

Different concentrations of 17 β -estradiol modulates apoptosis induced by interleukin-1 β in rat annulus fibrosus cells

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Abstract. Interleukin-1 β (IL-1 β) is a pleiotropic cytokine that mediates inflammatory and cell death activities. IL-1 β has been previously reported to induce apoptosis of intervertebral disc (IVD) cells in IVD degeneration. Accumulating data have suggested that post-menopausal women have a high incidence of IVD degeneration. It has therefore been proposed that estrogen may have a close association with IVD degeneration. Whether estrogen is able to protect IVD cells from apoptosis remains unclear. The present study aimed to examine whether 17 β -estradiol (17 β -E2) inhibited IL-1 β -induced apoptosis of rat annulus fibrosus (AF) cells. Additionally, the dose-response effect of 17 β -E2 on cell apoptosis was investigated. AF cells were isolated from male Sprague Dawley rats and cultured in complete medium. Following approximately two weeks, the AF cells were treated with IL-1 β (75 ng/ml) for 24 h, with a pretreatment of 17 β -E2 for 1 h. Apoptosis of AF cells was analyzed by annexin V/propidium iodide binding assay and morphological changes, together with an assessment of caspase-3 activity. Cell viability of the AF cells was determined by MTT assay. The level of apoptosis and caspase-3 activity in the AF cells was increased whereas the cell viability was decreased following treatment with IL-1 β (75 ng/ml), as compared with the control group. This effect was reversed by pretreatment with 17 β -E2, in a dose-dependent manner. The protective effect of 17 β -E2 was abolished by estrogen receptor antagonist ICI182, 780. These results indicate that 17 β -E2 protects rat AF cells from apoptosis induced by IL-1 β , in a dose-dependent manner.

Introduction

Chronic lower back pain is a common and debilitating disorder that accounts for societal economic losses. It is estimated that ~70% of the population will experience lower back pain during their life (1). Intervertebral disc (IVD) degeneration is the most prominent cause of chronic lower back pain (2). IVD degeneration can progress to disk herniation, spinal canal stenosis, and, in conjunction with facet joint arthrosis, degenerative spondylolisthesis. The mechanism of IVD degeneration, however, has not been fully elucidated. The of IVD degeneration process is considered to have a biochemical basis, involving inhibition of nuclear proteoglycan synthesis and enhanced matrix degradation (3). Previous studies have suggested that in the process of IVD degeneration, the loss of IVD cells due to excessive apoptosis is a central factor (4-6). It is therefore required to identify the causes of IVD cellular apoptosis. It has been demonstrated that numerous factors can lead to apoptosis of IVD cells, including abnormal mechanical stresses (7-9), interleukin-1 β (IL-1 β) (10) and serum withdrawal (11). If apoptosis of IVD cells can be inhibited, degradation of the IVD may be decelerated.

IVD tissue has been shown to produce inflammatory cytokines, including matrix metalloproteinases (MMPs), IL-1 β , IL-6, tumor necrosis factor- α (TNF- α), nitric oxide, and prostaglandin E2 (PGE2) (12,13). Among these cytokines, IL-1 β is a multifunctional inflammatory cytokine which functions in the progression of IVD cell apoptosis. IL-1 β is known as a proinflammatory cytokine since it has been shown that IL-1 β can increase the synthesis of matrix-degrading enzymes (MMP-2, MMP-3, MMP-13), decrease the synthesis of proteoglycan, collagen I and collagen II, and induce the expression of IL-6, cyclooxygenase-2, and PGE2 (12,13,15,16). Le Maitre *et al* (15) identified that in human IVD degeneration, there was local production of IL-1 β by native disc cells that could induce the cellular and matrix changes of IVD degeneration. Hoyland *et al* (14) showed that enzyme activity was upregulated by IL-1 β and reduced by its inhibitor, therefore placing IL-1 β as a key regulator of matrix enzyme activity in normal and degenerate IVD cells. It has been additionally reported that IL-1 β could induce apoptosis of AF cells in vitro (10). Previous studies have demonstrated that IL-1 β could enhance the effects of serum deprivation on

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rat annular cell apoptosis and IL-1 β sensitized rat IVD cells to Fas ligand-mediated apoptosis in vitro (17,18). Since serum may alter the effects of IL-1 β , culture medium without fetal bovine serum (FBS) was used in the present study.

