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Monstrous Epithelial Cell Formation in Seminal Vesicle After Cyclophosphamide Treatment

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ABSTRACT

Cyclophosphamide (CPA) is a nitrogen mustard alkylating, anticancer and immunosuppressive agent which is used to treat malignancies such as Hodgkin's disease, leukemia, lymphoma, breast and prostate cancer. While studying the effect of various doses (5mg, 7mg and 10mg/KgBW) of Cyclophosphamide on the reproductive tract of male rat some interesting cytotoxic features were observed in the seminal vesicle epithelium (SEV). Intraperitoneal injection of Cyclophosphamide resulted in the formation of monstrous (monster) protruding epithelial cells (MEC) of the seminal vesicle in a clustered pattern with acidophilic cytoplasm, hyperchromatic nuclei, lipochrome pigment in the cytoplasm, very large bizarre nuclei simulating carcinoma cells, spindle cells with multiple discrete areas of anaplastic epithelial-like cells. The seminal vesicles, an androgen dependent organ are among the most important male accessory gland, the formation of monstrous epithelial cell directly suggest anti-androgenic nature of CPA in concomitance with a fall in testosterone level.

KEYWORDS : Monstrous epithelial cell, seminal vesicle, Cyclophosphamide, antiandrogenic.

Introduction

The seminal vesicle (SV) is the among the most important male accessory glands contributing to about 60% of the seminal plasma, provides nutrients to spermatozoa and optimizes the conditions for transport, sperm motility, viability, elimination of non-viable spermatozoa from the uterus on both male and female reproductive tracts (Mann and Lutwak-Mann, 1981; Gonzales, 1994 and Almenara et al. 2000). Its secretion is also important for semen coagulation, stability of sperm chromatin, for potent suppression of immune activity in female reproductive tract, possesses 5α-reductase activity which converts testosterone (T) to dihydrotestosterone (DHT) (Gonzales and Villena, 2001; Troedsson et al. 2005). 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 seminal vesicle gland.

Materials and methods

Drug

Cyclophosphamide, an anticancer drug, 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

Adult, healthy Wistar rat, *Rattus norvegicus*, with average body weight of 250-300g obtained from Shree Farma, Bhandara were used for the study. Animals were maintained in the laboratory under an absolute hygienic condition as per the recommended ethical standards. They were housed in polypropylene box cages, bedded with rice husk and kept at constant temperature 28±2°C and relative humidity with 12h light: 12h dark cycle. They were fed with pelleted diet and water *ad libitum*.

Treatments

Various experiments with different dose regimens were performed for studying the toxicity of CPA on male reproductive system. The experimental protocol is depicted in Table-1.

Table-1: Experimental design for Cyclophosphamide dose treatments

Number of animals and sex	Treatment	Dose (mg/Kg BW/day)	Route	Duration
6males (Expt.)	CPA	5mg	I.P.	2 weeks
6males (Expt.)	CPA	7mg	I.P.	2 weeks
6males (Expt.)	CPA	10mg	I.P.	2 weeks
6males (Expt./ Controls)	Saline	Equal volume	I.P.	2 weeks

Expt. = Experimental, I.P. = Intraperitoneal, BW = Body Weight

Histological assessment

Animals were sacrificed using chloroform 24 hours after the last day of each experiment. Immediately the seminal vesicles 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.

Results

Histopathological Examination

Vehicle-treated control seminal vesicle

The accessory reproductive gland seminal vesicles are saccular glands consisting of a central cavity and peripheral pouches. The gland is encapsulated in thick connective tissue capsule. The normal luminal surface of seminal vesicles is a system of anastomosing glandular architecture oriented in various directions and lined by cuboidal to tall columnar epithelium which forms an intricate arrangement of mucosal folds that ramifies into secondary and tertiary folds. Few basal cells, almost rounded in shape and basal in position were also observed between the columnar epithelial cells. Secretion filling the lumen expanded the seminal vesicles resulting in distended alveoli, a scanty submucosal layer, and a thin smooth muscle layer lining each mucosal fold (figs. 1 and 2).

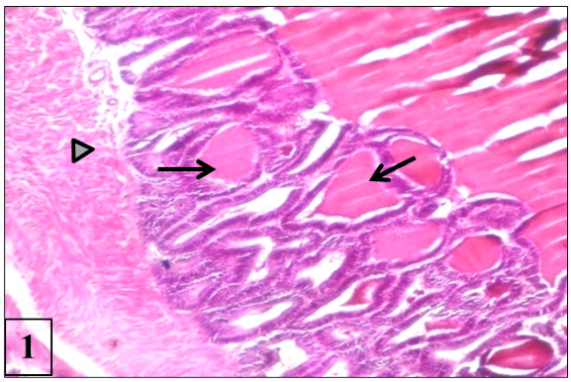

Fig.1: Vehicle treated seminal vesicle is a saccular gland composed of central cavity and peripheral pouches of anastomosing glandular structures filled with copious amount of secretion (arrow). The gland is encapsulated in thick connective tissue capsule (triangle) X 100.

