Effect of some chemotherapy drugs (vincristine and vinblastine) on females albino mice fertility and sex hormones level

تاثير بعض ادوية العلاج الكيمياوي (الفنبلاستين والفنكرستين) على خصوبة اناث الفئران ومستوى الهرمونات الجنسية

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Abstract

The aim of this study was to determine the effect of chemotherapy (vincristine and vinblastine) on the fertility and sex hormones level of female mice. Twenty four female albino mice were divided into three equal groups. The First group injected with vincristine 0.05 mg/kg intra-peritoneal daily for 3 weeks. The second group injected with vinblastine 0.05 mg/kg intra-peritoneal daily for 3 weeks. The third group treated with normal saline intra-peritoneal daily for 3 weeks as control group. Blood samples were collected by using heart puncture within plastic syringes and transferred to plastic tubes. Blood centrifuged and serum was harvested for hormonal assay. The animals in each group were killed by dislocation of cervical vertebrate in third week of the treatment. Ovaries weighed and fixed with Bouin solution for histological study. Results showed significantly (P<0.05) decreased in ovaries weight on groups treated with vincristine and vinblastine as compared with control group. The mean numbers and diameters of primary, secondary follicles and corpus luteium, and hormones level [follicular stimulating hormone (FSH), lutenizing hormone (LH), estrogen and progesterone] were significant declined in groups treated with vincristine and vinblastine as compared with control group. Results indicated that fertility and sex hormones level reduced in females treated with chemotherapy drugs vincristine and vinblastine.

المستخلص

هدفت الدراسة الحالية الى معرفة تاثير بعض ادوية العلاج الكيمياوي (فنكرستين و فنبلاستين) على ، خصوبة اناث الفئران ومستوى الهرمونات الجنسية فيها . استخدمت في هذه الدراسة 24 انثى من الفئران قسمت الى 3 مجاميع متساوية ، حقنت المجموعة الاولى بالفنكرستين 0.05 ملغم/كغم داخل الخلب البريتوني يوميا لمدة ثلاثة اسابيع . اما المجموعة الثانية فقد حقنت بالفنبلاستين 0.05 ملغم/كغم داخل الخلب البريتوني يوميا لمدة ثلاثة اسابيع . في حين حقنت المجموعة الثالثة بالمحلول الملحي الفسيولوجي كمجموعة سيطرة داخل الخلب البريتوني يوميا لمدة ثلاثة اسابيع . تم جمع نماذج الدم عن طريق ثقب القلب بواسطة محاقن بلاستيكية ووضعت في انابيب بلاستيكية في جهاز الطرد المركزي للحصول على المصل لغرض قياس الهرمونات . تم قتل جميع الحيوانات عن طريق فصل الرقبة بعد الاسبوع الثالث من التجريع ، وزنت المبايض ووضعت في سائل باون لغرض اجراء الدراسة النسيجية . اظهرت النتائج انخفاض معنوي (P<0.05) في معدل اوزان المبايض في المجاميع المعاملة بالفنكرستين والفنبلاستين مقارنة مع مجموعة السيطرة . وكذلك انخفضت معدلات اعداد واقطار الجريبات الاولية والثانوية والاجسام الصفراء ، ومستوى الهرمونات في الدم (الهرمون المحفز للجريبات والهرمون اللوتيني والاستروجين والبروجستيرون) في المجاميع المعاملة بالفنكرستين والفنبلاستين مقارنة

Key words: chemotherapy, vinblastine , vincristine , fertility, sex hormones

مع مجموعة السيطرة . اظهرت نتائج الدراسة ان الاناث المعاملة بادوية العلاج الكيماوي بالفنكرستين والفنبلاستين قد انخفضت خصوبتها و مستوى الهرمونات الجنسية لديها .

