Effect of vincristine and vinblastine on mice spermatozoa *in vitro*

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Abstract:

The aim of this project was to study the *in vitro* effect of antineoplastic drugs (vincristine and vinblastine) on mice spermatozoa. Eighteen adult (age 8-9 weeks) male mice were divided into three groups equally. The animals in each group were slain by cervical dislocation, the testes were removed and two tails of epididymides isolated. Spermatozoa were obtained from the two tails of epididymides by mincing in 500 µl TCM-199.The first group non-treated (unadded) as a control group, second group added 10 µg/ml of vincristine to TCM-199 and the third group added 10 µg/ml of vinblastine to TCM-199. After 10 minutes from added of vincristine and vinblastin measured the following test: spermatozoa activity, percentage dead spermatozoa and morphological abnormalities of spermatozoa. The obtained results indicated that vincristine and vainblastine showed significant reduction in activity, while increased in percentage of dead/live spermatozoa and morphological abnormalities of spermatozoa compared with the control group. Moreover, the data showed that vincristine and vinblastin had effect on spermatozoa *in vitro*.

Key words: vincristine , vinblastine , spermatozoa , *in vitro*

Introduction:

In vitro spermatogenesis, maturing germ cells in vitro, stimulating their differentiation into spermatozoa, would be particularly useful in patients who have received profoundly gonadotoxic therapy in whom the supporting Sertoli cells are unable to support spermatogenesis. Although restoration of fertility after in vitro spermatogenesis has been reported [1], it involved maturation of the later stages of spermatogenesis rather than stem cells; in vitro maturation of diploid stem cells into haploid spermatozoa appears unlikely to become technically possible in the near future [2].

Vinblastine and vincristine cause an arrest of mitotic and meioticdivisions to metaphase followed by cell death, which Naas more rapid after vincristine administration. Both alkaloids had aslight damaging effect on the pachytene spermatocytes. Large doses of both drugs primarily affected the Sertoli cells by destroying their microtubules and mitochondria. Vincristine specifically damaged the acrosomic system and the cytoplasmic bridges of the young spermatids [3].

Some investigators have used sperm in vitro assay to ascertain antioxidant effects on DNA integrity [4]. While others, have studied the effect of low molecular factor excreted by E.coli on spermatozoa [5]. In this case, the investigators have observed that this factor has the ability to immobilize spermatozoa in vitro. Moreover this test has several other applications in regard of infertility. It is routinely used to assay male infertility [2].

In the study Parvinen [6] main attention was focused to the early specific cellular effects of the vinca alkaloids vinblastine and vincristine,

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because their primary mechanisms of action are largely unknown. Most authors agree that vinblastine stops the mitotic cycle in metaphase [7], but vinb1a,,,tine has also been shown to kill interphase cells [8]. The suggested mechanisms of the action have been acylation [9]. alteration of cellular respiration [10], or inhibition of rR.NA- or protein synthesis [11]. In the mouse spermatogenesis, vincrsitine has been shown to have all inhibitory effect on thvinidine uridine and L-leucine.

The first study in this direction was made by [12], who studied the effects of vincristine on DNA, RNA and protein syntheses during mouse spermatogenesis, fractionating the cells by a velocity sedimentation technique. By this and a serial mating experiment they showed that vincristine in maximally tolerated doses affected all spermatogenic cell types with the possible exception of the mature spermatozoa. Vincristine also had a moderately depressing effect on RNA synthesis [13].

An important finding in this study is that although the chemical structures of vinblastine and vincristine are very similar, there exist marked differences in their effects in spermato genesis.Vincristine induces more severe alterations to the cells [14].

Our study indicates that the specific action of anticancer drugs can be rapidly screened by the simple transillumination of the freshly isolated unstained seminiferous tubules. The affected regions of the mitotic and meiotic cell cycles can be analyzed further by morphological methods. In addition to the effects of vines alkaloids on dividing and RNA synthesizing cells, these drugs have pronounced effects on the Sertoli cells, which may be an important cause of male sterility induced by vinblastine and vineristine [15].

The results reported in this study show that two antitumor drugs, vincristine and vinblastine, cause marked effects on spermatozoa in *in vitro* assay.

Materials and Methods:

Animals

Healthy adult albino male of Swiss albino strain were obtained from animal house Biotechnology Research Center/ AL-Nahrain University (18 male mice were used in this study), the age of the mice were in the range of 8-9 weeks old , and the weight in the range 25-30 grams. The animals were housed in small plastic cages, which were cleaned weekly by washing with soap and tap water and sterilized with 70% ethyl alcohol throughout the period of the study. The room temperature was maintained at (24±2) º C, and the animals were exposed to 14 hours light program.