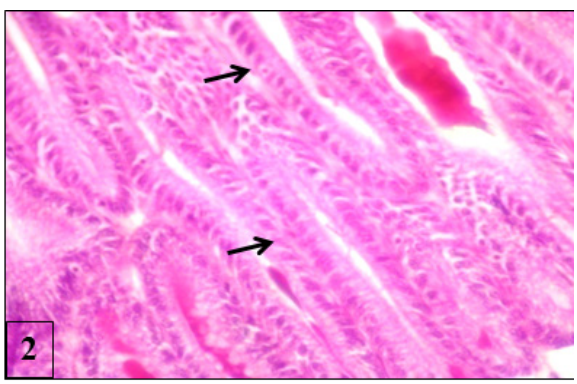

Fig. 2: Vehicle treated seminal vesicle: Mucosal folds lined by tall columnar epithelium (arrow). Few basal cells are present in between the columnar epithelial cells (arrow head) X 400.

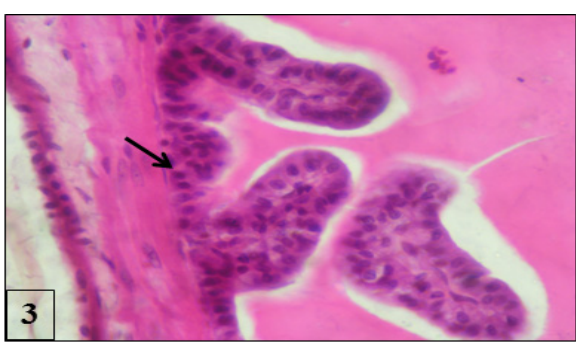

Fig.3: 5mg/KgBW treatment resulted into restriction in the ramification of the secretory epithelium, regression in the epithelium height, less secretory zone, therefore depletion in the quality of colloid, condensation, pyknosis and displacement of nuclei (arrow).

5mg/KgBW/day Cyclophosphamide treated seminal vesicle

5mg/KgBW Cyclophosphamide dose resulted in the restriction of the secretory epithelial cells of the seminal vesicle with regression in its height, depletion in the quality of the colloidal secretion, its condensation. The nuclei underwent pyknosis and displacement (fig.3).

7mg/KgBW/day Cyclophosphamide treated seminal vesicle

Further increase in the dose of Cyclophosphamide, 7mg/KgBW showed formation of monstrous epithelial cells (MEC) in the seminal vesicle in a clustered pattern with acidophilic cytoplasm, hyperchromatic nuclei, lipochrome pigment in the cytoplasm, very large bizarre nuclei simulating carcinoma cells (fig.4).

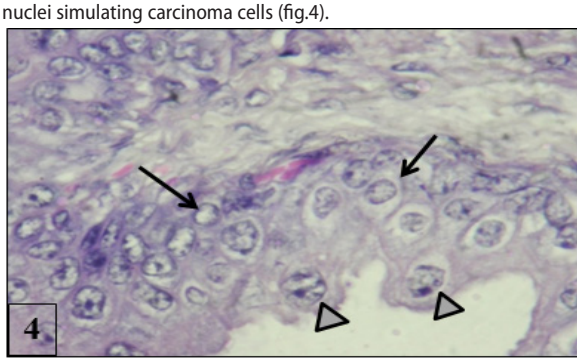

Fig.4: Protruding monstrous (monster) epithelial cells (MEC) (arrow) of the seminal vesicle in a clustered pattern with acidophilic cytoplasm, hyperchromatic nuclei, lipochrome pigment in the cytoplasm, very large bizarre nuclei (arrow head) simulating carcinoma cells X 1000.

10mg/KgBW/day Cyclophosphamide treated seminal vesicle

Further increase in the dose level resulted in the formation of autophagic vesicle in the mucosal folds of seminal vesicle. These sequestrated autophagic vesicles at advance stage of development underwent intra-lysosomal degradation bathing in the lumen containing recognizable fragments of cytoplasm (fig. 5 and 6).

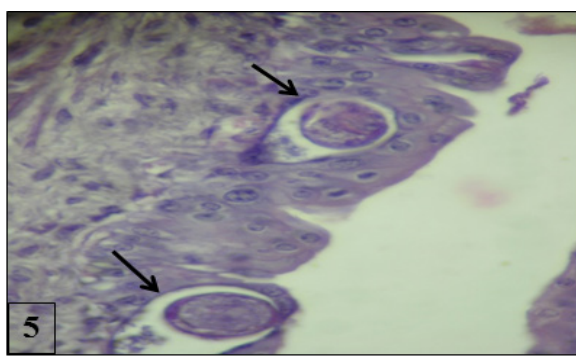

Fig.5: The cytoplasmic volume fraction of lysosomes or dense bodies limited by a single membrane, an early stage in the formation of autophagic vesicle in the mucosal fold (arrow) X 1000.

