CHONDROCYTE IS A SENSITIVE CELL TYPE TO DIOXIN EXPOSURE

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Introduction

Dioxin is a highly toxic environmental contaminant and is known to disrupt bone modeling and decrease bone mechanical strength.[1] While effects of environmental toxicants on bone are relatively well documented, possible effects of environmental chemicals such as TCDD on cartilage tissues is rarely understood. Recent epidemiology studies indicated that PAHs in the cigarette smoke has an association with rheumatoid arthritis (RA).[2] In particular, heavy smoker without a family history of RA showed a stronger association with RA than non-smoker. Rheumatoid arthritis is a chronic inflammatory condition of which the disease pathogenesis is characterized by cartilage degradation, production of reactive oxygen intermediates and proinflammatory cytokines, angiogenesis, etc.[3] Among the pathogenic factors, cartilage degradation is the most prominent feature. Chondrocytes are the sole residents of the cartilage and maintain cartilage integrity. These cells play an essential role in keeping the equilibrium between synthesis and degradation of cartilage matrix. Thus, analyzing the effects of chondrocyte upon the exposure of pathogens is important to understand the etiology of RA.

Since cigarette smoking is associated with RA and dioxin is an important component of cigarette smoke, we attempted to look into possible effects of dioxin in chondrocytes to understand the mechanism of environmental toxicant-derived arthritis. The expression of dioxin-responsive genes and RA-associated genes were examined in primary chondrocytes in culture.

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.[4]

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 isozymes, 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. Following RT reaction, PCR was carried out as previously described.[4]

Western Blot Analysis. 10 ug of whole cell lysates were immunoblotted as described previously.[4] PKC isoforms were detected using isoform-specific anti- PKC monoclonal antibodies for α, βII, γ, ε, λ, and ι (BD Transduction Laboratories, Lexington, KY). The blots were reacted with a peroxidase-conjugated anti-mouse IgG and detected by Super Signal (Pierce, Rockford, IL).

Results and Discussion

Dioxin altered mRNA expression of dioxin-responsive genes and other RA-associated genes in chondrocytes in culture. This is the first report on the effects of TCDD in the primary chondrocytes. Dioxin exposure for 24hr revealed mRNA induction of CYP1A1, CYP1A2 and CYP1B1, which are known as the most prominent marker genes with exposure to dioxin. The induction of these genes was dose-dependent. A considerable amount of the mediator gene mRNAs of dioxin toxicity, AhR and ARNT, were also expressed. The results indicate that chondrocyte may be a target cell type sensitive to the dioxin exposure and provide a scientific basis to elucidate the mechanistic pathway of battery of dioxin-responsive genes in the cartilage (Figure 1).

Cartilage degradation, the most prominent feature of RA, is preceded by the production of proinflammatory cytokines, reactive oxygen species and angiogenesis. The present study demonstrated that dioxin induced increases of IL-1β, iNOS, NF-κB, COX-II and VEGF mRNA levels. IL-1β is a major proinflammatory cytokine involved in cartilage destruction.[5] The aberrant production of proinflammatory cytokines such as IL-1β is strongly associated with RA pathogenesis. Chondrocytes possess a NADPH oxidase and produce NO, which is involved in the cartilage destruction.[6] NF-κB is a well studied nuclear transcriptional factor and is rapidly induced in response to chronic inflammation.[7] Increase of IL-1β or iNOS leads to induction of COX-II which generates PGE2, implicated in the pain and arthritic disease.[8] Mechanically induced expression of VEGF in articular cartilage is known to initiate degenerative process and finally leads to the destruction of joint system.[9] 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 (Figure 2).

In addition to genes associated with arthritic conditions, trichohyalin, aggrecan, HIF-1a and ALDH1a1 mRNA levels were also increased. Trichohyalin is a keratinization marker in epidermolytic hyperkeratosis and functions as an intermediate filament-associated protein.[10] Aggrecan is a major cartilage-specific extracellular matrix that maintains the phenotype of the differentiated chondrocytes. Both HIF-1a, and ALDH1a1 are associated with

hypoxic conditions.[11] While the significance of these findings remains to be elucidated, the altered expression of these genes suggest that dioxin may play an important role in understanding the biochemical events in chondrocytes and initiate more enthusiastic researches in the etiology of the connective tissue diseases (Figure 3). PKC plays a crucial role in the maintenance of chondrocyte phenotype.[12] Among the isozymes examined, levels of PKC-α, -βII, and -ε proteins were altered at 10nM TCDD (Figure 4). Altered expressions of certain PKC isozymes may play a pivotal role in the chondrogenesis process.

