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Research article

Changes in rat testis morphology and androgen receptor expression around the age of puberty

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ABSTRACT

Background: Androgens are the keystone in fertility and intact sexual functions in males. It exerts its actions via androgen receptors extensively present in testicular cells, only its presence in germ cells is controversial. The alteration of androgen receptors in different testicular cells is usually accompanied by different sexual disorders. On the other hand, many sexual disorders are treated with androgens. Puberty, being the juncture of hormonal blossom, is an important stage to evaluate the evolution of testicular cells including androgen receptors.

The aim of the work was to investigate the morphological and androgen receptor changes in different testicular cells during puberty in the rat testis using histological and immunohistochemical techniques.
Material and methods: This study was carried out on 45 male albino rats (Sprague-Dawley). The rats were divided into three age groups; group I (prepubertal) 21 days old, group II (peripubertal) 35 days and group III (postpubertal) 90 days old. The rat testes were examined histologically and immuneohistochemically. Cells and androgen receptors were counted using Leica Qwin 500 image analyzer computer system. Data were analyzed using univariate ANOVA and Bonferroni post hoc test.
Results: Histological examination of the different ages showed developmental changes of different testicular cells. Immunohistochemical examination revealed the presence of AR in spermatogenic cells in pubertal and postpubertal groups and partially in prepubertal group. AR was clearly expressed in both Sertoli and Leydig cells in the three groups. The maximum expression in Sertoli cells was at 90 days while that of Leydig cells was at the age of 35 days.
Conclusion: Androgen receptors should not be excluded as an effective factor on germ cells through its direct action on AR, clearly expressed in spermatogenic cells and its surge at the age of puberty. Studies and treatments should respect the AR expected levels according to age in other testicular cells as well. Sertoli cells show a linear increase of AR expression throughout life, while Leydig cells show a peak at the age of puberty.

1. Introduction

Fertility and intact sexual functions are the dream of males throughout lifetime. Accordingly, being the keystone in those features, androgens have always been a target for medical research. Androgens, excreted from the testis and to a lesser degree from the adrenals, produce their function through binding to androgen receptors (AR), leading to transactivation, resulting in the modulation of AR downstream gene expression (Chang et al., 2013). The high similarity in amino acid sequence of AR between rats and human, and the entirely identical sequences in the DNA binding domain and Ligand binding domain made the rat an excellent model for studying AR (Mc Ewan, 2004).

One of the most important organs as an androgen target is the testis itself. Androgen is essential for maintenance of reproductive functions (Zhu et al., 2000). Male mice lacking AR show incomplete germ cell development and lowered testosterone level, resulting in azoospermia and infertility (Xu et al., 2007). Androgen has been used for decades in the treatment of different masculine disorders,

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such as hypogonadism (Snyder, 1984), delayed puberty (Joss and Mullis, 1994) and even for the treatment of some hematological disorders (Ammus, 1989).

The expression of AR in germ cells has been controversial. Some studies claim the presence of AR (Kimura et al., 1993; Zhou et al., 1996), while other studies deny its appearance (Zhou et al., 2002; Tsai et al., 2006). However, both teams agreed that AR have been implicated in the process of gametogenesis. AR expression in Sertoli cells are needed in such a process. Failure of maturation of Sertoli cells in humans is associated with absent or weak AR expression (Regadera et al., 2001). AR expression in Leydig cells regulates hormonal production. Decreased number of working AR of Leydig cells causes spermatogenesis arrest mainly during the Spermatid stage (Tsai et al., 2006).

The aim of this work was to investigate the changes in the morphology and AR expression in germ, Sertoli and Leydig cells during pre-pubertal, pubertal and post-pubertal ages, using histological and immunohistochemical techniques, trying to clarify the changes occurring during puberty in the rat testis, which might be used as a guide in different treatments affecting that organ during this stage, especially those using androgen or androgen derivatives.

2. Materials and methods

2.1. Animals

This study was carried out on 45 Sprague-Dawley male albino rats, obtained from the animal house, Faculty of Medicine, Cairo University. The rats were housed at three rats/cage and maintained in a controlled environment with a free access to food and water. The age of the rats from the day of birth was recorded on the cage and they were divided into three groups (21, 35 and 90 days old), of 15 rats each. The age determination was done according to Shan et al. (1997).

Group I (GI) (pre-pubertal rats): 21 days old represents pre-pubertal rat, just after weaning.

