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# Long Term Teratogenic Effects of Mitomycin-C on the Second Gestation in Mice

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**Abstract.** The effects of a single injection of Mitomycin-C (MMC) on the fertility of female mice treated during the first pregnancy and its possible long term teratogenic effects on the following pregnancy have been studied. The drug was proven to be deleterious, teratogenic and growth suppressing to offspring of the first pregnancy ($F_{1A}$) and to embryos and fetuses of the second pregnancy ($F_{1B}$). The results also indicated that the effect of the drug was dose-dependent and a dose of more than 2.0 mg/kg MMC, significantly affected the parameters investigated. Treatment with MMC resulted in a decrease of mean litter size and body weight and an increase in exencephaly, abnormal growth of tail and hindlimb and umbilical hernia in the fetuses of the second pregnancy ($F_{1B}$) in a dose-dependent fashion. A precautionary delay in conception by humans desiring a child may be indicated from this study following MMC chemotherapy.

## Introduction

Mitomycin-C (MMC), also known as mutamycin or mitomycin, is a growth inhibitor and potent antitumor agent [1-7]. It has been reported to be cytotoxic, teratogenic and mutagenic [8-13]. MMC also produces sister-chromatid exchanges in bone marrow and testis of rats and mice and in human lymphocytes following *in vivo* treatment [14, 15].

Several studies have shown that when MMC is administered to pregnant rats [16, 17], mice [18-23] and chickens [13, 24], it produces teratogenic, lethal, growth suppressing and/or curative effects. The frequency and types of defects produced by MMC were both dose and time dependent. Moreover, MMC has been shown to have an antifertility effect on $F_1$ generation obtained from treated pregnant female mice

[20]. Since MMC is still in use as a chemotherapeutic agent in Saudi Arabian hospitals, the present study was undertaken to investigate the effects of MMC on fertility of female mice treated during the first pregnancy, and the possible long term teratogenic effects of such treatment on the following pregnancy.

## Materials and Methods

Adult Balb/c male and female mice used in this study were obtained from the Animal House, King Faisal Specialist Hospital and Research Center, Riyadh. The animals were housed in plastic boxes in an environmentally controlled room with a temperature of 22±1°C, a humidity of 45±5% and a light/dark cycle of 14/10 hrs. Mouse food (commercially available in Saudi Arabia) and water (via bottle) were offered *ad libitum* throughout the study period. In each box, 3-4 nulliparous females were caged together with a single male until a vaginal plug was found, then the female(s) was separated.

The day the plug was observed was counted as day 0 of gestation. On day 9 of gestation, dams (N= 5-16/group) were injected intraperitoneally (ip) with a single dose of 1.5, 2.0, 2.5, 3.5, 6.0, 8.0 or 10.0 mg/kg MMC solution (Bristol, Evansville, IN, U.S.A.) in sterile normal saline. Injection with MMC on gestational day 9 has been shown to induce malformations of viable fetuses [23]. A control group of mice was injected ip with an equivalent volume of normal saline. At parturition, the number of live and dead pups was recorded for each pregnant mouse. Each pup was examined macroscopically, both externally and internally for gross abnormalities.

The fertility of these dams was evaluated again by recaging them with males on day 10 of lactatoin. The commencement of pregnancy was determined as mentioned before, and the dams were then kept under daily observation. On gestational day 17, the dams were killed by cervical dislocation and the number of live and resorbed fetuses was recorded for each pregnant mouse. Each fetus was examined for gross abnormalities as performed earlier.

Data were analyzed statistically using SAS computer program and a 2×2 contingency table ($X^2$) for the actual numbers obtained [25].

## Results

The effects of MMC over two successive matings of one generation of mice ($F_{1A}$ and $F_{1B}$) are shown in Tables 1-3. Table 1 indicates that treatment with MMC at doses $\geq$2.5 mg/kg significantly ($P \leq 0.01$) decreased both the mean litter size and

body weight in offspring of the first mating. There was a strong negative correlation between MMC dose and mean litter size (r = –0.61), and between MMC dose and mean litter body weight (r=–0.74). Administration of ≥2.0 mg/kg MMC significantly (P≤0.01) increased the mean number of pup deaths at or shortly after birth and there was a moderate positive correlation (r=0.41) between MMC dose and the mean number of pup deaths. MMC treatment also resulted in abnormal development of tails and hindlimbs in surviving pups (Table 1).