Introduction

Successful therapy for children and adolescents with cancer therapeutics including ionizing irradiation and/or chemotherapeutic agents. These may produce damaged cell's DNA, resulting to lethal or sub lethal cells. These effects may be expressed in the gonads as sterility or germ cell DNA damage [1]. Sterility may be acute, or identified by the occurrence of premature menopause, DNA damage may be identified by an increased risk for chromosomal aberrations, single gene defects or major congenital malformations in the offspring [2]. Chemotherapeutic agents are used because their anti-tumor effects are commonly additive, but in many occasions their adverse effects are increased as well. Late complications associated with chemotherapy treatments, such as secondary malignancies and adverse effects on the female gonads, are assuming greater significance. Ovarian damage and failure are unfortunately common long-term side effects of curative chemotherapy [3]. Germ cell survival may be adversely affected by radiation therapy and chemotherapy [1, 4]. This study was carried out to investigate the treatment effects of vincristine and vinblastine on germ cell survival, fertility, as well as the effect of chemotherapy on sex hormone levels and ovarian histological changes.

Materials and Methods

Animals and treatment of Females

Twenty four adult of female albino mice (27-34) gm provided from Biotechnology Research Center/ AL-Nahrain University, maintained in the animal house on a 14:10 hour light: dark cycle. Animals were provided with food and water ad libitum. One week later, females were randomly divided into 3 treatment groups of 8 mice for each. First group injected with vincristine 0.05 mg/kg intra-peritoneal daily for 3 weeks. Second group injected with vinblastine 0.05 mg/kg intra-peritoneal daily for 3 weeks. Third group treated with normal saline intra-peritoneal daily for 3 weeks as control group. Blood samples were collected via heart puncture using plastic syringes and transferred to plastic test tubes. Samples were centrifuged and serum harvested for hormonal assay. The animals in each group were killed by dislocation of cervical vertebrate in third week post- treatment. Ovaries were weighed in week 3 post-treatment and fixed with Bouin solution to histology.

Hormones assay

Serum FSH, LH, Estrogen and Progesterone levels were determined by using miniVIDAS, (ELISA) for the hormonal assay.

Histological studies

The fixed ovaries placed in Bouin solution overnight, and processed for routine paraffin embedding. The ovaries were cut into 5-µm sections. Three serial sections per ovaries were mounted on slides, deparaffinized, rehydrated, and stained with hematoxyline - eosin stain. Sections of the ovaries were examined by light microscopy. Diameters of primary and secondary follicles and corpus luteium were assessed in each using a calibrated micrometer eyepiece [2].

Statistical analysis

Data were analyzed by one-way analysis of variance with ANOVA- test (LSD) [5]. Data are presented as means ± SE. The level of significance was $p < .05$.

Results and Discussion

The ovaries weight was significantly (P<0.05) decreased in mice treated with vincristine and vinblastine (0.10±0.062 , 0.09±0.052 mg respectively) as compared with control group (0.15±0.034 mg), Table (1).

Table (1): Ovary weight in mice treated with vinblastine and vincristine , and control group (Mean + SD)

Treatment groups	Ovary weight mg/ 100 mg B.W
Vinblastine 0.05 mg/kg	B 0.09±0.052
Vincristine 0.05 mg/kg	B 0.10±0.062
Control	A 0.15±0.034

Different superscripts within each column differ significantly (P<0.05)

A decreases in numbers of primary and secondary follicles as well as corpus leutium in mice treated with vincristine and vinblastine (2.30±0.58 , 2.12±0.64) ; (3.82±1.16 , 3.74±1.09) ; (1.64±0.63 , 1.42±0.58) respectively, compared with control group (4.20±0.76 , 6.52±1.31 , 3.04±0.79) respectively Table (2).

