Autophagocytosis of seminal vesicle epithelium with Cyclophosphamide
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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. Intraperitoneal injection of Cyclophosphamide (10 mg/KgBW for 2 weeks) resulted in an activation of lysosomal system of secretory cells in the rat seminal vesicle, elevated activities of lysosomal enzymes thus resulted in the formation of autophagic vesicles. The seminal vesicles, an androgen dependent organ are among the most important male accessory gland contributing to about 60% of the seminal plasma, the autophagic activity of CPA directly suggest its antiandrogenic nature in concomitance with a fall in testosterone level.

Keywords: autophagic activity, seminal vesicle, cyclophosphamide, antiandrogenic

Introduction
The seminal vesicles are among the most important male accessory glands contributing to about 60% of the seminal plasma (Mann and Lutwak-Mann, 1981). Seminal vesicular secretion is rich in fructose, proteins, prostaglandins, complex carbohydrates and enzymes involved in the clotting of the ejaculate (Gonzales and Villena, 2001). It also provides nutrients for the spermatozoa and optimizes the conditions for transport, sperm motility, viability, elimination of non-viable spermatozoa from the uterus in both the male and female reproductive tracts (Troedsson et al., 2005). The seminal vesicle is an androgen dependent organ in terms of both structure and function (Gonzales, 1994). 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).

While studying the effect of various doses of Cyclophosphamide on the reproductive tract of male rat some interesting cytotoxic features were observed in the seminal vesicle epithelium (SEV) with only the 10 mg/KgBW dose level which are described here. Perusal of literature on this aspect suggested us that such changes belong to the cell death Type-II or autophagic cell death category since it was characterized by sequestration of bulk cytoplasm and organelles in double or multi-membrane autophagic vesicles, and their subsequent delivery and degradation by the cell’s own lysosomal system (Eskelinen, 2004; Fensrud et al., 2004; Gozuacik and Kimchi, 2004). Type-II cell death is different from Type-I cell death which is characterized by cell shrinkage,
chromatin condensation, nucleosomal DNA degradation and fragmentation of the cell into so-called ‘apoptotic bodies. Type-III cell death also exists which is nonlysosomal vesiculate degradation and totally differs from Type-II cell death (Gozuacik and Kimchi, 2004).

Materials and Methods

Drug

The anticancer drug Cyclophosphamide (Endoxan-N, CAS no. 50-18-0), with the chemical formula C_7H_15Cl_2N_2O_2P and molecular weight, 261.086 g/mol, manufactured by Candila Healthcare Limited, Goa, India.

Experimental animals

Wistar albino rats (Rattus norvegicus) with average body weight of 250-300 g were used for the experiments. Animals were maintained in the laboratory under an absolute hygienic condition as per the recommended procedures by fulfilling all the necessary ethical standards. They were housed in polypropylene box type cages, bedded with rice husk and kept at constant temperature 28±2°C and relative humidity with 12 h light: 12 h dark cycle. They were fed with pelleted diet and water ad libitum.

Treatments

Many experiments with different regimens and durations were performed for studying the toxicity of CPA on male reproductive system, however, the autophagocytosis was observed only with 10 mg/KgBW dose, which is depicted in Table 1.

Histological assessment

The animals were sacrificed using chloroform 24 hrs after the last day of each experiment. Immediately the seminal vesicles were excised, fixed in Bouin’s fluid for 24 hrs and 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 5 μm sections. For routine histological study the sections were stained with Ehrlich’s haematoxylin and counter-stained with eosin.

Results

Histopathological studies: Vehicle-treated control

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. 1.1 and 1.2).