Chemicals

Commercially available vincristine and vinblastine sulphate were obtained from Cipla Ltd.

Treatments

18 adult (age 8-9 weeks) male mice were divided into three groups, each group contain 6 males. The animals in each group were sacrificed by cervical dislocation, the testes were removed and placed in a sterile disposable petridish containing 1 ml TCM-199, and then the epididymides were isolated. Spermatozoa were obtained from the two tails of epididymides by mincing in 500 µl TCM-199 by using microsurgical scissor and forceps, and maintained at 37°C in 5% CO2 incubator prior treatments. Spermatozoa were treated as follows after 10 minutes from treated:

Group A : Untreated control group.
Group B: Treated (added) 10 µg/ml vinblastine.
Group C : Treated (added) 10 µg/ml vincristine.

Microscopical examination

Spermatozoa were assessed according to WHO laboratory manual [16] for viability, percentage dead/live spermatozoas, motility and abnormalities.

1-The caudal epididymis was cut and placed in a petridish containing 500 µl of TCM-199 and minced by using microsurgical scissor and forceps.
Three drops of eosin stain was placed on the cleaned and dried slide.
2-One drops of diluted semen was added on the slide and mixed with the stain for 10 sec.
3-The mixture was left to stand for about 50sec.
4-The mixture , semen + colorants, was spread under a second slide or cover slide by drawing a film of the mixture as thinly and regularly as possible.
5-The slide was left in the warm place to dry and then was examined by the microscope.

Statistical analysis

Statistical analysis was performed to compare two different groups by using ANOVA-test. Statistical significance was determined at $P<0.05$ [17].

Results and discussion:

Single dose was used of vincristine and vinblastine 10 µg/ml and an assessment of their potential effect on spermatozoa is shown in table(1).

Table (1): In vitro effects of vinblastine and vincristine on spermatozoa activity of mice.

Treatment groups	Sperm activity%(mean±SD)
Vinblastine10 µg/ml	27.5±2.5 A
Vincristine 10 µg /ml	42.5±2.5 B
Control	60.0±7.3 B

Differences A, B, C are significant (P<0.05) to compared rows

Table (1) shows the percentage of sperm activity after in vitro treatment with both antineoplastic agents. Vincristine and vinblastine caused significant reduction in activity of spermatozoa as compared with control. However, vinblastine was more effective. The sperm activity reduced to 27.5 % and 42.5 % after vinblastine and vincristine treatments respectively .These results are in contrast with the reports showing that higher activity usually observed with vincristine against animal and tumor cells [18]. On the other hand vincristine caused lower survival of sperms (table 2) and higher morphological abnormalities (table 3).

Table(2): Percentages of dead mice spermatozoa after in vitro treatment with vincristine and vinblastine.

Treatment groups	Dead sperm %(mean±SD)
Vinblastinel0 µg /ml	68.0±4.0 A
Vincristine 10 µg /ml	59.5±0.5 B
Control	48.5±3.8 C

Differences A, B, C are significant (P<0.05) to compared rows

Table(3): Morphological abnormalities of mice spermatozoa after in vitro treatment with vinblastine and vincristine.

Treatment groups	Abnormalities %(mean±SD)
Vinblastine 10 µg /ml	27.0±3.0 A
Vincristinel0 µg /ml	31.5±0.5 A
Control	21.75±1.3 C

Differences A, B, C are significant (P<0.05) to compared rows

Morphological changes were observed, after ten minutes of treatment with vincristine and vincristine. Figure(1) showed the normal spermatozoa of mice prior treatment ; Whereas in Figure (2).