Table (2): Numbers of primary and secondary follicles and corpus leutium in ovaries of treated mice with vinblastine and vincristine , and control group (Mean ± SD)

Treatment groups	No. of primary follicles	No. of secondary follicles	No. of corpus leutium
Vinblastine 0.05 mg/kg	B 2.12±0.64	B 3.74±1.09	B 1.42±0.58
Vincristine 0.05 mg/kg	B 2.30±0.58	B 3.82±1.16	B 1.64±0.63
Control	A 4.20±0.76	A 6.52±1.31	A 3.04±0.79

Different superscripts within each column differ significantly (P<0.05)

Treatment with either vincristine or vinblastine exhibits a significant (P<0.05) reduction in diameter of primary (24.66±3.22 and 21.42±3.60mm) and secondary follicles (133.75±6.81 and 128.53±7.43mm) as well as corpus leutium (21.33±3.51 and 19.70±4.30 mm) compared with their control group (47.88±4.05 , 211.42±4.11 and 38.28±4.71 mm) respectively Table (3).

Table (3): Diameters of primary, secondary follicles and corpus leutium in ovaries of treated mice with vinblastine and vincristine , and control group (Mean ±SD)

Treatment groups	Diameters of primary follicles (mm)	Diameters of secondary follicles (mm)	Diameters of corpus leutium (mm)
Vinblastine 0.05 mg/kg	B 21.42±3.60	B 128.53±7.43	B 19.70±4.30
Vincristine 0.05 mg/kg	B 24.66±3.22	B 133.75±6.81	B 21.33±3.51
Control	A 47.88±4.05	A 211.42±4.11	A 38.28±4.71

Different superscripts within each column differ significantly (P<0.05)

High dose of chemotherapy and radiotherapy have radically increased survival rate of young-cancer patients, but major side effects on ovarian failure and infertility were observed [2,6]. The mechanism of primordial follicles damage induced by radio/ chemotherapy is presented as well as the role of apoptosis signaling pathways underlying destruction. Increased knowledge of these mechanisms could help to identify the potential effective inhibitors that can block the path of primordial follicles destruction and reduce ovarian failure rate [7]. The long-term results

following chemotherapy and radio- therapy treatments show reduced follicle stores or ovarian atrophy. However, the acute effects and the direct mechanisms are only partially understood. Recent studies have stressed the role of apoptotic pathways underlying germ cell destruction. A good knowledge of the mechanisms involved in ovarian damage caused by radio/chemotherapy is important in order to introduce agents that block or reduce damage effectively [2, 8].

Treatment of the female with anti-cancer drugs prior to conception might affect the outcome of subsequent progeny due to drug-induced defect in the ova itself, such as an effect on the DNA or chromosomal proteins, or due to an effect due to presence of the drug in the follicular fluid. There are three main mechanisms of female reproductive toxicity: non genetic (e.g., due to the presence of drug in follicular fluid), genetic (e.g., gene mutation or chromosomal abnormality), and epigenetic (e.g., effect on gene expression, genomic imprinting or DNA methylation). The female reproductive system has a number of unique properties that help us interpret some of the mechanisms underlying female-mediated drug effects. Germ cells in the ovaries show one of the highest mitotic activities of any tissue in the body [4, 9].

In order to visualize and study the direct mechanism of chemotherapy-induced ovarian damage and primordial follicles injury, healthy human cortical ovarian slices were exposed *in vitro* to therapeutic doses of vincristine and vinblastine. Histology and immunohistochemical staining showed that chemotherapy induced pregranulosa cell swelling, marked pregranulosa cell nuclear swelling and primordial follicles architecture disruption with disappearance of the lumen and its oocyte. Positive apoptotic staining was obtained in the pregranulosa cells exposed to chemotherapy but not in controls [10, 11].

Recent studies have initiated mapping the apoptosis signaling pathway underlying germ cell destruction by chemotherapy, and identifying key genes and proteins as potential inhibitors to block the path of primordial follicles destruction. A study by [12] has shown that in mice the chemotherapeutic agent doxorubicin induces apoptosis in the primordial follicles and the first steps of apoptosis occur in the pregranulosa cells. The protein p53 was not required for drug-induced oocyte destruction; however, members of the caspase gene family were required for oocyte death [12]. The pathways of K element regulation showed that potassium efflux during ovarian cell death appears early in oocytes and granulosa cells and may regulate a number of apoptotic events including caspase activity and internucleosomal DNA cleavage [13].

