J. Ecophysiol. Occup. Hlth. 15 (3 & 4), 2015, 129–132

DOI : 10.15512/joeoh/2015/v15i3-4/121597

Effect of Anticancer Drug Etoposide (VP-16) on Epididymis of Male White Rat

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Abstract: *To study ultra-structure changes in epididymis caused by an etoposide(VP-16) with the help of electron microscope. The etoposide (VP-16) is in use as one of the regimen in cyclical chemotherapeutic treatment for several kind of cancer. Etoposide as an anticancer drug, 1mg/kg/day was administered intra-muscular for two months. The animals were anaesthetized with ether and excised testis and epididymis for electron microscopic studies. Etoposide treatment resulted. The goal of theses study was to determine. In Caput epididymis as compared with the control cell, the supranuclear cytoplasm shows hypertrophied, dilated and distorted Golgi cisternae and vesicles are filled with moderately dense material. The Principal cells of epididymis shows increase in abudance, as well as size of lysosomal bodies and their appearances in the basal cytoplasm in the principal cell of the caput and cauda epididymis indicate argumented endocytosis. In the present study it has been shown that the vesicle dilated due to treatment with etoposide contain an electron dense material providing evidence for formation of secretory granules enclosed in membranous vesicles in the transfer of the golgi apparatus of principal cells. The vesiculation/ vaculation of golgi apparatus to the opening up of golgi area observed in the principal cell of etoposide treated rat because a toxic manifestation of the golgi apparatus as a heightened activity. In conclusion our result shows that adverse effect of etoposide on epididymis can be distinguished after two months treatment.*

Keywords: Epididymis, Caput, Cauda Principle Cell.

Introduction

The etoposide (VP-16) is in use as one of the regimen in cyclical chemotherapeutic treatment for several kind of cancer like testicular cancer, small cell bronchogenic carcinoma, malignant lymphoma. The testicular alterations induced by etoposide have also been thoroughly studied.

Materials and Methods

In wistar strain of rat, drug Etoposide (VP-16)1mg/kg/day was administered intramuscular for two months. The animals were anaesthetized with ether and excised testis and epididymis for electron microscopic studies. The tissues were sliced into 1mm piece fixed with 3% glutaraldehyde for 2 hours for and were washed thrice in 0.1 M Cacodylate buffer. The tissue was rinsed briefly in buffer and post osmicated in 1% OSO_4 (osmic acid) for one hour. The tissue was dehydrated in ascending series of alcohol followed by propylene oxide and embedded in resin, which was polymerized at 60°F. Subsequently, the blocks were prepared in araldite, 1um sections were cut with glass knife on ultra-microtome, mounted on glass slides, stained with buffered toludine blue solution and studied under light microscope. Ultra-thin sections were of selected blocks were cut and lead citrate for final viewing, sections were observed and photographed on a Jen-100s Jeol Electron Microscope.

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The ultra-structure observations of the caput epididymis of the control rat revealed the principle or tall columnar cells, which constituted the epithelium. The basal region of the epithelium, resting on the smooth muscular layer is presented in the small pyramidal shaped basal cells, lodged between the bases of the columnar cells are clearly visible. The intraepithelial lymphocytes also known as halocells with their irregular shaped nucleus and dense chromatin content are also visible. Very few organelles are found in the basal cells and halocells. The mid part of the principle cell consisted of nucleus and the cytoplasm below it contained a dense network of endoplasmic reticulum composed of thin saccules and studded with ribosomes. The nucleus on the other side is flanked by mitochondria with well-formed cristae (Akbarsha M.A *et al*., 1998a). The junctional complexes between the two columnar cells are clearly visible. The apical end of the columnar cell show numerous sertiocilia extending downward up to some distance in the luminal area. The cell surface between the seriocilia is irregular and exhibits numerous small pinocytotic vesicles and secrection filled vacuoles (Akbarsha M.A *et al*., 1998b). An extensive endoplasmic reticulum and Golgi apparatus formed a large network connected with numerous vesicles and multivesicular bodies containing small granules. The luminal side of the caput epididymidis displayed the transverse sections of the maturing spermatozoa. The electron microscopic observation of the basal region of the principle cells of the cauda epididymis of control rat revealed a pyramidal showed basal cell. The basal portion of the principle cells, all resting upon thick muscular coat. The basal cells possess a nucleus with much heterochromatin material the cytoplasm contain only a small number of organelles like a few dense bodies and profiles of endoplasmic reticulum is present. The mitochondria are seen scattered throughout the cytoplasm.

