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17B–ESTRADIOL SUPPRESSES PROLIFERATION OF FIBROBLASTS DERIVED FROM CARDINAL LIGAMENT IN PATIENTS WITH OR WITHOUT PELVIC ORGAN PROLAPSE (POP)

Hypothesis / aims of study

Estrogen plays an important role in growth, differentiation, and extracellular matrix synthesis in various tissues. It is also critical for the development and maintenance of reproductive tissues. Nuclear estrogen receptors (ER) mediate most of the actions of estrogens, which results in transcriptional activation and suppression, integration of intracellular signaling pathways, and control of cell cycle progression. Estrogen replacement therapy (ERT) is one of the non-surgical options of POP treatment, which may help to limit further weakness of the muscles and other connective tissues that support the uterus. Although ERT has been used in the treatment of urinary stress incontinence and POP, results are inconclusive [1] and the precise role of estrogen on the pathogenesis of POP is not fully understood. Previous studies have shown that estrogen can mediate two diverse growth responses: inhibition or proliferation [2]. The purpose of this study was to investigate the effect of 17β–estradiol (E2) on proliferation of fibroblasts derived from cardinal ligament in patients with or without POP.

Study design, materials and methods

Study design: Seven subjects with POP and seven normal subjects were recruited for study. Those subjects who have concomitant malignant pelvic diseases or those receiving ERT were excluded from the study. Subjects unwilling to participate were also excluded. Informed consent and IRB approval were obtained from the participating institution and subjects.

Materials and methods: During vaginal hysterectomy for patients with POP or abdominal hysterectomy performed for nonprolapse/nonmalignant indications in controls, small branches of the cardinal ligaments were extracted for cell culture. Seven cell strains of cardinal ligament fibroblasts derived from patients with POP (P1 to P7) and control patients without POP (C1 to C7) were established as described [3]. Fibroblasts at passages 2 to 4 were used for studies.
The fibroblasts were first characterized by light microscopy and immunostaining with monoclonal anti-human fibroblast surface protein (Clone 1B10) (Sigma Chemical Company, St. Louis, MO). The growth rate of the fibroblasts from patients with POP was compared with that of control by MTT assay. Four cell strains from each patient (P2, P3, P4 & P6) and control (C1, C2, C4 & C5), with age not statistically different ($p>0.05$), were treated with different concentrations of E2 (Sigma Chemical Company, St. Louis, MO) (0, 10^{-4}, 10^{-8} ,10^{-9} & 10^{-10}M) in phenol red-free MEM plus 2% charcoal treated FBS and incubated for 48 hr. The effect of E2 on cell proliferation was then measured by MTT assay. The statistical comparisons were performed using ANOVA. A value of $p<0.05$ was considered significant.

Results

In the cell growth study of both patient (P1 to P7) and control (C1 to C7), the growth rate of all the seven patient fibroblasts was lower than that of control fibroblasts (except compared to C2). E2 suppressed cell growth in both patient and control cardinal ligament fibroblasts as shown in Graph A. At super-physiological E2 concentration (10^{-4}M), both patient and control fibroblasts showed decrease in cell number. In addition, the percentage of decrease in cell number in patient fibroblasts was significantly higher than that of controls ($p<0.001$). Compared to vehicle control (0M E2), both patient and control fibroblasts showed slower cell growth at physiological E2 concentrations (10^{-8}, 10^{-9} & 10^{-10}M). However, the percentage of increase in cell number in the patient fibroblasts was significantly lower than that of controls ($p<0.001$).

Interpretation of results

We showed that the overall growth rate of patient cardinal ligament fibroblasts was slower than that of controls. In addition, E2 suppresses cell proliferation of both patient cardinal

ligament fibroblasts and controls, with the most predominant inhibition effect in patient fibroblasts.

Concluding message

Cell proliferation of cardinal ligament fibroblasts from patients with POP is lower than that of controls. This suggests that slower fibroblast proliferation may contribute to the development of POP. E2 suppresses cell proliferation of cardinal ligament fibroblasts derived from patients with POP and controls. Our results suggest that ERT may not be an effective and beneficial treatment for POP by promoting the cell proliferation of cardinal ligament fibroblasts, but may even inhibit the growth of the cells.

References

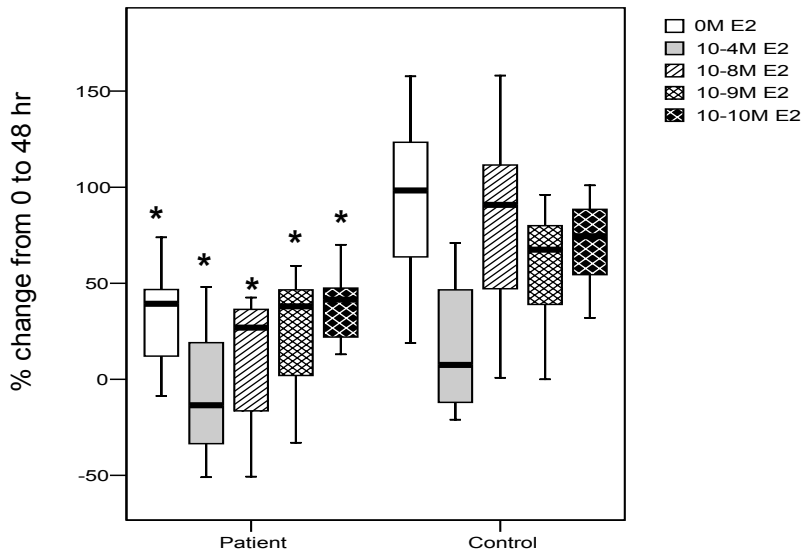
[1] Hormonal influence on the urinary tract. Urologic Clinics of North America. 1994; 22:629-639

[2] Estrogen regulation of endothelial and smooth muscle cell migration and proliferation. Arteriosclerosis Thrombosis and Vascular Biology. 2002; 22: 1585-1590

[3] Decrease in elastin gene expression and protein synthesis in fibroblasts derived from cardinal ligaments of patients with prolapsus uteri. Cell Biology International. 1997; 21: 605-611

Graphs

(A)



Box plot showing the percentage change of viable cell number of fibroblasts from cardinal ligament in patients and controls added with different concentrations of E2 (0, 10^{-4}, 10^{-8} ,10^{-9} & 10^{-10}M) after 48hr incubation.