Estrogen hormone is considered to be key in the preservation of quality of life. There is evidence that estrogen affects multiple tissues and organs in humans, as well as female sexual features (19,20). Studies have shown that, compared with males, female subjects have a higher incidence of IVD degeneration as (21). Compared with untreated post-menopausal women, estrogen-replete females maintain higher and therefore healthier IVDs as (22). It has been reported that female rats have a tendency to develop IVD degeneration following an ovariectomy, and estrogen supplementation can halt the development of this ovariectomy-associated disc degeneration (23). Taken together, these studies demonstrate that estrogen hormone is closely associated with IVD degeneration. In addition, 17 β -estradiol (17 β -E2) can protect pancreatic β -cells, synovial fibroblasts and human chondrocytes from apoptosis (24-26). Whether 17 β -E2 can protect IVD cells from apoptosis, however, has not been reported.

There is evidence showing that nucleus pulposus (NP) tissue retains notochordal cells throughout the lifespan (27), and an in vitro study has shown that notochordal cells in the IVD can interact with NP cells and is able to affect annulus fibrosus (AF) cells (28). By contrast, cells in the AF share a similar phenotype. In an effort to maintain the homogeneity of cells cultured in vitro and exclude interactions between different cell types, the present study used only the cells from the rat AF.

The present study examined whether 17 β -E2 inhibited IL-1 β -induced apoptosis in rat AF cells, and the dose-response effect of 17 β -E2 on cell apoptosis.

Materials and methods

Materials. Collagenase type II, trypsin, 17 β -E2, ICI182,780 and Dulbecco's modified Eagle's medium (DMEM) high glucose medium were purchased from Sigma-Aldrich (St. Louis, MO, USA). IL-1 β was purchased from PeproTech (Rocky Hill, NJ, USA). Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium/F12 (DMEM/F12) were obtained from HyClone (HyClone Laboratories, Logan, UT, USA). Hank's Balanced Salt Solution (HBSS) was bought from Gibco-BRL (Grand Island, NE, USA). The Cell Proliferation kit I (MTT) was purchased from Solarbio (Beijing Solarbio Science & Technology Co., Ltd, Beijing, China), the Annexin V/propidium iodide binding kit was obtained from Multi Sciences Biotech. Co., Ltd. (Hangzhou, China) and the Caspase-3 activity assay kit was obtained from Beyotime (Beyotime Institute of Biotechnology, Shanghai, China).

Primary disc cell isolation. Male Sprague Dawley rats (weighing 180-240 g) were obtained from the Laboratory Animal Center of Hebei Medical University (Hebei, China). The Animal Care and Experimental protocols conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health and were approved by the Animal Ethics Committee of Hebei Medical University. The rats were sacrificed by intravenous administra-

tion of 150 mg/kg pentobarbital sodium, and the rat lumbar IVD (L1-L5) were harvested immediately in a sterile environment. The muscle tissues and ligaments around the IVD and inner NP were removed, and the outer AF was obtained through an operating microscope to ensure the identity of the tissue. The AF tissues were then placed into DMEM/F12 and were cut into small pieces (<1 mm³). To isolate the cells, the AF tissues in the DMEM/F-12 were digested with 0.25% collagenase type II for ~1 h in a water bath at 37°C. Subsequently, the tissue was additionally digested with 0.2% trypsin (including 0.02% EDTA) for ~5 min at 37°C. Following the two-step enzyme digestion, the suspension was filtered through a 70 μ m mesh. The filtered cells were then washed twice with Hank's Balanced Salt Solution. Finally, the AF cells were added to DMEM/F12 media, supplemented with 15% FBS, 100 U/ml penicillin G, and 100 μ g/ml streptomycin and cultured at 37°C in a humidified atmosphere of 95% air and 5% CO₂. The medium was changed every two days. The AF cells were passaged three times prior to being collected for use.

Cell culture and drug treatment. When the cell culture became 80-90% confluent, the cells were digested and subcultured into appropriate culture plates and cultured as previously described. When the cell confluence in each well reached 80-90%, the medium was replaced with DMEM-high glucose medium without FBS, phenol red, penicillin and streptomycin. Six experimental groups were established, consisting of one control, one induction and four treatment groups. In the four treatment groups, 0.1, 1, and 10 μ mol/l 17 β -E2, and 10 μ mol/l 17 β -E2+10 μ mol/l ICI182,780 was respectively added to the medium (without FBS). The cells were cultured for 1 h and then 75 ng/ml IL-1 β was added to the induction group and the four treatment groups. Cultures without the addition of IL-1 β , 17 β -E2 and ICI182,780 acted as the control group. All the groups were cultured in a humidified atmosphere with 5% CO₂ at 37°C for 24 h.

