MEASUREMENT OF DNA DAMAGE IN URINARY BLADDER TRANSITIONAL CELLS INDUCED BY LOW ESTROGEN LEVELS: INSIGHTS ON THE MENOPAUSAL STATUS

Hypothesis / aims of study
The bladder can be considered a target organ for the actions of estrogens. Decreases in circulating estrogen after menopause have been associated with bladder dysfunctions. Moreover, ovariectomized rats have been used as the animal model for the study of oxidative stress in uterus. Oxidative stress due to reactive oxygen species (ROS) can cause oxidative damage to cells such as urothelial cells. Cells have a number of defense mechanisms to protect themselves from the toxicity of ROS. If this evidence is correct, treatment with an antioxidant should be beneficial in ovariectomized rats. We set out to study whether the vitamin E supplementation influences oxidative stress as well as DNA urothelial damage in castrated female rat bladder.

Study design, materials and methods
The project was approved by the University Ethical Committee. A total of 30 female Wistar rats were divided into three groups of 10 animals; group I got a sham operation and was sacrificed after 30 days; group II was ovariectomized and sacrificed after 30 days without any replacement of vitamin E; group III was ovariectomized and got on a supplementation with 1,000 IU/kg vitamin E/once a week, IM, for 30 days. After 4 weeks of procedure the rats were anesthetized and the bladders were rapidly excised, frozen and stored at −70°C for analysis of the alkaline Comet assay that was performed on the single cell suspensions from lymphocytes and urothelial cells. We also determined the plasma concentrations of 8-isoprostane to confirm the presence of oxidative stress. Statistical significance was evaluated with Prism 4.0 (USA) using Anova.

Results
The oophorectomy and alpha toopherol supplementation did not present any effects on the bladder volume and bladder wall thickness of the animal in the groups. The concentration of alpha tocopherol was 0.5ng/mg wet weight in group I, 0,58ng/mg wet weight in group II and 1,7ng/mg wet in group III (p < 0.05 compared group III to others). The 8-isoprostane assays via the commercial kit described previously demonstrated that the values for group II (oophorectomised without alpha tocopherol replacement; mean±SD: 4.56±0.76 pg/ml) were statistically greater than those for group I (sham; mean±SD: 1.34±0.23 pg/ml) and group III (oophorectomised with alpha tocopherol supplementation; mean±SD: 2.04±0.35 pg/ml).

The analysis of the Tail Moment of lymphocytes demonstrated no differences among the groups. However, the analysis of the Tail Moment of urothelial cells showed that the ovariectomized group presented a significantly DNA damage when compared to sham as well as ovariectomized group with vitamin E supplementation (figure).

Interpretation of results
Our results demonstrated that bilateral oophorectomy in rats raised the plasma levels of 8-isoprostane in these animals, thus characterising the systemic generation of free radicals. We observed that DNA damage could be identified using the Comet assay on the urothelial cells, thus, we can suppose that either the levels of oxidative stress were insufficient to provoke damage in the lymphocytes or bilateral oophorectomy does not induce a systemic damage identified by Comet assay. Furthermore, we have observed that alpha-tocopherol supplementation after oophorectomy avoids DNA fragmentation in urothelial cells.

Concluding message
Based on the data, it can be stated that low oestrogen levels are associated with the induction of free radicals formation; and these free radicals would be at least partially responsible for the urothelial lesions. It can be also suggested that the use of antioxidants would have a protective role in states of hypoestrogenism, thereby avoiding diseases stemming from urothelial damage.

References
1. Urology (2002);60:64-70.

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