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ANTIOXIDANT SUPPLEMENTATION INHIBITS THE PROCESS OF APOPTOSIS IN THE BLADDER WALL SUBJECTED TO ANDROGEN DEPRIVATION

Hypothesis / aims of study

As the size of the elderly population increases, many middle-age and elderly people are developing health problems [1]. The relationship between androgen deprivation and age-related urinary disturbance has been examined and the age-induced disease bladder dysfunction has been reported [2]. Data have been shown on the relation between the lower levels of testosterone and the cells death as a component of muscle atrophic induced by castration, that corroborate with the hypothesis that there is an androgen sensibility in different body cells and that can induce morphological and functional alterations in those cells [1,2]. The main of this study was to evaluate the effects of hypogonadism on the bladder wall induced by castration in male rat. Furthermore, the alpha-tocoferol supplementation was used to evaluate its protection effects on the apoptosis process.

Study design, materials and methods

Thirty-two male Wistar rats (250-300g) were used in this experiment. The animals were divided into 4 groups: Group I: control group (sham procedure n=8); Group II: subjected to bilateral orchiectomy and sacrificed eight weeks after the procedure (n=8); Group III: alpha-tocoferol supplementation 4 weeks previously bilateral orchiectomy and sacrificed after 8 weeks (n=8) and Group IV: alpha-tocoferol supplementation 4 weeks previously castration and 8 weeks after the procedure (n=8). All rats were sacrificed and samples of blood were collected for analysis of testosterone and 8-isoprostane. The bladder was removed at the vesical neck level and was fixed in 10% formalin and processed into paraffin for immunohistology. Immunohistochemistry was performed on 5µm sections. The caspase-3 actived was detected by the technical describes previously [3] modify for the analysis of the results through stereological methodology. Nonparametric methods were used for tests of stastiscal significance. This study was approved by Ethical Committee of University, where the project was developed.

Results

The concentrations of serum testosterone at the end of the experiment in groups II, II and IV were less than 20pg/mL. The analysis of 8-isoprostane showed statically significance in group II compared to other ($p<0.0003$) (figure 1). The volumetric density of caspase-3 showed a significant difference when the groups were compared ($p=0.0023$). Group II presented the highest level of cells death compared to other (figure 2 and 3).

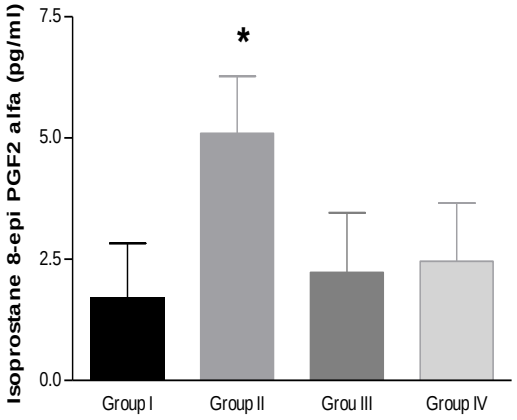


Figure 1: Graph of serum levels of 8-isoprostane; *p<0.003.

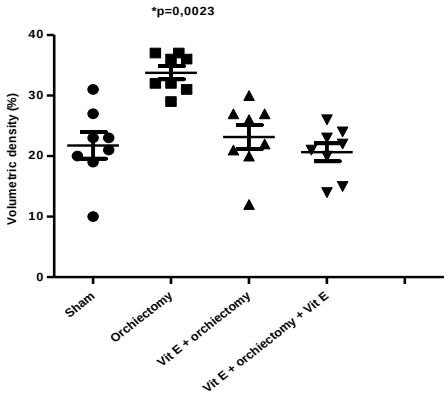
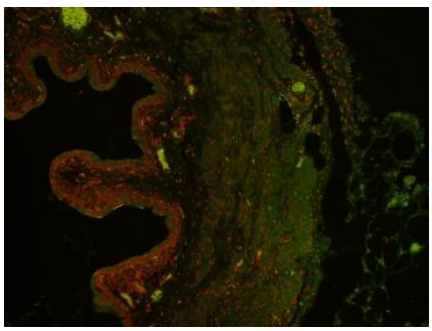
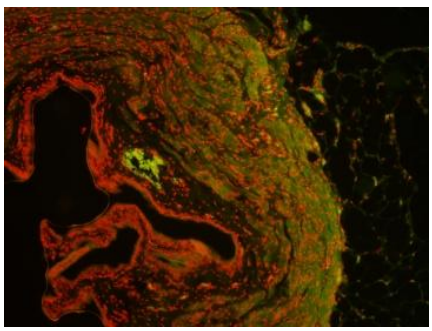


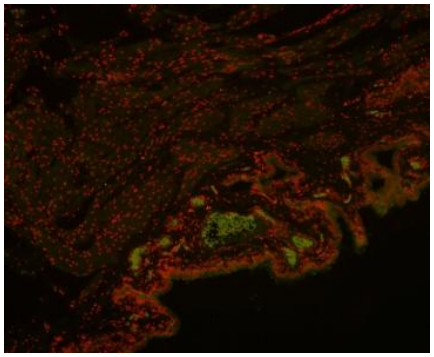
Figure 2: The volumetric density of apoptotic process determines by caspases-3 analysis in different groups



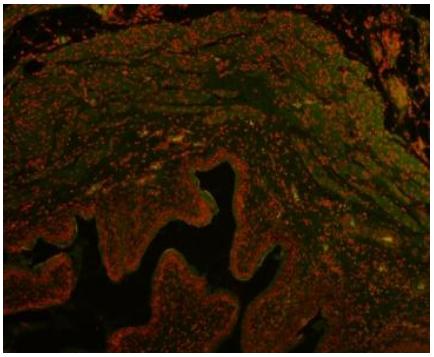
(a)



(b)



(c)



(d)

Figure 3: Microscopic fluorescence Immunohistochemistry for the detection of active caspase-3. Increase 10x, groups I (a), II (b), III (c) and IV (d). The positive reaction is visualized in green. Nuclei are observed in red.

Interpretation of results

Our results demonstrated that the apoptotic process in the bladder wall was more expressive in castration group than others. It was associated with induction of markers for oxidative stress (8-isoprostane). The alpha-tocopherol supplementation significantly attenuated the oxidative stress and cell death in the bladder. These results suggested that male castration caused oxidative stress and that may be involved in the induction of apoptosis. Furthermore, the administration of an antioxidant significantly reduced apoptosis in the bladder subjected to androgen deprivation.

Concluding message

The generation of free radicals is at least partially involved in the induction of cell death in the bladder subjected to androgen deprivation. Moreover, the administration of an antioxidant produced a significant protective effect. Thus, the present study provides a rationale for further studies on the relationship between testosterone and its role on the bladder dysfunction in the aging process.

References

[1] Nature 408: 239-247 (2000)
[2] Aging Male 5: 74-86 (2002)
[3] Histochem Cell Biol 105: 261-267 (1996)

Specify source of funding or grant	**None**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**COMMITTEE OF ETHICAL IN RESEARCH OF FEDERAL UNIVERSITY OF SÃO PAULO**