Morphological observation. Cells were sub-cultured in 6-well plates at 2x10⁵ cells/well in complete culture medium. Following 24 h treatment, coverslips with adherent cells were observed under a fluorescence inverted microscope (Olympus IX50). Three areas of 200x200 pixels in one sample were randomly selected from the image to observe the morphological changes in the apoptotic cells.

Cell viability assay. Cell viability was measured using an MTT assay. The MTT assay relies on the observation that succinate dehydrogenase in the mitochondria of the viable cells revert MTT into a visible dark-blue formazan reaction product, which provides an indirect measure of cell viability. Cells were distributed into 96-well plates at 5,000 cells/well and maintained for one day in complete media with 15% FBS. The next day, the medium was replaced with DMEM-high glucose medium (200 μ l/well, without FBS). After 24 h treatment, 20 μ l of MTT (5 mg/ml), dissolved in phosphate-buffered saline (PBS), was added to each well. Following a 4 h incubation in a humidified atmosphere with 5% CO₂ at 37°C, the plates were centrifuged at 1,900 x g for 10 min. The medium was then removed and replaced with dimethyl sulfoxide (150 μ l/well). The plates were placed onto a shaker for agitation at

a low speed for 10 min. Finally, the solubilized mixture was measured by reading the absorbance at 490 nm wavelength using a microplate reader.

Apoptosis assay. Cells were sub-cultured in 6-well plates at 2×10^5 cells/well with complete culture medium. Following 24 h treatment, cells still attached to the plate and those present in the supernatant were collected together and resuspended in cold binding buffer. The apoptotic incidence was detected using an Annexin V/fluorescein isothiocyanate (FITC) apoptosis detection kit. Apoptosis was determined by staining cells with both Annexin V/FITC and propidium iodide (PI), according to the manufacturer's instructions. Annexin V/FITC was used to quantitatively determine the percentage of cells undergoing apoptosis based upon the loss of membrane asymmetry in the early phases of apoptosis. In apoptotic cells, the membrane phospholipid phosphatidylserine is translocated from the inner leaflet of the plasma membrane to the outer leaflet, thereby exposing phosphatidylserine to the external environment. Cells that were positively stained with Annexin V/FITC and negatively stained for PI were therefore considered to be undergoing apoptosis. Cells that were positively stained for both Annexin V/FITC and PI were considered to be undergoing necrosis (29,30). The cells were then stained with $5 \mu\text{l}$ Annexin V/FITC and $10 \mu\text{l}$ PI, followed by the addition of $500 \mu\text{l}$ binding buffer for 15 min at room temperature in the dark. The samples were analyzed by flow cytometry (FCM) within 1 h.

Measurement of caspase-3 activity. Caspase-3 activity was assayed using the caspase-3 activity assay kit, based upon the ability of caspase-3 to convert acetyl-Asp-Glu-Val-Asp-p-nitroanilide into the yellow formazan product, p-nitroaniline. Cells were placed in 6-well plates at 2×10^5 cells/well and treated as above. The cells were then lysed with lysis buffer ($100 \mu\text{l}/2 \times 10^6$ cells) for 15 min on ice following washing with cold HBSS. The mixture was composed of $10 \mu\text{l}$ cell lysate, $80 \mu\text{l}$ reaction buffer and $10 \mu\text{l}$ of 2 mM caspase-3 substrate and was incubated in 96-well microtiter plates at 37°C .

