THE EFFECT OF MITOMYCIN C ON SOME IMMUNOLOGICAL AND CYTOGENETICAL PARAMETERS IN ALBINO MALE MICE

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Abstract

The present study was carried out to shed light on the effect of Mitomycin C on some immunological and cytogenetical parameters in albino male mice. The effects of the drugs were investigated after one day treatment with a single dose(0.005 mg/ml). All treatments were paralleled by controls. The results revaled that the dose of MMC significantly decreased the total count of leucocytes, lymphocytes and neutrophils, while the monocytes and eosinophils showed no significant differences, as compared to controls.Such observation was positively correlated with the phagocytic index and plague forming cells. Moreover, the mitotic index showed significantly decreased, as compared with controls. In contrast, the spontaneous formation of micronuclei and chromosomal aberration in the bone marrow cell was significantly increased. The results showed that the MMC had immune suppressive effect. Also, the genotoxic and mutgenic effects was observed.

Introduction

That certain chemicals have different toxicities with respect to the various stages of the cell division cycle has been observed in several types of mammalian cells in culture (1). In general, many anticancer drugs are shown to be mutagenic and carcinogenic due to their ability to chemically modify DNA(2). The Mitomycin C is a family of aziridin-containing natural products isolated from streptomyces (3), it was first isolated by wall associates in 1958 (4). It has antibiotic and cytotoxic cancer chemoth-erapeutic action used in the clinical treatment of several human malignancies (5, 6). In addition to cancer therapy, MMC is a well-recognized antifibroblastic drug (7). The compound is heat stable, has a high melting point and freely soluble in organic solvents (8). The present study was designed with the aim to evaluate the Mitomycin C effect on some immunological and cytogenetical parameters in albino male mice.

Materials and Methods

Albino male mice (Mus musculus) were the tested animals, which were 9-10 week old at the beginning of experiments, and during the experiments, they had free access to water and food (ad libitum).

Mitomycin C was obtained from (Sigma Chemicals, USA) at concentration of (20 mg) MMC was prepared by dissolving (2 mg) Mitomycin C powder in (4 ml) of distilled water to make a stock solution , and from this solution (1 ml) was taken and dissolved in (99 ml) of distilled water to make the concentration of (0.005 mg/ml) to be used in mouse studies , and then sterilized by filtration and kept at (4°C) until being used .

The animals in this experiment were treated with dose of MMC in a short time. In the first experiments , the animals (number =12) were given orally a single dose of MMC for one day , and in the next day, they were dissected to assess RBC's, total and differential counts of leucocytes and phagocytic index (PI) . In the second experiments, the animals (number-12) were given a single dose of MMC for one day, and in the next day, they were dissected to assess mitotic index(MI)micronucleus formation (MN) and chromosomal aberration (CA). The latter two experiments were paralleled with two control groups ,in which the MMC was replaced with distilled water for each experiment.

Total and differential counts of leucocytes, RBC'S, phagocytic index (PI) and plague forming cells were the parameters of immunological evaluations. These experiments were done

according to Hudson and Hay (9). For mitotic index (MI), the cells were obtained from the bone marrow of animals after treatment with colchicin and at the same time the chromosomal aberrations were determined in 25 well-spread metaphase (10).

The micronucleus formation was examined in bone marrow cells that were obtained from the femur of animals (11).

Statistical Analysis

Differences between means were assessed by the least significant difference (LSD) by employing the computer programme SPSS. The difference considered significant if the probability level was less than 0.05.

Results and Discussion

Table (1) shows the changes in R.B.C's count in mice treated with MMC, the R.B.C's numbers were decreased non significantly ($P<0.05$) it reached (6.17 cells /cu.mm.blood) when compared with controls (6.95 cells/cu.mm.blood). Also, the number of total leucocyte count decreasing significantly in group treated with MMC (5.8 cel Is/cu. mm. blood) when compared withcontrols (7.8cells/cu.mm.blood). Similar decrease was observed in the count of lymphocytes neutrophils, monocytes and eosinophils in the treated animals compared to control Table (1).

Table (1)
The effect of Mitomycin C on some immunological parameters in albino male mice.

