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EFFECT OF ESTROGEN ON OXIDATIVE STRESS RESPONSE OF CARDINAL LIGAMENT FIBROBLASTS FROM WOMEN WITH OR WITHOUT PELVIC ORGAN PROLAPSE.

Hypothesis / aims of study

Production of reactive species, including free radicals, is an integral part of human metabolism. Because of its high potential to damage vital biological systems, reactive species have now been incriminated in aging and in more than 100 disease states. Estrogen has been proposed to exert antioxidant effects in *in vitro* models as well as in many biological systems. It has been shown to prevent oxidative stress induced apoptosis of endothelial cells. Lately, oxygen radical absorbance capacity (ORAC) assay has been used by different laboratories and has provided substantial information regarding the antioxidant capacity of biological fluids and tissues. On the other hand, the presence of isoprostanes has been recognized as a stable marker of *in vivo* oxidative stress. Given that reproductive hormones may influence the progression of Pelvic Organ Prolapse (POP) and isoprostanes production could be a good index for assessing in vivo oxidative stress in prolapse tissues. We examined the total antioxidant capacity in the cardinal ligament fibroblasts from women with and without POP. And to investigate the *in vitro* oxidative stress response and antioxidant effects of estrogen in *in vitro* culture system.

Study design, materials and methods

Study design:

Nine subjects with POP and nine normal subjects were recruited. Subjects who have concomitant malignant pelvic diseases or that receiving hormone replacement therapy were excluded from the study. Informed consent and IRB approval was obtained from the participating institution and subjects.

Materials and methods:

After informed consent was obtained, small biopsy of cardinal ligaments were obtained from POP patients and control subjects who underwent gynaecological surgeries. Tissue samples were then washed in phosphate-buffered saline solution, cut into approximately 1 mm^3 fragments and placed into tissue culture dish for primary explanation. Fibroblast cells were grown to confluence as described (1). Cells at passages 2 to 4 were used for studies. Cells at proliferative phase were grown in serum-free medium for 24 hours before the addition of 17β-estradiol (Sigma Chemical Company, St. Louis, MO). After different times of incubation (24 to 96 Hours), culture medium was removed and stored at −80°C until assayed. Protein concentration in culture wells was measured by the Bradford assay. The total antioxidant capacity and isoprostanes level of cultured cardinal ligament fibroblasts was quantified by the oxygen radical absorbance capacity (ORAC) assay and ELISA (Cayman Chemistry, US) in culture medium, respectively. ANOVA was applied for comparisons of means. $P \leq 0.05$ was considered significant.

Results

Cardinal ligament derived fibroblasts from POP and control were identified by their characteristic long, needle-shaped morphology and the presence of fibroblast surface protein by immunocytochemistry using a specific antibody (monoclonal anti-human fibroblast surface protein [Clone 1B10], Sigma Chemical Company, St. Louis, MO). There were no significant difference between the total antioxidant capacities of fibroblast derived from POP patient (61±17mmol Trolox equivalents/mg protein) and control cardinal ligament fibroblasts (58±9mmol Trolox equivalents/mg protein) as determined by the ORAC assay. A sustained, spontaneous production of isoprostanes in POP patient and control (exposed only to vehicle) was observed. The baseline level of isoprostanes produced in culture medium of POP patients were significantly higher at 1hr, and 24hr than controls ($p<0.05$; Figure 1). Exposure

to physiological concentrations of E2 for 1hr did not modify isoprostanes production. Exposure to E2 decreased isoprostane production only after 24hr in patient; whereas E2 induced reduction of isoprostanes was significant only after 48hr in control cases (Figure 2).

Interpretation of results

We find that fibroblast derived from cardinal ligament with POP is associated with an increased isoprostanes production, indicating that oxidative stress could be a probable mechanism for pelvic organ prolapse. Our results clearly indicate that physiological concentration of estrogen (10^{-8}M) caused a dose-dependent decrease in spontaneous cardinal ligament fibroblast production of isoprostanes. The time-course analysis, indicated that E2 effects on isoprostanes are evident only after 24 hr, suggests a genomic action of E2 mediated through a steroid-receptor complex. Although, the antioxidant effects of E2 have been attributed to the aromatic hydroxyphenol structure of the A ring (2). An antioxidant mediated by estrogen receptor cannot be ruled out.

Concluding message

Our results demonstrate antioxidant effects of physiological concentration of E2. Augmentation of isoprostanes production reveals new insights into the molecular mechanisms involved in development of genitourinary prolapse.

Reference

(1) 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.

(2) Antioxidant and prooxidant actions of estrogens: potential physiological and clinical implications. Semin Reprod Endocrinol. 1998;16:309-14.

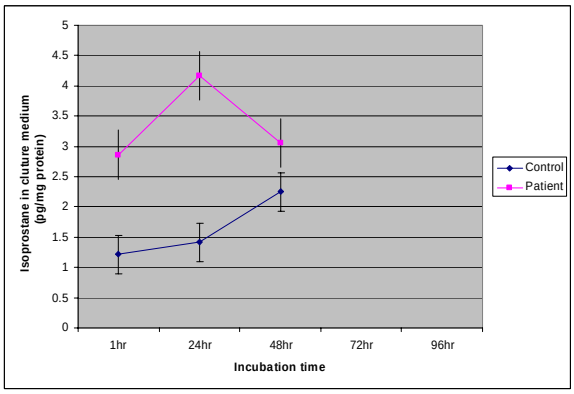

Figure 1. Time course of estradiol effects on spontaneous production of isoprostanes by cultured cardinal ligament fibroblasts. Cells were exposed to estradiol for 1 to 96hr, culture medium was collected, and isoprostane concentration was measured.

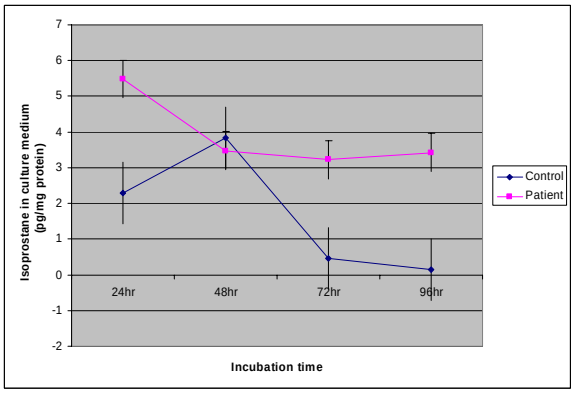

Figure 2. Effect of estradiol-induced reduction of isoprostanes production by cultured cardinal ligament fibroblasts (CLF). Culture CLFs were exposed to 10^{-8}M estradiol. Values are means±SE of 3 duplicate determinations.