Impact of vinblastine sulphate on sperm parameters and histological changes of the seminiferous tubules in mice

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Abstract:

Background:
Vinblastine Sulphate (VBL) is administered with other anticancer drugs for treatment of metastatic testicular carcinoma.

Objective:
The aim of this study was to investigate the effects of different concentrations of vinblastine on the outcome of sperm parameters and histological changes of the seminiferous tubules in mice.

Methods:
Thirty five healthy mature male mice (7 -8) weeks old and weight of (23- 25) g were involved in the present study. They were divided into six treated groups (0.092, 0.14, 0.18, 0.23, 0.28 and 0.4 mg/kg) with control group (5 mature males mice/group) according to periods of orally administration of each week throughout 6 weeks. Males were scarified at the end of administration period for assessment of sperm function parameters. The testes were obtained and prepared for histological sections. Diameter of seminiferous tubules and thickness of layers of proliferated spermatogenic cells were assessed for all groups of male mice.

Results:
A significant decrease ($P<0.05$) was observed in the sperm concentration after treatment with the doses 4, 5 and 6 when compared with control group and highly significant decrease ($P<0.001$) in the sperm viability percentage was appeared for all doses groups when compared with control group. On the other hand, highly significant decrease ($P<0.001$) appeared in the sperm motility after treatment with all doses when compared with control group.
Diameter of seminiferous tubules and thickness of proliferated spermatogenic cells layers were decreased gradually throughout periods of orally vinblastine administration. Highly significant decrease ($P<0.001$) in the diameter of seminiferous tubules and thickness of proliferated spermatogenic cells layers was appeared following the treatment with high doses when compared with control.

Conclusion:
High doses of vinblastine sulphate have negative effects on sperm parameters and histological feature testis in mice.

Key words: Vinblastine Sulphate, sperm parameters, seminiferous tubules.

Introduction

The formation of mature spermatozoa is one of the most essential functions in life. A concerted sequence of events is needed to proliferate, maintain and mature germ cells starting with spermatogonial stem cell and culminating in mature gametes (1). This is owing to the fact that spermatogenesis is an intricate and highly specialized process evolved to suit the individual particularities of each sexual species (2). The site of spermatogenesis is the male gonad. Therefore, spermatogenesis summarizes all events that transform basic spermatogonia into highly specialized mature spermatozoa within the testis. Spermatogonia derive from primordial germ cells which, after entering the testis, develop into gonocytes (1).

In the testis, the germ cells are located in tubules of which their inner side is covered by the seminiferous epithelium containing somatic Sertoli cell which provide nourishment and support cells of the germ line. Before a gamete can leave the testis, it has to pass through several stages of maturation. These processes include meiotic multiplication and propagation of the spermatogonial stem cells, meiotic recombination of genetic material and testicular maturation of spermatozoa (3).

Vinblastine Sulphate is anticancer drug that considered as microtubule inhibitors and it is structurally related compounds derived from the periwinkle plant (Vinca rosea). So it referred to as the vinca alkaloids (4). Moreover, VBL was administered with bleomycin and cisplatin for the treatment of metastatic testicular carcinoma. As well as it is used in the treatment of systemic dgkins and - dgkins lymphomas and some solid tumors e. g. breast and lung cancer (5).

Mechanism of action for VBL is cell-cycle specific and phase specific, because they block mitosis in metaphase. Also, it binding to the microtubular protein, tubulin and blocks the ability of tubulin to polymerize to form microtubules. Instead, paracrystalline aggregates consisting of tubulin dimmers and the alkaloid drug are formed. The resulting dysfunction spindle apparatus, frozen in metaphase, prevents chromosomal segregation and cell proliferation (4).

Few experimental studies have examined the effects of VBL on histological changes of testis in human or laboratory animals. Therefore, the aim of this study was to investigate the influence of different concentrations of VBL for different administration periods on some histological parameters of testis and sperm parameters in mice.

Materials and Methods

1. Animals:

Thirty five mature male mice of Swiss albino strain at an age (7 -8) weeks old weighing (23-25g) were obtained from the animal house at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University. The animals were housed in plastic cages (5 mice/cage) measuring about (29X15X12) cm.

Animals were kept in air conditioned room with an optimum temperature of 24±2 ºC and exposed to about 12- 14 hours/day light program. Water and food were locally prepared and the consisted of available constituents that fulfill the mouse dietary requirements.

2. Dose preparation and experimental design:

Vinblastine Sulphate solution was calculated depending on recommended human body surface area (BSA) doses (3.7, 5.5, 7.4, 9.25, 11.1 and 18.5 mg/m2 BSA). The doses was diluted with normal saline to obtain different doses of Vinblastine Sulphate (0.092, 0.14, 0.18, 0.23, 0.28 and 0.4 mg/kg) in 0.1 mL/mice per week during 6 weeks.