Statistical analysis. Values are presented as the mean \pm standard deviation. Statistical analyses were performed using the SPSS 13.0 statistical software program (SPSS, Inc., Chicago, IL, USA). The means of apoptotic incidences among groups, as well as the absorbances among groups were compared by one-way analysis of variance, followed by pairwise comparison using the Student-Newman-Keuls-q test. All statistical tests were two-sided and $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Morphological changes of apoptotic AF cells induced by IL-1 β and the protective effects of 17 β -E2. Using a fluorescence inverted microscope, apoptotic cells exhibited plasma membrane blebbing, cell shrinkage and nuclei condensation. A total of 75 ng/ml IL-1 β was used for this experiment. Few apoptotic cells were observed in the control group. As compared with the control group, treatment with IL-1 β (75 ng/ml) induced more apoptotic cells. Pretreatment with

17 β -E2 (10 $\mu\text{mol/l}$) resulted in a decrease in this effect, with the number of apoptotic cells less than those treated with IL-1 β (75 ng/l; Fig. 1). Fig. 1D demonstrated that estrogen receptor antagonist ICI182, 780 (10 $\mu\text{mol/l}$) could revert the protective effects of 17 β -E2 (10 $\mu\text{mol/l}$). There was no significant difference between the IL-1 β (75 ng/l) and IL-1 β (75 ng/l) + 17 β -E2 (10 $\mu\text{mol/l}$) + ICI182, 780 (10 $\mu\text{mol/l}$) groups (Fig. 1).

Effect of 17 β -E2 on cell viability in AF cells damaged by IL-1 β . An MTT assay for cell viability was used to determine whether 17 β -E2 could prevent cell death in cells exposed to IL-1 β , and whether 17 β -E2 (0.1, 1, 10 $\mu\text{mol/l}$) pretreatment could increase cellular proliferation in a concentration-dependent manner. As shown in Fig. 2A, as compared with the control group, treatment with IL-1 β alone caused an $\sim 45\%$ decrease in cell viability ($P < 0.001$). The cell viability in the 17 β -E2 + IL-1 β group was significantly increased, as compared with the IL-1 β group. There were no significant changes in cell viability between the IL-1 β group and 17 β -E2 + IL-1 β + ICI182, 780 group. The inhibitory effect of 17 β -E2 was abolished by the application of the estrogen receptor antagonist ICI182, 780. Taken together, these results showed that 17 β -E2 can protect cells from apoptosis induced by IL-1 β . Cell growth was inhibited following treatment with IL-1 β , as compared with the control group ($P < 0.001$). The cellular proliferation of IL-1 β -induced apoptotic cells was significantly increased after pretreatment of cells for 1 h with 17 β -E2 at concentrations of 0.1 $\mu\text{mol/l}$ (0.421 ± 0.021), 1 $\mu\text{mol/l}$ (0.537 ± 0.027) and 10 $\mu\text{mol/l}$ (0.629 ± 0.031 ; Fig. 2B). Therefore, 17 β -E2 pretreatment increased cellular proliferation in a concentration-dependent manner.

Effect of 17 β -E2 on IL-1 β -induced AF cells apoptosis. It was further investigated whether 17 β -E2 could inhibit IL-1 β -induced apoptosis in cells and whether 17 β -E2 (0.1, 1, 10 $\mu\text{mol/l}$) pretreatment could decrease apoptosis in a concentration-dependent manner. The level of apoptotic cells was determined by double staining with Annexin V/FITC and PI. The apoptotic ratio of cells was calculated as a percentage of apoptotic cells/total cells (Table I). Annexin V/FITC and PI labeled cells were quantified by FCM, thus allowing for discrimination between viable/intact cells (Annexin V-PI-), early apoptotic (Annexin V+PI-) and late apoptotic or necrotic cells (Annexin V+PI+). As is shown in Fig. 3, the percentage of apoptosis increased in cells following treatment with IL-1 β alone (19.9%). Cells pretreated with 17 β -E2 showed a reduced rate of apoptosis (12.3%). To further elucidate the potential contribution of 17 β -E2, an estrogen receptor antagonist ICI182, 780, was used. When cells were incubated with both 17 β -E2 and ICI182, 780, the protective effects of 17 β -E2 were reduced (18.0%). To investigate the association between the concentration of 17 β -E2 and the protective effects by 17 β -E2, three different concentrations of 17 β -E2 (0.1, 1 and 10 $\mu\text{mol/l}$) were used. Statistical analysis showed that pretreatment with increasing concentration of 17 β -E2 reduced the rate of apoptosis in a concentration-dependent manner (12.3, 9.5, 5.3%).

Effect of 17 β -E2 on caspase-3 activity in AF cells induced by IL-1 β . Caspase-3 is a critical mediator of apoptosis. As shown in Fig. 4A, caspase-3 activity was increased in cells following

Table I. Effect of 17β-E2 on IL-1β-induced apoptosis.