The present study demonstrated that chondrocyte is a sensitive cell type responding to dioxin exposure. Since PAHs from heavy smoking is closely associated with the outcome of rheumatoid arthritis, our results may provide a solid basis to understand the pathogenesis of joint diseases and pursue further studies in the future. In addition, the results may shed a new light in understanding environmental toxicant-induced RA and enable us to assess the risks of dioxin with respect to a broader spectrum of diseases

Acknowledgment

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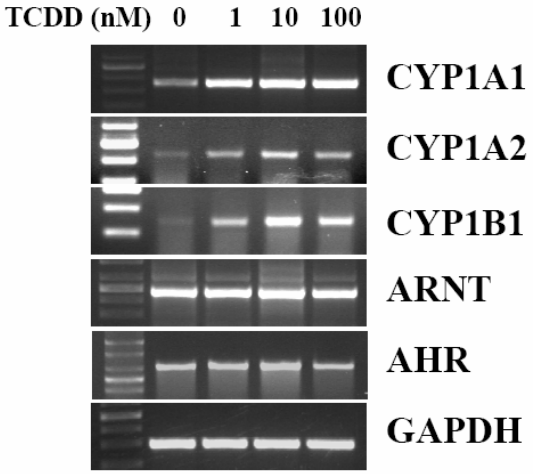

Fig.1. RT-PCR analysis of dioxin-responsive genes following 24 h exposure of TCDD

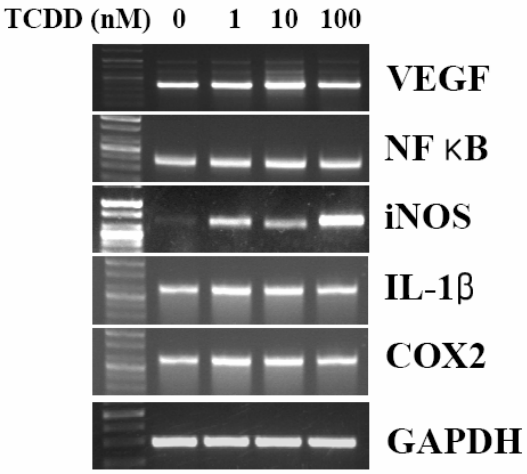

Fig.2. RT-PCR analysis of RA-associated genes 24h exposure of TCDD

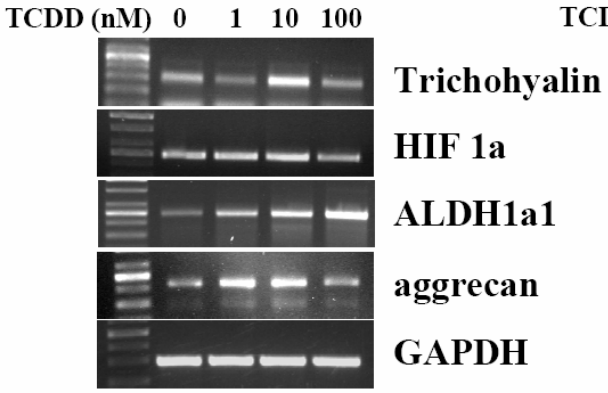

Fig.3. Altered expression of mRNAs in chondrocytes following TCDD exposure

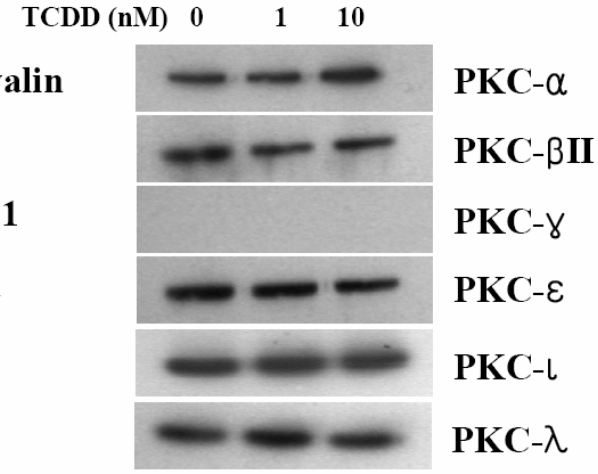

Fig.4. Western blot of PKC isozymes in chondrocytes with 15min exposure of TCDD