Clinical and Experimental Rheumatology 2000; 18: 743-745.

Effects of estrogen on interleukin-6 production in rheumatoid fibroblast-like synoviocytes

T. Kawasaki, T. Ushiyama, K. Inoue, S. Hukuda

Department of Orthopedic Surgery, Shiga University of Medical Science, Seta, Otsu, Shiga, Japan.

Taku Kawasaki, MD, Postgraduate student of Orthopedic Surgery; Toshio Ushiyama, MD, Assistant Professor of Orthopedic Surgery; Koji Inoue, MD, Associate Professor of Orthopedic Surgery; Sinsuke Hukuda, MD, Professor of Orthopedic Surgery.

Please address correspondence and reprint requests to: Dr. T. Kawasaki, Department of Orthopedic Surgery, Shiga University of Medical Science, Seta, Otsu, Shiga, 520-2192, Japan.

Received on August 13, 1999; accepted in revised form on September 8, 2000.

Key words:
Rheumatoid arthritis, fibroblast-like synoviocytes, interleukin- 6.

ABSTRACT

Objective

In view of the importance of estrogen and IL-6 in the pathogenesis of rheumatoid arthritis (RA), the effects of 17 β-estradiol (E2) on interleukin (IL)-6 production in cultured rheumatoid fibroblast-like synoviocytes were investigated.

Methods

Cultured fibroblast-like synoviocytes obtained from RA joints were treated with the vehicle (control), as well as 10^{-8} and 10^{-6} M of E2, with or without IL-1β stimulation. After 72 hours of culture, the levels of IL-6 in supernatants were measured by ELISA.

Results

Treatment with 10^{-8} and 10^{-6} M of E2 had no effect on the constitutive production of IL-6 in cultured fibroblast-like synoviocytes. However, E2 increased IL-1β-induced IL-6 production in a dose-dependent manner, with a mean 12.5% increase with 10^{-8} M of E2 ($p = 0.048$) and 33.4% with 10^{-6} M ($p < 0.0001$) versus the control.

Conclusion

Estrogen up-regulates IL-1β-induced IL-6 production in cultured fibroblast-like synoviocytes, possibly contributing to the enhancement of rheumatoid inflammation in synovial tissues.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disorder that frequently occurs in women when the level of estrogen is high. However, in older age when estrogen levels decrease in women, both men and women equally develop the disease (1). These clinical observations suggest the role of estrogen in rheumatoid inflammation (2).

Among various cytokines taking part in rheumatoid inflammation, interleukin (IL)-6 is considered to play an essential roles in joint damage. IL-6 levels in RA synovial fluid are elevated and related to disease activity (3). On the other hand, estrogen modulates IL-6 production via an estrogen receptor (ER)-dependent mechanism in various cells. For bone tissues, 17β-estradiol (E2) was found to downregulate IL-1β-induced IL-6 production in bone marrow stromal cells (4) and a human osteoblastic cell line (5), whereas it up-regulated IL-1β-induced IL-6 production in human articular chondrocytes (6). Although previous studies have detected the presence of ER and estrogen binding in human synovial cells by an estrogen binding assay (7), immunoprecipitation (8), and reverse transcription-polymerase chain reaction (RT-PCR) (9), there is no known study that has addressed the effects of estrogen on IL-6 production in synovial cells, which are important constituents of joint structure.

The present study was conducted to examine the effects of estrogen on IL-6 production in rheumatoid fibroblast-like synoviocytes. The function of these cells represent those of other synovial cells, namely macrophage-like synovial cells and lymphocytes (10).

Materials and methods

Subjects

Synovial tissues were obtained from 8 RA patients (3 men and 5 women, average age 59.4 years, range 30-78 years) who underwent a knee synovectomy or total knee arthroplasty. The patient characteristics are shown in Table I. The diagnosis of RA was established according to the American College of Rheumatology criteria (11). None of the patients had received hormone replacement therapy or intra-articular corticosteroid injections for at least one month before surgery.

