Effect of methylene blue on intra-abdominal adhesion formation in rats
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ABSTRACT
Background: Adhesion formation represents an important clinical challenge in gastro-intestinal surgery. In the late era, methylene blue (MB) has been claimed to play a helping role in combating such a problem. In the present study, we investigated the effects of MB administered intraperitoneally on the surgically-induced adhesions in rats and tried to clarify whether its impact is concentration-dependent.

Material & Methods: Seventy adult male Wistar rats were used. They were allocated into seven groups, ten rats each: one control, two sham-operated and four experimental ones. Peritoneal adhesions were evoked by scraping the cecal serosa in the four experimental groups which were given different concentrations of MB, intraperitoneally. The animals of the control group were similarly dealt but were given saline. The rats of the sham-operated groups were given either MB or saline but without any serosal damage.

Results: After the elapse of two weeks, the resulting adhesions, inflammation and fibrosis were evaluated. In the rats treated with MB 1%, adhesions, inflammatory process and fibrosis scores were similar to those in the sham-operated rats and were significantly lower than those in all other groups.

Conclusion: It could be concluded that MB, 1%, had the best anti-adhesion potential.

1. Introduction

The occurrence of peritoneal adhesions is a serious surgical postoperative drawback.2,3 These adhesions represent a major cause of intestinal obstruction and of primary and secondary infertility in women. Several treatment strategies have been claimed to reduce the incidence of such adhesions. These include peritoneal lavage with proteolytic enzymes, giving anticoagulants and agents that interfere with collagen deposition, application of tissue plasminogen activator to enhance the peritoneal fibrinolytic activity, prevention of tissues apposition by oils, polymers, or resorbable membranes and eventually induction of peristalsis by prostigmine. However, the expected benefits of such methods in lessening the peritoneal adhesions remain unclear.

In many ways, adhesion formation, more or less, simulates an inflammatory process and numerous mediators may be implicated in its pathogenesis and so the antioxidant and anti-inflammatory properties of vitamin E, glucocorticoids and aspirin, are postulated to hinder the adhesion formation.

Since its discovery as the first synthetic anti-malarial agent by Ehrlich in 1891, methylene blue (MB) has been utilized in several clinical fields, including the management of septic shock, renal stones and encephalopathy. It has been claimed that its intraperitoneal application may alleviate the postoperative adhesions.

The present study was carried out with an intent to throw light on the effects of different concentrations of MB on the process of peritoneal adhesions formation and also to define its minimum dose that can effectively prevent the formation of such adhesions.

2. Materials and methods

Seventy adult Wistar male rats, weighing between 300 and 350 g were used in the present study. They were handled in accordance with guidelines of animal welfare in the animal house of Mansoura Faculty of Medicine, Egypt. All animals were housed under the same conditions and were allowed food and water ad libitum. The animals were randomly categorized into seven groups, ten rats each. These groups were one control, two sham-operated and four experimental: 1, 2, 3 and 4. Talc-free gloves were used. The surgical field was prepared with 1% of antiseptic povidine-iodine solution. A 3–4 cm long midline abdominal incision was made; the cecum was mobilized and placed onto a wet gauze. Punctuate hemorrhages were generated by scraping the cecal serosa to induce adhesions in the animals of the four experimental groups. Then, MB solution 2 ml was instilled into the peritoneal cavity before closure, in an increasing concentration (0.5%, 1%, 5% and 9% to the groups 1, 2, 3, and 4 in succession). The control group rats were similarly dealt but were treated with saline 2 ml, intraperitoneally.
sham-operated rats had a similar laparotomy but without induction of adhesions. The animals of one of these sham-operated groups were treated with MB, 1% while those of the other, with saline. The abdominal incision was then closed in two layers with continuous 3/0 nylon. The rats resumed their preoperative routine until the fourteenth postoperative day, when they were killed by an overdose of ether. The abdominal cavity was inspected through a U-shaped incision and adhesions were identified, counted, and graded using the classification described by Nair et al.,\(^5\) Table 1, by two independent investigators, who were unaware of to which group the rats belonged. In addition, samples of adhesive bands, were analyzed microscopically. They were fixed in 10% neutral formalin for 24 h, dehydrated, cleared and embedded in paraffin wax. Paraffin sections were cut at a thickness of 6 μm and stained with hematoxylin and eosin (H&E). Histo-morphological findings were assessed with respect to the severity of interstitial fibrosis (IF) and inflammatory cell reaction (ICR). The extent of ICR was evaluated and graded on a scale as follows: 1 for mild, 2 for moderate and 3 for severe. The intensity of fibrosis was examined in 10 randomly-selected high power fields (HPF). The scores were between 0 and 4: 0, no fibrosis; 1, less than 10%; 2, 10–30%; 3, 30–50%; 4, above 50%. A comparison of the groups was carried out using the non-parametric Kruskal–Wallis test followed by Mann–Whitney U statistics, to detect the statistically significant differences among the groups. Data were presented as mean ± SD. Analysis was performed using SPSS version 12.