**Table 1. Effect of MMC treatment on day 9 of gestation of the first mating ($F_1$)**

| MMC dose (mg/kg) | No. of females | Litter size Mean+(Range) | Litter body wt. (gms) (mean±S.E.) | No. of dead pups per litter Mean + (Range) | % of abnormalities observed $ |
|---|---|---|---|---|---|
| Control | 35 | 9.80(4-14) | 1.39±0.04 | 0.43(1-3) | None |
| 1.5 | 15 | 8.73(6-12) | 1.35±0.06 | 1.60(1-7) | 16T |
| 2.0 | 15 | 9.20(4-13) | 1.23±0.06* | 3.47(1-10)** | 75; 5L |
| 2.5 | 16 | 6.69(3-13)** | 1.12±0.06** | 6.56(2-13)** | 100T; 20L |
| 3.5 | 13 | 6.46(3-10)** | 1.13±0.07** | 6.46(3-10)** | 100T; 30 L |
| 6.0 | 16 | 3.19(4-12)** | 0.76±0.06** | 2.94(4-12)** | 100T |
| 8.0 | 15 | 5.87(2-13)** | 0.81±0.06** | 5.87(2-13)** | 100T |
| 10.0 | 5 | —— | —— | —— | —— |

* Statistically different from the control at p ≤ 0.05
** Statistically different from the control at p ≤ 0.01
$ T = Abnormal tails L = Abnormal hindlimbs

The effect of the treatments on dams in their second pregnancy is shown in Table 2. There was no significant difference in duration of gestation in the < 10 mg/kg groups. However, duration of gestation in the 10 mg/kg group was significantly (P≤ 0.05) longer than the control. There was a very weak positive correlation between MMC dose and duration of gestation (r=0.07). Animals treated with doses ≥ 6.0 mg/kg of MMC showed a significant (P≤ 0.01) decrease in mean body weight gain during the period from day 0 to day 17 of the second mating. There was a strong negative correlatoin between MMC dose and mean body weight gain (r = –0.5).

As shown in Table 3, animals treated with ≥ 3.5 mg/kg of MMC showed a significant (P ≤ 0.01) decrease in both the mean number of implantation sites and mean number of live fetuses observed on day 17 of the second mating. There was a moderate negative correlation (r = –0.34) between MMC dose and mean number of implantation sites, and a strong negative correlation (r=–0.56) between MMC dose

and mean number of live fetuses. The mean body weight of live fetuses was significantly ($P \leq 0.01$) lower in dams which were treated with doses of 2.0, 2.5 and 3.5 mg/kg of MMC than the control. Moreover, abnormal development was observed in fetuses obtained from the second mating of dams treated with MMC. The incidence of exencephaly, abnormal tails, abnormal hindlimbs and umbilical hernia was increased in dose dependent fashion as shown in Table 3.

**Table 2. Effect of MMC treatments on the time required for mating and on the body weight gain during the period from day 0 to day 17 of the second mating**

| MMC dose (mg/kg) | No. of females | Time required for mating in days (mean ± S.E.) | Body wt. gain (gms) (mean ± S.E.) |
|---|---|---|---|
| Control | 35 | 3.60±0.49 | 20.22±0.79 |
| 1.5 | 15 | 4.60±0.75 | 17.15±1.20 |
| 2.0 | 15 | 4.53±0.75 | 17.53±1.20 |
| 2.5 | 16 | 2.94±0.73 | 17.71±1.16 |
| 3.5 | 13 | 2.23±0.81 | 17.64±1.29 |
| 6.0 | 16 | 4.81±0.73 | 13.21±1.16** |
| 8.0 | 15 | 4.13±0.75 | 14.55±1.20** |
| 10.0 | 5 | 6.40±1.30* | 2.28±2.08** |

*Statistically different from the control at $p \leq 0.05$
**Statistically different from the control at $p \leq 0.01$

**Table 3. Effect of MMC treatment on fetuses taken on day 17 of the second mating**

| MMC dose (mg/kg) | No. of females | No. of implantation sites (mean ± S.E.) | No. of live fetuses (mean ± S.E.) | % Resorption | Body wt. of live fetuses (gms) (mean ± S.E.) | % of abnormalities observed $ |
|---|---|---|---|---|---|---|
| Control | 35 | 11.49±0.34 | 11.06±0.47 | 3.7 | 0.77±0.03 | 1.0E |
| 1.5 | 15 | 10.00±0.51** | 9.40±0.72* | 6.0 | 0.66±0.05 | 2.0E |
| 2.0 | 15 | 10.53±0.51 | 10.13±0.72, | 3.8 | 0.59±0.05** | None |
| 2.5 | 16 | 10.44±0.50 | 10.06±0.70 | 3.6 | 0.59±0.05** | 1.2E;0.6T; 0.6L |
| 3.5 | 13 | 9.77±0.55** | 8.77±0.78** | 10.2** | 0.63±0.05** | 2.4E;2.4L |
| 6.0 | 16 | 9.50±0.50** | 6.13±0.70** | 35.5** | 0.73±0.08 | 1.3E;3.3H; 1.3T;5.9L |
| 8.0 | 15 | 10.20±0.51* | 7.33±0.72** | 28.1** | 0.76±0.05 | 5.2E;0.7T; 0.7L |
| 10.0 | 5 | 7.40±0.89** | 0.00±0.00** | 100.0** | —— | —— |