Culture of rheumatoid synovial cells

Synovial tissues obtained during surgery were immediately minced into small pieces and washed in PBS, before being incubated with 1.5 mg/ml of collagenase (Sigma Chemical Co., St. Louis, MO) in serum free Dullbecco's modified Eagle medium (DMEM, Gibco Laboratories, Grand Island, NY) for 1 hour at 37° C. The cells were then filtered through a nylon mesh and resuspended in phenol red-free DMEM (GIBCO) with 10% heat inactivated fetal bovine serum (FBS, Genzyme Co., Cambridge, MA), penicillin (100 U/ml), and streptomycin (100 μg/ml) in a 5% CO_2 incubator.

Effects of 17β-estradiol on IL-6 production

At confluence of the synovial cell cul-

Table I. Levels of IL-6 produced by cultured synovial cells. Means of triplicates (pg/ml) and %IL-6 (in parentheses).

Pt	Sex	Age	Duration of RA (yrs.)	IL-1β absent Control	IL-1β absent E_2 10^{-8}M	IL-1β absent E_2 10^{-6}M	IL-1β present Control	IL-1β present E_2 10^{-8}M	IL-1β present E_2 10^{-6}M
1	M	78	20	65.8 (100)	50.8 (77.2)	79.1 (120.2)	19,770 (100)	20,270 (102.5)	23,570 (119.2)
2	F	78	21	34.0 (100)	26.5 (77.9)	28.0 (82.4)	20,540 (100)	21,300 (103.7)	24,870 (121.1)
3	F	59	25	71.7 (100)	71.7 (100.0)	126.7 (176.7)	14,720 (100)	15,280 (103.8)	17,700 (120.2)
4	F	59	15	325.0 (100)	266.3 (81.9)	231.7 (71.3)	33,530 (100)	35,870 (106.7)	39,230 (117.0)
5	M	43	7	63.7 (100)	80.0 (125.6)	92.8 (145.7)	11,170 (100)	12,400 (111.0)	16,270 (145.7)
6	M	74	20	55.0 (100)	65.0 (118.2)	53.3 (96.9)	5,870 (100)	7,370 (125.6)	8,300 (141.5)
7	F	54	6	38.3 (100)	18.3 (47.8)	58.3 (152.2)	10,700 (100)	11,000 (108.4)	15,170 (141.8)
8	F	30	7	100.5 (100)	105.5 (105.0)	95.5 (95.2)	2,780 (100)	3,850 (138.5)	4,470 (160.8)
Mean				94.3 (100)	85.5 (91.7)	95.7 (117.6)	14,890 (100*)	15,990 (112.5*)	18,700 (133.4*)
SD				95.5	78.3 (25.4)	62.7 (37.4)	9,730	9,970 (12.8)	10,780 (16.2)

* By one-way ANOVA, there was a significant difference between the 3 groups at the level of $p < 0.05$ ($p < 0.0001$ between control vs 10^{-6} M of E_2, $p = 0.048$ between control vs 10^{-8}M of E_2 by Fisher's PLSD).

tures from the 8 RA patients, the medium was removed and the cells were passaged by trypisinization (0.05% trypsin/0.53 mM EDTA, Gibco), before being washed twice with PBS buffer and seeded in triplicate in 12-well culture plates at 3 x 10^4 cells/well. Subsequently, the first passage cells were incubated in fresh medium containing 1% FBS and various agents: the vehicle (0.01% ethanol), or 10^{-8} M or 10^{-6} M of E2 (Sigma Chemical Co., St. Louis, MO), with or without 0.1 ng/ml of IL-1β (recombinant human interleukin-1β, Genzyme Co.), for 72 hours. After incubation, the supernatants were collected, centrifuged, and stored at -70°C until the IL-6 concentrations were measured.

