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THE EFFECT OF MITOMYCIN C ON POSTOPERATIVE PERITONEAL ADHESIONS IN MALE RATS

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Abstract--*Postoperative peritoneal adhesions contribute to high morbidity and mortality rates. Fibroblast proliferation is one of the risk factors. Mitomycin C (MMC) is known to inhibit fibroblast proliferation. We herein evaluate the effect of MMC on the peritoneal adhesion in the animal model of peritoneal irrigation.Sixty-four male Wistar rats underwent a midline incision laparotomy and then were randomly assigned to eight groups. The peritoneal cavity was rinsed with injectable normal saline (NS), MMC at doses of 0.5 or 1 mg/kg with or without NS, followed by an immediate suction. The peritoneal cavity in three groups was rinsed with NS or MMC at doses of 0.5 or 1 mg/kg for 3min before suction. After 6 weeks, a laparotomy was again performed on each rat andthe adhesions were scored macroscopically withCanbaz scoring system.Animals treated with immediate MMC 1 or 3 mg/kg had the lowest incidence of postoperative adhesion. The adhesions in groups receivedeach dose of MMC, with or without saline,were significantly reduced compared with the saline group. The adhesion score was significantly lower in MMC groups (0.5 and 1mg/kg) vs. each dose of MMCs +saline or each dose of MMCs for 3min. Notably, the adhesion score was not reduced with MMC 1mg/kg (3min) compared with NS (3min), while adhesions were significantly decreased with MMC 0.5mg/kg (3min). There was no difference between NS and NS (3min).Immediate peritoneal rinsing with MMCduring the laparotomy seems to be effective in preventing the formation of postoperative adhesions.*

Keywords: *Mitomycin C, postoperative, peritoneal adhesions, Rat*

I INTRODUCTION

There are many cases of abdominal surgeries around the world for various reasons. Peritoneal adhesion is one of the common complications of these surgeries. After surgery, a layer of fibrin covers all the surfaces in the abdomen, which is exacerbated by a foreign body (sterile gas, talcum powder, gloves, suture residue, etc.) and ischemia(1).

After a while, plasminogen is secreted and destroys the fibrin strands.For unknown reasons, plasminogen secretion may be suppressedand the fibrin strands not be degraded resulting in migration of fibroblasts to the injury site and formation of fibrous adhesion bands. Abdominal adhesions occur in 1/4 of patients undegoingabdominal surgery, especially extensive surgery, pelvic surgery or multiple abdominal surgeries. They

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are the reason for asymptomatic inflammation in 1/3 of cases foundin an autopsy, and can kink, twist, pull, or compress the intestines and other abdominal organs contributing to significant complications and symptoms. Accordingly, abdominal adhesions are the most common cause of small bowel obstruction and may cause chronic abdominal or pelvic pain impairing one's quality of life. They also seem to be associated with infertility in 20-25% of patients(1-3).

Unlike recent advances in surgical techniques, there is no effective strategy to prevent postoperative adhesions. Application of solid or liquid physical barriers (e.g. oxidized regenerated cellulose, seprafilm, and polyethylene glycol)(3) and improving the intestinal microflora seem to be effective in reducing adhesion formation(4, 5). Systemic nonsteroidal anti-inflammatory drugs, corticosteroids, antihistamines, antibiotics, calcium channel blockers, colchicine, fibrinolytic agents, and vitamins have not shown satisfying results in human trials despite animal studies(6). Hence, there is still a need for innovating novel adhesion-reducing adjuvants.

Fibroblasts play a key role in adhesion formation, thus antifibrotic drugswill be effective in reducing abdominal fibrous bands(7). Mitomycin C (MMC) is an antimetabolite agent that was first extracted by Wakaki from Streptomyces caespitosus in 1985. It inhibitsthe synthesis of RNA, DNA, and proteins. Therefore, it is widely used to treat colorectal, stomach, cervix, breast, lung, head and neck and bladder cancers(8). Due to its known antifibrotic effects, it has been used in eye surgeries especially glaucoma surgeriesto minimize scar formation by inhibiting fibroblasts activity(9, 10). With the same mechanism, inhaled MMCpreventspostoperative scar-induced strictures in pediatric aerodigestive tract surgeries with minimal adverse effects(11). Interestingly, we have recently shown that MMCis effective to prevent the formation of postoperative peritoneal adhesions in rats(12). Here, we aim to extend our previous findings by evaluating the effect of MMC alone or with normal saline (NS) on reducing adhesions; and identifying the best time for peritoneal rinsing.

