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THE EFFECTS OF CYCLOPHOSPHAMIDE ON THE FREQUENCY OF MICRONUCLEUS, BIOCHEMICAL PARAMETERS, OVARIAN AND UTERINE HISTOLOGY IN RATS

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ABSTRACT: Cyclophosphamide(CTX) is one of the most effective cytotoxic and immunosuppressive agent. It has many toxic side effects, including risk of secondary malignancy and myelosuppression. This compound has been shown to produce ovarian failure in humans and ovarian damage in animals treated for short or long time periods. In this study, the effects of cyclophosphamide(CTX) on the micronucleus frequency, urine and blood parameters, ovarian and uterine histology were investigated. 20 Sprague Dawley female cycling rats were divided into 2 groups: Group I(Control) was injected with 0.3ml/day physiologic saline (ip) . This injection was administered at the same time every day for a period 21 days. Group II(CTX) was injected (ip) with 50 mg/kg /day loading dose of CTX for the first two days of the experiment, then 5 ml/kg/day treatment dose of CTX for the remaining 19 days. As a result of the experiment, micronucleus frequency, SGOT and SGPT activities, creatinine clearance, urine creatinine, urea nitrogen, uric acid, urine volume, and pH levels increased in Group II, according to the control group. Histologically, a few of the primary follicle, and a large number of the corpus luteum were obtained in the ovaries, and decreasing gland and endometrium proliferation were determined in the uterine tissue of the group II animals.
[Key Words: Cyclophosphamide (CTX), Micronucleus, Ovarian, Uterine, Rat]

INTRODUCTION

Cyclophosphamide (CTX), an alkylating agent, has been used for cancer chemotherapy as a immunosuppressant drug [1,2,3,4]. CTX is used in the treatment of malignant diseases such as non-Hodgkin's lymphomas, acute lymphoblastic leukaemia, Behçet diseases, carcinomas of the lung, breast, cervix and ovaries, neuroblastoma, retinoblastoma and other neoplasm's of childhood [5,6,7]. CTX is metabolised to active toxic metabolites, acrolein and phosphomide mustard (PAM). PAM binds to the DNA and disturbs the fundamental mechanism concerned with cell proliferation; in particular DNA synthesis, transcription and cell division. The effects of the CTX, although dependent on proliferation, are not cell-cycle-specific, and it may act on cells at any stage of the cycle. Additionally, CTX is highly leukemogenic, so it causes deletions of chromosomes and well-known mutagen in many groups of animals in vivo and in vitro [8,9,10].

CTX, either alone or in combination with other drugs, has been shown to produce gonadal failure in humans and animals [5,6,11,12]. CTX causes infertility [11] and a 63% reduction in the number of small follicles in the ovaries of mice [13]. Furthermore, CTX increases the frequency of micronucleus and decreases cell number in a dose-related manner, due to chromosome aberrations thought to arise from chromosome breakage [3].

The aim of this study was to investigate the effects of CTX on the frequency of micronucleui, some biochemical parameters and ovarian and uterine histology.

MATERIALS AND METHODS

In this study, 20 Sprague Dawley mature female cycling rats, (aged, 2.5-3 months, weighing 200-300 gr.) were used. Prior to utilization of any animal, normal estrous cyclicity was confirmed by vaginal cytological smear quantitating the nuclear cytoplasmic ratio and the cellular characteristics. Regular cycling animals with 4- or 5- day estrous cycles were enrolled in our study and these rats were divided into 2 groups: **Group I(Control)** was injected with 0.3ml/a day physiologic saline (ip) . This injection was administered at the same time every day during the 3 weeks (21 days). **Group II(CTX)** was injected (ip) 50 mg/kg /a day loading dose of CTX for the first two days of the experiment. Then, a 5 mg/kg/a day treatment dose of CTX for the remaining 19 days.

The animals were kept in an air-conditioned room and fed ad libitum. At the end of the study, urine was collected during a 24 hour period from all animals. Under ether anaesthesia, blood was collected by cardiac puncture into heparinized tubes. Ovarian and uterine tissue samples were collected into neutral formaline.

