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Bisphenol-A interferes with estradiol-mediated protection in osteoarthritic chondrocytes

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ABSTRACT

Aged women have a higher risk of osteoarthritis (OA) due to estrogen (E2) loss at menopause. Studies suggested that E2 inhibits nuclear factor (NF)-κB activity which is increased in arthritis pathogenesis. Other studies revealed that external E2 reduces the expression of matrix metalloproteinases (MMPs) in osteoarthritic chondrocytes, and attenuates the pathogenesis of OA. Bisphenol-A (BPA) is an important industrial material, and an endocrine-disrupting chemical (EDC) that binds E2 receptors and interrupts the hormone signaling. It is unknown how BPA affects E2 functions and influences E2-mediated protections in OA. In this study, we investigated the effects of E2 and BPA on nitric oxide (NO) production, NF-κB activation, and MMP-1 expression in chondrosarcoma SW1353 cells and primary human osteoarthritic chondrocytes. Among the tested chemicals, BPA reduced NO production and cell viability of chondrosarcoma cells, but had little effects on osteoarthritic chondrocytes. Using HPLC–UV, we observed BPA in the serum and synovial fluid of OA patients. In primary chondrocytes, modest concentrations of E2 reduced interleukin (IL)-1β-dependent NF-κB activation and MMP-1 expression. BPA antagonized these protective effects of E2 in a concentration-dependent manner. In conclusion, BPA interferes with E2's functions in chondrocytes and may promote OA.

1. Introduction

Osteoarthritis (OA) is characterized by the degeneration of articular cartilage, marked by the breakdown of matrix proteins. This leads to the development of fibrillations, fissures, and ulcerations of articular cartilage surfaces. Chondrocytes can produce interleukin (IL)-1β that induces the expression of matrix metalloproteinases (MMPs), aggrecanases, and other catabolic proteins (Attur et al., 2000; Moos et al., 1999). Cartilage degradation is mediated by MMPs, which specifically cleave matrix proteins (Mort and Billington, 2001). There is extensive evidence that among MMPs, MMP-1 (collagenase 1), MMP-3 (stromelysin 1), and MMP-13 (collagenase 3) are particularly involved in the OA process (Mengshol et al., 2002; Tetlow et al., 2001). Chondrocytes in OA cartilage may continuously be exposed to autocrine, paracrine, and other catabolic factors at high local concentrations. These factors induce the synthesis of MMPs, aggrecanases, cytokines, nitric oxide (NO), and prostaglandins, and may regulate their responses.

Hormonal changes occurring around menopause have long been thought to affect the occurrence of OA (Felson and Zhang, 1998). Epidemiological studies suggest that estrogen (E2) loss may be accompanied by an increase in the prevalence and incidence of knee and hip OA. A role for E2 in OA is consistent with the larger incidences in female than in male patients over 50 years of age with hip, knee, or finger OA. The two E2 receptors (ERα and ERβ) were identified in normal and osteoarthritic cartilage, indicating that cartilage can respond to E2s. Subsequently, identification of the two ERs in chondrocytes provided further evidence that the cartilage is sensitive to E2s (Ushiyama et al., 1999). Studies of OA in postmenopausal women with and without hormone replacement therapy provide strong support for the beneficial effect of E2s in OA (Nevitt et al., 1996). In the presence of tumor necrosis factor (TNF)-α, the secretion of MMP-1 is significantly reduced by 17β-estradiol; however, E2 exerted no significant effect on MMP-3, MMP-13, or TIMP-1

Abbreviations: OA, osteoarthritis; E2, estrogen or estradiol; NF, nuclear factor; MMP, matrix matelloproteinase; NO, nitric oxide; IL, interleukin; ER, estrogen receptor; IKK, IκB kinase; EDC, endocrine-disrupting chemical; BPA, bisphenol-A; DDT, 1,1-Bis(4-chlorophenyl)-2,2,2-trichloroethane; TCDD, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; PCB77, 3,3′,4,4′-Tetrachlorobiphenyl; PCB126, 3,3′,4,4′,5-Pentachlorobiphenyl.

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expression (Lee et al., 2003). Furthermore, Richette and colleagues showed the biphasic effect of estradiol (Richette et al., 2004). When added in combination with IL-1β to cartilage *in vitro*, estradiol modulates IL-1β-induced PG degeneration and MMP expressions. At a low concentration of estradiol (0.1 nM), IL-1β-induced effects were inhibited, whereas they were enhanced at a high concentration (10 nM).