The epithelium contains principal cells, halocell and basal cells. The principal cells are tall columnar cells with elongated basal nucleus and luminal surface is covered with steriocilia. As compared with the control cell, the supranuclear cytoplasm shows hypertrophied, dilated and distorted Golgi cisternae and vesicles are filled with moderately dense material. The apical cytoplasm contains numerous profiles of vesiculated rough endoplasmic reticulum with de-segregation of ribosomes and dense secretory granules. The lysosomes and multi-vesicular bodies are increased in their size. The mitochondria are balloon shape and undergo hypertrophy following treatment with etoposide (VP-16).

The halo cells are seen in between the principal cells. The cytoplasm contents of cell organelles similar to control except that there are numerous cisternae of vesiculated rough endoplasmic reticulum and lysosomes. There are well developed tight junctional complexes between neighboring cells.

The cauda epididymis of etoposide treated rats consists of principal and halo cells. The principal cells are moderate in height with folded nucleus. The principal cells of cauda epididymis contains numerous hypertrophied, dilated and distorted golgi cisternae and vesicles. The supranuclear cytoplasm is rich in smashed rough endoplasmic reticulum. The apical cytoplasm shows secretory vesicles, vacuoles and lysosomes. Infranuclear region of principal shows irregular nucleus with heterochromatin material is highly dispersed which is arranged towards the periphery. Nucleolus is marginalized. The most prominent change noticed in the principal cell of cauda epididymis is accumulation in the cytoplasm of spherical or oval membrane bound bodies containing a darkly stained matrix in which an electron dense material occurred peripherally or centrally. In the supranuclear apical cytoplasm, the multivesicular bodies (MVB) were more abundant. Several lysosomal bodies are seen in associating with MVB.

Results and Discussion

The principle cell of the epididymal epithelium is concerned with true major function, *viz.* Endocytosis and secretion. A number of large dense supranuclear located spherical membrane bound bodies, identify cytochemically as lysosomes, were characteristics of principal cell (Robaire and Hermo, 1988). Using traces it was established that such lysosomes formed due to the association between MVB's and the small coated vesicles containing hydrolytic enzymes packaged in the golgi apparatus (Abe *et al.,* 1983). The increase in abudance, as well as size of lysosomal bodies and their appearences in the basal cytoplasm in the principal cell of the caput and cauda epididymis indicate argumented endocytosis. Earlier study reported that etoposide treatment cause regenerative changes in the seminiferous epithelium and the cell fragments thus formed reach the epididymis (Stanley and Akbarsha, 1992a, 1992b; Stanley *et al.,* 1995; Averal *et al.,* 1995, Akbarash *et al.,* 1996). It could be suggested that the principal cell is concerned with the removal of such fragment. Moreover, it may be because of a direct toxicity of the drug on the cellular constituents, resulting in the triggering of the intracellular homeostatic machinery for scavenging the damaged cellular constituents. The extensively developed RER in the basal and perinuclear cytoplasm sparsely granulated ER in the supranuclear cytoplasm, the large golgi apparatus formed of many stacks of saccules and the associate vescicular elements in the supra nuclear area are the machinery for extensive protein synthesis and secretion (Robaire and Hermo, 1988) and glycosylation of the secretory proteins (Flickinger, 1979, 1985). VCR treatment may agument the secretion of certain specific proteins as reflected in the appearance and accumulation of small coated vesicles, containing secretion granules, in the golgi apparatus. It is known that such vesicles deliver hydrolytic enzymes to the MVB's (Friend, 1916). VCR action is causing extensive damage to lydig cell and the resultant decrease in the circulating levels of androgens has been reported (Stanley and Akbarash 1984; Akbarash *et al.,* 1995). The epididymis in general and the principal cell in particular is androgen dependent (Robaire and Hermo, 1988). Androgen withdrawal is known to cause extensive changes in the principal cell (Robaire and Hermo, 1988). Thus the change in the principal cell of etoposide treated rats may reflect a manifestation of the etoposide induced hypoandrogenic status. Effect of etoposide treatment on the mitochondria of the epididymal epithelium cell is one of the characteristic changes noticed in the epididymal epithelium of etoposide VP16 treated rat was the swelling of mitochondria collapse of their cristae and accumulation of intramitochondrial bodies in the Principal cell. According to Walker *et al.,* (1965). Mild to gross swelling of mitochondria and appearance of flocculent and sometimes granular, densities in the matrix are clear indication of cell injury. Such changes are the consequences of marked increased in the permeability of mitochondrial membrane. Several toxicants like carbon tetrachoride ammonium carbonate, alcohol (Ghatiyali, 1979) causing mitochondrial pathology. Fernandez (2001) stated that the detoxification pathways results in to the damage of mitochondria and ER. Thus the present study provides the clear evidence of a least the early pathological changes in the tall columnar cells of epididymal epithelium to the toxic manifestation of etoposide. Effect of etoposide VP16 treatment of the golgi complex of the principal cell is one of the important ultrastructural changes taking place in the epithelium of epididymis. Golgi apparatus is the most prominent organ in the epididymal principal cell, particularly in the more proximal parts. It occupies the lower part of the supranuclear region and is formed of stacks of saccules and associated small vesicles running parallel to the long access of the cell (Hermo *et al*., 1991). In the present study it has been shown that the vesicle dilated due to treatment with etoposide contain an electron dense material providing evidence

