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

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Abstract: *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. Etoposide (VP-16), an anticancer drug, 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 testicular tissue revealed that the severe atrophic changes, suggestive arrest at the germinal cell level as well as at the Sertoli cell level. The cellular integrity of all is drastically reduced with a severe nuclear alteration also taking place. The observations showed that damage of early spermatogonia and the later stages of spermatogenesis were completely eliminated. The leydig cells revealed state of work induced hypertrophy as is evident from the secretory activity but the organelles show a condition of extreme overburdened state just prior to state of collapse. In conclusion our result shows that adverse effect of etoposide on testis especially on spermatogonia can be distinguished after two months treatment. The electron microscopic features show that it leads towards the male fertility disorder.*

Keywords: Testis, Spermatogonia, Sertoli Cell, Leydig Cell, Epididymis, 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 alteration induced by etoposide were thoroughly assessed by Kodata T., Chikazawa and Takahashi N. (1989). To study ultrastructure changes in male testis and epididymis caused by an etoposide (VP-16) with the help of electron microscope

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 pieces fixed with 3% glutaraldehyde for 2 hours and then they were washed thrice in 0.1 M Cacodylate buffer. The tissues were rinsed, briefly, in buffer and post osmicated in 100 per cent OSO_4 (osmic acid) for one hour, thereafter were dehydrated in ascending series of alcohol followed by propylene oxide and embedded in resin, which was polymerized at 60^0F. 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 of selected blocks were cut and lead citrate for final viewing, sections were observed and photographed on a Jen-100s Jeol Electron Microscope.

Results and Discussion

The seminiferous tubule is enclosing by one or more layer of myoid cells or peritubular cells constituting the myoid layer of the lumina propria. It is lined by a complete stratified epithelium, composed of two types of cells, the supporting cells and spermatogenic cells. The supporting cells are of single kind called Sertoli cells. Whereas the spermatogenic cells includes several morphogically distinguishable types of spermatogonia, primary spermatocytes, secondary spermatocytes and the spermatid and spermatozoa. The sertoli cell is rested on the basal lamina and extended upwards through the full thickness of the epithelium. The cytoplasm contains numerous slender elongated mitochondria oriented parallel to the longitudinal access of the cell and other cellular organelles like smooth surface tubules, few membrane limited lysomes and occasional lipid droplets are observed. The junctional complex on the boundary separating the sertoli cells from the neighboring cells is prominently visible. In the spermatogenic cells, the first to be encountered are the spermatogonia. The type B spermatogonia with a round nucleus and heavily stained chromatin material are visible. Further downwards towards the lumen a number of conjoined spermatids are visible which are the result of the division of secondary spermatocytes. These spermatids possessed a spherical nucleus with pale staining, finely granular chromatin. The cytoplasm is less electron dense and spermatid at the periphery are lined by mitochondria some of the stages of spermatids showing complete acrosome formation. As bit further downwards the lumen spermatid being transformed into the Spermatozoa are observed.

The first sign of differentiation of the specific component of the spermatozoan and the appearance of a few small granules within the golgi apparatus. The phase four or the maturation phase of the spermatid is clearly visible. The nucleoplasm shows coarseness, the limiting membrane of the acrosome vesicle has increases its areas of adherence to the nuclear envelope and forma thin fold that spread over the pole of nucleus. In the meantime the acrosome granule disappeared and contributed itself to then formation of the acrosomal cap. The nucleus by this attains the flattened pyriform shape characteristic. The cytoplasm of the maturing spermatid is well lined by mitochondria along the peripheral border. Apart from the well-formed Golgi body and the mitochondria tiny secretory filled smooth edged tubules, chromatid bodies and endoplasmic reticulum are also visible. Frequently, transverse sections of various stages of spermatozoa; through the different parts are also noticed between the spermatids.