II Methods and Materials

Peritoneal adhesion model

The study was an experimental randomized controlled animal trial in which Sixty-four male Wister Albino rats weighting 250–300 g were randomly assigned to eight groups (Table 1). All the animals had unlimited access to food and water. The same surgeon performed all the surgical procedures. Animals were anesthetized with intraperitoneal (i.p.) injection of ketamine hydrochloride (30 mg/kg). A heating lamp preserved the body temperature during the surgery. Animals were allowed to breathe spontaneously. The abdomen was then shaved and prepared with povidine iodine solution before the 5-cm vertical midline incision through the abdominal cavity. Care was taken to avoid gross bleeding from the injured sites. The peritoneal space was rinsed with 10 ml of solution in accordance with the study group. Immediately or after three minutes, the solution was suctioned. The muscle-fascial layer and the skin were closed with a single layer continuous absorbable 4/0 and 4/0 silk suture, respectively. Then, the rats were evaluated for any postoperative complications daily during the first week and every three days thereafter for up to six weeks. No perioperative antibiotics or analgesics were used since there were no symptoms associated with infection or pain(12).

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Postoperative peritoneal adhesion scoring

The animals were anesthetized 6 weeks after surgery. A blinded observer did a laparotomy to explore the abdomen and score the severity of the peritoneal adhesions according to the Canbaz scoring system (Table 2)(13).

Statistical analysis

The primary outcome was a two grades reduction in adhesion formation. A sample size of 8 rats/group were sufficient to achieve an 80% power to detect a difference of two grades in the Canbaz scoring system. Statistical analyses were performed using SPSS 24 (Chicago, Illinois, United States). Numerical data were presented as mean ± standard deviation (SD). A T-test, one-way analysis of variance (ANOVA)and Chi-square test wereused to compare the mean Canbaz score and the incidence of adhesion formation between the study groups. A P value less than 0.05 was considered statistically significant.

Ethics Statement

All the experiments were conducted under the approved guidelines of Semnan University of Medical Sciences and Guide for the Care and Use of Laboratory Animals (NIH, 1985). The Animal Care and Treatment Committee approved this study.

III RESULTS

There was no case of wound dehiscence or death. Groups 3 (MMC 1 mg/kg, 2(25%)) and 6 (MMC 0.5mg/kg, 2(25%)) had the lowest incidence of postoperative adhesions compared with groups 1 (NS, 8(100%)) and 2 (NS 3min, 8(100%)) ($p<0.05$). The adhesion score was lower with immediate rinsing with MMC 1mg/kg vs. MMC 0.5mg/kg, while the difference was not significant. Additionally, our data showed that groups treated with immediate rinsing with both doses of MMC (1mg/kg=0.25±0.46, 0.5mg/kg=0.62±1.4) had significantly lower adhesion scores compared with NS (3.25±1.38), NS (3min)(3.62±1.06) and MMC 1mg/kg (3 min) (3±1.85). We thus suggest that prolonged exposure with a high dose of MMC increases the incidence and severity of peritoneal adhesions. The data also revealed that MMC (0.5 or 1mg/kg) concurrently with NS significantly decreased the adhesion score. However,this treatment did not significantly reduce the incidence of the formation of adhesions compared with the NS group (4 (50%) in MMC1mg/kg +NS or 5 (62.5%) in MMC0.5mg/kg +NS vs. 8(100%) in NS group, $p>0.05$). There was no significant difference in the severity and incidence of adhesions between rinsing with any dose of MMC concurrently with NS and MCC alone (Table 1).

IV DISCUSSION

Our findings show that immediate rinsing the peritoneum with MMC at both doses of 0.5 or 1 mg/kg was effective in reducing the adhesion band formation and its severity. Interestingly, the application of MMC with NS significantly decreased the severity of postoperative adhesions; although, it was not effective to reduce the incidence of adhesion band formation compared to the saline group. Moreover, the data represent no protective effects of 3min rinsing with any doses of MMC.

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Following an injury to the peritoneum and stimulation of the subsequent healing process beginswith an inflammatory responseinitiated bythe release of histamine and vasoactive agents resulting inincreased permeability of vessels, secretion of fibrin-rich fluid into the peritoneal cavity and the temporary fibrin matrix formation(14). Fibrin formation is suggested to be the end-product of the coagulation cascade when thrombin converts fibrinogen into fibrin remnants(15). The impaired balance between plasminogen activators and inhibitors disrupts the peritoneal tissue repair process. As a result, inflammatory cells such as monocytes and plasma cells migrate into the fibrous tissue and the subsequent proliferation of collagen-secreting fibroblastsorganizes the temporary fibrin matrix(6). Additionally, fibroblasts secrete angiogenic factors resulting in new blood vessel formation. Permanent adhesion bands are formed after these newly vascularized tissues are covered with peritoneum(6, 16).