ELISA IL-6 assay

IL-6 concentrations in the supernatants were measured using an ELISA Kit (Genzyme Co.) and expressed in pg-ng/ml relative to the standards provided with the test kit. IL-6 levels were determined from the mean value of triplicate samples. Thus, a total of 144 samples (8 patients x replicates x conditions of E2 (0, 10^{-8} M, 10^{-6} M) x conditions of IL-1β (0, 0.1 ng/ml)) were measured. Percent IL-6 (%IL-6) was defined as the IL-6 level x 100/IL-6 level of the control (without E2).

Statistical analysis

All values were expressed as the mean ± standard deviation (SD). Comparison of %IL-6 between different groups was performed by one-way analysis of variance (ANOVA). Fisher's protected least significant difference (PLSD) was applied when ANOVA was significant. Differences were considered significant at $p < 0.05$. When appropriate, Student's t-test was also used.

Results

Table 1 shows the levels of IL-6 produced by cultured fibroblast-like synoviocytes and %IL-6 under various conditions. When the cells were incubated without IL-1β (unstimulated), %IL-6 was 91.7 ± 25.4 (mean ± SD) for a physiological dose of E2 (10^{-8} M) and 117.6 ± 37.4 for a pharmacological dose of E2 (10^{-6} M) versus 100 (control). No significant differences were found between the three groups by one-way ANOVA. When the cell were incubated with IL-1β (stimulated), %IL-6 was 112.5 ± 12.8 (mean ± SD) for a physiological dose of E2 and 133.4 ± 16.2 for a pharmacological dose of E2. There were significant differences between the three groups ($p = 0.048$, control versus 10^{-8} M; $p < 0.0001$, control versus 10^{-6} M; $p = 0.021$, 10^{-8} M versus 10^{-6} M by Fisher's PLSD). Levels of IL-6 produced by fibroblast-like synoviocytes and %IL-6 were compared between men (n = 3) and women (n = 5) under various conditions (unstimulated/stimulated, addition of 0, 10^{-8} M or 10^{-6} M of E2). However, no significant differences were seen between men and women by Student's t-test (data not shown).

Discussion

The present study demonstrated that both a physiological and a pharmacological dose of E2 increases the level of IL-1β induced IL-6 production in fibroblast-like synoviocytes from RA joints. IL-6 is a multifunctional cytokine that is closely related to the disease activity of RA patients (3). It has been shown to stimulate both hormonal and cellular immunity (12, 13), increase such acute phase proteins as C-reactive protein (14), and induce bone resorption by regulating the proliferation and differentiation of osteoclasts (15). The present results therefore suggest that estrogen contributes to the enhancement of rheumatoid inflammation in synovial tissues.

In contrast, estrogen has been shown to be protective with regard to the risk of osteoporosis in RA, and its administration has been shown to have favorable effects in postmenopausal women with RA (16-20) with respect to subjective joint symptoms, without significant improvements in laboratory markers such as the erythrocyte sedimentation rate and C-reactive protein level (19,20). The effects of estrogen on IL-6 production seem to vary, depending on the target cell phenotypes. In human osteoblastic cells, estrogen is known to inhibit IL-6 production (4, 5), whereas it increases IL-1β induced IL-6 production in human chondrocytes (6). Since responses to estrogen vary between target tissues, the beneficial effects of estrogen administration in RA patients would equal the

overall sum of varied responses.

Estrogen levels differ according to gender. Thus, the proinflammatory effects in fibroblast-like synoviocytes induced by estrogen may be significant in women, but less in men. As suggested by Cutolo (2), androgens, which have immunosuppresive effects, may have significant effects in men, when androgen levels decrease with age.