Table (4) showed significantly ($P<0.05$) decreased in serum FSH and LH concentrations of mice treated with vincristine and vinblastine (0.63 ± 0.04, 0.60 ± 0.04 mIu/ml); (0.71 ± 0.02, 0.74 ± 0.03 mIu/ml) respectively compared with control group (1.21 ± 0.04, 1.02 ± 0.72 ng) respectively.

Table (4): Serum concentrations of FSH and LH in mice treated with vinblastine and Vincristine, and control ones (Mean ± SD)

Treatment groups	Progesteron mIu/ml	Estrogen mIu/ml
Vinblastine 0.05 mg/kg	A 0.35±0.06	B 20.98±8.31
Vincristine 0.05 mg/kg	A 0.34±0.03	B 22.03±7.45
Control	A 0.37±0.02	A 36.81±6.82

Different superscripts within each column differ significantly (P<0.05)

The use of chemotherapeutic agents is necessary in the treatment of many malignancies that occur in young men, including Hodgkin's lymphoma, ovulatory cancer, and acute lymphocytic leukemia [14,15] Chemotherapeutic agents have the potential to damage both germ cells.

Inhibins and activins are glycoproteins produced by the granulosa cells that belong to the transforming growth factor-b family. Inhibins have a regulatory effect on pituitary FSH synthesis and secretion. Activins act as functional antagonists of inhibin to stimulate FSH synthesis and secretion [16]. Serum dimeric inhibin B is regarded as a direct measure of ovarian reserve, as it is mainly secreted by the granulosa cells of pre-antral follicles [17]. Low levels of both inhibin A and B are typical in women with premature ovarian failure and postmenopausal women [18]. [19] Suggest that a fall in the inhibin B concentration might be an earlier marker for limited ovarian reserve than an elevated FSH and LH concentrations.

Mice treated with vincristine and vinblastine significant (P<0.05) reduced estrogen concentration (22.03±7.45 , 20.98±8.31 mIu/ml respectively) as compared with control group (36.81±6.82 mIu/ml) Table (5).

Table (5): Serum concentrations of estrogen and progesterone of mice treated with vinblastine and vincristine , and control group (Mean± SD)

Treatment groups	FSH mIu/m	LH mIu/m
Vinblastine 0.05 mg/kg	B 0.60±0.04	B 0.74±0.03
Vincristine 0.05 mg/kg	B 0.63±0.03	B 0.71±0.02
Control	A 1.21±0.04	A 1.02±0.72

Different superscripts within each column differ significantly (P<0.05)

With the decline of the follicle pool, serum levels of E2 were decreased [20]. However, the condensed follicular phase length in older women might be the result of an advanced follicular recruitment by cycle day 3. This early dominant follicle selection is expressed by high serum concentrations of estradiol. It has been shown in a population receiving assisted-reproduction treatment in which GnRH analogues were not administered that increasing day 3 estradiol concentrations are associated with decreasing oocyte numbers and pregnancy rates [21]. Estradiol measurement is also useful when obtained concurrently with FSH levels, because values > 80 pg/mL indicate disrupted folliculogenesis, which does not allow accurate interpretation of FSH measurements [21]. Other authors have not found any correlation between estradiol concentrations and the ovarian reserve [22].

Progesterone concentration in serum of treated mice with vincristine and vinblastine were no significant reduced (0.34±0.03, 0.35±0.06 mg respectively) as compared with control group (0.37±0.02) Table (5). In mice, progesterone was found to have a protective effect when administered 1 week before the start of vincristine and vinblastine and during the treatment [23]. Results indicated that the females treated with chemotherapy drugs (vincristine and vinblastine) reduced fertility and sex hormones level.

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