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Histopathological Study of The Rat Bulbo-Urethral Glands After Cyclophosphamide Treatment

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Abstract

A bulbourethral gland, also called a Cowper's gland is one of two small exocrine glands in the reproductive system of male mammals. In rodents the bulbo-urethral gland produces a mucous secretion that supports the survival of spermatozoa and provide a medium through which they can move through the reproductive ducts for semen coagulation and urethral lubrication, for ejaculation of viable sperm into the female reproductive tract and hence during fertility. Present study was carried out to study the cytotoxic effect of an anticancer drug Cyclophosphamide (CPA) on the bulbo-urethral glands of rat. After intraperitoneal administration of Cyclophosphamide, the bulbo-urethral glands showed regressive changes in the gross appearance as well as histopathological architecture and physiology as evident by an increase in fibro-muscular tissue causing shrinkage and compactness of tubules, reduction in the acinal size and atrophic changes in the secretory epithelium leading to reduction in the secretory activity. These changes were dose and duration dependent and directly suggest cytotoxic nature of CPA.

Keywords: Bulbo-urethral glands, Cowper's glands, Cyclophosphamide, cytotoxic.

Introduction

The bulbo-urethral or Cowper's glands are male accessory sex glands which are present in most mammals but absent in aquatic mammals and a few carnivores (Mitsumori and Elwell, 1994; Dyce et al. 1999). It appears as a single pair of multi-lobular tubular or tuboalveolar glands or small appendages of male genital tract. They are situated in the urogenital diaphragm deep to the bulb of the penis between the ischiocavernosus and the bulbocavernosus (bulbospongiosus) muscles, embedded within the deep transverse perineal muscle, and form part of the pelvic floor. The accessory ducts of the Cowper's gland join and enter the posterior urethra directly or drain into the main duct. Bulbourethral glands produce a mucous secretion that supports the survival of spermatozoa and provides a medium through which they can move through the reproductive ducts (e.g. epididymis, vas deferens, ejaculatory duct and urethra), for semen coagulation and urethral lubrications, since in the absence of the coagulant, spermatozoa are not transported through the cervix to the site of fertilization, for ejaculation of viable sperm into the female reproductive tract and hence during fertility (Dunker and Aumuller, 2002; Chughtai et al. 2005; Adebayo et al. 2009; Dimitrov et al. 2011; Akosman et al. 2013; Mawhinney and Mariotti, 2013).

Cyclophosphamide belonging to the class of Oxazaphosphorines, is a bioactivated metabolite and alkylating agent that show cytostatic effects by forming covalent DNA adducts. Treatment with cytotoxic chemotherapy is associated with significant reproductive damage and alkylating agents are the most common agent implicated in the development of infertility (Vaisheva et al. 2007). The goal of present study is to elucidate impact of Cyclophosphamide treatment on bulbo-urethral glands.

Materials and Methods

Drug

Cyclophosphamide (Endoxan), with the chemical formula $C_7H_{15}Cl_2N_2O_2P$ and molecular weight, 261.086 g/mol., manufactured by Candila Healthcare Limited, Goa was used.

Experimental Animals

Wistar albino rats (*Rattus norvegicus*) with an average body weight of 250-300g obtained from Department of Biochemistry, R. T. M. Nagpur University, Nagpur were used for the study. Animals were housed in polypropylene box type cages, bedded with rice husk under controlled environmental condition and humidity-controlled with free access to food and water. All experimental procedures were carried out under strict compliance with Institutional Animal Ethical Committee (IAEC) according to guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA).

Treatments

Animals were allowed to acclimatize for a period of week before being treated. They were selected randomly and divided into different

groups with six animals in each group, vehicle-treated control group and three experimental groups. The experimental group animals were administered with 5mg, 7mg and 10mg/KgBW/day of CPA for 2 weeks intraperitoneally and the vehicle-treated group with equal volume of saline.

Histological assessment

Animals were sacrificed 24 hours after the last day of each experiment. Immediately the bulbo-urethral glands were excised, fixed in Bouin's fluid for 24hrs and post preserved in 70% alcohol. The tissues were dehydrated by passing through graded series of alcohol, cleared in xylol and after embedding in paraffin blocks were prepared and cut in numerous parallel 5µm sections. For routine histological study the sections were stained with Ehrlich's haematoxylin and counter-stained with eosin.

Observations

Vehicle-treated control

The rat bulbo-urethral or Cowper's glands are small paired glands partially embedded in the skeletal muscle fibres of the urinogenital diaphragm. Each gland is divided into several lobes and opens with a single duct onto the floor of the cavernous part of the urethra. The lobes consist of secretory end-pieces or alveoli that open directly, or through small ducts, into wide cavities called ampullae. The principal ducts originate from the confluence of the ampullae of the various lobules. In the control vehicle-treated rat most of the alveoli are cyst-like dilated. The alveoli and ampullae are bound by tall glandular cells with flattened basal nuclei. The cytoplasm is full of secretion granules. These granules are bound by a unit membrane, but large irregular masses are also seen in the collecting ducts which are lined by a single layer of cuboidal cells. Some granules have dense corn-like structure. The secretory epithelium is so tall that it nearly fills the lumen. Small amounts of stromal and smooth muscle cells surrounds each acinus. The striking feature of the secretory epithelium is the secretory vacuoles showing filamentous or reticular texture (fig.1).

