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Effect of GnRH on Vincristine-Induced Spermatogenic Defects on Sertoli Cell and Defect Produced on the Blood- Testis Barrier (BTB): A Morphological Study

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ABSTRACT

Drugs which are used in chemotherapy to cure cancer, generated destruction effects in sensitive of organs as testies. These substances are disrupted Spermatogenesis. The aim of this Study is investigating protective effects of cetrorelix from side effects of Vincristine. 30 adult male mice were divided into 3 equal groups as: control, vincristine (V) and vincristine + cetrorelix (V+C) groups. A single dose of Vincristine was injected as ip at 1.5 mg/kg. In V+C group cetrorelix injection was started one week before vincristine treatment. Mice in groups were sacrificed 35 days after vincristine injection. Half of testicular specimens were fixed in 2% glutaraldehyde (EM studies), one another of theirs were fixed in boueins fixative (LM studies); Electron microscopic study showed that in control group sertoli cell cytoplasm lie was on BL and there is BTB between them. In (V) group study showed that there were large spaces between sertoli cells and between sertoli and BL. Blood- Testis Barrier were so irregular. In (V+C) group sertoli cells were similar to control group; According to the result it is concluded that GnRH antagonist administration before cancer treatment could partially prevent the side effect of anticancer drugs.

Key words: Vincristine; chemotherapy; GnRH; sertoli cells; Electron microscopic; Blood- Testis Barrier **(**BTB).

INTRODUCTION

Drugs which are used in chemotherapy to cure cancer generated destruction effects in sensitive of organs as testies. These substances are disrupted Spermatogenesis[1-7]. Spermatogenesis is influenced by radiotherapy and cancer cytotoxic drugs[7, 8]. vincristine used for treatment of various malignancies including cancer of testis, ovary, lung, bladder and Hodgkin and non-Hodgkin lymphoma[9-10]. The previous studies showed the side effects of chemotherapeutic drugs such as azoospermia after treatment[1, 3, 11]. The side effects of vincristine on the spermatogenesis when used as anticancer drugs are known[12]. In comparison to normal tissue, tumors are characterized by uncontrolled division. The process of cell division- normal or cancerous cell- is through the cell cycle. The cell cycle goes from the resting phase, through active growing phases and then to mitosis (division). The ability of chemotherapy to kill cancer cells depends on its ability to halt cell division. Therefore chemotherapy is most effective at killing cells that are rapidly dividing. Unfortunately, chemotherapeutic agents can not differentiate between the cancerous and the normal cells. It has been shown that apoptosis is one of the mechanisms in cell destruction following chemotherapy[13-15]. On the other hand, it is known that spermatogenesis is affected by FSH, LH and testosterone and suppression of them could suppress spermatogenesis. Thus it is logical to conclude that suppression of gonadotropins during chemotherapy would protect spermatogenesis by inhibition of their proliferation. For the first time, Glode et al. showed that disturbance of Hypothalamo – hypopheseal axis and reduction the FSH and LH secretion during chemotherapy

resulted in promotion of spermatogenesis after chemotherapy[16]. There are different methods for inhibition of Hypothalamo – hypopheseal axis including usage of agonist and antagonist of GnRH[17-19]. Vincristine are used in a variety malignancies and its effect on spermatogenesis is well studied[12, 20]. And also makes changes in the number of Sertoli cells, Reports on the number of Sertoli cells to chemotherapy are contradictory[21-23]. But there is not enough study about Ultrastractural alterations. Since Ultrastractural studies are useful for evaluation of intercellular junction and the blood-testis barrier (BTB). Therefore we decided to study the Ultrastractural changes induced by vincristine and the inhibitory effect of Cetrorelix, as an antagonist of GnRH on Changes made of following treatment with vincristine.

The aim of the present study is to investigate the action mechanism of vincristine in its influence on Sertoli cells and defect produced on the blood- testis barrier (BTB) and investigates the protective effect Cetrorelix, in reducing the damaging effects of vincristine.