ERα was shown to inhibit NF-κB activity in an E2-dependent manner at nanomolar concentrations of E2 in various cell lines (Hsu et al., 2000). The NF-κB family of transcription factors controls various aspects of the immune and skeletal systems, as well as inflammatory responses (Viatour et al., 2005). The NF-κB signaling pathway is present in all types of cell and consists of canonical and non-canonical pathways. In the canonical pathway, NF-κB dimers are inactive in the cytoplasm owing to their interaction with the inhibitor, IκB, such as IκBα (Baeuerle and Baltimore, 1988). Numerous signals, including cytokines, chemokines, components of the bacterial cell wall, growth factors, and B and T cell receptor antigens, lead to activation of an IκB kinase (IKK) complex. Activated IKK phosphorylates IκB, leading to its proteosomal degradation, which enables NF-κB transcription factors to be translocated to the nucleus. Optimal induction of NF-κB target genes also requires phosphorylation of NF-κB proteins, such as p65, within their domain by a variety of kinases in response to distinct stimuli (Viatour et al., 2005).

Large amounts of bisphenol-A (BPA) are produced worldwide. There are a total of 12 nations manufacturing BPA commercially, especially the USA, Germany, Japan, and Taiwan (Tsai, 2006). BPA is used extensively in epoxy resins lining food and beverage containers and polycarbonate plastics in many consumer products. Widespread and continuous exposure to BPA, primarily through food but also through drinking water, dental sealants, dermal exposure, and inhalation of household dusts, is evident from the presence of detectable levels of BPA in more than 90% of the US population (Calafat et al., 2005, 2008). In Taiwan, BPA was detected as much as up to 4.23 μg/l (18.5 nM) in water collected from the Kao-Ping River and its tributaries (Chen et al., 2009). The biological effects of BPA on experimental animals were shown to have endocrine disruptive activities (Takeuchi et al., 2004; Tsai, 2006). Most of the studies focused on well-documented estrogenic activities such as uterotrophic effects, decreasing sperm production, simulation of prolactin release, promotion of cell proliferation in a breast cancer cell line, alteration in the onset of sexual maturity in females and change in the development of male reproductive organs, and influence on preimplantation development. BPA is considered to be a weak environmental estrogen with only 1/1000th ER-mediated transcriptional activities (Witorsch, 2002), leading some to suggest that its presence in our environment is relatively harmless. Several studies also suggest that the interaction of the ERs with E2 or BPA is different, and it is likely that BPA induces a unique conformation of ERs (Kuiper et al., 1997; Nikula et al., 1999; Routledge et al., 2000; Wade et al., 2001). However, picomolar concentrations of both E_2 and BPA caused changes of a cellular process other than the genomic effect (Wozniak et al., 2005). More studies may be required to clarify the effects of BPA on E2-mediated functions.

The influence of BPA on OA by interfering with E2 regulation has not yet been reported, and the concentration of BPA in synovial fluid has not been investigated, either. Although BPA was thought to be metabolized rapidly in the human body (Vandenberg et al., 2007), a recent study suggested a longer than expected half-life for BPA (Stahlhut et al., 2009). Continuous exposure to BPA may cause metabolism imbalance in chondrocytes.

In a screening of five endocrine-disrupting chemicals (EDCs), we identified BPA which significantly affected chondrosarcoma cell activities. Further studies using human primary chondrocytes were conducted to test the effects of BPA and E2 on IL-1β-dependent NO production, NF-κB activation and MMP-1 expression. Our results suggest a role of BPA in promoting OA via interfering with E2's functions in chondrocytes.

2. Materials and methods

2.1. Preparation of charcoal-stripped fetal bovine serum (FBS)

We incubated charcoal and dextran (Sigma) in 0.25 M sucrose, 1.5 mM $MgCl_2$, and 10 mM HEPES (pH 7.4) at final concentrations of 0.25% and 0.0025%, respectively, at 4 °C overnight. Then we took a volume of the dextran-coated charcoal equivalent to that of the serum which was to be stripped and centrifuged it (at 500 × *g* for 10 min) to pelletize the charcoal. Finally, we replaced the supernatant with the same volume of FBS (Invitrogen) and incubated it overnight.

2.2. Cartilage samples and chondrocytes isolation

Cartilage was obtained from the knee cartilage of OA patients at the time of knee replacement arthroplasty. Primary chondrocytes were released from articular cartilage treated with 0.1% hyaluronidase for 15 min, followed by 0.5% proteinase for 30 min, then 0.2% collagenase in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL) containing 10% charcoal-stripped FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin at 37 °C overnight. Isolated chondrocytes were placed in 100-mm culture dishes. Primary chondrocytes were cultured at 37 °C in a 5% CO_2 incubator, with culture medium being changed every 48–72 h.