	Group					
	Control	IL-1β	17β-E2 ^a	17β-E2 ^a	17β-E2 ^a	17β-E2 ^a + ICI182,780
Dose	75 ng/ml	0.1 μmol/l	1 μmol/l	10 μmol/l	10 μmol/l	10 μmol/l
Apoptosis (%)	3.47±0.25	18.80±0.99	12.97±0.61	8.67±0.74	6.00±0.61	18.57±0.51

^aGroups with IL-1β (75 ng/ml) pretreatment. Percentage of apoptotic cells are expressed as the mean ± standard deviation. 17β-E2, 17β-estradiol; IL-1β, interleukin-1β; ICI182, 780, estrogen receptor antagonist.

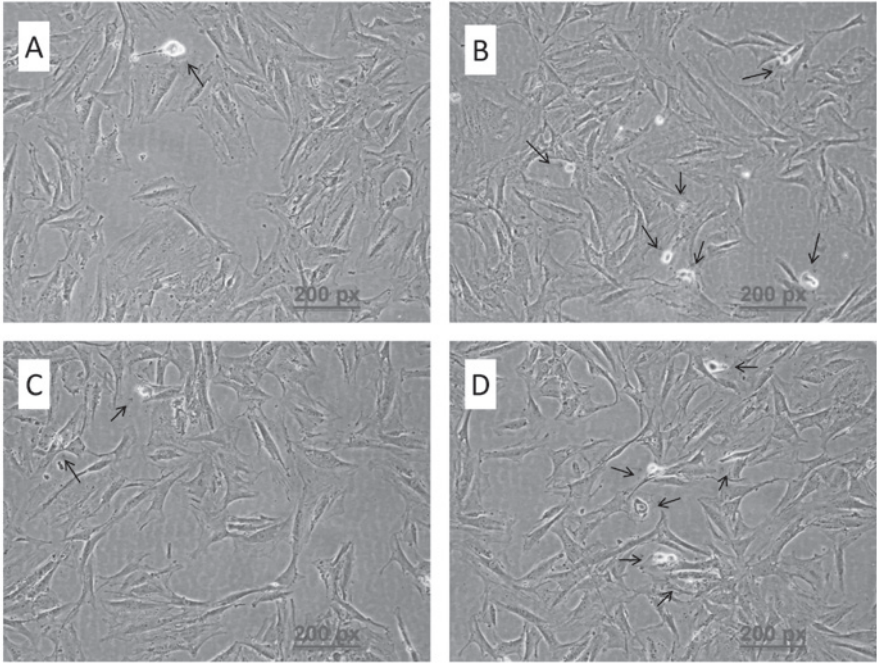


Figure 1. Morphological changes in rat annulus fibrosus (AF) cells. (A) Phase-contrast photomicrographs of AF cells cultured in serum-free media. (B-D) Phase-contrast photomicrographs of AF cells cultured in serum-free media stimulated with IL-1β, IL-1β + 17β-E2, IL-1β + 17β-E2 + ICI182, 780, respectively. Apoptotic cells were characterized by plasma membrane blebbing, cell shrinkage and nuclei condensing, as indicated by the black arrows. Scale bar=200 μm; original magnification, x100. 17β-E2, 17β-estradiol; IL-1β, interleukin-1β; ICI182, 780, estrogen receptor antagonist.

