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The effect of methylene blue on peritoneal adhesion formation

Peritonda adezyon oluşumuna metilen mavisinin etkisi

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Background/aims: *Postoperative adhesions following abdominopelvic surgery may cause significant morbidity. In this study, the effect of different doses of methylene blue in the formation of adhesions and the role of allopurinol in revealing the possible mechanism of action was evaluated.* ***Methods:*** *Seventy five male Wistar-Albino rats weighing 200g-220g. were divided into five groups of 15 rats each. After laparotomy, cecal serosal abrasions were performed. In Group I, 1ml of saline was administered intraperitoneally, while Group II, III and IV were given 1mg/kg, 5mg/kg and 9mg/kg of intraperitoneal methylene blue respectively. In Group V after 1 ml of saline administration at surgery, allopurinol 30 mg/kg/ day was administered postoperatively for 14 days. On day 14, laparotomies were repeated. Adhesions were graded and tissue samples were taken from incisions and adhesions. Hydroxyproline contents representing adhesions were measured quantitatively.* ***Results:*** *Low dose methylene blue (Group II) caused inhibition of adhesion formation when compared with that of other groups ($p<0.05$). With a higher dose (Group III), this effect disappeared and methylene blue actually induced adhesion formation ($p<0.05$) at the highest dose (Group IV). Allopurinol treatment (Group V) also provoked adhesion formation when compared with Group I ($p<0.05$).* ***Conclusions:*** *Methylene blue has diverse effects on postoperative adhesions in a dose-dependent manner. While with low doses there is evidence of inhibition, it promotes adhesion formation at higher doses. As allopurinol also provokes adhesion formation, it is thought that the preventive role of methylene blue may not be due to its free oxygen radical inhibitor effect but the mechanism has yet to be clarified.*

Key words: Methylene blue, adhesion.

Amaç: *Abdominopelvik cerrahi sonrası gelişen adezyonlar büyük morbidite nedeni olabilir. Bu çalışmada adezyon oluşumunda metilen mavisinin çeşitli dozlarının etkisi ve olası mekanizmayı açıklamada allopurinolün rolü araştırılmıştır.* ***Yöntem:*** *200–220 gr ağırlığında 75 erkek Wistar-albino rat her birinde 15 ratın bulunduğu 5 gruba ayrılmıştır. Laparatomi sonrası çekal serozal abrazyon oluşturularak I. ve V. Gruba 1 ml serum fizyolojik II, III ve IV. Gruba sırasıyla 1mgr/kg, 5mgr/kg ve 9mgr/kg metilen mavisi intraperitoneal olarak verilmiştir. V. Grup ayrıca 14 gün süreyle 30 mgr/kg/g allopurinol almıştır. Ondördüncü gün relaparatomi yapılarak adezyonlar sınıflandırılmıştır. İnsizyon ve adezyonlardan doku örnekleri alınarak kantitatif ölçüm amacıyla hidroksiprolin düzeylerine bakılmıştır.* ***Bulgular:*** *Düşük doz metilen mavisi (Grup II) diğer gruplara göre adezyon oluşumunu önlemektedir ($P<0.05$). III. grupta bu etki ortadan kalkmakta hatta metilen mavisi adezyon oluşumunu en yüksek dozda (Grup IV) arttırmaktadır. Allopurinol alımı (Grup V) I. Grupla karşılaştırıldığında adezyon oluşumunu provoke etmektedir ($P<0.05$).* ***Sonuç:*** *Metilen mavisi doza bağımlı olarak postoperatif adezyonlarda farklı etki göstermektedir. Düşük dozlarda adezyon oluşumunu inhibe etmekte, yüksek dozlarda ise arttırmaktadır. Allopurinolün adezyon oluşumunu arttırması nedeniyle metilen mavisinin koruyucu etkisinin bu maddenin serbest oksijen radikallerini azaltıcı etkisine bağlı olmadığı düşünülmüş ancak mekanizma aydınlatılamamıştır.*

Anahtar kelimeler: Metilen mavisi, adezyon.