2.3. Chondrocytes culture and treatment

The human chondrosarcoma SW1353 cell line and primary human articular chondrocytes were seeded in 100-mm culture dishes. SW1353 cells were cultured in Leibovitz's L-15 medium (Invitrogen) with 10% charcoal-stripped FBS at 37 °C. Primary human chondrocytes were cultured in phenol red-free DMEM with 10% charcoal-stripped FBS with 5% CO_2 at 37 °C. The culture media contained 1% penicillin–streptomycin solution, 1% sodium pyruvate, and 4 mM L-glutamine (Invitrogen). Media were replaced every 2 days. For the experimental design, before chemical treatment, the culture medium of SW1353 was replaced with 5% charcoal-stripped FBS in phenol red-free L-15 medium. The culture medium of primary human chondrocytes was replaced with phenol red-free DMEM without charcoal-stripped FBS.

Cells were treated with EDCs, Estradiol (Sigma) and recombinant IL-1β (Invitrogen) as indicated to test NO production and cell viability, and determine the involved signaling pathways. EDCs including PCB77, PCB126, BPA, DDT, and TCDD were dissolved in DMSO, and used 1:1000 to treat cells. All experiments were performed at least three times.

2.4. Measurement of nitric oxide (NO) production

We used the Griess reaction for NO detection (Green et al., 1982). Briefly, after cells were treated with stimulants, 100 μl of supernatant of culture medium were collected into a 96-well clear microplate. And then 100 μl of Griess reagent (Sigma) were added to each well. After 3–5 min, the optical density was measured at 540 nm and compared to the external standard curve.

2.5. MTT assay

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) (Sigma) is reduced to purple formazan in the mitochondria of living cells. Cells were incubated in 24-well plates for 24 h, and then treated with starvation medium containing various concentrations of stimulants. After incubation, the medium was replaced with 100 μl of an MTT mixture composed of an MTT solution and starvation medium in a ratio of 1:9. Cells were then incubated in normal culture conditions for 2 h before being analyzed at an absorbance of 540 nm.

2.6. SDS-PAGE and Western blotting

Whole-cell lysates were obtained from SW1353 cells or primary human articular chondrocytes using 50 μl of golden lysis buffer. Cellular proteins at 50 μg each were separated by SDS-PAGE. After electrophoresis, the proteins were transferred onto PVDF membranes. Then the membranes were blocked with Tris-Buffered Saline Tween-20 (TBST) containing 5% BSA at room temperature for 1 h. After blocking, membranes were incubated with primary antibodies at 1:500 to 1:10,000 in TBST at room temperature for 1–2 h, and washed with the same buffer three times for 10 min each. The membranes were then incubated with secondary antibodies at 1:5000 in TBST at room temperature for 1 h, and washed three times. Blots were developed in chemiluminescence horseradish peroxidase (HRP) substrate (Millipore, Billerica, MA).

Antibodies specific for MMP-1 were purchased from CHEMICON (Temecula, CA). Immunoglobulin G (IgG) and IκBα antibodies were purchased from Santa Cruz

(Santa Cruz, CA). Antibodies specific for phospho-IκBα and phospho-p65 were purchased from Cell Signaling Technology (Danvers, MA).

2.7. Purification of BPA from serum and synovial fluid

To extract BPA from serum, we added 20 μl of glucuronidase/sulfatase (type H-2 from Helix pomatia, Sigma) and 60 μl of 2 M acetate buffer (pH 5.0) into 1 ml of serum, and incubated it at 37 °C for 3 h. One ml of synovial sample was treated with 50 μl hyaluronidase solution, and the mixture was incubated at 37 °C for 30 min. For solid-phase extraction, samples were applied to an Oasis HLB (Waters, Milford, MA). To condition and equilibrate the cartridge, we injected 1 ml of ethyl ether, followed by 1 ml of methanol (Merck, Whitehouse Station, NJ) and 1 ml of distilled water. Samples were injected into the Oasis HLB cartridges (at a flow rate of <1 ml/min). Lipids were removed from the column with 10% methanol. Finally BPA was eluted with 1 ml of methanol/acetonitrile (3:1).

2.8. HPLC conditions

The HPLC system, consisting of an LC model I (Waters) with an ODS column (4.6 mm × 250 mm $5C_{18}$-MS-II Cosmosil) and a UV detector, was used for the BPA analysis. The solvent system used was acetonitrile–water (40:60). The flow rate was 1.0 ml/min. The injection volume was 50 μl. BPA was detected by a UV detector at 228 nm. Different concentrations of pure BPA in methanol were used as standards. Normal serums with added BPA were used as controls.

2.9. Statistical analysis

The mean and standard deviation (SD) were used to illustrate the results from at least three data sets of each experiment. Statistical significance ($p < 0.05$) was assessed using Student's test or one-way analysis of variance, followed by a post hoc analysis using Dunnett's test when appropriate.