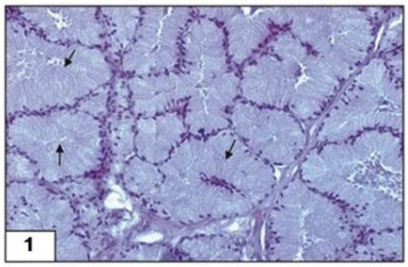

Fig. 1: Vehicle treated control- Note normal appearance of ducts from the acini (arrow) which are end products of the normal ducts separating the various lobules. These ducts open into normal appearing central ampulla before they open into the exocrine duct. X400

5mg/KgBW/day CPA for 2 wks treatment

This treatment resulted into regressive changes in the gross appearance as well as histopathological architecture. Moderate enhancement in the amount of fibro-muscular mesenchyme caused moderate shrinkage and compactness of the tubules, partial atrophic changes in the secretory epithelium, appearances of large vacuoles in the lumen, vacuolation in the epithelium suggestive of reduction in the functional activity (fig. 2).

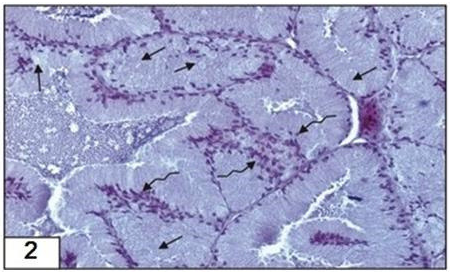

Fig. 2: 5mg/KgBW/day CPA for 2 wks treatment resulted into regression in the height of secretory epithelium, reduction in the secretion as appeared by presence of vacuoles (arrow). Most of the acini are devoid of secretion such acini shows vacuolated granular cytoplasm with congestion of nuclei due to regression of epithelium (wavy arrow) X 400.

7mg/KgBW/day CPA for 2wks treatment

An enhancement in the regressive changes as evident by further shrinkage of the secretory acini, their compactness, loss of lumen and hence loss of secretion from most of the acini, however, secretion is partially restored in some of the acini, abundancy in the quantity of inter-acinal connective tissue mesenchyme, notable feature being accumulation of eosinophilic globules in the lumen. The accumulation of eosinophilic secretory granules and eosinophilic

globular secretion in the lumen directly indicates degenerative effect (fig. 3).

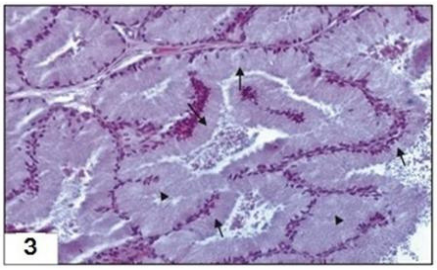

Fig. 3: 7mg/KgBW/day CPA for 2 wks treatment showed regression in the height of secretory epithelium (arrow), reduction in the secretion, lack of secretion (arrow head). The secretory cells have lost their normal architecture as evident by disturbance in the placement of nuclei and even in their number. An increment in the quantity of inter-acinal mesenchyme also points the regressive changes X 400.

10mg/KgBW/day CPA for 2wks treatment

Notable feature being the cystic dilation of some acini with eosinophilic secretion, however, the impact of this dose was severe as noticed by extreme shrinkage obliteration of lumen in most of the acini, when lumen was there it was found to be empty, all the secretory epithelial cells found to loss their original texture due to loss of cellular boundary, appearance of granulated eosinophilic secretion and mixing of nuclei, an increase in the amount of stromal and smooth muscle cells. Similarly the disturbance in general architecture was supplemented by enormously thickened fibro-muscular capsule encasing the gland. The gross visibility of the epithelial cytoplasm was filamentous or reticular texture. (fig.4).

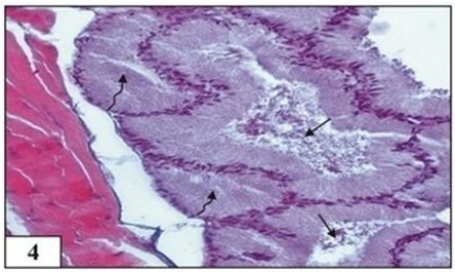

Fig. 4: 10mg/KgBW/day CPA for 2 wks treatment - The acini show reduction in size, loss of secretory material (arrow), noticeable feature being the merging of epithelial cells into a mass with pyknotic nuclei (wavy arrow), some tubules have obliterated lumen X 400.

