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Androgen therapy reverses injuries caused by ethanol consumption in the prostate: Testosterone as a possible target to ethanol-related disorders

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ABSTRACT

Aims: Chronic ethanol consumption leads to reproductive damages, since it can act directly in the tissues or indirectly, causing a hormonal imbalance. Prostate is a hormone-dependent gland and, consequently, susceptible to ethanol. The potential of testosterone therapy in the ethanol-related disorders was investigated in the prostate microenvironment.

Main methods: UChB rats aged 90 days were divided into 2 experimental groups (n = 20): C: drinking water only and EtOH: drinking 10% (v/v) ethanol at >2 g/kg body weight / day + water. At 150 days old, 10 rats from each group received subcutaneous injections of testosterone cypionate (5 mg/kg body weight) diluted in corn oil every other day for 4 weeks, constituting T and EtOH + T, while the remaining animals received corn oil as vehicle. Animals were euthanized at 180 days old, by decapitation. Blood was collected to obtain hormone concentrations and ventral prostate was dissected and processed for light microscope and molecular analyses.

Key findings: Ventral prostate weight, plasma testosterone and DHT and intraprostatic testosterone concentrations were increased after testosterone treatment. Plasma estradiol level was reduced in the EtOH + T. Inflammatory foci, metaplasia and epithelial atrophy were constantly found in the prostate of EtOH and were not observed after hormonal therapy. No differences were found in the expression of AR, ERβ and DACH-1. Additionally, testosterone treatment down-regulated ERα and increased the e-cadherin and α-actinin immunoreactivities.

Significance: Testosterone was able to reverse damages caused by ethanol consumption in the prostate microenvironment and becomes a possible target to be investigated to ethanol-related disorders.

Introduction

In addition to inciting social and economic problems, ethanol and its metabolites provoke disorders in several organic systems [11]. Animal models have helped to explain some of the biologic aspects of alcohol ingestion by humans. Among these animal models are the UChA and UChB strains of rats, which are the oldest and only rat strains that remain in inbreeding and therefore represent a unique model for understanding the basis of alcoholism-linked characteristics, such as those found in alcohol-related human diseases [20].

The prostate is an accessory gland that is often affected by benign prostatic hyperplasia and cancer [14]. In rodents, the ventral and dorsal lobes are the first to respond to morphologic and inflammatory alterations [30]. Ethanol can act directly in the testis, modifying testosterone production [33,36], and indirectly in the hypothalamic–pituitary–testis axis, reducing LHRH and testosterone production by Leydig cells [31,36]. As a consequence of androgen decrease, changes occur in the prostate, such as involution, epithelial atrophy, apoptosis and reduction of androgen receptor (AR) expression [31,36]. Besides the decreasing of plasma testosterone level often related to chronic ethanol, Sáttolo et al. [32] and Cândido et al. [4] also observed severe morphologic injuries, including intraepithelial neoplasia, stromal hypertrophy and the presence of inflammatory cells in the prostate of alcoholic animals.

Testosterone and dihydrotestosterone (DHT) have essential roles in the development, proliferation, differentiation, maintenance and physiology of the prostate [9,27]. It is known that estrogens are also involved

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in the normal and abnormal growth of the prostate in different species [1]. Circulating testosterone is converted to estrogen in many tissues by aromatase enzyme [5], and its cellular actions are mediated by two receptors, estrogen receptor α (ERα) and β (ERβ). In the prostate, ERα is expressed mainly in the stroma and is almost absent in the epithelium, whereas ERβ is highly expressed in the epithelium (reviewed in [35]). Both receptors act at particular times during development with specific and mostly opposite roles in the prostate [24]. Therefore, hormonal prostate control involves intricate events that depend on the balance between steroid hormones.

According to Yatkin et al. [42], an imbalance between androgen and estrogen concentrations (i.e., changes in the androgen/estrogen ratio) may be the essential factor responsible for triggering the inflammatory process in the diseased prostate. Increasing estrogenic stimulation in the prostate is associated with reactivation of prostate growth, inflammation [8], neoplastic transformations [16] and, perhaps, cancer [2]. Fávaro and Cagnon [9] proposed a relationship between ethanol and estrogenic stimulation, showing an increasing of ERα expression in the prostate of rats submitted to chronic ethanol consumption, compromising the prostatic hormonal balance, which is a crucial factor to maintain the morphological and physiological features of this organ.

Therefore, the aim of this study was to evaluate the effects of ethanol on the prostate microenvironment, focusing on hormonal and morphological parameters and to verify if the testosterone could be used as a therapy on the ethanol related-disorders in the ventral prostate of UChB adult rats.