3. Results

3.1. BPA reduces NO production and cell viability in SW1353 chondrosarcoma cells

Proinflammatory cytokines increased the synthesis of NO through the inducible enzyme, iNOS (Palmer et al., 1993; Stadler et al., 1991). Studies showed that the *in vivo* selective inhibition of iNOS reduces the symptoms of inflammation and biochemical abnormalities of affected joint tissues (Connor et al., 1995; Pelletier et al., 1996, 1998). Therefore, the effects of five EDCs on NO production were screened on the SW1353 chondrosarcoma cell line. NO production was assessed by measuring nitrite concentrations in the culture media after treatments. Our results revealed that NO production of SW1353 varied in response to the EDCs; however, it was decreased significantly with high levels of BPA (Fig. 1A). Note that the cell viability in the presence of 100 μM BPA was only 30% of the untreated cells (Fig. 1B). Thus BPA influences NO production only at 10 μM.

In order to assess whether the EDCs affect chondrocytes viability, SW1353 cells were treated with the EDCs for 24 h. After treatment, an MTT assay was used to measure cell viability at 540 nm. Our data revealed that four of the five EDCs significantly reduced SW1353 cell viability at high concentrations, and surprisingly 100 μM BPA had the best effect (Fig. 1B). BPA is structurally similar to E2 which is able to reduce iNOS expression and inhibit NO production in articular chondrocytes (Richette et al., 2004, 2007); however, BPA may functionally interferes with E2 signaling and is eventually harmful to the cells.

3.2. BPA is present in the serum and synovial fluid of OA patients

To determine whether BPA is present in human bodies, we first measured concentrations of BPA in blood samples from patients with OA or immune-rheumatism. We used the samples from immune-rheumatic patients for comparison because patients with OA and immune-rheumatism share similar phynotype and symptoms, yet they undergo distinguishing pathogenesis. Using HPLC–UV, we were able to set the BPA standards (data not shown)

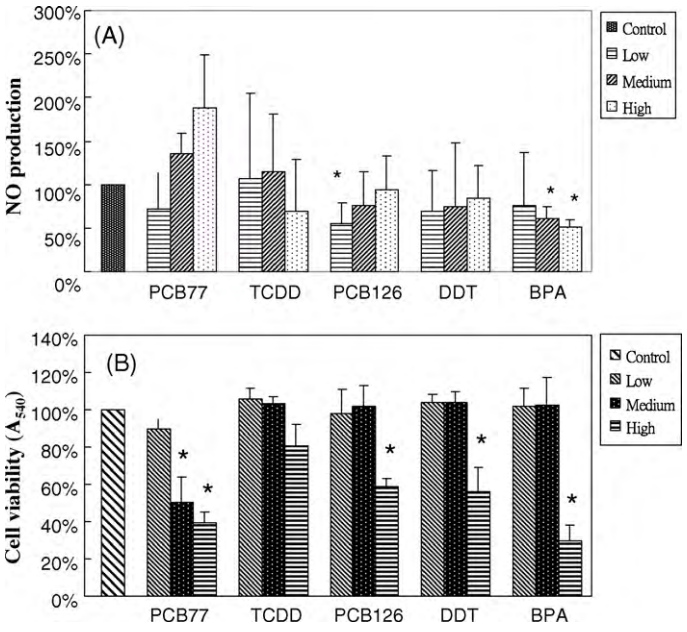

Fig. 1. Effects of endocrine-disrupting chemicals (EDCs) on SW1353 chondrocytes. SW1353 human chondrosarcoma cells were treated with five different EDCs for 24 h. Three different concentrations of each chemical were used as follows (low, medium, and high): PCB77, 0.1, 1, and 10 μM; TCDD, 0.1, 50, and 100 nM; PCB126, 0.1, 1, and 10 μM; DDT, 1, 10, and 100 μM; and bisphenol-A (BPA), 0.01, 1, and 100 μM. Control: solvent only. (A) Nitric oxide production was detected by the Griess reaction, and values were normalized to the controls. (B) Cell viability was determined by an MTT assay and detected at an absorbance of 540 nm. $^*p < 0.05$.

and determine BPA concentration in patient samples. BPA was detected in the serum of all three tested OA patients, while it is not detected in patients with immune-rheumatism (Fig. 2A). Furthermore, BPA was also detected in synovial fluid of the tested patient undergoing knee replacement arthroplasty. The concentration was higher in the synovial fluid (54.8 nM) than in the serum (23.3 nM; Fig. 2A) of the patient. The data support a role of BPA in OA pathogenesis.