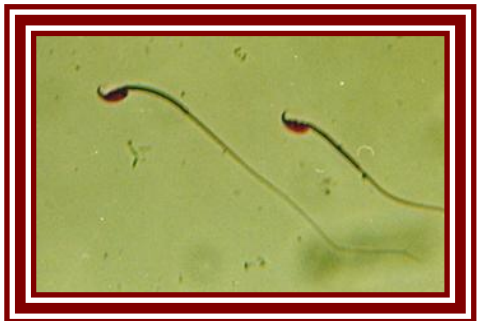

Fig. (1): Normal morphological appearance of mice spermatozoa

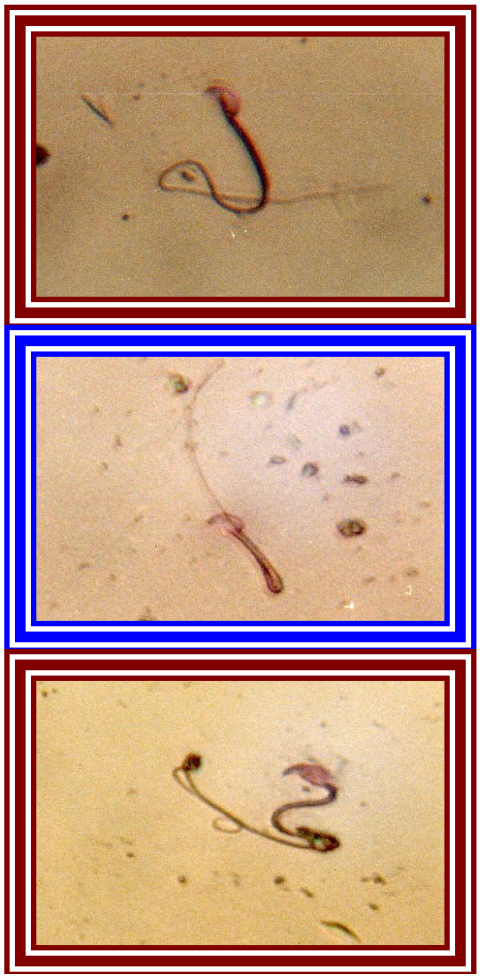

Fig. (2): Abnormal appearance of mice spermatozoa after treatment with 10 µg/ml vincristine or vinblastine

Morphological changes in spermatozoa were observed as a result of vincristine and vinblastine treatment. The reported assay and their results might be useful for studying the effects on male fertility of chemical, drugs, food additives and other compounds, considering the ease and availability of spermatozoa for such analysis, we suggest to add in vitro analysis of spermatozoa to other assays for assessments of drugs, e.g:mutagenicity, carcinogencity and teratogenicity assays.[19,20].

In the mouse spermatogenesis , vincristine has been shown to have an inhibitory effect on thymidine , uridine and L-leucine incorporation in all testicular cell types, accompanied with decrease in fertility [21]. Vincristine and vinblastine has also been demonstrated to increase the amount of abnormal sperm cell in mice[22,23].

When mice spermatozoa were exposed to Vincristine and vinblastine In vitro, they underwent a series of changes that produced apoptotic bodies [24]. Apoptosis also plays a significant role in the process of normal germ cell depletion [25], so that the existence of a genetic predetermined pathway has been suggested that can be aberrantly activated by chemotherapeutic drugs [26]. As a logical consequence, the use of apoptosis inhibitors could potentially stop the apoptotic process.

Maturing germ cells In vitro, stimulating their differentiation into spermatozoa, would be particularly useful in patients who have received profoundly gonadotoxic therapy in whom the supporting Sertoli cells are unable to support spermatogenesis. Although restoration of fertility after In vitro spermatogenesis has been reported [27], it involved maturation of the later stages of spermatogenesis rather than stem cells; in vitro maturation of diploid stem cells into

haploid spermatozoa appears unlikely to become technically possible in the near future [28].

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تاثير الفنكرستين والفنبلاستين على الحيوانات المنوية للفئران خارج الجسم

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الخلاصة:

الهدف من اجراء هذا البحث هو دراسة تاثير بعض الادوية المضادة للسرطان (فنكرستين والفنبلاستين) على الحيوانات المنوية للفئران خارج الجسم الحي.استخدمت في هذه الدراسة 18 من ذكور الفئران البالغة تتراوح اعمارها بين 8-9 اسابيع، قسمت الحيوانات الى 3 مجاميع كل مجموعة تحتوي على 6 ذكور. تم قتل جميع الحيوانات بطريقة فصل الرقبة وتم عزل الخصى وذيلي البربخ, عزلت الحيوانات المنوية عن طريق هرسها في 500 مايكروليتر من الوسط الزرعي المحور 199. تركت المجموعة الاولى من غير علاج (عدم اضافة) كمجموعة سيطرة، اضيف الفنكرستين 10 مايكروغرام/مل الى الوسط الزرعي النسيجي المحور -199 في المجموعة الثانية اما المجموعة الثالثة فتم اضافة 10 مايكروغرام/مل من الفنبلاستين الى الوسط الزرعي النسيجي المحور -199. بعد مرور 10 دقائق من اضافة الفنكرستين والفنبلاستين الى الحيوانات المنوية تم قياس الفحوصات التالية: حيوية الحيوانات المنوية، النسبة المئوية للحيوانات المنوية الميتة/الحية والتشوهات الشكلية للحيوانات المنوية. اظهرت النتائج انخفاض معنوي ($P<0.05$) في حيوية الحيوانات المنوية المعاملة بالفكرستين والفنبلاستين و زيادة في النسب المئوية للحيوانات المنوية الميتة/الحية والتشوهات الشكلية للحيوانات المنوية مقارنة مع مجموعة السيطرة. ان المعاملة بالفكرستين والفنبلاستين لها تاثير سلبي على الحيوانات المنوية خارج الجسم الحي.