for formation of secretory granules enclosed in membranous vesicles in the transfer of the golgi apparatus of principal cells (Hamilton D.W *et al*., 1971). The vesiculation/ vaculation of golgi apparatus to the opening up of golgi area observed in the principal cell of etoposide treated rat because a toxic manifestation of the golgi apparatus as a heightened activity. Endocytotic uptake of cell fragments by the apical portion of the principal cell and their processing in the membrane vesicles and MVB's and lysosomes have also been seen in the present study. These cell fragments arrive at the epididymis from the testis. There is concomitant increase in the lysosomal bodies in the apcal cytoplasm of principal cells (Flikinger C.J *et al*., 1978). Robaire and Hermo (1988) proposed that secrectory granules form the golgi apparatus in the form of small coated vesicles are discharging the content in the lumen whereas granules in the form of a small uncoated vesicles for discharging the content in the lumen or for association with the endosome to form a multivesicular bodies which subsequently associated with lysosome for enzymatic digestion. The prominent electron dense secretory granules in the trans phase of golgi vesicles and the abundance of lysosomes in the principal cells of etoposide VP16 treated rat correlated w ith the endocytosis of the cell fragment and would imply it as a manifestation of heightened activity in the golgi apparatus (Akbarsha M.A *et al*., 1988c).

Halo Cell-Halo cells are considered as a derivation of lymphocyte (Goyal and Williams, 1991). The halo cells are considered as concerned with surveillance of sperm antigens 1975, Flickinger, 1997 (Robaire, 1999). It has also been reported that with increase in age the blood epididymis barriers might become leaky attracting more Halo cells into epididymis epithelium (Roabire, 1991). In the Goat epididymis they showed that halo cells were usually confined to basal area and also been seen throughout the epithelium. They possess dense osmophilic nucleus and clear cytoplasm devoid most organelles except for few ribosomes, mitochondria and occasional dense granules. Corresponding observation were seen in present work.

In conclusion our result shows that adverse effect of etoposide on epididymis especially on The prominent electron dense secretory granules in the trans phase of golgi vesicles and the abundance of lysosomes in the principal cells of etoposide VP16 treated rat correlated with the endocytosis of the cell fragment and would imply it as a manifestation of heightened activity in the golgi apparatus can be distinguished after 2 months treatment. The electron microscopic features shows that it leads towards the male fertility disorder.

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