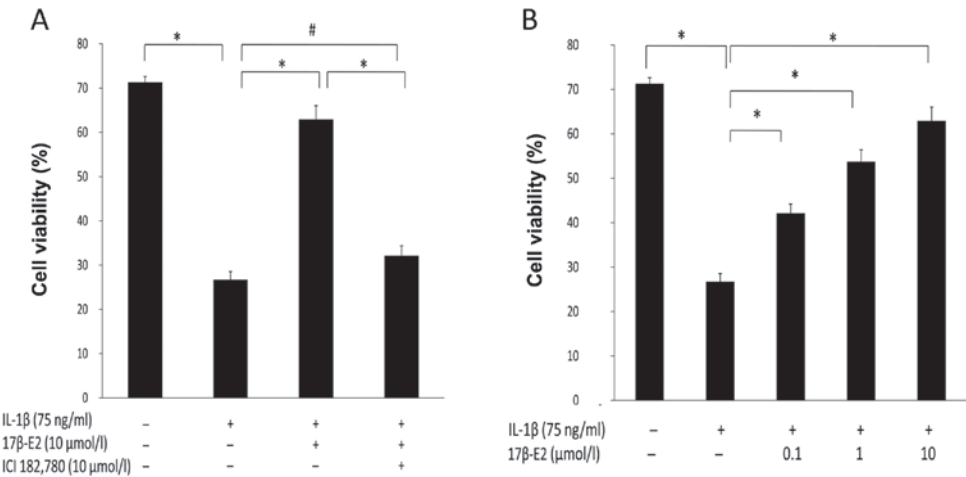


Figure 2. Evaluation of cell viability. The cell viability was measured by MTT assay. (A) The rat annulus fibrosus (AF) cells were incubated in serum-free media or stimulated with IL-1β (75 ng/ml), IL-1β (75 ng/ml) + 17β-E2 (10 μm/l), IL-1β (75 ng/l)+17β-E2 (10 μm/l) + ICI182, 780 (10 μm/l). (B) Cells were treated with IL-1β (75 ng/l) for 1 h alone or followed by treatment with differing doses of 17β-E2 (0.1, 1, 10 μmol/l). *P<0.001; #P>0.05. It was identified that 17β-E2 could prevent cell death in AF cells exposed to IL-1β, and 17β-E2 (0.1, 1, 10 μmol/l) pretreatment could increase AF cell proliferation in a concentration-dependent manner. Error bars represent the mean ± standard deviation. IL-1β, interleukin 1β; 17β-E2, 17β-estradiol; ICI182, 780, estrogen receptor antagonist.