We then cultured primary human chondrocytes from cartilage tissue of OA patients, and determined the effects of BPA on cells. According to a previous report, the low-dose range of BPA for *in vitro* studies was <50 ng/ml (<50 ppb; <219 nM; 1 nM = 0.228 ng/ml) in organ or tissue culture medium (Welshons et al., 2006). We used BPA ranging from 0.01 nM to 10 μM to treat cells, and then checked their NO productivity and cell viability. Our results showed that BPA had little effect on NO production in human primary chondrocytes (Fig. 2B). Also the cell viability did not significantly change in the presence of BPA (Fig. 2C). The cells may have been accommodated to BPA in the tissue of OA patients and were less responsive to *in vitro* BPA treatments.

3.3. Effects of E2 and BPA on IL-1β-stimulated NO production

It was shown that IL-1β can stimulate NO production (Palmer et al., 1993; Stadler et al., 1991) and may be linked to BPA and E2 function in primary chondrocytes. NO production by human primary chondrocytes in response to dose-dependent IL-1β stimulation was assayed. The results showed that 5 ng/ml IL-1β significantly induced NO production, although higher concentrations did not influence further (Fig. 3A). The effects of IL-1β concentration on NO production in human arthritic chondrocytes are not clear at this point.

Cells were then analyzed for the dosage effects of BPA and E2 on IL-1β-dependent NO productivity. Human primary chondrocytes were co-treated with 5 ng/ml IL-1β and various concentrations of

(A)

Patient source	Immune-Rheumatism		Osteoarthritis		
Serum BPA (nM)	N.D.	N.D.	**17.5**	**23.3**	**18.1**

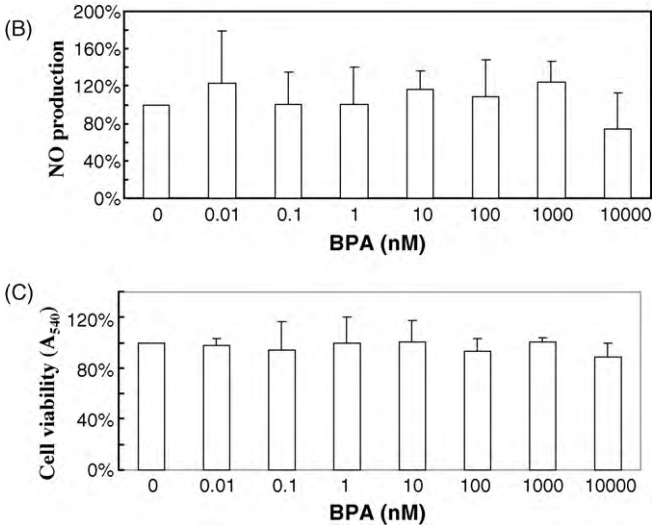

Fig. 2. Bisphenol-A (BPA) in human body fluids and effects on primary chondrocytes. (A) Concentrations of BPA in serum of patients with immune-rheumatism or osteoarthritis. N.D., not detected. (B) and (C) Primary cultured chondrocytes were treated with BPA at various concentrations for 24 h; for NO productivity (B) and cell viability (C) were assayed.

BPA or E2. The results showed that both E2 and BPA suppressed IL-1β-dependent NO production at 10 nM (Fig. 3B and C). In the contrary, NO production was restored at high concentrations of BPA. Overall these data indicated that E2 and BPA individually can negatively regulate NO production in human primary chondrocytes. This effect is in an IL-1β-dependent manner, and may only be seen at certain concentrations of BPA and E2. High BPA may eventually lead to stress responses of chondrocytes, and is destructive to cartilage tissue.

3.4. Effects of E2 and BPA on NF-κB activity

The NF-κB family of transcription factors controls inflammatory responses. Previously, the effects of E2 on NF-κB inhibition were investigated (Hsu et al., 2000). Further evidence that the inhibition is due to inactivation of IL-1β by E2 was also reported (Richette et al., 2004, 2007). Here we further examined the effects of BPA on NF-κB activities (Fig. 4). In articular chondrocytes, the addition of 5 ng/ml IL-1β activated p65, a subtype of NF-κB, within 15 min. Simultaneously, we observed the phosphorylation and degradation of IκBα, an NF-κB inhibitor (Fig. 4A), suggesting effective activation of NF-κB by IL-1β. The activation was inhibited by E2 at 60 min because the level of p-p65 was less and IκBα was more in the presence of E2 than in its absence (Fig. 4B). When BPA was added together with IL-1β and E2, the level of p-p65 dramatically increased at 15 min of treatment, although at 60 min it dropped to a similar level as treated with only IL-1β and E2 (Fig. 4B).