Material and methods

Animals and experimental design

Forty adult UChB male rats were bred and maintained at the Department of Anatomy, Bioscience Institute of Botucatu, UNESP — Univ Estadual Paulista. When the UChB rats reached 60 days old, they were submitted to a selection process for ethanol preference. Thus, during 20 days of selection, the rats were given a choice between two bottles containing either water *ad libitum* or 1:10 (v/v) ethanol. After this period, rats exhibiting ethanol consumption at 2–6 g/kg body weight/day were selected for this experiment according to Mardones and Segovia-Riquelme [23]. The selected rats, at 80 days old, were divided into two groups (n = 20): the EtOH group, receiving ethanol 1:10 (v/v) and water *ad libitum*, and the control group (C), receiving only water. At 150 days old, 10 rats of each group received subcutaneous injections of testosterone cypionate (Deposteron®, 5 mg/kg body weight) diluted in corn oil, every other day over 4 weeks at a consistent time each day (8:00–8:30 am) [32,34]. Rats receiving injections constituted the T and EtOH + T groups, while the other twenty (10 rats/group) males received just corn oil (Fig. 1).

All rats were housed in polypropylene cages with laboratory-grade pine shavings as bedding, maintained under a controlled temperature (23 °C ± 1 °C) and day/night cycle (12 h/12 h), and provided filtered tap water and rat chow *ad libitum*. Experimental protocols followed the ethical principles in animal research of the Brazilian College of Animal Experimentation (208-CEEA).

Food intake and organ weights

Feeding content was prepared in lots of 5 days, always at the same time of the day (15 h) using a marked test tube and analytical balance (Ohaus Traveler; Ohaus Corporation, México, D.F., México). The profile food ingestion (caloric value of standard chow = 2930 kcal/kg) was assigned according to the standards of necessary care. The animals were weighed at the beginning and end of the experiment to perform the calculation of the body weight gain. At the end of treatment, the ventral prostate and seminal vesicle were dissected and weighed. The determination of the body and organ weight was carried out using an analytical balance (Owa Labor, Oschatz, Germany).

Sex hormone assay

Plasma testosterone, dihydrotestosterone (DHT) and estradiol

At 180 days old, the rats were euthanized in a CO_2 chamber followed by decapitation. Blood samples were collected from the trunks of decapitated rats into heparinized tubes at the time of death (between 9:00 and 11:30 am). Afterwards, plasma was obtained by centrifugation at 1,200 × *g* for 15 min at 4 °C and stored at −20 °C until it was assayed. Testosterone and DHT levels were determined by a double-antibody radioimmunoassay using Coat-A-Count® (Diagnostics Products Corporation, Los Angeles, USA). All samples were dosed in the same assay to avoid inter-assay errors. The intra-assay variation was 1.75%, and the results were in ng/mL. Estradiol levels were assayed by chemiluminescence (Elecsys Kit — Roche®, Basileia, Swiss; Estradiol E2 II, test sensitivity: 5 pg/mL, linearity: 4.300 pg/mL). The assays were performed at the Neuroendocrinology Laboratory, Dental School of Ribeirão Preto, University of São Paulo — USP.

Intraprostatic testosterone

After euthanasia, the ventral prostate was rapidly removed, and tissue samples of 150 mg were immediately frozen in liquid nitrogen and stored at −80 °C. The tissue fragments were homogenized (9500 rpm) with PBS for 2 min in "tube A," and diethyl ether was added. The tissues were then placed on the vortex for 2 min. The homogenate was incubated at room temperature and the liquid phase transferred into "tube B". The precipitate from tube A was resuspended with diethyl ether, homogenized, and incubated in dry ice, and the liquid phase transferred to tube B. Tube B was left in the fume hood overnight to evaporate all of the volatile components. The next day, PBS was added, the specimen was aliquoted and the testosterone levels were determined by double-antibody radioimmunoassay using Coat-A-Count® (Diagnostics Products Corporation, Los Angeles, CA, USA). The assays were performed at the Neuroendocrinology Laboratory, Dental School of Ribeirão Preto, University of São Paulo — USP.

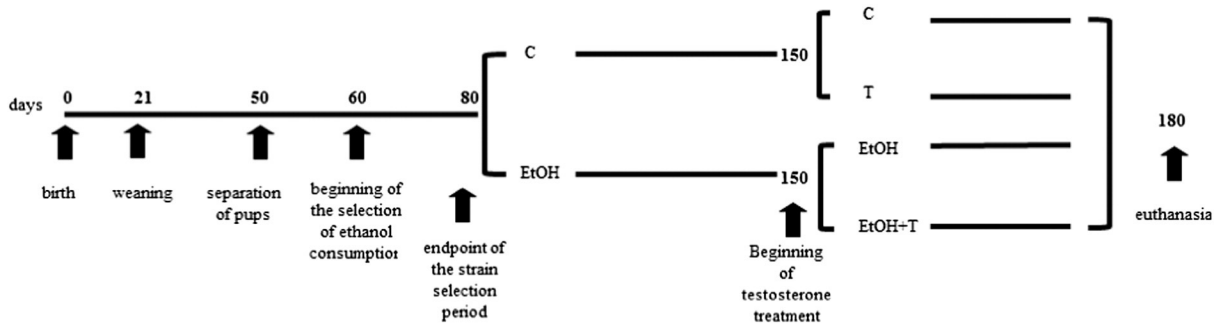

Fig. 1. Chronological scheme for overall chronic treatment.