METHOD AND MATERIAL

In the present study 30 adult male mice aging 6-8 weeks were used. The mice were divided into 3 equal groups as: control, exp C, exp (C+V) group.in exp C group a single dose of vincristine was injected intra peritoneal at 1.5mg/kg. And in exp (C+V) group, cetrorelix injection was started one week before vincristine treatment and continued for 3 more weeks. The dosage was selected as reported by previous studies. After 35 days from beginning of treatment, all animals were scarified in 3 groups (Spermatogenic cycle is 35 days). The testes were removed from the abdominal cavity and separated from the epididymis with care by using a surgical blade and then half of testicular specimens were fixed in 2% glutaraldehyde and prepared for EM studies. A sample were established using double-fixing, and in the hydration and replacement process and impregnated with resin, were molded and for studies of EM, was Trimming and was Painting, The thin sections were studied with LEO 906 TEM. And one another of testicular specimens were fixed in boueins fixative for 48 hours; Paraffin sections and staining with (H and E) were prepared for light microscope studies. Sertoli cells calculated by the methods of descriptive statistics (The average ± Standard deviation) and test (Kruskal - Wallis) and using SPSS-13 statistical software and statistical analysis were examined.

RESULTS

Optical microscopy

Showed that in the group receiving vincristine, the thickness of testicular germinal epithelium was greatly reduced, as well as there

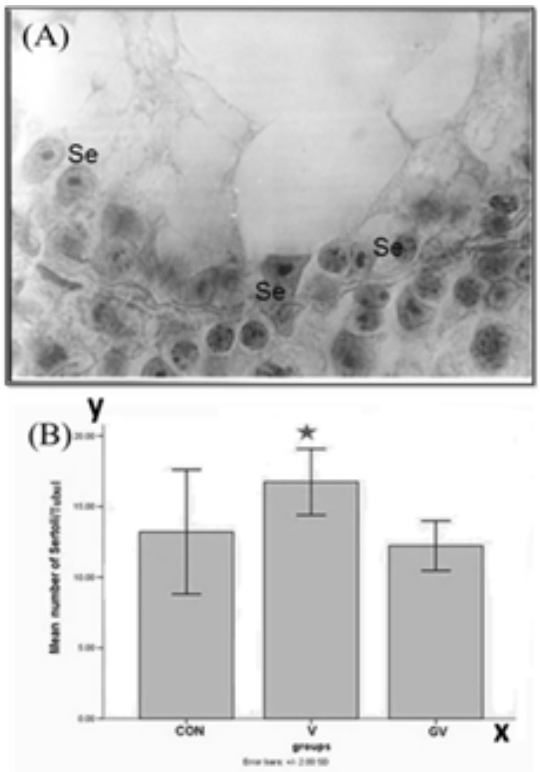

Fig. 1: Light microscope and Histomorphometric evaluation observations of seminiferous tubules in three Experimental groups, A: in the group receiving vincristine, in some seminiferous tubules were down only sertoli cells, B: Comparison the number of Sertoli cells in the three Experimental groups (Transverse axis represents the three groups participating in the Experimental and The longitudinal axis indicates the average number of Sertoli cells in the seminiferous tubules in three groups),(Error bars = 0.002 Standard deviation)

was a lot of empty spaces between the cells of the basal membrane, which likely were gone in some seminiferous tubules were down only sertoli cells) Fig 1A). The number of sertoli cells in the seminiferous cross-section of 20 tubes per sample was counted .the average number of these cells in the control group (13/20 ± 2/20) and group (V) (16/85±1/14) and in group (V+C) (10/20±1/13). Compare the figures showed that the number of Sertoli cells in the group (V) than the control group significantly increased (P=0.002). But in the group (V+C) similar Sertoli cells in the control group. The statistical analysis of the number of Sertoli cells in groups (V) and (V+C) showed that the number of Sertoli cells in the group (V) was significantly higher in group (V+C) (p=0.002) (Fig 1B.)

Electron microscopic

Study showed that in control group sertoli cells had euchromatic nucleus with obvious nucleolus and cytoplasm of the cells were on the basement membrane (BM) (Fig 2A). A connection between sertoli cells and the spermatogonia were observed fully and clearly indicate the establishment of the blood- testis barrier (BTB). In larger magnification BTB consists of three parts: 1) Adjacent Sertoli cell membranes that are woven together and centrally located.2) Flat endoplasmic reticulum in both parties. 3) Clusters of filamentary are located between the endoplasmic reticulum membranes and Sertoli cells. (Fig 2B).EM study showed that in the group receiving vincristine Spermatogonia and Sertoli separated by vast spaces of the basement membrane and also the vast spaces between and within the sertoli cells and spermatogonia were seen, In this group BTB - clearly thinner and irregular than the control group were seen (Fig 2C) EM study in the group (V+C) showed that, BM was a regular basis, but still empty space in between Sertoli cells and spermatogonia can be seen. The study of the BTB in this group reflects the components of the similarity of this barrier with the control group, but the endoplasmic reticulum forming the barrier is seen as swollen. (Fig 2D)

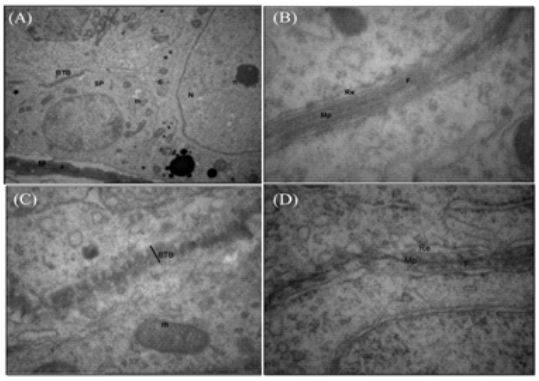

Fig. 2: Electron microscope observations of seminiferous tubules in three experimental groups. A: In control group sertoli cells had euchromatic nucleus with obvious nucleolus, B: In control group clearly indicate the establishment of the blood- testis barrier (BTB), C: in the group receiving vincristine (BTB), clearly thinner and irregular than the control group was seen, D: in the group (V+C) BM was a regular basis and (BTB) in this group reflects the components of the similarity of this barrier with the control group

DISCUSSION

The aim of this study was to evaluate the adverse effects of vincristine as an anti-cancer drug to little changes of testicular germinal epithelium and the ability Cetrorelix (the antagonist GNRH) was to inhibit these effects. Studies show that Spaces between Sertoli and germ cells and between the cells and the basal membrane was observed. Such changes as separation of the basement membrane of spermatogonia and Sertoli cell is in fact signs of change before the apoptosis. In fact, the connection between Sertoli cells and germ cells is to increase Lifetime germ cells. Newton and colleagues in 1993 showed that in vitro intercellular connections are done through cadherin germ cells are increased Lifetime germ cells[24]. Cadherin in fact an important molecular system is the reaction between Sertoli cells and germ control and contributing to the survival of germ cells. It is thought that the connections made through cellular cadherin causes inbound Intracellular messages Which ultimately leads to control differentiation, migration and cell survival[25, 26]. Sertoli cells play an important role in normal spermatogenesis and in different ways have contact with Spermatogenic cells. Also Sertoli cells have receptors for FSH[27]. and testosterone[28], That the role of these hormones than the Sertoli cells function shows. The emergence of space between Sertoli cells and the