In another experiment with 16-h incubation, 5 ng/ml IL-1β activated p65 in both primary (Fig. 5A, left panel) and SW1353 (Fig. 5A, right panel) chondrocytes. It is similar to the 15-min treatment (Fig. 4A). Interestingly both the levels of p-p65 and p-IκBα were increasing as E2 concentration raised. It seems likely that low levels of E2 protect chondrocytes; however, high E2 induce stress to the cells instead. When BPA was introduced in addition to IL-1β and

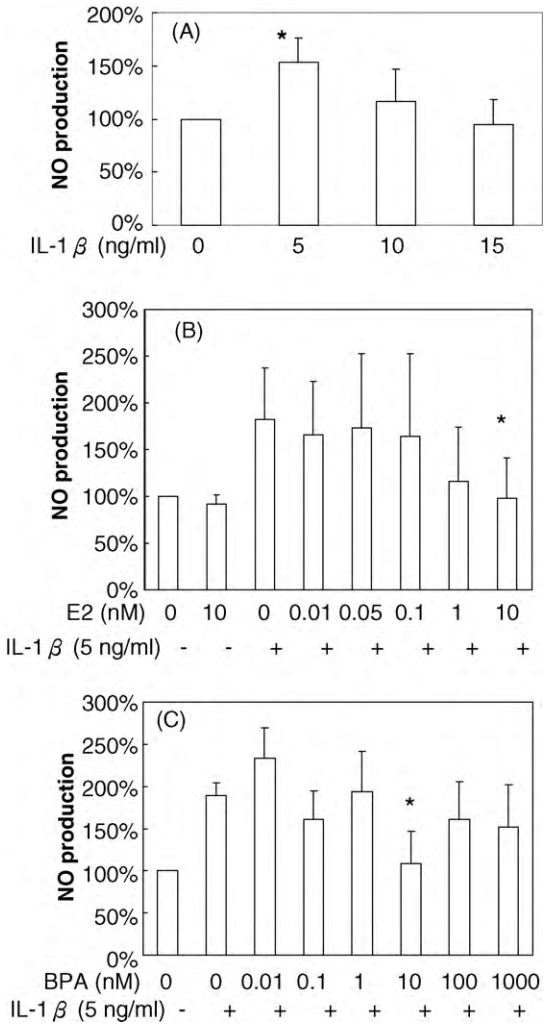

Fig. 3. Nitric oxide (NO) production in response to interleukin (IL)-1β and effects of the addition of bisphenol-A (BPA) or estradiol (E2). NO production was measured by the Griess reaction. Values were normalized to the untreated controls. (A) Primary human chondrocytes were treated with 0, 5, 10, or 15 ng/ml IL-1β for 16 h. (B) and (C) Cells were treated with 5 ng/ml IL-1β and various concentrations of E2 (B) or BPA (C) for 16 h.

low E2, indeed p-p65 levels were increased in a dose-dependent manner (Fig. 5B). These results support a role of BPA in activating NF-κB.

3.5. BPA interferes with E2's function of suppressing MMP-1 expression

In order to explore the effects of BPA and E2 on OA pathology, we analyzed MMP-1 expression in response to IL-1β and also E2 and BPA in chondrocytes at 16 h. There was little change in MMP-1 level when the cells were treated with only E2 at 10 nM (Fig. 5A). As expected, 5 ng/ml IL-1β was able to induce the expression of MMP-1 in both primary (Fig. 5A, left panel, and 5B) and SW1353 (Fig. 5A, right panel) chondrocytes. With the addition of E2 at concentrations of 0.1∼1 or 0.05∼0.1 nM in primary or SW1353 cells, MMP-1 expression was nearly suppressed to basal levels as in the absence of IL-1β (Fig. 5A). With 10 nM E2 in addition to IL-1β, however, MMP-1 expression increased. It is similar to another report that a low dose of E2 suppresses MMP-1 expression through inhibiting IL-1β activity in rabbit chondrocytes, while at high concentration E2 enhances the IL-1β effects (Richette et al., 2004).