In the present work, thirty five male mice were divided into seven major groups (included one group as control) depending on different doses of Vinblastine Sulphate (males were classified into 5 mice weekly). The period of the experiment was unified for each major group during 6 weeks. Oral route of administration was used for treatment with Vinblastine Sulphate.

3. Collection of mouse spermatozoa

The collection of mouse spermatozoa occurred after scarification by cervical dislocation of male mouse. Then, isolated epididymes from testes and taken both left and right cauda (tail of epididymis) considered site of acquire capacity for spermatozoa (6), and washed with normal saline then they were put in a small well of Petridish contained (1mL) essential medium then cauda were minced by microsurgical scissor about 200 times until getting a homogenized solution which contained the spermatozoa (7).

4. Assessment of mouse spermatozoa:

One drop taken about 10µL of spermatozoal suspension was mounted placed on a warm microscopical class slide and covered with a cover slip (22 x 22 mm), then left for one minute in an incubator to stand before microscopic examination.

Calculations were as assessed as mentioned in the human experiment study. Mouse spermatozoal parameters were assessment about 10 of randomly selected microscopical fields under a high power magnification of 40X objective (8). Parameters of mouse spermatozoa include: sperm concentration, sperm motility (%), progressive sperm motility (%), normal sperm morphology (%), sperm agglutination (%) and sperm viability (%).

5. Histological changes Study:

At end of administration period, both testes were obtained and cleaned from adipose tissues. Then, both testes were weighted and fixed in 10% formalin processed routinely, embedded in paraffin and stained with hematoxyllin and eosin for histological examination as mentioned by Bancroft and Steven (9). By ocular micrometer measured diameter of seminiferous tubules and thickness of layers of proliferated spermatogenic cells were assessed for all groups of male mice as described in details by Zedan, (7).

Statistical analysis:

Means and standard error of mean (mean + SEM) were determined using descriptive statistical methods. The data were statistically analyzed by multiple analysis of variance (MANOVA) and LSD to compare among different means of groups using special statistical computerized package SPSS (statistical Package of Social Science version 15).

Results

The results of oral treatment with different doses of Vinblastine Sulphate on mouse sperm parameters are shown in the table (1). At the end of administration period, the effect of VBL showed significant ($P<0.05$) decrease of treated groups with (dose 4, dose 5 and dose 6) compared to the control group in sperm concentration. The percentages of total sperm motility, sperm progressive motility, sperm agglutination and sperm viability revealed a highly significant ($P<0.001$) decrease in all treated groups as compared with the control group. Moreover, sperm motility grade C % was gradually affected and shown highly significant ($P<0.001$) increase by using high doses in treated groups (Dose5 and Dose6) as compared with control group. Sperm motility grade D percentage showed highly significant ($P<0.001$) increase for all treated groups as compared to the control group. Lastly, decline appeared in normal sperm morphology percentage after treatment with different doses of VBL but do not reach to the significant value.

Table (2) shows the effects of Vinblastine Sulphate administration for orally administration period on diameter of seminiferous tubules and the thickness of layers of proliferated spermatogenic cells. The diameter of seminiferous tubules for groups treated with dose 4 and dose 5 was highly significant decreased ($P<0.001$) as compared to the control group and other treated groups. On the other hand, the thickness of layers of proliferated spermatogenic cells was gradually decreasing for all treated groups and reach to the significant value. However, the thickness of layers of proliferated spermatogenic cells appeared non significant differences ($P>0.05$) among the control and treated groups for administered Vinblastine Sulphate except for dose 5 group appeared highly significantly decreased ($P<0.001$) as compared to the control and other treated groups. Moreover, the diameter of seminiferous tubules and thickness of layers of proliferated spermatogenic cells for group dose 6 were irregular shape and severe damage appeared when compared to the control group and other treated groups, this damage which caused cannot measuring this parameters.

Table (1): Effects of orally administrated Vinblastine Sulphate doses after end of administration period on mouse sperm parameters.

Sperm parameters		Vinblastine doses groups						
		Control	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
		45.4 A ± 1.63	42 A ± 3.74	39 A ± 1.87	40.6 A ± 1.96	37 B ± 3.36	36.2 B ± 2.84	35.2 B ± 2.94
Sperm concentration ($X10^6$ sperm/mL)		82.6 A ± 1.66	66 C ± 1.87	64 C ± 1.87	63 C ± 3.67	54 C ± 7.31	51 C ± 1.87	49.5 C ± 1.22
Sperm motility Proyressive percentage (%)	A+B	56 A ±1.87	42 C ± 2.55	35 C ± 4.18	31 C ± 4.64	19 C ± 2.45	13 C ± 5.15	7 C ± 3
Sperm motility non progressive percentage (%)	C	28 A ± 2.55	30.4 A ± 1.63	31 A ± 3.32	33 A ± 2	35 A ± 5.24	43 C ± 3.74	56 C ± 1.87
Immotile sperm (%)	D	16.4 A ± 1.57	24.8 C ± 1.66	36 C ± 1.87	39 C ± 3.67	46 C ± 7.31	46 C ± 2.45	48 C ± 1.22
Normal sperm morphology percentage (%)		52.5 A ± 1.81	51.5 A ± 1.8	51.2 A ± 1.8	50 A ± 1.78	48.5 A ± 1.7	48.5 A ± 1.7	47 A ± 1.68
Sperm agglutination percentage (%)		31 A ± 3.67	24 C ± 1.87	18.6 C ± 0.98	17 C ± 1.22	15 C ± 1.58	12.8 C ± 0.97	12.8 C ± 0.97
Non-progressive motility (%)		78 A ± 1.4	56 C ± 1.87	56 C ± 2.55	55 C ± 3.16	43 C ± 3.39	40 C ± 3	37 C ± 1.22