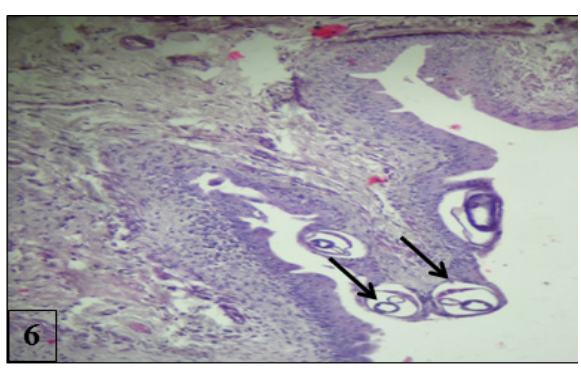

Fig.6: Mucosal fold demonstrating autophagic vacuoles at advanced stage of development (arrow) X 400.

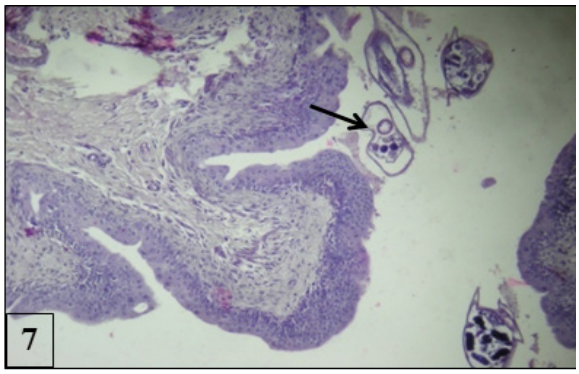

Fig.7: Sequestrated autophagic vacuoles at late stage of intra lysosomal degradation bathing in the lumen containing recognizable fragments of cytoplasm (arrow) X 400.

Discussion

Primary function of the SV is to synthesize proteins that contribute to the seminal plasma which is important for the sperm transport and nutrition of sperm as well as (in rodents) the formation of a copulatory plug after ejaculation (Pang et al. 1979; Peitz and Olds-Clarke, 1986). SV has a highly convoluted pseudostratified columnar epithelium with active protein secretory machinery. Also secretes fructose, prostaglandins, metallothionein-1 (Mt-1), and transglutiminase-4 (TGM4) (Jonsson et al. 2006; Kawano and Yoshida, 2007; Balaji et al. 2008). SVs secretory functions are androgen dependent (Cunha and Donjacour, 1987), however, castration or any chemical insult results in involution due to, cytological degeneration, apoptosis, gross reduction in secretion. Alteration in androgen-estrogen balance can also affect adult male accessory sex organs resulting in aberrant histological changes and even prostatic squamous metaplasia (Rivas et al. 2003; Bianco et al. 2006) as noticed in the present work.

In this context it was observed that Cyclophsphamide treatment showed monstrous (monster) protruding epithelial cells (MEC) of the seminal vesicle in a clustered pattern with acidophilic cytoplasm, hyperchromatic nuclei, lipochrome pigment in the cytoplasm, very large bizarre nuclei simulating carcinoma cells, spindle cells with multiple discrete areas of anaplastic epithelial-like cells. It was found that above results were in accordance with previous workers, Kuo and Gomez, 1981; Habanec,1984; Trainer, 1992; Rosai, 2004 and Terada, 2011. Kuo and Gomez in 1981 named these cells "monstrous epithelial cells" and stressed that these cells should not been mistaken for carcinoma. According to Habanec, 1984; Kuo and Gomez, 1981; Rasai, 2004; Terada, 2011, MEC is a degenerative change. Previous studies have also suggested that MEC is degenerative or hormone-related phenomenon or due to aging similar to Arias-Stella, 1958 a phenomenon of the endometriasis (Kuo and Gemez, 1981; Trainer, 1992).

Similarly induction of autophagocytosis by Cyclophosphamide resulted in an activation of the lysosomal system of the secretory cells in the rat seminal vesicle epithelium (SVE), and hence an elevation of the activities of lysosomal enzymes, formation of large autophagic vacuoles, sequestration of rough endoplasmic reticulum and part of Golgi apparatus. Since the formation of monstrous cells is a degenerative state it may be considered as the basic stage in the formation of autophagic vesicles and thus in the induction of autophagocytosis of seminal vesicle epithelium.

Deduced from the formation of monstrous cells and vesicles it is hypothesized that loss of intracellular material during autophagocytosis diminishes the intracellular concentration of substances required for cell division below their effective threshold. The prerequisites of this mechanism may be sufficient distribution capacity of the stroma for androgen, as well as transporting capacity for the metabolic precursor of basal cells to the secretory cells, thereafter sloughing of these secretory cells separates them from these auxiliary structures (stroma and basal cells) and thus enables the basal cells to divide (Aumüller et al. 1981; Sastry and Kashmri, 2012; Sastry and Kashmri, 2013).

From the foregoing it is concluded that the autophagic cell death induction by Cyclophosphamide via the formation of degenerative monstrous cells as well as other anticancer agents underlines the potential utility of its induction as a new cancer treatment modality.

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