Cyclophosphamide treated group

10 mg/KgBW Cyclophosphamide dose resulted in the formation of autophagic vesicles. The early stage in the formation of the autophagic vesicle was characterized by the cytoplasmic volume fraction of lysosome or dense bodies limited by a single membrane at various regions of the mucosal folds. Mostly these were in the vicinity of the stroma and not peripheral (Fig.1.3). Similarly some of the mucosal folds hanging into the lumen revealed presence of large autophagic vesicles at advance stage of growth (Fig. 1.4). These large peripheral advanced vacuoles were characterized by a closed double membrane, a phagophore or isolation membrane, bound vacuoles containing cytosole.
Table 1. Experimental design for Cyclophosphamide dose treatments

<table>
<thead>
<tr>
<th>Number of animals and sex</th>
<th>Treatment</th>
<th>Group</th>
<th>Dose (mg/Kg BW/day)</th>
<th>Route</th>
<th>Duration weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 males (Experimental)</td>
<td>Cyclophosphamide</td>
<td>E1</td>
<td>10 mg</td>
<td>I.P.</td>
<td>2</td>
</tr>
<tr>
<td>6 males (Expt./Controls)</td>
<td>Saline</td>
<td>E2</td>
<td>Equal volume</td>
<td>I.P.</td>
<td>2</td>
</tr>
</tbody>
</table>

I.P. = Intraperitoneal, BW = Body Weight

Fig. 1. (1). Vehicle treated seminal vesicle: 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. (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. (3) 10 mg/KgBW CPA treated seminal vesicle: 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 400. (4) Mucosal fold demonstrating autophagic vacuoles at advanced stage of development (arrow) X 400. (5) Large autophagic vacuoles or membrane limited bodies (arrow) causing expansion of the lysosomal compartments due to intralysosomal degradation (triangle) X 1000. (6) Note sequestrated autophagic vacuoles at late stage of intralysosomal degradation bathing in the lumen containing recognizable fragments of cytoplasm (arrow) X 400.

or organelles, an autophagosome causing expansion of the lysosomal compartments due to intralysosomal degradation (Fig. 1.5). At the late stage of intralysosomal degradation autophagic vacuoles were found to be sequestrated in the lumen containing recognizable fragments of cytoplasm (Fig. 1.6).
Discussion

Seminal vesicular secretion is important for semen coagulation, sperm motility, stability of sperm chromatin and suppression of immune activity in female reproductive tract (Gonzales and Villena, 2001). In the present study intraperitoneal injection of an anticancer drug 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. Deduced from our findings 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).

Similar to our observations a wave of autophagic vacuole formation at early and advanced stages in seminal vesicle epithelium (SVE) were also reported after incubation in vitro upto 8 h in medium 199 or Krebs-Ringer bicarbonate buffer (Kovács and Kovács, 1977a, 1977b) or p-chlorophenylanlanine (pCPA) methylester (Aumüller et al., 1981) or in murine following administration of Triton X-100 or vinblastine (Kovács et al., 1987) or in a medium containing 0.03% Triton-100 (Kovács et al., 2000) or occurrence of autophagic vesicles may be a natural phenomenon (Kovács, 1982). It was also demonstrated that the suppression of this phenomenon is possible by the administration of some exogenous drugs such as vinblastine sulphate, leupeptin, cycloheximide and their different combinations or estrogen acetate (Kovács and Kovács, 1980; Kovács, 1982; Kovács, 1983). The wave of autophagic vacuole formation was further characterized by a short transient rise and their subsequent transformation into advanced forms filled with degrading cytoplasmic fragments and thereafter sloughing of these secretory cells into the lumen separating them from auxiliary structures (stroma and basal cells) and thus enabling the basal cells to divide since the high proliferative activity of SVE might be a footprint for seminal vesicle epithelial cancer (Qu et al., 2003; Yue et al., 2003). In the same context autophagy has multiple physiological functions in multicellular organisms, such as protein degradation, organelle turnover (Gozuacik and Kimchi, 2004), an important survival mechanism during short-term starvation, degradation of some nonessential components, cells get nutrients for vital biosynthesis reactions, cell homeostasis in muscle, liver and pancreas (Tanaka et al., 2000; Eskelinen et al., 2003), as well as to development, growth regulation and longevity (Melendez et al., 2003) and hence the present study also emphasizes Type II apoptosis or autophagic cell death (Gozuacik and Kimchi, 2004). From the foregoing it is concluded that the autophagic cell death induction by Cyclophosphamide as well as other anticancer agents underlines the potential utility of its induction as a new cancer treatment modality.

References