The testicular tissue revealed that the severe atropic changes suggestive arrest at the germinal cell level as well as at the Sertoli cell level. The cellular integrity of all is drastically reduced with a severe nuclear alteration also taking place (Matsui *et al*., 1993). The nucleoplasm of Sertoli cell is very electron lucent and the nuclear shape has also altered. The Sertoli cell appears markedly shrunken that is its cytoplasm also revealed degenerative changes. There is a pronounced vaculation, presence of polyribosomes forming body are dense. Large lipid droplets are present in the cytoplasm. The Golgi droplets rough endoplasmic reticulum, and mitochondria directly responsible from secretory activity are form lacking on their activity seen totally suppressed. The nuclear regulation of the Sertoli cell seems to be suppressed in the divided Sertoli cell region towards the lumen few scattered secretion filled secretory vesicles are observed.

As result of hyper Sertoli cell inactive the other adjoining germinal cell namely the spermatogonia B, early spermatocytes also appear affected. The cytoplasm has lost its electron integrity and become severe electron lucent. There is a large accumulation of lipids, zebra lipid like structure seen. Prominent change in mitochondria, Golgi zone and

endoplasmic reticulum is visible with onset of vaculation (Lampe H *et al*., 1997). All these indicate change of cytoplasm that takes place when the testis tubular control of the germinal epithelium is obstructed due to hypertrophied Setroli cell. No concretely shaped germinal epithelium is present even all the way towards the lumen. The leydig cells revel state of work induced hypertrophy as is evident from the secretory activity but the organelles show a condition of extreme overburdened state just prior to state of collapse. All processes of active cellular transport across the cellular membrane are visible namely exocytosis, numerous exocytosis vesicles, being pinched off numerous secretion filled vesicles abourding near the cell membrane border and sequestrated secretion in the vesicles. The mitochondria are in an overburdened exhaustive state. Some of being devoid of cristae, significantly reduced rough endoplasmic and Golgi activity, forming phase of dense bodies and lysosomes are point towards the suppressed state of the cellular activity attend in the region towards the cellular periphery. Between the nuclear-adjacent cytoplasm shows secretory activity gets undisturbed, as there is prominent Golgi network, near more appearing motochonria and secretion filled secondary vacuoles. The Ledig cells present a picture of the indicator of the sacculation-suppression action though vesiculated to the surrounding peripheral cytoplasm.

Etoposide (VP-16) treated rat showed severe atrophic changes and suggested arrest at the germinal cell level and the sertoli cell level as well. The lysosomal activity had brought about cytoplasmic degeneration in sertolli cell. The Golgi complex, rough endoplasmic reticulum and mitochondria directly responsible for secretion shows the suppressed activity (Abraham S.K *et al*., 1983).

This implies that these all are drug induced changes. Impaired sertoli cell cytoplasm also implies that these all are drug induced changes. Similar ultra-structure changes were observed by Takahashi *et al*., (1994) in etopside treated rat. Sertoli cell vacuolation is common feature of those animals which are treated with the drugs having effect on testicular functions. CPA, DMPA, estrogen for instance are drugs which are known induce violation in Sertoli cells (Avari 1990) in the present study also we have noticed similar kind of vacuolations in sertoli cells under the effect of etoposide. Thus we would like to expand the already known mode of action of drug as a Topoisomarase-II inhibitor to an agent which hampers spermiogenesis by affecting Sertoli cell (Mahecha *et al*., 2000). This cell type is the principle testicular site action for FSH. Many actions of FSH are replaced by testosterone in the adult male. These effects on Sertoli cells and testicular functions leads to a significant impairment in the fertility of the exposed rats. (Albro *et al*., 1989). Similar changes were observed by Kodata *et al*., (1989) revealed the cytoplasmic vacuolation in the Sertoli cell due to the toxic effect of etoposide drug. In present work etoposide drug shows a visible alteration in the endoplasmic reticulum and Golgi as well as mitochondria with the onset of vacuolation. This impaired Sertoli cell function leads to a decreased production of the androgen binding protein which is transported via rete testis fluid to the epidedymis. They observed the testicular atropy with suppression of spermatogenisis and tubular atrophy. Most of the findings for a reference drug, VCR were similar to those for VP-16 (Akbarsha *et al*., 1996 and Akbarsha and Averal, 1996). Hyperactive sertoli cells inactive the other germinal cells namely spermatogonia B and early spermatocytes. Chemotherepeutic agents manifest their cytotoxic effect by interrupting obligatory cell process involving DNA synthesis and foliate metabolism. These effects are multiplied in rapidly multiplying malignant cells. Testicular injure, including both injure to germinal epithelium and to a lesser extent, the leydig cells, occur with the most chemotherapeutic regimens. Germ cells are responsible for the development of mature spermatozoa and the

continuation of the spermatogenic stem-cell line. Chemotherapeutic agents damage both cell lines, resulting in azoospermia shortly after the onset of treatment; recovery occurs after the variable time period as the stem-cell line regenerates from surviving spermatogonia. The ultimate return of the fertility depends on the degree of damage inflicted to the primary spermatogonia. Complete elimination of spermatogonia can occur after the treatment with alkylating agents and result in azoospermia and infertility (Fox B *et al.*, 1963). The etoposide treated rat revealed that the severe atropic changes suggested the arrest at the germinal cell level as well as the sertoli cell level. Several anticancer drugs show similar changes. Tsunenari *et al.*, (2000) studied the testicular toxicity of Adrimycin (ADR) observed after a single intravenous administration disappearance of seminiferous epithelial cells was observed histopathologically. Mahacha *et al.*, (2000) studied after exposure of male rats to cyclophosphoamide a commonly used as anticancer drug has shown progeny out come in male germ cell phase specific manner. Ito K *et al.*, (2000) studied the testicular toxicity of nitrofuzone after 2 and 4 weeks. NF- administered animal shows that seminiferous tubules were severely atrophied due to a total absence of spermatids and degeneration of spermatids and degeneration of spermatocytes. Cytotoxic effect of Doxorubincin hydrochloride was observed by Swada *et al.*, (1994). Induce oligozoospermia in mice. Almost all germ cells disappeared but the leydig cell remain unchanged. These result suggested that Doxorubincin is known for the damage spermatogenesis in rats at dose dependent manner mainly at early states of spermatogonia (stages A1, A2 & A3). From their observations they stated that the higher dose of Doxorubincin can cause temporary supperssion of spermatogenisis.

Both the types of spermatogonia are highly sensitive to irradiation (Carig and Jakson, 1962) and their proliferative capacity can be inhibited or the cell can be permanently destroyed by exposure to cytotoxic chemicals. Various types of chemicals like Diesters of methanol, sulphonic acid shows selective antispermatogonial action. A single spermatogonia was found to be in G1 stage, the cells dying in the next mitosis. Similar alteration are seen due to the action of etoposide drug in present work which is supported by Kodata *et al.*, 1989. Chemotherupatic agents manifest their cytotoxic effect by interrupting obligatory self-process involving DNA synthesis and foliate metabolism. These effects are multiplied in rapidly dividing malignant cells. The high mitotic rate of germ cell makes the male g gonad highly susceptible to the toxic effects of chemotherapy. Testicular injury including both injury to germinal epithelium and to a lesser to Leydig cells, occur with most chemotherapeutic regiment. Germ cells are responsible for the development of mature spermatozoa and continuation of the spermatogenic stem-cell line. Chemotherapeutic agent damage both cells lines, resulting in azoosperma shortly after the onset of treatment; recovery occurs over a variable time period as the stem cell line regenarete from surviving spermatogonia. The ultimate return of the fertility depends on the degree of damage inflicted to the primary spernatogonia. Complete elimination of spermatogonia can occur after the treatment with alkylating agents and result in azoospermia and infertility. Agents that are relatively sparing to the spermatogonia have a more rapid recovery period. In present study ultra-structure of Leydig cell reveal the state induced hypertrophy as well as organelle shows a condition of extreme overburdened state just prior to a state of collapse (Clermont, Y *et al.*, 1968). Damage to the Leydig cells, which are responsible for the production of testosterone, is due to direct cytotoxic effects or injury to hypothalamic-pitutary-testicular axis. Leydig cells have a slow mitotic rate and are relatively spared from the toxic effect of chemotherapy. Testosterone maintain libido, has anti anabolic effect on bone and skeleton

muscle, and is essential for spermatogenesis. Histopathological findings in the testis range from mid hypospermatogenis to germ cell fibrosis and leydig cell injury. The recovery of germ cells, fertility, and sexual function in men subjected to the damaging effect of chemotherapy is highly variable.

