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Ohata R[1], Kinouti S[1]
1. Tottori University Hospital

FFECTS OF ESTROGEN REPLACEMENT TO POSTMENOPAUSAL RATS ON THE EXPRESSION OF APOPTOTIS-RELATED GENES IN THE BLADDER

Aims of Study

The decrease in serum level of estrogen that occurs in menopause causes metabolic and atrophic alterations in many organs. Postmenopausal urogenital atrophy causes pollakiuria, urinary urgency, stress and urge incontinence, dysuria and so on. Previous investigations have demonstrated the presence of estrogen receptors in the human urinary tract. This supports the practice of giving estrogen treatment to menopausal women with lower urinary tract dysfunction. Estrogen has been found to improve lower urinary tract function in postmenopausal women (1). Several studies have indicated that estrogen regulates cell survival and death factors. Estrogen administration decreases apoptosis (2). In the present study which uses female rats after menopause, the effects of estrogen replacement therapy on their bladders were investigated, including the effects on the expressions of bcl-2 and bax.

Methods

Sixteen-month-old female Wistar rats were divided into 5 groups. In group □, no hormonal treatment was carried out (control group;n=8). In group□ & □, a silastic tube containing estradiol (E2) dissolved in sesame oil at 10 μg was subcutaneously placed for 2 weeks(group□) or 4 weeks(group□) without an ovariectomy (low dose E2 replaced group: group□;n=8, group□;n=8). In group □ & □, E2 was placed in the form of silastic capsules containing 2.5 mg β-E2 for 2 weeks(group□) or 4 weeks(group□) .(high dose E2 replaced group: group□;n=8, group□;n=8). All animals were kept in an air-conditioned room lighted 12 h a day, and allowed access to food and water ad libitum. Plasma E2 concentrations were measured at 16 month old in control group and, 2 weeks(group□,□) or 4 weeks(group□,□) after hormone replacement. After 2 or 4 weeks replacement, the bladder was removed and weighed after emptying the urine. Four bladders per group were embedded in paraffin and used for hematoxylin and eosin staining (HE), and also immunohistochemical staining by monoclonalantibody against Bcl-2 or Bax. Remaining 4 bladders per group were frozen in liquid nitrogen, total RNA was extracted, and semiquantative RT-PCR analysis of mRNA expression of *bcl-2*, *bax* andβ-*actin* was performed. In control group, same experiments were done after 4 weeks of no replacement.

Results

Plasma E2 levels ranged from 15 to 45 pg/ml in low dose replaced group, from 100 to 330 pg/ml in high dose replaced group. These levels were significantly higher than those in control group. Bladder weight was significantly heavier in the E2 replaced groups, but there was no significant difference among the low-dose and high-dose replaced groups.

There was no significant difference between control and E2-replaced rats in the HE stains of the bladder.

Immunostained bladders showed no difference between control and E2-replaced rats in reactivity against Bcl-2 protein. In contrast, against Bax protein, mucosal region of control rat showed immunoreactivity, whereas E2-replaced rats showed little reactivity.

Upon mRNA analysis, the expressions of *bcl-2* mRNA showed no difference among groups. The expressions of *bax* mRNA were significantly suppressed in groups □, □, □ as compared with the control group. In group □, the expression of *bax* mRNA was almost the same level as that in the control group.

Conclusions

This study showed that E2 administration to the postmenopausal rats suppressed the expression of *bax* mRNA, but did not suppress the expression of *bcl-2* mRNA. These results suggest that E2 administration affects the expression of apoptosis-related genes and suppresses apoptotic action, and that these changes could consequently alter voiding behavior.