basement membrane can be prevented from reaching FSH and testosterone Sertoli cells and ultimately lead to disturbances in the function of these cells. On the other hand, Sertoli cells isolated from Spermatogenic cells can prevent messages from reaching paracrine Sertoli cells in spermatogenesis cells and spermatogenesis function is impaired. Our study also showed that the number of Sertoli cells was increased in the group treated with vincristine. Sertoli cells are too closely associated with germ cells and create a suitable environment for their reproduction and conservation of spermatogenesis cells[29]. Therefore, any change in the sertoli cells can lead to infertility[30]. According to the androgen receptor, estrogen and FSH on Sertoli cells, depends on the activity of these cells to Hypophysis hormones and factors in testicular[31]. any changes in the Hypophysis hormones and intra-testicular factor affects the function of these cells. It is shown that the chemotherapy in human amount of C-K18 increases in Sertoli cells (there are not the markers of normal Sertoli cell). Finally this marker inactivates the Sertoli cells and impact on the quality of spermatogenesis[32]. Reports on the number of Sertoli cells to chemotherapy are contradictory. The number of Sertoli cells to chemotherapy does not change[21]. The numbers of Sertoli cells followed by chemotherapy to reduce their copper. Other studies have shown that the proliferation of Sertoli cells after radiation therapy and is Cryptorchidism[22, 23]. Increase the number of Sertoli cells in a recent study in the group receiving vincristine justification. In our study Sertoli cells increased which is probably due to the balance of apoptosis, because of apoptosis in normal conditions, the balance between cell populations[33]. Treatment with vincristine which reduces spermatogenic cells, and the balance of apoptosis contributes to proliferation compensated and increased the number of Sertoli cells. Our findings indicate a change in the BTB in the group treated with vincristine. BTB have an important role in normal spermatogenesis. And causes the body's immune factors do not have access on dividing spermatogenic cells, and these cells evolve in an environment free of antigens. The blood- testis barrier (BTB) creates components supplied by neighboring Sertoli. Vincristine causes changes in Sertoli cells that this change can cause changes in the function of these cells and eventually cause changes in the structure of connections between Sertoli cells (BTB).

In the experimental group (C + V) that with vincristine, both as a GnRH antagonist Cetrorelix received study spermatogenesis epithelium showed that Cetrorelix (antagonist of GnRH) partially inhibits the adverse effects of vincristine. In support of our findings, Udagawa has shown that treatment with GnRH analogs improve spermatogenesis recovery following chemotherapy[34] and Meistrich et al. have shown that following radiotherapy, the administration of GnRH agonists and antagonists before and after spermatogenesis disorder have a protective effect[19]. It is known that the secretion of FSH and LH increases after chemotherapy and destruction of germinal epithelium[34, 35]. Besides, secretion of testosterone increases after exposure to reprotoxic substance[36]. Increased testosterone result in suppression of membrane bound stem cell factor (SCF) expression which is necessary for spermatogenesis[34]. Thus it appears that reduction of intratesticular testosterone would protect spermatogenesis. This explains the action mechanism of GnRH antagonists, i.e. using GnRH antagonist before treatment leads to reduction of FSH, LH and testosterone. Consequently, reduction of these hormones suppresses spermatogonial proliferation. Since non-dividing cells are less prone to toxic effects of chemotherapeutic agents, spermatogenic cells would not be affected during their non-proliferating period. In agreement with this postulation, Shetty et al. have demonstrated that testosterone-therapy following radiotherapy, will inhibit spermatogenesis improvement[37]. Meistrich et al also demonstrated that with reduction of testosterone can improve spermatogenesis after exposure to reprotoxic sub testosterone[38]. It is also shown that in rats undergone radiotherapy protocol elevation of FSH level result in inhibition of spermatogonial differentiation and steradial therapy following radiotherapy could improve spermatogenesis, after radiotherapy, by suppressing the inhibitory effect of testosterone[37-39]. Suppression of gonadotropins and testosterone has also been demonstrated to improve the damaged spermatogenesis[36]. Our results indicate that administration of vincristine, as an anticancer drug in mice, destroyed the number and function of

Sertoli cells and a change in the structure of cell connections testicular (BTB).and Administration of cetrorelix prior and along with chemotherapy could partially protect Sertoli cell function and thus improve connections between Sertoli cells (BTB) by acting on hypothalamic gonadal axis.

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