References

1. INOUE K, SHICHIKAWA K, NISHIOKA J, HIROTA S: Older age onset rheumatoid arthritis with or without osteoarthritis. *Ann Rheum Dis* 1987; 46: 908-11.
2. CUTORO M: The roles of steroid hormones in arthritis. *Br J Rheumatol* 1998; 37: 597-601.
3. HOUSSIAU FA, DEVOGELAER JP, VAN DAMME J, DE DEUXCHAISNES CN: Interleukin 6 in synovial fluid and serum of patients with rheumatoid arthritis. *Arthritis Rheum* 1988; 17: 469-73.
4. POTTRATZ ST, BELLIDO T, MOCHARLA H, CRABB D, MANOLAGAS SC: 17β-estradiol inhibits expression of human interleukin-6 promoter-receptor constricts by a receptor-dependent mechanism. *J Clin Invest* 1994; 93: 944-50.
5. KASSEM M, HARRIS SA, SPLSBERG TC, RIGGS BL: Estrogen inhibits interleukin-6 production and gene expression in a human osteoblastic cell line with high levels of estrogen receptors. *J Bone Miner Res* 1996; 11: 193-9.
6. GUERNE PA, CARSON DA, LOTZ M: IL-6 production by human articular chondrocytes. Modulation of its synthesis by cytokines, growth factors, and hormones *in vitro*. *J Immunol* 1990; 144: 499-505.
7. CUTOLO M, ACCARDO S, VILLAGGIO B *et al.*: Presence of estrogen binding sites on macrophage-like synoviocytes and CD8+, CD29+, CD45RO+ T lymphocytes in normal and rheumatoid synovium. *Arthritis Rheum* 1993; 36: 1087-97.
8. USHIYAMA T, INOUE K, NISHIOKA J: Expression of estrogen receptor related protein (p29) and estradiol binding in human arthritic synovium. *J Rheumatol* 1995; 22: 421-6.
9. CUTOLO M, ACCARDO S, VILLAGGIO B *et al.*: Androgen and estrogen Receptors are present in primary cultures of human synovial macrophages. *J Clin Endocrinol Metb* 1996; 81: 820-7.
10. CUTOLO M, SULLI A, BARONE A, SERIOLO B, ACCARDO S: Macrophages, synovial tissue and rheumatoid arthritis. *Clin Exp Rheumatol* 1993; 11: 331-9.
11. ARNETT F, EDWORTHY S, BLOCH D *et al.*: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
12. HIRANO T, TAGA T, NAKANO N *et al.*: Purification to homogeneity and characterization of human B-cell differentiation factor (BCDF or BSFp-2). *Proc Natl Acad Sci USA* 1985; 82: 5490-4.
13. TAKAI Y, WONG GG, CLARK SC, BURAKOFF SJ, HERRMANN SH: B cell stimulatory factor-2 is involved in the differentiation of cytotoxic T lymphocytes. *J Immunol* 1988; 140: 508-12.
14. KISHIMOTO T: The biology of interleukin-6. *Blood* 1989; 74: 1-10.
15. TAMURA T, UDAGAWA N, TAKAHASHI N *et al.*: Soluble interleukin-6 receptor triggers osteoclast formation by interleukin-6. *Proc Natl Acad Sci USA* 1993; 90: 11924-8.
16. SAMBROOK P, BIRMINGHAM J, CHAMPION D *et al.*: Postmenopausal bone loss in rheumatoid arthritis: Effect of estrogens and androgens. *J Rheumatol* 1992; 19: 357-61.
17. VAN DEN BRINK HR, VAN EVEDINGEN AA, VAN WIJK MJ, JACOBS JW, BIJLSMA JW: Adjuvant oestrogen therapy does not improve disease activity in postmenopausal patients with rheumatoid arthritis. *Ann Rheum Dis* 1993; 52: 862-5.
18. HALL GM, DANIELS M, DOYLE DV, SPECTOR TD: Effects of hormone replacement therapy on bone mass in rheumatoid arthritis patients treated with or without steroids. *Arthritis Rheum* 1994; 37: 1499-505.
19. HALL GM, DANIELS M, HUSKISSON EC, SPECTOR TD: A randomized controlled trial of the effect of hormone replacement therapy on disease activity in postmenopausal rheumatoid arthritis. *Ann Rheum Dis* 1994; 53: 112-6.
20. MACDONALD AG, MURPHY EA, CAPELL HA, BANKOWSKA UZ, RALSTON SH: Effects of hormone replacement therapy in rheumatoid arthritis. *Ann Rheum Dis* 1994; 53: 54-7.