Micronucleus assay on rat blood:

Blood culture was prepared by adding 0.5-0.8 ml heparinized blood obtained from the rats, to 5 ml supported RPMI 1640 medium (85 ml RPMI 1640 with L-glutamine, 15 ml fetal bovine serum, 1 ml heparin, 1.2 ml penicillin/streptomycin and 1 ml phytohemagglutinin) in a culture tube. The culture tubes were incubated at 37°C under 5% CO_2 atmosphere for 72 h. After a 44 h. incubation, 150 µl of cytochalasin B was added to each tube. After a total of 72 h incubation, the cultures were transferred to centrifuge tubes and santrifuged at 1300 rpm for 8 min. Supernatant was removed and added 5 ml of prewarmed 0.075 M KCl into each tube and then the tubes were incubated at 37°C for 25 min. After the incubation period, the tubes were centrifuged at 1300 rpm for 8 min. Supernatant was removed, mixed thoroughly and added to 5 ml of fresh fixative made up of 1 part acetic acid to 3 parts methanol. This step was repeated four times. At the end of the harvesting process, four slides were prepared from each animal. The slides were stained with 5% Giemsa solution. 10 animals were used from each group and 1000 cells per sample were screened for micronucleus frequency.

Serum and urine biochemical parameters were measured spectrophotometrically with UV. 1600 Shimadzu.

Paraffin sectioned (4µm) ovarian and uterine tissue samples were stained with haematoxylin eosin. All of the slides were examined with an Olympus Pm 10 ADS photomicroscope.

For the determination of statistical significance t-test analysis and Mann-Whitney U test was used.

RESULTS

After the microscopic examination, the increase of micronucleus frequency in group II (cyclophosphamide injected rats) was found significantly with respect to the control group ($P<0.001$)(**Table I**).

In the II^{nd} group, serum SGOT ($P<0.001$) and SGPT ($P<0.001$) activities, urine creatinin ($P<0.001$), creatinine clearance ($P<0.001$), urea nitrogen ($P<0.01$), uric acid ($P<0.05$), urine volume ($P<0.05$) and pH level ($P<0.05$) were significantly increased according to the control group (**Table I**).

Histologically in the II^{nd} group, low numbers of primary follicles (**Figure 1**), a large amounts of corpus luteoms (**Figure 2**) and single-layered epithelium formation were observed .

In the II^{nd} group, uterinal gland tissue was decreased according to the control group (**Figure 3**). It was determined that uterus endometrium was stopped at the proliferation phase and didn't pass into the secretion phase.

DISCUSSION

Cyclophosphamide (CTX) has been used extensively for the treatment of different

cancers. Because CTX generates to the cross links in the DNA chain, it causes an increase of micronucleus counts [9,10]. CTX is increased to micronucleus formation on the rat bone marrow [3,14,15,16] and human lymphocyte culture [17]. Proudlock R. J. et al. indicated that CTX increased the micronuclei density of the rat bone marrow and peripheral blood, and they found a relatively low number of micronucleated cells in rat blood rather than bone marrow cells [18]. Our study results are in agreement with these results.

Ghooh S. et al. reported that activities of SGOT and SGPT were elevated significantly in the liver, kidney and serum after cyclophosphamide treatment [19]. The other study (Ghosh S. et al.) also reported raised SGOT and SGPT levels raise in a patient, reported with small cell lung cancer, who was treated with cyclophosphamide, vincristine and etopside [20]. We determined a significant increase in the serum SGOT, SGPT activities according to the control group.

Funauchi M. et al. investigated the effects of 500 mg/m^2 cyclophosphamide on the creatinin clearance levels in 11 lupus nephritis patients. They found that creatinin clearance increased in one group of patients (n=7), while it decreased in the other group (n=4) after 6-9 courses [21]. In another study, serum creatinine levels were found to be increased in 20 acute myelocytic patients following 6-8 months of treatment with CTX, vincristine and prednisolone [22]. In the study of Bokser et al's, Cyclophosphamide was given at a loading dose of 50 mg/kg followed by 5 mg/kg/day for 30 days. At the end of this study (after 3 months), the number of follicles decreased by half with CTX [6]. In the Ataya et al's study, after the rats were suppressed with GnRH, CTX was given for 21 days. At the end of treatment, CTX produced a significant reduction in the total number of follicles, according to the control and CTX + GnRHa groups [5]. In the present study, a few primary follicles and a large number of the corpus luteum were observed in the ovaries, and decreasing gland and endometrium proliferation were determined in the uterus tissue of the IInd group.