* Statistically different from the control at $p \leq 0.05$
** Statistically different from the control at $p \leq 0.01$
$ E=Excencephaly; T=Abnormal tails; L=Abnormal hindlimbs; H=Umbilical hernia

## Discussion

The present study demonstrated the deleterious, teratogenic and growth suppressing effects of MMC on litters obtained from dams treated with the drug during the first pregnancy. Similar observations have been reported by several authors in various species [16-24 and 26-28].

The results of the present study also indicate that MMC has a long term effect on fertility and can influence the outcome of the second pregnancy of treated dams. The disruption of implantation, the increased number of resorbed and dead embryos and fetuses and the teratogenic effects observed in fetuses of the second mating are indicative of this long term effect of MMC. It is possible that some of the amounts of MMC in the treated females might not be inactivated or eliminated fast enough, particularly in those treated with larger doses of the drug, and the amounts remaining could be the major factor affecting the parameters investigated in the second pregnancy. An antifertility effect of MMC on $F_1$ generation obtained from treated female mice has been reported [20]; histologically the testes of $F_1$ mice were deficient in germ cells and possessed many tubules containing Sertoli cells only, while females had small ovaries with about half as many follicles as the controls. As far as we know, this is the first report of such antifertility and teratogenic effects of MMC on the second pregnancy of treated females.

It is known that MMC inhibits DNA synthesis *in vitro* and *in vivo* and such inhibition of DNA synthesis in rapidly proliferating embryonal tissues could be a major initiating factor in the malformations and lethality produced by MMC [6, 7, 13]. Hence, the malformations and lethality produced in the present study may be the results of MMC action on DNA synthesis in rapidly proliferating embryonal tissues.

Therefore, it is concluded that MMC has toxic, growth suppressing and teratogenic effects on the offspring of first gestation as well as on the embryos and fetuses of the second gestation. However, further studies are needed involving detection of MMC or its metabolite(s) in dams during the second pregnancy and/or in the following generation(s). A similar study of MMC injected into only male mice and follow ups of male fertility, and any growth suppressing and teratogenic effects in offspring would also be of interest.

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# التأثيرات التشويهية طويلة الأمد لعقّار الميتومايسين على فترة الحمل الثانية للفئران المخبرية

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(سُلِّمَ في ٦ جمادى الآخرة ١٤١٣هـ، وقُبل للنشر في ٢٥ ذي الحجة ١٤١٣هـ)

**ملخص البحث.** لقد تمت في هذا البحث دراسة تأثير عقار الميتومايسين على خصوبة إناث الفئران المخبرية Balb/c وذلك بمعالجتها بالحقن في التجويف البطني بالجرعات ١,٥، ٢,٠، ٢,٥، ٣,٥، ٦,٠، ٨,٠، أو ١٠,٠ مغم/ كغم من وزن الجسم، والمحضّرة في محلول مِلْحي فسيولوجي، خلال اليوم التاسع من الحمل الأول. كما تمت دراسة التأثيرات التشويهية طويلة الأمد المحتملة لهذا العقّار على الأجنّة المتحصل عليها من الحمل الثاني. ولقد أوضحت نتائج هذه الدراسة أن لعقّار الميتومايسين تأثيرًا سامًا ومشوهًا ومثبطًا للنمو على أفراد نسل الولادة الأولى وعلى الأجنّة المتحصل عليها من الولادة الثانية. فقد برهن العقّار أن له تأثيرًا مُشَوهًا بعيد المدى حيث أدى إلى زيادة ملموسة في نسبة تَشَوُّهات تعرية المخ وتشوهات الذيل والأرجل الخلفية، وكذلك في نسبة حدوث فتق الحبل السري في أجنّة الولادة الثانية. وأن كل تلك التأثيرات تزداد بصورة اضطرادية مع زيادة الجرعة المعالجة بها الأمهات خاصةً من الجرعة ٢,٠ مغم/ كغم من وزن الجسم.