Estrogen stimulates gene expression and protein production of osteoprotegerin in rheumatoid synovial fibroblasts

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INTRODUCTION:
Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with immune system abnormalities and hyperplasia of the synovial tissues. RA causes the destruction of cartilage and sub-chondral bone, leading to joint destruction and deformities [1]. Destruction of cartilage and bone in RA is partly mediated by proteinases, such as metalloproteinases, secreted from activated synoviocytes and chondrocytes [2]. However, recent studies suggest that bone-resorbing osteoclasts formed in synovial tissues play an important role in the bone destruction in RA [3, 4]. It is also reported that estrogen is a novel secreted member of the tumor necrosis factor (TNF) receptor family that acts as a decoy receptor of RANKL and negatively regulates osteoclastogenesis. It has been shown that RANKL is an essential factor for osteoclastogenesis in the rheumatoid synovial tissue and that OPG may prevent the bone erosion in joints with RA [5].

Meanwhile, previous studies in vivo and in vitro have indicated that sex hormones influence a number of potential processes involved in the pathogenesis of RA [6, 7]. Among the sex hormones, estrogen is essential for bone growth, bone development, and the maintenance of bone health in adulthood. It has been suggested that estrogen may exert its anti-resorptive effects on bone in part by stimulating OPG expression in osteoblasts [8].

Therefore, in view of the importance of estrogen, OPG, and RANKL in the pathogenesis of cartilage and bone destruction in RA, we investigated the effect of 17β-estradiol (E2) on OPG and RANKL in HFLS cells.

MATERIALS AND METHOD:
Cell Preparation: Synovial tissue samples from patients with RA at the time of TKA surgery were minced and dissociated using 0.2% of collagenase. Fibroblasts obtained were cultured in DMEM containing 10% FCS and antibiotics. These cells were used at third or fourth passage as HFLS cells.

Total RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR): 5.0 X 10^6 cells were inoculated into the 6-well plates. When the cultured HFLS cells reached confluence, the conditioned media was replaced by fresh media in the presence of E2. After 6, 12, 24, and 48 hours of incubation, total RNA was isolated with using RNeasy Mini kit (QIAGEN, Hilden, Germany). The RNA was quantified by spectrophotometry, and 1μg of total RNA was reverse-transcribed to first-strand cDNA with MuLV reverse transcriptase (Perkin Elmer, Foster City, CA) and oligo dT primer. RT-PCR was then carried out with specific primers for RANKL, OPG, and glyceraldehyde-3-phosphate dehydrogenase (GADPH).

ELISA OPG assay: 1.0 X 10^5 cells were inoculated into 24-well plates. When the cultured HFLS cells reached confluence, the cells were stimulated with E2 or Tamoxifen, a selective estrogen receptor modulator. After 6, 12, 24, and 48 hours of incubation, the concentration of OPG in the supernatants was measured using OPG enzyme-linked immunosorbent assay (ELISA) system (Immunodiagnostik, Bensheim, Germany). The concentration of OPG was determined from the mean value of triplicate samples. Statistical analyses were performed by one-way ANOVA and Fisher's PLSD. P values less than 0.05 were considered significant.

RESULT:
Effect of estrogen on OPG and RANKL mRNA expression: The time-course study revealed that 10^{-5} M of E2 enhanced OPG mRNA expression as early as 3 hours after treatment, and that the effect of E2 reached the maximum between 6 and 12 hours (data not shown). Dose-response study with various concentrations at 12 hours revealed that E2 increased OPG mRNA expression at concentration of 10^{-6} and 10^{-5} M, but did not enhance RANKL mRNA expression at concentration between 10^{-6} and 10^{-7} M (Fig. 1).

Effect of estrogen on OPG protein secretion: OPG protein secreted by HFLS cells in the presence of 10^{-5} M E2 was statistically higher than control at 48 and 72 hours (p<0.05) (Fig. 2A). Treatment with E2 at concentration of 10^{-5} and 10^{-4} M increased OPG protein secreted by HFLS cells at 48 hours (p<0.05) (Fig. 2B). Tamoxifen that acts as a weak estrogen agonist on bone in vivo did not stimulate OPG protein secretion in HFLS cells (data not shown).

DISCUSSION:
Estrogen regulates inflammatory cytokines and proteinases, such as Interleukin 6 and matrix metalloproteinases, and is considered to mediate the destruction of cartilage and bone in RA partly through the cytokines and the proteinases [7]. OPG and RANKL are also important for the bone destruction, but the effect of estrogen on OPG and RANKL expression in HFLS cells was not revealed before.

In this study, we found that estrogen treatment stimulated the production of OPG in rheumatoid synovial fibroblasts in vitro. On the other hand, estrogen treatment did not stimulate the gene expression of RANKL. The relative levels of RANKL and OPG expression are likely to be important in determining whether osteoclasts will form or not [9]. These data suggest that estrogen may prevent the bone erosion by stimulating HFLS cells to produce OPG.

REFERENCES: