

Effect of Selective Estrogen Receptor Modulator/Raloxifene Analogue on Proliferation and Collagen Metabolism of Tendon Fibroblast

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INTRODUCTION:

Hormonal alterations after menopause lead to profound changes in various connective tissues, including bone, skin, vascular tissues, pelvic organs, and muscles. Alterations of collagen metabolism mediated by estrogens are considered to play an especially/particularly critical role in degradation of tissues containing collagen. Like bone and skin, tendons and ligaments are mainly composed of type I collagen, and tendon fibroblasts were shown to express estrogen receptors. It remains controversial, however, whether estrogen affects collagen metabolism of tendons and ligaments.

It is also unknown whether selective estrogen receptor modulators (SERMs) affect collagen metabolism of tendons and ligaments though it has been shown to have therapeutic effects on many of postmenopausal issues involving connective tissue degradation such as osteoporosis, dermal degradation, and vascular sclerosis. Since SERMs display an unusual tissue-selective pharmacology, it may have an adverse effect to estrogen on the metabolism of tendons and ligaments.

In the present study, we aimed to elucidate how estrogen and SERM/raloxifene analogue affect the proliferation and extracellular matrix (ECM) metabolism in tendon fibroblasts.

METHODS:

Cell culture: Primary cultures of female Sprague-Dawley rat Achilles tendon cells were used as tendon fibroblasts. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with dextran-coated charcoal-treated fetal bovine serum (FBS) to minimize the influence of sex hormones contained in untreated FBS. Ascorbic acid (50µg/ml) was added to culture medium for secreted collagen assays. The experimental protocol was approved by the institutional animal study committee.

Cell proliferation assay: Cell proliferation was measured using a BrdU incorporation assay (24 hr) and a WST-8 colorimetric assay (2, 6 days). Cells were cultured at various concentrations of 17β-Estradiol (E₂) or raloxifene analogue (LY117018) and either vehicle or estrogen receptor alpha blocker (ICI 182,780, 100 nM).

Reverse transcription and real-time polymerase chain reaction analysis: Cells were cultured at various concentrations of E₂ or LY117018 and either vehicle or ICI 182,780 (10⁻⁷ M) for 8 hr. Total RNAs were extracted from the samples and then 1 µg of RNA was reverse-transcribed and real-time PCR was performed using specific primers against tendon extracellular matrix (ECM) related genes.

Immunocytochemistry: Cells were cultured with or without 10⁻⁹ M E₂ or 10⁻⁷ M LY117018 for 48h and were fixed with 4% paraformaldehyde and Triton X, and incubated with rabbit anti-rat polyclonal antibodies against type I or type III collagens and tropoelastin. Bound antibodies were observed by the peroxidase detection system.

Measurement of total soluble collagen: Total secreted soluble collagen was quantified using the Sircol collagen assay. Cells were cultured with E₂ (10⁻⁹ M) or LY117018 (10⁻⁷ M), and either vehicle or ICI 182,780. The total collagen levels were measured at 48 hr or 72hr.

Western blotting: Cells were cultured with 10⁻⁹ M E₂ or 10⁻⁷ M LY117018, and either vehicle or ICI 182,780 (10⁻⁷ M). After incubation for 48 h, the supernatants were collected and electrophoresed and transferred onto a PVDF membrane. The membranes were blocked and then incubated with type I or type III collagens antibodies and MMP-13 antibodies. The bands were detected by the chemiluminescent method.

Statistics: Analysis of variance and Dunn's post hoc test were performed.

RESULTS:

Effect of E₂ and raloxifene on tendon fibroblast proliferation

Neither E₂ nor LY117018 significantly affected the proliferation of tendon fibroblasts in both the BrdU assay (24h) and the WST-8 colorimetric assay (2, 6 days).

Effect of E₂ and raloxifene on mRNA expression of tendon ECM-related genes

Neither E₂ nor LY117018 changed mRNA expression level of type I collagen significantly, but both of them significantly upregulated the mRNA expression level of type III collagen and elastin, and the

estrogen antagonist ICI 182,780 attenuated these changes. Both E₂ and LY117018 upregulated the mRNA expression level of MMP-13 in a dose-dependent manner and these changes were also attenuated by ICI 182,780. E₂ did not significantly increase the mRNA expression of TIMP-1, but LY117018 at a concentration of 10⁻⁸ M significantly upregulated the mRNA expression of TIMP-1, and ICI 182,780 treatment attenuated this upregulation.

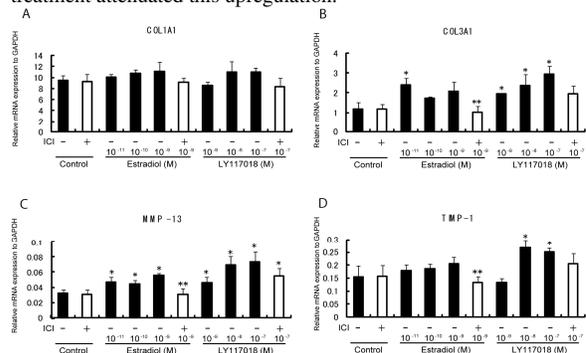


Fig. 1 mRNA expression of collagen metabolism related genes in tendon fibroblast cells treated with various concentrations of E₂ or LY117018 and either vehicle (solid bar) or ICI 182,780 (open bar)

Immunocytochemistry of type I and type III collagens and tropoelastin

Type I collagen was highly expressed in the fibroblasts and there was no significant difference in the area or density of staining among control, E₂-treated, and LY117018-treated cells. In contrast, E₂- and LY117018-treated cells exhibited much stronger and extensive staining for type III collagen and tropoelastin compared to the untreated controls.

Effect of E₂ and raloxifene analogue on amount of collagens and MMP secreted in the culture medium

With regard to total soluble collagen in cell culture supernatants, neither E₂ nor LY117018 significantly affected collagen levels for 48 hr and 72 hr in the Sircol collagen assay.

In western blotting experiments, exposure of fibroblasts to E₂ or LY117018 did not change the intensity of the band for type I collagen, but the band intensity for type III collagen and MMP-13, however, increased with 10⁻⁹ M E₂ or 10⁻⁷ M LY117018 treatment and this effect was attenuated by 10⁻⁷ M ICI 182,780.

DISCUSSION:

The results of the present study suggest that both estrogen and SERM/raloxifene analogue affect collagen metabolism in tendon fibroblasts. Although neither E₂ nor LY117018 significantly changed the expression of type I collagen, which is a main component of the tendon ECM, both E₂ and LY117018 increased the expression of MMP-13, which is implicated in collagen degradation of the rat tendon ECM. Because structural and material properties of the tendons and ligaments are maintained by the balance between collagen synthesis and degradation, these results indicate that estrogen deficiency retards collagen turnover and that treatment with either E₂ or SERM recovers MMP-13 expression to the premenopausal level.

An interesting finding was that both E₂ and LY117018 increased the expression of type III collagen and elastin, which account for less than 10% of the tendon ECM but are considered to govern tendon elastic properties. Although increased type III collagen content is reported to weaken the tensile strength of tendons, the expression level of type III collagen and elastin induced by E₂ and LY117018 may be normal level and these may contribute to physiological level of elasticity in tendons and ligaments.

These findings indicate that estrogen deficiency might retard tendon ECM remodeling and alter tendon elasticity. Treatment with raloxifene may help to recover these changes to premenopausal levels, but further in vivo studies are required.

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