INTRODUCTION

Following abdominopelvic operations, almost 95% of patients are shown to have adhesions at subsequent surgery (1). Peritoneal adhesions are the major cause of intestinal obstruction (2) and approximately 30-40% of patients who require abdominal reoperation have adhesion related intestinal obstruction (3). Peritoneal adhesions are also the leading cause of primary and secondary infertility in women (4). A recently published survey documented that 5.7% of all readmissions were classified as being directly related to adhesions with 3.8% managed operatively (5). It is therefore important to recognise the possible consequences of postoperative adhesions to patients, surgeons, and the health system. Given the dimensions of this problem, the clinical pre-

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vention of peritoneal adhesions has become one of the most studied issues in medicine. However, no method of clinical therapy has appeared to date as the radical and final solution of the problem. Of the few number of drugs that have been used in experimental studies, those which have shown some beneficial effects include hyaluronic acid (6) and halofuginone, an inhibitor of collagen type I synthesis (7).

Methylene blue (MB), a low molecular weight, partially liposoluble vital dye, has been proposed as a new therapeutic option in the reduction of surgery-induced peritoneal adhesions by Galili et al (8). They found that MB was very effective in preventing formation of peritoneal adhesions in contrast to the study of Prien et al (9) which documented increased adhesions with this dye. In this study, we evaluated the effect of different doses of MB and also aimed to clarify the possible mechanism of action of allopurinol, a xanthine oxidase inhibitor for toxic oxygen radicals.

MATERIALS AND METHODS

This study was performed at the laboratories of the Surgical Research Department of Ankara University School of Medicine and the Biochemistry Department of Hacettepe University School of Medicine. Seventy five male Wistar-albino rats were housed under environmentally controlled conditions at 20 °C and 30-70% relative humidity with 12 h dark /12 h light provision and access ad libitum to tap water and standard dairy pellet chow. The guiding principles in the Care and Use of Laboratory Animals together with the recommendations from the Declaration of Helsinki were strictly adhered to at all times.

The animals were divided into five groups, consisting of 15 rats each. Following 12 hours of fasting, the rats were anesthetized with 50 mg/kg sodium pentobarbital. The animals were allowed to breathe room air spontaneously. The surgical field was prepared with 1% of antiseptic povidine-iodine solution. After midline laparotomy, the cecum was mobilized and placed onto a wet gauze. Punctuate hemorrhages were generated by scraping the cecum serosa to induce adhesions. Prior to closure of the abdomen, the rats were administered either 1ml of saline (Group I), or 1mg/kg (Group II), 5mg/kg (Group III), and 9mg/kg (Group IV) of MB (Merck, USA) intraperitoneally. In Group V, after 1 ml of saline administration at surgery, rats were treated with allopurinol (30 mg/kg p.o.) for 14 days. The rats were killed on the 14th postoperative day. Laparotomy was repeated and tissue samples from the incisions were obtained. Adhesions were graded blindly by two independent observers as follows: absent (0); thin, easily separable (1); fibrotic, requiring sharp dissection (2); extensive, dense adhesions (3) as described in the literature by Evans (10). Adhesions were also sampled for analysis.

Measurement of the hydroxyproline content is considered to be a sensitive and objective method for adhesion study (11). All tissue samples were stored at –30°C and hydroxyproline content was determined spectrophotometrically according to Bergman's modified Stegman method (12). The data were calculated as mgr hydroxyproline content per mg of tissue.

Statistical analysis of adhesion grades among the five groups was performed using the Kruskal-Wallis test followed by Mann- Whitney U statistics and hydroxyproline content of the groups was analysed using one-way analysis of variance (ANOVA) with Bonforini correction. $P < 0.05$ was accepted as statistically significant.

RESULTS

Analysis of the grading of adhesions documented significant differences between the groups (Figure 1). Adhesion severity was found to be the lowest in Group II (1mg/kg MB) (1.7 ± 0.2) and when compared with that of the others, it was significantly lower ($p<0.05$). There was no significant difference between the saline group (Group I) (2.3 ± 0.3) and Group III (5 mg/kg MB) (2.4 ± 0.2), while there were significant increases in both Group IV (9 mg/kg MB) and Group V (Allopurinol) when compared with that of the saline group ($p<0.05$ for both).