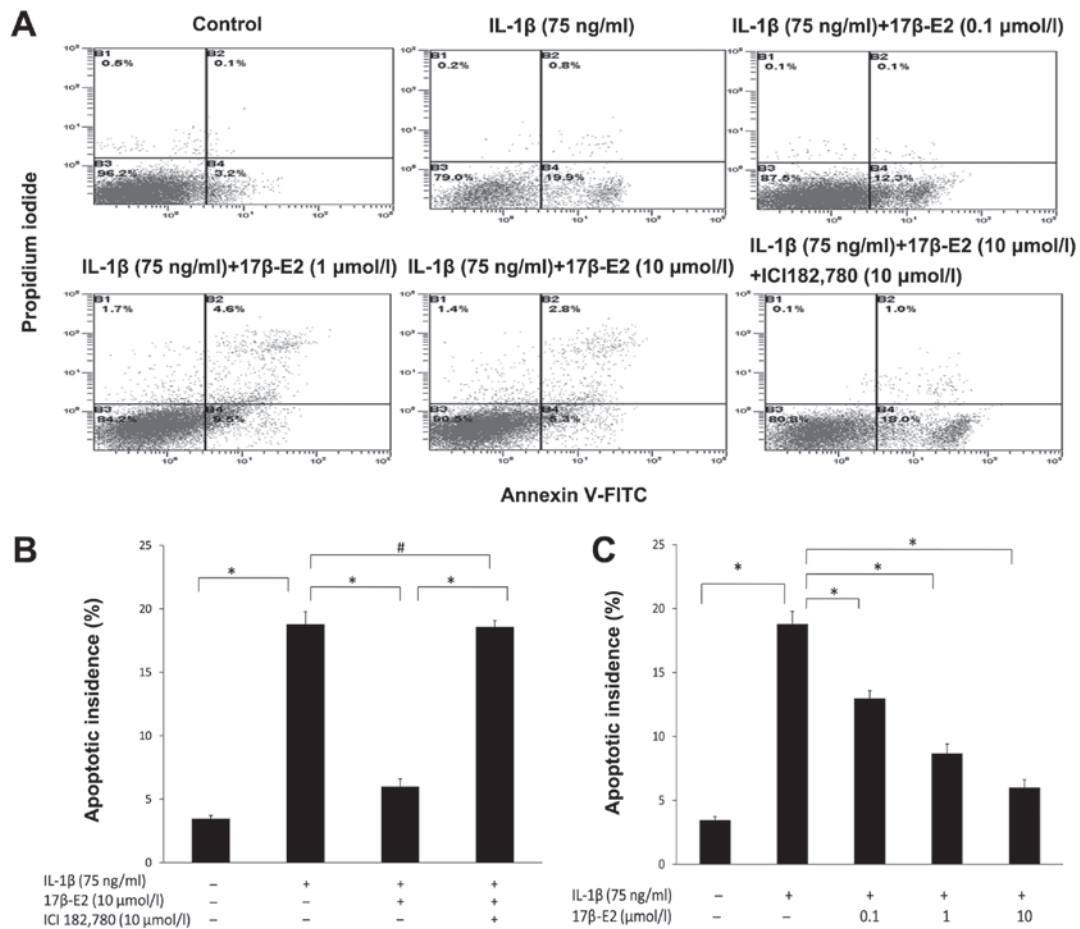


Figure 3. Evaluation of apoptotic incidence. (A) Representative graphs obtained by flow cytometry analysis following double staining with Annexin V/FITC and propidium iodide. (B and C) The apoptotic incidences of rat annulus fibrosus (AF) disc cells cultured with IL-1 β and stimulated with ICI182, 780 or various concentrations of 17 β -E2. 17 β -E2 could inhibit IL-1 β -induced apoptosis and 17 β -E2 (0.1, 1 and 10 μ mol/l) pretreatment could decrease AF cell apoptosis in a concentration-dependent manner. Error bars represent the mean \pm standard deviation. *P<0.001; #P>0.05. FITC, fluorescein isothiocyanate; IL-1 β , interleukin 1 β ; ICI182, 780, estrogen receptor antagonist; 17 β -E2, 17 β -estradiol.

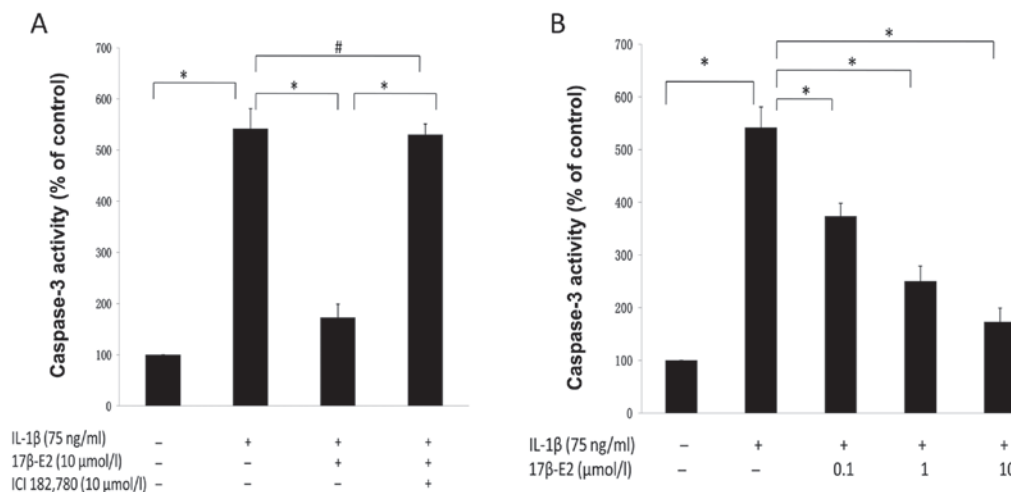


Figure 4. Changes in caspase-3 activity. The caspase-3 activity of rat annulus fibrosus (AF) cells cultured with IL-1 β and stimulated with ICI182, 780 or various concentrations of 17 β -E2 (0.1, 1 and 10 μ mol/l). The figure shows that 17 β -E2 could decrease IL-1 β -induced activity of caspase-3 and 17 β -E2 (0.1, 1, 10 μ mol/l) pretreatment could decrease the AF cells activity of caspase-3 in a dose-dependent manner. Error bars represent the mean \pm standard deviation. *P<0.001; #P>0.05. IL-1 β , interleukin-1 β ; ICI182, 780, estrogen receptor antagonist; 17 β -E2, 17 β -estradiol.

treatment with IL-1 β alone, as compared with the control cells. However, cells pretreated with 17 β -E2 showed a reduc-

tion in this ability. Addition of estrogen receptor antagonist ICI182, 780 resulted in elimination of the protective effects

of 17 β -E2. Fig. 4B shows that cells pretreated with increasing concentrations of 17 β -E2 reduced caspase-3 activity in a concentration-dependent manner.

