the presence of BSO, the GSH depleting agent. The result further supports that dioxin induces ROS generation (Fig. 2).

Treatment of chondrocytes with dioxin induced the translocation of PKC isoforms. Among the isoforms examined, PKC-delta showed the most significant translocation from the cytosol to the membrane fractions. The translocation was blocked by the treatment of NAC or trolox, suggesting that this translocation is mediated via the ROS generation (Fig. 3). In human articular chondrocytes, PKC-delta activation is known as a principal rate-limiting event in the *b*FGF-dependent stimulation of MMP-13[9]. Thus, our results suggest that dioxin-derived ROS generation is involved in the activation of PKC-delta, which may lead to the stimulation of the cartilage degrading enzyme, MMP-13. It is evident from this study that dioxin plays a role in the detrimental effects of joint diseases thru the ROS generation and the alteration of PKC signaling pathway. Further studies are warranted to elucidate the mechanism of dioxin-derived cartilage damages including the effects of MMP and TIMP. Our results may shed a new light in understanding environmental toxicant-induced joint diseases and enable us to assess the risks of dioxin with more solid scientific mechanism of action.

Acknowledgment

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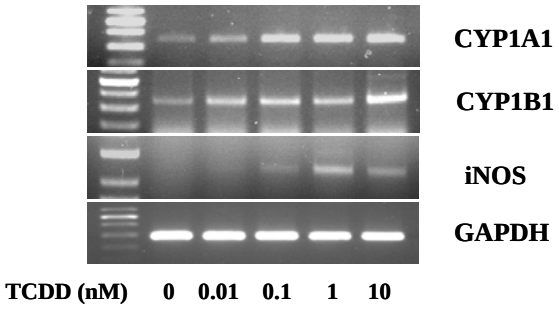

Fig. 1. RT-PCR analysis of CYP 1A1, 1B1 and iNOS mRNA following 24hr exposure of TCDD

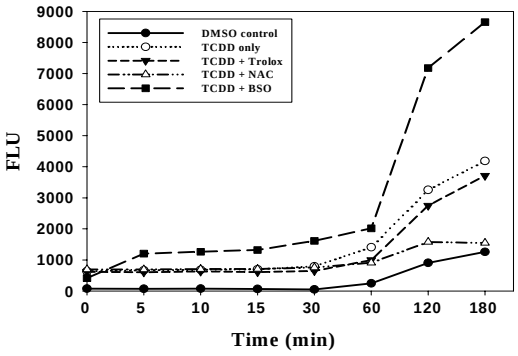

Fig. 2. ROS generation of TCDD in the presence of TCDD (1nM) Trolox (100 μM), NAC (10mM) or BSO (10mM)

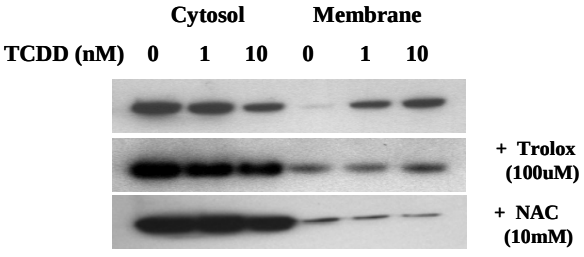

Fig. 3. Western blot analysis of TCDD-indeuced PKC-δ Translocation in presence of Trolox or NAC