Parameters		Mean + standard Error	
		Distilled water	Mitomycin C
Blood cell Count $\times 10^3$ ($c^{el}ls/$ cu-mm)	RBC	6.95+0.2	6.17+0.2
	WBC	7.8+0.11	5.8+0.30*
	Lymphocyte	5.3+0.40	3.6+0.46*
	Neutrophils	3.1+0.40	2.0+0.15*
	Monocyte	0.3+0.01	0.2+0.01
	Eosinophils	0.1+0.03	0.07+0.01
Phagocytic Index (%)		10.8+0.7	6.3±0.5*
Plaque Forming		36.0+4.8	29.0+3.8*

****significant difference from control (P<0.05).***

The phagocytic index decreased significantly ($P<0.05$) it reach (6.3%) in mice treated comparing with (10.8%), in controls Table (1). Also, Table (1) shows the changes of plaque forming cells (PFC) in group of mice treated with MMC, and a significant differences was revealed as compared to the controls.

The total count of leucocytes gives an overall picture of the immune system function, but the differential count may specify some function (12). However, the decrease counts of leucocytes in this study may be refect that MMC inhibition protein biosynthesis and it reduced mitosis of cells and that it inhibited the body's immune response.

Play an important regulation role in antigen processing and monokine production (13). In living creatures, which are exposed to a mutagen factor, the probability of defects is increased with severe inhibition to immune system (14). These results were agreed with the results of (12,15) who found that the MMC had cause reduction in leucocyte counts and differential count.

Table (2) have shown that dose of MMC caused a significant reduction ($P<0.05$) in mitotic index (5.7) after one day treatment when compared with controls. The MMC induced the formation of micronuclei in the bone marrow cell, and reached a mean of 12.11% , which was significantly higher than the spontaneous formation of such micronuclei in the with MMC showed a high frequency of chromosomal aberration (6.3%) difference was revealed as compared to controls Table (2).

Table (2)
The effect of Mitomycin C on some cytogenetical parameters in albino male mice.

Parameters	Mean + Standard Error	
	Distilled water	Mitomycin C
Mitotic Index	9.0+0.8	5.7+4*
Micronucleus(%)	0.0	12.11+2*
Chromosomal Aberrations(%)	0.0	6.3+8.

****significant difference from control (P<0.05).***

Many studies reveal that Mitomycin C has genotoxic properties in bacteria in mammalian cells in vitro in Drosophila melanogaster and in mammals in vivo (16,17). However, in our study, the results showed that the mean value of the MI rate of mice treated was (5.7%). This is lower may be related to the proteins required for mitosis which were not produced at the same quantities, or the drug may cause the death of bone marrow cells, or the mitotic activity of the cell which affected with MMC could not that MMC increase micronuclei and chromosomal aberration, these results were agreed with the results of AL_Halbosiy et al.(20) who found that MMC had cause reduction in MN and CAs of mouse bone marrow cells.

Mitomycin C has been found to be carcinogenic in rats and mice. At doses the recommended clinical dose in man, it produces a greater than 100 percent increase in tumor incidence in male Sprague-Dawley rats, and a greater than 50 percent increase in tumor incidence in female Swiss mice (21).

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الخلاصة

لقد أجريت هذه الدراسة من اجل تسليط الضوء على بعض التأثيرات المناعية والوراثية لعقار المايتومايسين سي على خلايا نقي العظم في ذكور الفئران البيض. قيمت هذه التاثيرات بعد يوم واحد من المعاملة بالعقار ولجرعة. اظهرت النتائج بان العقار كان فعالاً في خفض العدد الكلي والتفريقي لخلايا الدم البيض والبلعمة والخلايا المكونة للويحات معنوياً .كذلك اظهرت النتائج انخفاضا معنوياً في معامل الانقسام مقارنة بالسيطرة. وبالمقابل فان الدراسة سجلت ارتفاعاً في التردد التلقائي لمعدل بكوين النوى الصغيرة ومعدل الزيغ الكروموسومي. اظهرت النتائج امتلاك عقار المايتومايسين سي تاثير مثبط للجهاز المناعي بالاضافة الى تاثيرات سمية وتطفيرية.

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