Values mean ± S.E.M.
Different letters mean a significant difference between treated groups with control group.
A : Non significant.B : Significant (P<0.05).C : Significant (P<0.001).

Table (2): Effects of different doses of Vinblastine Sulphate on diameter of seminiferous tubules and Thickness layers of proliferated spermatogenic cells (micrometer (μ m)).

parameters	Vinblastine doses groups						
	control	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
Diameter of semniferous tubules	7.42 A ± 0.41	7.14 A ± 0.39	7.01 A ± 0.35	6.92 A ± 0.34	4.86 B ± 0.12	4.06 B ± 0.18	Irregular shape and Severe damage of seminiferous tubules
Thickness layers of proliferated spermatogenic cells	2.34 A ± 0.26	2.12 A ± 0.1	2.08 A ± 0.13	2.02 A ± 0.12	1.66 A ± 0.12	1.2 B ± 0.17	

Values mean ± S. E. M.
Different letters mean a significant difference between treated groups with control group and other treated groups.
A: Non significant.
B: Significant (P<0.001).

Discussion

The results in the present study revealed highly significant affects of mouse sperm parameters for different doses of Vinblastine Sulphate as compared to the control group, except normal sperm morphology percentage (did not reach the significance level) with slight decrease appeared. On the other hand, diameter of seminiferous tubules and thickness layers of proliferated spermatogenic cells showing highly significantly decrease after treatment with high doses of VBL as compared to the control group through orally administration period.

This finding is due to Vinblastine Sulphate is anticancer drug that considered as microtubule inhibitors, using for the treatment of metastatic testicular carcinoma (4, 5). Chemotherapy can directly damage spermatogenesis temporarily or permanently (10). Kallio *et al* (11) mentioned the effects of VBL on in vivo and *in vitro* meiosis in male rats showed an increased rate of detached chromosomes. Cell proliferation was inhibited, as shown by the number of cells arrested at metaphase during the first meiotic (MI) or second meiotic (MII) division. VBL was found to be a potent inducer of cell death (11). Also it is produced identical morphological patterns of response in the seminiferous tubules resulting in arrest of germ cell mitosis and meiosis and a rapid depletion of the microtubules normally found within the Sertoli cell as well as Leydig cell dysfunction with a low incidence of recovery (12, 13).

Some vital structures in the living cells affected by VBL like microtubules. Microtubules are dynamic components of the cell cytoskeleton that participate in many important cellular processes, including mitosis, cell motility, morphogenesis, and organelle transport (14). VBL which is known to bind to microtubule ends has been shown to inhibit the persistent forward motion of the growth cone. This data strongly suggests that molecules that bind to the ends of microtubules contribute to the regulation of microtubule behavior in living cells (15). Usually VBL bind tubulin and cause dissociation of the microtubule apparatus (16). Thus binding of VBL to tubulin at a microtubule end may induce a conformational change in the molecule of tubulin to which it is

bound, which increases the affinity of the tubulin for adjacent tubulin molecules in the microtubule lattice. In VBL - treated cells, both the average duration of a pause (a state of attenuated dynamics where neither growth nor shortening could be detected) and the percentage of total time spent in pause were significantly increased because mitotic slowing and mitotic abnormalities induced by this drug is a direct result of suppression of dynamics of mitotic spindle microtubules (17). Moreover, VBL is cytotoxic to primary spermatocyte and a highly potent spindle poison that produces a high number of shortened and monopolar spindle as well as multiple chromosomal loss (18, 19).

Scientific truth for mechanism of VBL has approved adverse effective on spermatogenesis including percentage of sperm concentration, sperm motility and sperm viability. These results in present study agreement with Jagetia (20), as well as it is referred through mouse administration VBL showed significant decline in testicular weight whenever increasing doses of VBL and long time administrated. Also, significant changes in the relative percentages of different germ cell populations included elongated spermatids, round spermatids, spermatogonia, gonial cells and primary spermatocytes (20). From the results of the present study, high doses of VBL administration impact significantly reduction for diameter of seminiferous tubules and thickness of layers of proliferated spermatogenic cells.

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