As a result, although there is a remedial effect of the chemotherapeutic drug CTX, it caused an increase of the micronuclei density, serum SGOT, SGPT, creatinin clearance, urine creatinine and urea nitrogen levels. In addition to these, it caused a decrease in follicle counts. Therefore, the dosage and administration frequency of CTX used for therapy is selected carefully.

Table I. Frequency of micronucleus some serum and urine parameters(Mean±SD)

GROUPS	n	Frequency Of micronucleus (Per 10000 cells)	SERUM			Creatinie Clearence (mg/dl)	URINE				
			SGOT (u/l)	SGPT (u/l)	Creatinine (ml/min)		Creat. (mg/dl)	Urea N (mg/dl)	Uric Acid (mg/dl)	Volume (ml)	pH
I Group (control)	10	15.5±3/3	11.22±4.18	4.55±2.4	6.22±0.47	0/01±0/00	21.23±4..	612.6±	0.25±0.19	5.98±0.81	9.14±0.11
II Group (CTX)	10	37.6±5.06 ***	57/8±12.93 ***	21.5±11.	5.86±0/41	0.04±0.02 ***	49.38±11 ***	1223.2± **	0.67±0.55* *	8.22±2.4 *	8.27±1.1 *

*:p<0.05 **:p<0.01 ***:p<0/001

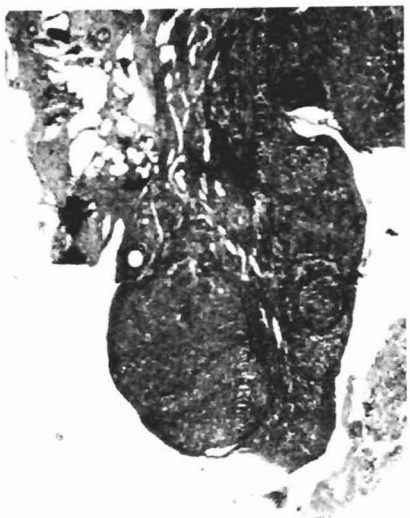

Figure. 1. Low numbers of primary follicles in the ovary II[nd] group (H&E, x 13,2).

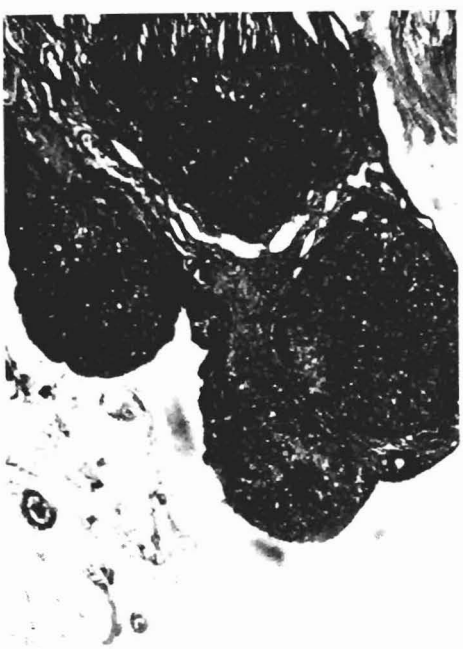

Figure. 2. A large amount of corpus luteums in the ovary II[nd] group (H&E, x 13.2).

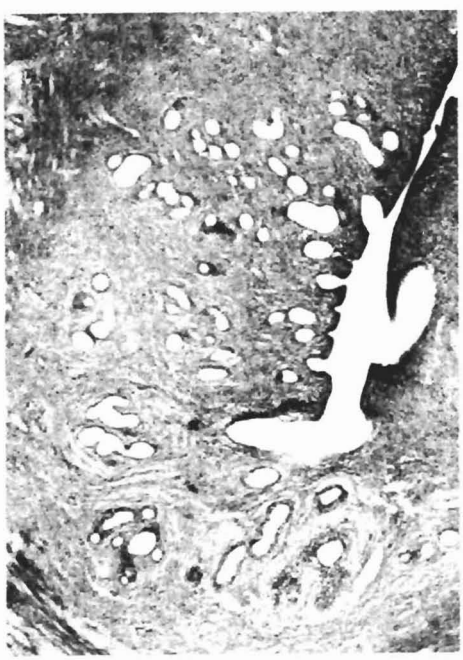

Figure. 3. Decreased uterinal gland proliferation the uterus in the II[nd] group (H&E, x 33).

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