MMC is found to induce the fibroblasts apoptosis, including human subconjunctival and corneal fibroblasts, and mouse embryonic fibroblasts (17) by which it seemsto be effective in reducing peritoneal adhesion formation. MMC is shown to significantly induce fibroblasts apoptosis leading to suppression of collagen production and epidural fibrosis after laminectomy. Downregulating the expression of miR-200b in human fibroblasts and activating the expression of the apoptotic proteins e.g. PARP and Bax are the mechanisms underlying MMC-induced fibroblasts apoptosis (17).

Due to its anti-fibrous effect, MMC 0.5mg/cc has been widely used in eye and aerodigestive system surgeriesto minimize the scar formation(11, 18). However,the application of MMC at the dose of 0.5mg/cc in the peritoneal cavity is fatal for animals. The peritoneum is a wide highly absorbable barrierso that the drugs should be administered at lower doses compared to eyeball(12). In line with previous experimental findings, in the two groups received MMC at doses of 0.5 and 1mg/kg, the severity and incidence of adhesions werereduced (7, 12)with no mortality or side effect was noticed. Furthermore, our data showed that the mixture of MMC+ saline was as effective as MMC alone in reducing the adhesion severity, while it was not effective in decreasing the incidence of adhesion formation. Therefore, rinsing with NS following the MMC application may decrease its efficacy.

MMC is an anti-tumor drug that can interfere with natural wound healing. Increased dose or duration of exposure to intraoperative MMC is suggested to have greater effectiveness while increases the risk of complications as well(19, 20). Adachi W *et al*.administered MMC and cis-diamminedichloroplatinum to patients with gastric cancer to inhibit peritoneal recurrence after radical surgery. Various degrees of extensive peritoneal adhesion were found in34.8% of patients who underwent relaparotomy (21). H Lee *et al.* showed that MMC has dose-dependent toxicity that significantly lowers cell viability with an increase in the dose(19, 20). Hence, intraoperative MMC is usually administered for a few minutes in eye surgeries (19). Our data also showed that increased duration of peritoneal rinsing with MMC (0.5 or 1mg/kg) to 3 min does not significantly reduce the severity of incidence of adhesion formation compared with controls. M Jansen *et al.* found that 0.67 mg/kg MMC adsorbed on 37.5 mg activated carbon increases adhesion formation in rats after laparotomy and small bowel anastomosis. They also found that MMC causes concentration-dependent cytotoxicity in vitro(22). The mixture of MMC with activated carbon could explain their discrepantfindings with MMC.

We conclude that peritoneal rinsing with MMC for few seconds prevents postoperative adhesion formation. Increased duration of exposure to MMC up to 3 min reverses its protective effects. Hence, additional research is

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needed to identify the optimum dose and a safe protocol for intraperitoneal MMC in humans as well as its long-term effects.

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Table 1: The incidence and severity (Canbaz score) of peritoneal adhesions

Group#	**Group** (n=8)	**Incidence rate** n (%)	**Significant difference groups *** (One way ANOVA)	**Adhesion score** (Mean±SD)	**Significant difference groups *** (t-test)
1	NS	8 (100%)		3.25±1.38	
2	NS (3min)	8 (100%)		3.62±1.06	
3	MMC 1mg/kg	2 (25%)	Group1 (NS) Group2 (NS (3min)) Group4 (MMC 1mg/kg (3min))	0.25±0.46	Group1 (NS) Group2 (NS (3min)) Group4 (MMC 1mg/kg (3min))
4	MMC 1mg/kg (3min)	6 (75%)		3±1.85	
5	MMC 1mg/kg+ NS	4 (50%)		0.62±0.74	Group1 (NS) Group2 (NS (3min)) Group4 (MMC 1mg/kg (3min))
6	MMC 0.5mg/kg	2 (25%)	Group1 (NS) Group2 (NS (3min)) Group4 (MMC 1mg/kg (3min))	0.62±1.4	Group1 (NS) Group2 (NS (3min)) Group4 (MMC 1mg/kg (3min))
7	MMC 0.5mg/kg (3min)	5 (62.5%)		0.75±0.7	Group1 (NS) Group2 (NS (3min)) Group4 (MMC 1mg/kg (3min))
8	MMC 0.5mg/kg+ NS	5 (62.5%)		0.62 ±0.51	Group1 (NS) Group2 (NS (3min)) Group4 (MMC 1mg/kg (3min))
Total		40 (62.5%)		1.59±1.25	

n, noun; SD, standard deviation; NS, normal saline; MMC, mitomycin C; min, minute

Table 2: Canbaz scoring system

Grade	**Observation**
0	No adhesions
1	One single adhesion
2	Mild adhesions
3	Moderate adhesions
4	Severe adhesion that adhere to abdominal wall

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