

Methylene Blue Prevents Post-Surgical Adhesion Formation in the Rabbit

H. Hodjati, T. Kazerooni¹, N. Tanideh²

Abstract

Peritoneal adhesions are challenging in abdominal surgery. In spite of different kinds of treatments still no definitive and effective treatment is suggested. To determine the prevention effects of intraperitoneal (ip) methylene blue in post surgical adhesion formation 44 rabbits were randomly divided in two groups of equal number. Group I received an intra peritoneal injection of a 1% suspension of methylene blue diluted with Talc powder, and group II received ip injection of diluted Talc powder. Five months later both rabbits of groups were evaluated for severity of adhesion. The mean scores for adhesions for groups I was 0.9 ± 0.88 which was significantly lower than 3.72 ± 1.32 for group II ($p < 0.05$). It is therefore, concluded that intraperitoneal administration of methylene blue decreases the rate of post surgical adhesion formation in the rabbit.

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Keywords • Methylene blue • Talc powder • adhesion formation • rabbit

Introduction

One of the main problems arising after abdominal surgery is formation of post-surgical adhesion. Adhesion between loops of bowel may cause abdominal pain, whereas adnexal adhesion may impair fertility.¹ Numerous attempts have been made to reduce or prevent the incidence of peritoneal adhesions, but all have had limited success.² These include the removal of fibrin exudates by peritoneal lavage with or without the use of proteolytic enzymes, anticoagulants, steroids, etc. and cytotoxic agents.^{2,3} Adhesion formation in many ways is an inflammatory process with the release of cytokine.^{2,4,5}

Many drug regimens such as heparin, glucocorticosteroids, proteolytic enzymes, antibiotics, iodine solution and 32% dextran have been tried systematically and intraperitoneally to prevent adhesion formation.^{6,7} Methylene blue is also known to inhibit the generation of oxygen-derived free radicals.⁸ In this study the effect of methylene blue on the prevention of talc powder induced peritoneal adhesion formation has been investigated.

Material and methods

Forty-four mature white New Zealand, rabbits weighing 1.5-2kg were randomly divided into two groups of equal size. Experiments were performed in conformity with the University Research Council guidelines for conducting animal studies. The animals of group I received an intraperitoneal (ip) injection of a suspension of 1% solution of methylene blue (5mg/kg) and diluted talc powder (1g/kg) under sterile conditions,

Departments of General & Vascular Surgery, Obstetric & Gynecology,¹ and Pharmacology,² Shiraz University of Medical Sciences, Shiraz, Iran.

Correspondence:

Talieh Kazerooni MD,
Department of Obstetric & Gynecology,
Shiraz University of Medical Sciences,
Shiraz, Iran.

Tel/Fax: +98 7116288427

E-mail: kazeront@sums.ac.ir

whereas, group II only received a suspension of talc powder (1g/kg). Five months later, animals of both groups were killed painlessly (under deep anesthesia) and the severity of adhesion was evaluated after laparotomy. Adhesions were scored by our own definition as demonstrated in Table 1.

Table 1: Score grading used for the interpretation of the severity of the adhesion

Site of adhesion	Score
Subhepatic, or sub-diaphragmatic and liver, or left subdiaphragmatic	1
Inter bowel loops	2
Bowel loops and abdominal wall	3

The sum of the scores was calculated for each rabbit. For example, if a rabbit had intrabowel plus subhepatic adhesion formation, it got a score of three. Quantitative grading of adhesions was performed by two independent observers assigned to each group.

Statistical analyses

The score grading of absence or formations of adhesions were compared in both groups and their differences were statistically analyzed using Mann-Whitney U test and Chi-square.

Results

The total score of adhesions in groups I and II were 20 and 82 respectively, which was statistically significant ($P < 0.01$). After laparotomy, nine rabbits of group I had not any adhesions whereas, adhesion was present in all rabbits of group II. The mean of scores for adhesion in both groups were 0.9 ± 0.88 and 3.72 ± 1.32 respectively ($P < 0.05$).

Discussion

The result of this study is demonstrating that in this experimental animal model intraperitoneal administration of methylene blue decreased the incidence and extent of peritoneal adhesions. The highly significant protective effect of methylene blue is in contrast to the ability of the nitric oxide synthetase inhibitor in preventing peritoneal adhesion.^{10,11} It is possible that the interference of methylene blue with free-radical generation may be related to the decline in adhesion formation.² Adhesions are also formed after laparoscopic surgery, although it is not as common as after laparotomy.¹ In a recent study we bypassed other causes of adhesion formation such as trauma to visceral serosa by injection of methylene blue and talc powder in to the peritoneal cavity. Methylene blue is one of the safest anti-

adhesion drugs. Kluger, Gul and their colleagues showed that 1% concentration of methylene blue had the best anti-adhesion potential, while lower concentrations of methylene blue were only partially effective and its much higher concentrations may cause severe adhesions.^{12,13} On the other hand, methylene blue has been shown to be non-toxic and can be safely administered in a wide range of concentrations.¹⁴ This was demonstrated in a treatment protocol for human suffering from cyanide poisoning who received 1-2mg/kg methylene blue (repeat doses if needed) without developing any adverse effects.¹⁶ Prien et al. have demonstrated that high concentrations (9%) of methylene blue can activate macrophages and induce adhesion.¹⁴ Literature review showed only one reported case of inflammatory peritonitis with ascitis after methylene blue dye infiltration following a diagnostic laparoscopic procedure.¹⁵ The patient was treated empirically with corticosteroids.¹⁵ Prien et al. have compared the effect of methylene blue and indigo carmine on adhesion formation in a mouse model.¹⁴ They concluded that both of them appear to induce adhesion formation as well as macrophage activation in vivo. However, most of the literature review is in favor of the protective effect of methylene blue in relation to adhesion formation.^{8,9,11}

Conclusion

As shown in our study, no complications developed in any of the rabbits and although this is a preliminary study, it seems that this harmless substance may become an important preventive material for the surgery.

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