Group II (GII) (peri-pubertal rats): 35 days old represents the expected age of puberty.

Group III (GIII) (post-pubertal): 90 days old represents the post-pubertal or late adolescent age.

2.2. Paraffin-section preparation

Rats were sacrificed using a high dose of Ether. Both testis were carefully dissected from the epididymis, then taken out rapidly, fixed in Bouin's fixative for 36 h at + 4 °C, then dehydrated in ascending grads of ethanol, cleared in xylene, embedded in paraffin wax and prepared for hematoxylin and eosin (Hx & E) staining for histological examination of general architecture of rat testes. Five specimens per each group were subjected to immunohistochemical examination for AR detection as to their existence, degree and distribution.

2.3. Immunohistochemical preparation for androgen receptors immunostaining

The androgen receptor is a member of the steroid superfamily of ligand-dependent transcription factors. For immunohistochemistry, 5 μm thick sections were cut, mounted on poly-L-lysine-coated slides and heated in an oven at 60 °C for 1 h to promote adherence to the slide. The sections were dewaxed in xylene and then rehydrated in descending grades of ethanol. Endogenous peroxidase was blocked by 15 min incubation in 3% H_2O_2 in methanol. An antigen retrieval step was performed by heating the sections, immersed in 0.01 Methyl citrate buffer at pH 6.0, for 5 min in a 600 W microwave oven. After heating, the material was left to cool down to room temperature for 20 min after which the slides were washed in phosphate buffered saline (PBS). After antigen retrieval, sections were incubated with 10% non-immune goat serum for 10 min to block the nonspecific binding. Counterstaining was performed with Mayer's Hx&E for 3 min, and then sections were rinsed with distilled water (Timurkaan et al., 2012). Immunohistochemistry procedures were performed using a Rabbit polyclonal Antibody Cat. # RB-9030-R7 (7.0 ml) (Ready-to-Use for Immunohistochemical Staining). This antibody was obtained from Thermo Fisher Scientific, Fermont, USA, and is suitable for staining of formalin-fixed, paraffin-embedded tissues.

A negative control slide was prepared from one specimen using a non-immune solution instead of AR antibody.

AR was examined in the three monitored groups: spermatogenic, Sertoli and Leydig cells. Cells were reported to be negative if receptor staining intensity did not differ visibly from that of negative control section. The degree of stain was designated by semi-quantitative analysis (analysis of 10 microscopic fields at 400× magnification). According to the intensity of brown stain in different testicular cells, the positive stain was graded into mild, moderate and marked immunostain. Because of the controversy of its presence, positively stained spermatogenic cells were classified into positive and negative only.

2.4. Image analysis

Sections were examined microscopically using an eyepiece of 10× and an objective piece of 40×, i.e. at magnification of 400×. Ten fields were randomly examined to get 10 readings per slide of different testicular cells (spermatogenic, Sertoli and Leydig cells), a mean value is calculated per slide then measurement of the mean value per each group was done ($n = 15$ for histological examination and $n = 5$ for immunohistochemical examination).

Data were obtained using a Leica Qwin 500 image analyzer computer system, England. The image analyzer consisted of an Olympus BX40 microscope, colored Panasonic video camera, and colored monitor, hard disc of Leica IBM personal computer connected to the microscope, and controlled by the Leica Qwin 500 software. Images were manually corrected for brightness and contrast then converted to 8-bit monochrome type; color threshold was then performed automatically, programming was done to measure the brown area percentage of AR of different cells to the total area of the microscopic field as following: Area percentage ranging between1 and 3 means for mild, 4 and 6 means for moderate and 7 and 10 means for marked immuno-reactivity.

2.5. Statistical analysis

The data were coded, entered and processed on an IBM-PC compatible computer using SPSS (version 20). Differences among the three groups were analyzed with Univariate ANOVA and Bonferroni post hoc test. p-values less than 0.05 were considered significant, and less than 0.001 were considered highly significant, while other values were considered non-significant.

3. Results

3.1. Histological results

3.1.1. Group I (21 days)

Light microscopic examination of the rat testis specimens of group I exhibited the seminiferous tubules containing different types of cells; Spermatogonia type A appeared as an oval cell with a scanty cytoplasm and deeply stained nucleoplasm, spermatogonia type B appeared as a small cell with spherical nucleus and more abundant cytoplasm; both were resting on the basement