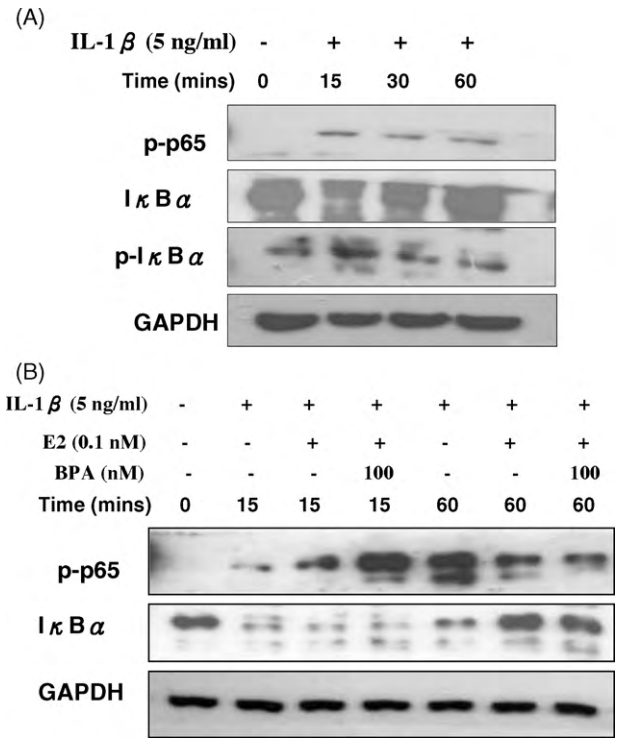

Fig. 4. Interleukin (IL)-1β-stimulated nuclear factor (NF)-κB activation in response to estradiol (E2) and bisphenol-A (BPA). Human articular chondrocytes were used. (A) Cells were treated with IL-1β alone for 0, 15, 30 and 60 min. (B) E2 and BPA were used in addition to IL-1β. p-p65, phosphorylated and activated p65 (a subtype of NF-κB); IκBα, an inhibitor of NF-κB; p-IκBα, phosphorylated and inactivated IκBα.

Whether BPA interferes with the protective effect of E2 on osteoarthritic chondrocytes is an important issue and a focus of this study. We treated primary chondrocytes with BPA in addition to 5 ng/ml IL-1β and 0.1 nM E2. As shown in Fig. 5B, 100 and 1000 nM BPA increased MMP-1 expression which is similar to the effect of ICI 182780, the known estrogen receptor antagonist. Thus BPA reversed the effect of E2 on suppressing IL-1β-dependent MMP-1 expression.

4. Discussion

OA is a gradually progressing disorder of mammalian joints, characterized by the destruction of articular cartilage, which results in discomfort and dysfunction of the affected joint. The pathologic changes during the development of OA are obviously similar and include proteoglycan degradation at the early stage, followed by type II collagen degradation, leading eventually to localized or complete loss of the cartilage matrix.

Great quantities of BPA are produced worldwide. Studies showed that BPA leaks from food containers, plastic bottles, and some dental sealants. Because of the high possibility of exposure to BPA, the influence on human health is obvious. To date, there has been no study determining BPA concentrations in synovial fluid. We obtained blood samples and synovial fluid from OA patients at the time of knee replacement arthroplasty, and found the presence of BPA in those fluids.

We discovered that the environmental contaminant, BPA, can promote OA. BPA increases MMP-1 expression and NF-κB activity in chondrocytes. It seems controversial that BPA decreases both NO production and cell viability of chondrosarcoma cells, but had no effect in osteoarthritic chondrocytes. Note that different cell types may respond to BPA differently. In fact, BPA was able to reduce IL-1β-induced NO production in primary chondrocytes at a low concentration, but restored NO at high concentrations. NO regulation may be different in chondrosarcoma cells and osteoarthritic chondrocytes. In endothelial cells, BPA stimulates NO synthesis through a non-genomic estrogen receptor-mediated mechanism (Noguchi et al., 2002), and increases cell death (Bredhult et al., 2009). However, suppression of NO production by BPA was found in macrophages which cellular activities were down-regulated as well (Ji Young and Hye Gwang, 2003; Pyo et al., 2007; Yoshitake et al., 2008). Indeed, the existence of BPA in OA patients' body flu-