3. Results

All animals survived the experiment and reached the end-point of observation in an apparently healthy condition except two animals which died, one in group 4 (on the 1st postoperative day) and the other in group 1 (on the 4th postoperative day). The only noticeable change was that rats treated with MB developed green urine for the first postoperative day. The induced adhesions were well-formed, filamentous and broad. They existed between the peritoneum and the intestine. Several animals had loops of bowel joined by adhesions. Adhesion grades 2, 3 or 4 were considered to be substantial, while animals with grades 0 or 1 were considered to be adhesion-free. All the animals of the sham-operated groups had an adhesion index, lower than 0.6 ± 0.1, regardless of the intra-peritoneal treatment. This index for the control group was 2.6 ± 0.3, while those of the experimental groups 1, 2, 3 and 4 were 2.1 ± 0.2, 0.7 ± 0.1, 3.3 ± 0.3 and 3.8 ± 0.2 in succession (Fig. 1, Table 2).

All the animals of the sham-operated groups gained an inflammation score lower than 0.4 ± 0.1, while this score for the control animals was 2.1 ± 0.2 and those of experimental groups 1, 2,
3 and 4 were 1.8 ± 0.3, 0.5 ± 0.4, 2.7 ± 0.4, and 2.9 ± 0.3 in succession (Figs. 2 and 3; Table 2).

All the animals in the sham-operated groups got a fibrosis score, lower than 0.5 ± 0.1. This score for the control group was 2.7 ± 0.4, while those of experimental groups 1, 2, 3 and 4 were 1.9 ± 0.4, 0.7 ± 0.2, 3.7 ± 0.4 and 3.9 ± 0.3 in succession (Fig. 4, Table 2).

The findings of the MB, 1% rats (group 2) did not differ significantly from the sham-operated groups. However, it was significantly different (p < 0.001) from the other groups treated with MB and from the control. The differences between the MB, 0.5% (group 1) and the other groups were not significant (p < 0.07).

4. Discussion

Adhesions most frequently complicate the intra-abdominal operations. They may lead to intestinal obstruction and infertility.23 Although several manoeuvres have been alleged to lessen the adhesion formation including the use of anti-inflammatory agents, antioxidants, anticoagulants, fibrinolytics, and mechanical barriers, the golden standard has not been confirmed yet.24–26 MB has been utilized in numerous clinical fields including the management of both cyanide and nitrite poisoning as well as urinary tract infections.27 It antagonizes the effects of nitric oxide and it also competitively inhibits the reduction of molecular oxygen to superoxide by acting as an electron acceptor for xanthine oxidase. Thus, the use of MB is postulated to be an effective antioxidant in cases of ischemia/reperfusion injury.28 The mechanism by which MB exhibits its antioxidant effect appears to differ from that of “classical” antioxidants such as vitamin E in that MB primary effect seems to be a direct inhibitory effect on endogenous NADPH-consuming oxidase such as NADPH oxidase and xanthine oxidase,13 rather than a scavenging effect. MB has been used to induce lipid peroxidation in the presence of light by increasing singlet oxygen formation,29 and at least one published report indicates that extracellular MB leads to enhanced superoxide production.30