Discussion

The present study examined the effects of 17 β -E2 on IL-1 β -induced apoptosis in AF cells. The data provide evidence that 17 β -E2 attenuated IL-1 β -induced apoptosis in AF cells, which may improve the survival and proliferative capacity of AF cells under inflammatory stress. These data are in agreement with the study of Hattori *et al* (26), in which similar concentrations of TNF- α were used to induce cell death in human chondrocytes.

Morphological observations and FCM were used to identify whether AF cells underwent apoptosis following exposure to IL-1 β . It was observed that the effects of IL-1 β on the number of apoptotic cells were prevented upon pretreatment with 17 β -E2. This protective action of 17 β -E2 was in a concentration-dependent manner. The results of the MTT assay verified that preincubation with 17 β -E2 could increase the viability of AF cells.

Previous studies in human IVD degeneration has identified that IL-1 β expression is derived from native disc cells (15). The inflammatory cytokine IL-1 β has a crucial function in IVD degeneration including promotion of apoptosis in IVD cells. Three concentrations of IL-1 β (40, 75, 150 ng/ml) were selected to induce apoptosis in AF cells. The results of the FCM indicated that IL-1 β (40 ng/ml) generated 3.4% apoptosis and there was no significant difference between the other two groups (20.4, 19.9%). A concentration of 75 ng/ml IL-1 β was subsequently used to induce cellular apoptosis. Serum contains various growth factors, including insulin-like growth factor-1 (IGF-1). IGF-1 can inhibit IL-1 β -induced apoptosis in growth plate chondrocytes from metatarsal bones and pancreatic β -cells (31,32). Cells were therefore cultured in media without FBS in order to eliminate the effects of serum. The results of the MTT assay and FCM demonstrated that IL-1 β could induce cell apoptosis and suppress proliferation in AF cells.

Estrogen is an essential sex hormone in males and females. Estrogen has effects on the reproductive system, as well as neurotransmitter release, bone structure, cognitive function and blood vessels (33,34). Understanding the importance of estrogen has significant implications. The beneficial effects of estrogen have been previously investigated. Huppmann *et al* (35) showed that 17 β -E2 attenuated hyperoxia-induced apoptosis in astrocytes. Ronda *et al* (36) demonstrated that 17 β -E2 has an anti-apoptotic function in skeletal muscle cells. Whether 17 β -E2 can prevent apoptosis in AF cells has not been previously investigated. To the best of our knowledge, the present study is the first to demonstrate that 17 β -E2 can prevent apoptosis induced by IL-1 β in AF cells. Further evidence has suggested that phenol red, a pH indicator widely used in growth medium, exhibits minor oestrogenic activity similar to the effect of steroid hormones (37). Wesierska-Gadek *et al* (38) showed that phenol red added to the culture medium strongly promoted the cell proliferation and cell cycle progression of human cells expressing the estrogen receptor. To eliminate the effects of phenol red, medium without phenol red was used. The results

from the present study indicated that 17 β -E2 has the ability of protecting AF cells from apoptosis, and pretreatment of AF cells with the estrogen receptor antagonist ICI182, 780 could attenuate the protective effects of 17 β -E2. Higher concentrations of 17 β -E2 (0.1-10 μ mol/l) exerted a stronger protective effect. Taken together, these results support our conclusion that 17 β -E2 can protect IL-1 β -induced apoptosis in AF cells.

There are several limitations to the present study. Firstly, only three concentrations of 17 β -E2 were selected, and additional concentrations are required to further investigate the dose-response effect of 17 β -E2 on cellular apoptosis. Secondly, the study design only observed the cellular effects after 24 h treatment. It is necessary to investigate additional timepoints in order to determine whether 17 β -E2 reduces the rate of apoptosis by IL-1 β in a time-dependent manner. Thirdly, rat AF cells were the only cell type investigated in this study, which cannot fully represent human AF cells. Finally, additional studies are necessary to further elucidate the signaling mechanisms which mediate the anti-apoptotic action of 17 β -E2 in AF cells.

In conclusion, the results of the current study have shown that incubation of rat AF cells with 17 β -E2 can counteract cell damage induced by IL-1 β . Pretreatment of cells with 17 β -E2 could decrease AF cell apoptosis in a concentration-dependent manner. The present study demonstrates that the inflammatory cytokine, IL-1 β , could induce AF cell apoptosis and that the absence of estrogen may be an important mediator of IVD degeneration in post-menopausal women. This may be of relevance to develop novel therapies associated with IVD in post-menopausal women.

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