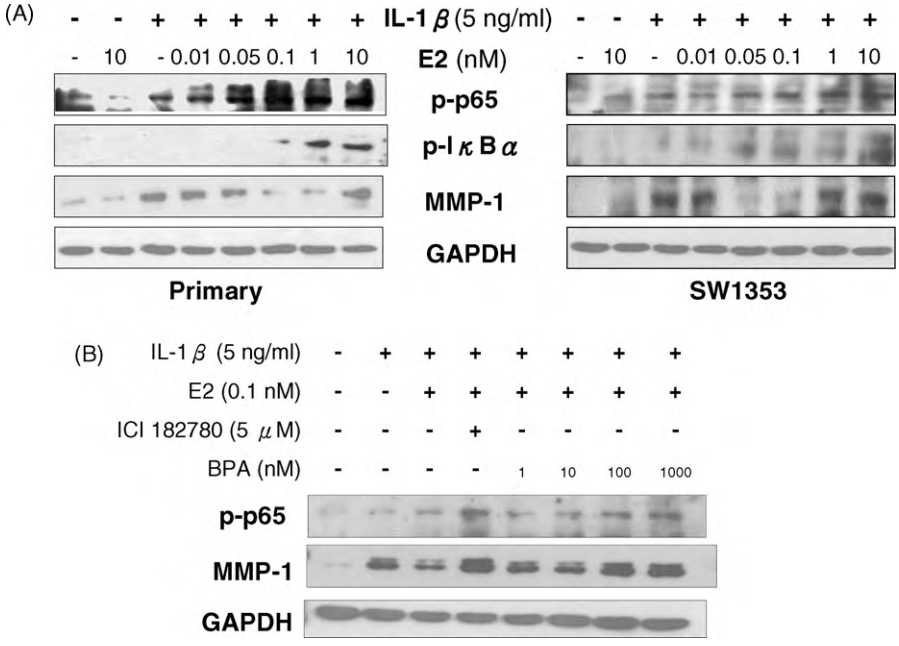

Fig. 5. Interleukin (IL)-1β-induced matrix metalloproteinase (MMP)-1 expression in response to estradiol (E2) and bisphenol-A (BPA). MMP-1 expression and NF-κB activation were analyzed by immunoblotting. Cells were treated for 16 h and the lysates at 50 μg each were used. (A) Primary (left panel) and SW1353 (right panel) chondrocytes were treated with 5 ng/ml IL-1β and various concentrations of E2. (B) Human primary chondrocytes were treated as indicated. ICI 182780 is an estrogen receptor antagonist, and was used as a control. p-p65, phosphorylated and activated p65 (a subtype of NF-κB); p-IκBα, phosphorylated and inactivated IκBα (an inhibitor of NF-κB).

ids, and its effects on cell viability, MMP-1 expression and NF-κB activities of chondrocytes suggest a role of BPA in promoting OA.

In our findings, the OA-promoting effects of BPA at levels which can be found in human body fluids (10–100 nM) mimic those of E2 at a high level (10 nM). In chondrocytes, BPA induced MMP-1 expression and NF-κB activation, which were also observed in the cells with either no E2 or high E2 treatments (Fig. 4). May those pathological concentrations of BPA mimic the high E2 effects? Obviously, it is not the case. When compared to E2, BPA binds to the ERs differently and has only 1/1000th affinity to the ERs (Routledge et al., 2000; Witorsch, 2002). In other words, the activity of 100 nM BPA to bind ERs may be similar to that of E2 at 0.1 nM, a concentration that reduces MMP-1 expression. Furthermore 100 nM BPA had no effect on reducing NO production in primary chondrocytes, while high E2 did (Fig. 3). Therefore our data favor a role of BPA in inhibiting E2 signaling, and/or BPA antagonizes other E2 functions in chondrocytes.

The effect of E2 on IL-1β-stimulated rabbit articular chondrocytes is controversial (Richette et al., 2004, 2007). At a low concentration of E2 (0.1 nM) IL-1β-induced effects are inhibited, whereas they are enhanced at a high E2 concentration (10 nM). High concentrations of E2 can reduce NO levels by impairing p65 transport to the nucleus, and can reduce the DNA-binding activity of p65 (Richette et al., 2007). In our study, we found that 10 nM E2 reduced NO production, but interestingly also enhanced MMP-1 expression and NF-κB activity after 16 h of treatment. High levels of E2 can cause an imbalance of the metabolism and catabolism of cartilage *in vivo* and *in vitro* (Richette et al., 2004). The mechanism of the dual effect of E2 requires further research.

More than the role of being an agonist of estrogen receptor, BPA is also an antagonist of the aryl hydrocarbon receptor (AhR) at concentrations of 10^{-5} to 10^{-4} M (Bonefeld-Jorgensen et al., 2007). The AhR is the receptor of EDCs, such as polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), and polychlorinated dibenzp-p-dioxins (PCDDs). After ligand binding, the AhR complex is translocated into the nucleus, and binds to the dioxin-responsive element (DRE), which is upstream of the CYP1A1 gene. Other reports showed that exposure to BPA disrupts expression of the AhR and related factors in the uterus (Nishizawa et al., 2005a), but induces the expression of the AhR in the cerebrum, cerebellum, and gonads (Nishizawa et al., 2005b). Furthermore, transfection of the ER in MDA-MB-231 cells restored the responsiveness of the AhR (Thomsen et al., 1994). Resulting from crosstalk with E2, the AhR may exhibit inhibitory effects on the ER (Safe and Wormke, 2003). The role of BPA in AhR-ER crosstalk may be one of the important mechanisms of E2 regulation in chondrocytes.

Conflict of interest

The authors declare that they have no competing interests.

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