MB has been accepted as a relatively nontoxic and safe dye and doses up to 7 mg/kg have been used in humans with cyanide poisoning.27 In the current investigation, different concentrations of MB have been evaluated. The highest accepted dose is 9 mg/kg while the lowest one is 0.5 mg/kg.31 The present study demonstrated that MB has diverse effects on the formation of peritoneal adhesions depending on the dose utilized. While inhibiting adhesions at a dose of 1 mg/kg, MB promotes adhesions at higher doses. Our results also suggest that a lower concentration of that dye (MTB 0.5%) couldn’t inhibit the adhesion formation. The data of two previous studies on the effect of MB on formation of peritoneal adhesions are conflicting. While, Galili et al.21 claimed that intraperitoneal administration of MB decreases the incidence and extent

<table>
<thead>
<tr>
<th>Groups</th>
<th>Adhesion score (mean ± S.D.)</th>
<th>Inflammation score (mean ± S.D.)</th>
<th>Fibrosis score (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (MTB 0.5%)</td>
<td>2.1 ± 0.2</td>
<td>1.85 ± 0.3</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>Group 2 (MTB 1%)</td>
<td>0.7 ± 0.1*</td>
<td>0.5 ± 0.4*</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Group 3 (MTB 5%)</td>
<td>3.3 ± 0.3</td>
<td>2.7 ± 0.4</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>Group 4 (MTB 9%)</td>
<td>3.8 ± 0.2</td>
<td>2.9 ± 0.3</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>control Group</td>
<td>2.6 ± 0.3</td>
<td>2.1 ± 0.2</td>
<td>2.7 ± 0.4</td>
</tr>
</tbody>
</table>

*Statistically significant difference P < 0.05.

Fig. 2. Photomicrographs of inflammatory cell infiltration. (A), grade 1: A photomicrograph showing a mild fibrosis and inflammatory cell infiltration (H&E ×200). (B), grade 2: A photomicrograph showing a moderate fibrosis and inflammatory cell infiltration (H&E ×100).

Fig. 3. A photomicrograph showing grade 3 inflammation (H&E ×100).
of peritoneal adhesions. Prien et al. found that mice injected intraperitoneally with MB 9% developed adhesions, probably through the activation of macrophages.22 The findings of this current investigation can be considered as compatible with those two previous conflicting reports in the sense that it demonstrates the dual effect of MB depending on the dose utilized. Many studies concluded that the concentrations of MB of more than 1% increase adhesion formation.23 According to the present results, the effects of MB is dose dependent and the optimum being 1%. It has deleterious effects in higher or lower concentrations. These findings were clinically supported by those reported by Nadler et al. They reported that high concentrations of MB could produce pericardial anemia complicating the treatment of methemoglobinemia, haemolysis, reticulocytosis and slight anemia.34 Matsuoka et al. found that MB could induce contraction of isolated rabbit aorta by the release of endogenous nor-adrenaline. However, an intravenous dose of 1 mg/kg elicited increased vasoconstriction without increasing the heart rate, cardiac output, or oxygen delivery and consumption.35 MB is absorbed through the peritoneal lining as was evidenced, in the present work, by the greenish discoloration of the rats’ urine. So, remote effects on other organs may be expected, particularly if high concentrations were used. Further investigations on various groups of higher animals are to be done to draw a more vivid picture for the effects of MB on postoperative adhesions. However, on the level of the present study results, volume of 1% of the dye could play a beneficial role to diminish the induced adhesions.

References