Discussion

The bulbo-urethral or Cowper's glands are male accessory sex glands (Dyce et al. 1999), usually appear as a single pair of multi-lobular tubular or tuboalveolar glands or small appendages of male genital tract. They are involved in the immune defense of the genitorurinary tract (Riva et al. 1990), play a role in fertility and secrete many glycoproteins, including Prostate specific antigen PSA (Chughtai et al. 2005 and Akosoman et al. 2013). Spring-Mills and Hafez, 1980 described mucous as well as serous types of cells in the alveoli. These glands secrete mucosubstance, but also some neutral mucosubstnaces. The acid mucosubstances consist mainly of sulfated acid mucosubstances and a smaller amount of carboxylated acid mucosubstances (Sialo mucin) (Sirigu et al. 1993). The secretions of the bulbo-urethral glands are emitted at the beginning of the ejaculatory phase and it is conceivable that, besides secretions from the prostate, some components of the first seminal fraction (Gonzales, 2001; Hernandez et al. 2007; Kawano and Yoshida, 2007; Balaji et al. 2008) could be produced by these glands. These glands can be affected by neoplastic, infectious, stone and especially cystic disease syringocele (Chughtai et al. 2005 and Melquist et al. 2010).

Histologically, the gland is covered by a capsule of connective tissue with smooth and skeletal muscles. These glands consist of closely juxtaposed acini composed of polygonal epithelial cells with abundant clear cytoplasm. Trabeculae from the capsule divide the gland into lobules. A section through the gland reveals tightly packed round to oval-shaped glandular alveoli which constitute the secretory unit and are surrounded by thin connective tissue derived from the interlobular connective tissue. The secretory epithelium is simple columnar cells arranged round a small central lumen which nearly fill the lumen. Although the nuclei of some of the cells show dorso-ventral compression, most of the cells have round basal nuclei with prominent nucleolus and poorly stained cytoplasm. The bulbo-urethal gland of the vehicle treated control rat is a lobular gland and centrally in each lobe, there is a duct, the ampulla, into which the alveoli drain, either directly or via a very short and narrow ductule. The above described histological structures of the vehicle-treated control are in consonance with the other mammals (Sirigiu et al. 1993; Mitsumori and Elwell, 1994; Roić et al. 2005 and Adebayo et al. 2009). The cytoplasm is full of secretory granules. There is only one large secretory duct lined by a single layer of cuboidal cells into which all the ampullae open and this

leave the gland at one end. Myoepithelial cells are present around the secretory cells. A striking feature of Cowper's gland is the large number of secretory vacuoles showing filamentous or reticular texture (Parr et al. 1993) and prominent Golgi complex (Parr et al. 1993). The filamentous appearance of the secretion in the present study represents a second secretory component because they fuse with the mucous droplets and the resulting mixture is discharged into the lumen. The mucuous cells lining the alveoli show presence of mucus droplets as in our study (Riva et al. 1990; Dünker and Aümüller, 2002).

The histological structures are further strengthen by the ultrastructural studies (Riva et al. 1988; 1990). These showed that the parenchyma of the Cowper's glands consists of (a) secretory tubules and alveoli lined by typical mucoid cells at different stages of secretory cycle, and (b) an excretory duct system. Secretory elements were also observed in the ducts, marked by the presence of secretory small dark granules in the cytoplasm (Riva et al. 1988).

As stated by Pedron et al. 1997 that Cowper's glands are affected by neoplastic, infectious, stone and especially cystic disease: syringocele (Dünker and Aümüller, 2002; Chughtai et al. 2005), our results are in consonance with their observations, thus in the CPA treated rats, we observed reduction in the size of acini, regression in the height of the columnar epithelium (5mg, 7mg, 10mg, 100mg/KgBW) which nearly filled the complete acinus in the vehicle-treated group, reduction in the amount of secretion or even total loss (7mg and 10mg/KgBW) from most of the tubules. The reduction or the shrinkage of the acini is in equivalence with the increase of fibro-muscular tissue defining stromal-epithelial interactions (Blanchere et al. 2001) as well as Dünker and Krieglstein, 2000 have shown expression of transforming growth factors beta (TGF-β) which are important in areas undergoing morphogenetic events, for instance those involving epithelial-mesenchymal interactions or differentiation. Similarly the flattened basal nuclei showed pyknosis and displacement. The above mentioned changes also points to androgen dependency of the gland, castration leads to apoptotic changes in the epithelium (reduction in DNA and protein content) but administration of exogeneous testosterone restores the original condition (Boronikhina and Iatskovskii, 2006). Similarly in the present study a co-relation between the testosterone level at different dose levels with histopathological architecture of the Cowper's gland has been done and thus it was found that low testosterone causes more pathological effect on Cowper's gland.

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