In our studies we have seen the damage of early spermatogonia and the later stages of spermatogenesis are seen to be completely eliminated. Our observation are in agreement with Sawada *et al.,* (1994) and Manable (1997). As far as spermatogonia are concerned with but regarding the leydig cells morphology are hinting to the opposite and contrary. Leydig cells under the etoposide treatment is found to be severely damage as reviewed from the electron microgram. Severe vacoulations has set in due to highly distended cisternae of RER. Many of this cisternae are showing the clear evidence of pinocytosis. The secretion filled cisternae and rapid pinocytosis increase episode of mitochondrial break are the changes suggestive of hypertrophy of Leydig cells to the point of collapse goes hand in hand. In conclusion our result shows that adverse effect of etoposide on testis especially on spermatogonia can be distinguished after two months treatment. The electron microscopic features shows that it leads towards the male fertility disorder

References

Abraham, S.K. and Fränz, J. (1983) Induction of sister-chromatid exchanges by chemotherapeutic drugs in spermatogonia of mice: effects of procarbazine, adriamycin, cyclophosphamide and mitomycin C. *Mut. Res*., **108**, 373–381.

Akbarsha, M.A. and Averal, H.I. (1996) Spermatotoxic efffect of vincristine: an ultrastructural study in the rat. *Biomed. Lett*., **54,** 239–241.

Albro, P.W., Chapin, R.E., Corbett, J.T., *et al.* (1989) Mono-2-ethylhexyl phthalate, a metabolite of di-(2-ethyl-hexyl)phthalate, causally linked to testicular atrophy in rats. *Toxicol. Appl. Pharmacol.,* **100,** 193–200.

Avari, K.M. (1988) Effect of depot metroxyprogesteron acetate and testosterone ethane ton the testis of albino rats, ultrastructure and biochemical studies. *Indian J. Exptl. Biol.,* **30,** 1118–1127.

Clermont, Y. and Bustos-Obregon, E. (1968) Reexamination of spermatogonial renewal in the rat by means of seminiferous tubules mounted "in Toto". *Am. J. Anat*., **122,** 237–248.

Fox, B., Jackson, H., Craig, A.W. and Glover, T.D. (1963) Effect of alkylating agents on spermatogenesis in the rabbit. *J. Reprod. Fert*. **5,** 13–22.

Ito, K. *et al.* (2000) collaborative work to evaluate toxicity on male reproductive organs by repeated dose studies in rat. *J. Toxicol. Sci*. 195–201.

Kodata, T., Chikazawa and Takahashi, N. (1989) Toxicological study of etoposide (VP-16) in rats with special emphasis on testicular alteration. *Toxicol Lett*., **45,** 185–194.

Lampe, H., Horwich, A., Norman, A., Nicholls, J. and Dearnaley, D.P. (1997) Fertility after chemotherapy for testicular germ cell cancers. *J. Clin. Oncol*., **15,** 239–245.

Mahecha, A., Hales, B.F. and Robaire, B. (2000) Expression of stress response genes in germ cells during spermatogenesis. *Biol. Reprod*., **65,** 119–112.

Matsui, H., Toyoda, K., Shinoda, K., Okamiya, H., Furukawa, F., Kawanishi, T. and Takahashi, M. (1993) Quantitative histopathological study on the adriamycin testicular toxicity in rats. *Bull. Nat. Instt. Health Sci*., **111,** 39–46.

Swada, T. *et al.* (1994) Secretion and epidermal growth factor in mice with oligospermia caused by Doxorubicin hydrochloride. *Andrologia*, **26,** 151–153.

Takahashi, M. (1994) Atlas of seminiferous tubules for evaluation of testicular toxicity in rats, mice and dogs. Soft Science, Tokyo.