The hydroxyproline content of the incisions varied within a narrow range: 6.25 ± 1.48, 7.97 ± 1.94, 7.51 ± 0.90, 7.89 ± 0.84, 6.24 ± 1.15 in Groups I-V respectively (Figure 2). There was no significant difference when the groups were compared with each other ($p>0.05$ for any combination).

When the hydroxyproline content of the adhesions was analysed, significant differences between the groups were noted (Figure 3). There was a significant decrease in Group II (l mg/kg MB) and a significant increase in Group IV (9 mg/kg MB) when compared with that of other groups ($p<0.05$).

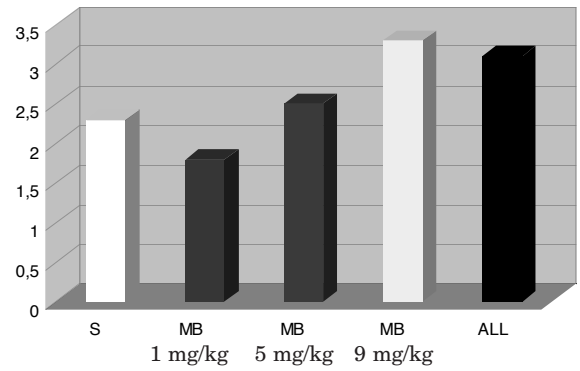

Figure 1. Comparison of severity of adhesion formation. (*) Indicates significant difference (P<0.05) versus all groups (Mann-Whimey U). (**) Indicates significant difference (P<0.05) versus saline, 1 mg/kg methylene blue and 5 mg/kg methylene blue groups (Mann-Whitney U). S: Saline; MB:Methylene Blue; ALL: Allopurinol.

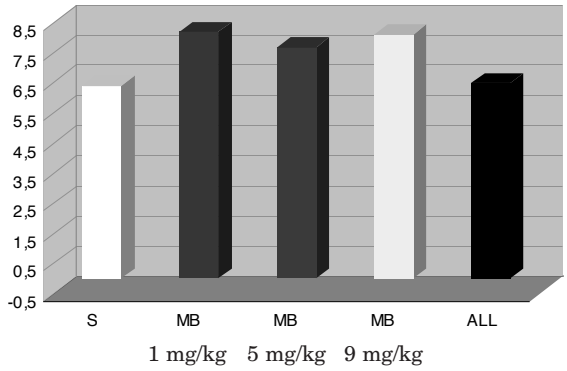

Figure 2. Comparison of incisional hydroxyproline levels (mg/mg tissue). S: Saline; MB:Methylene Blue; ALL: Allopurinol.

Although there was a decrease in Group V (allopurinol) when compared with the saline group, this was not significant (p>0.05).

DISCUSSION

Methylene blue has been used in medicine for the treatment of cyanide poisoning, methemoglobinemia, nitrite poisoning and urinary tract infections (13). After discovery of its two unrelated and important effects on the organism, it became a new potential drug. These effects are a) by blocking the nitric oxide (NO) binding sites of guanylate cyclase, it antagonizes the effects of NO (14) and b) it competitively inhibits the reduction of molecular oxygen to superoxide by acting as an electron acceptor for xantine oxidase. Thus the use of methylene blue is suggested to be an effective antioxidant in the setting of ischemia/reperfusion injury (13).

To date, the use of a wide range of doses of MB has been evaluated. It 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 (15). The highest safe dose of this dye was accepted as 9 mg/kg in our study. The lowest dose (1 mg/kg) was chosen on the basis of information in the literature that demonstrates the dye's potency at this dose (8) and the intermediate dose (5 mg/kg) was selected arbitrarily.

The present study demonstrated that MB has diverse effects on the formation of peritoneal adhesions depending on the dose used. While inhibiting adhesions at lower doses, it promotes adhesions at higher doses. The data of two previous reports on the effect of methylene blue on formation of peritoneal adhesions are conflicting. While Prien et al (9) suggested that MB activates macrophages and promotes adhesions, Galili et al (8) claimed that intraperitoneal administration of MB decreases the incidence and extent of peritoneal adhesions. The data presented in this study can be considered as compatible with those two previous conflicting reports in the sense that it

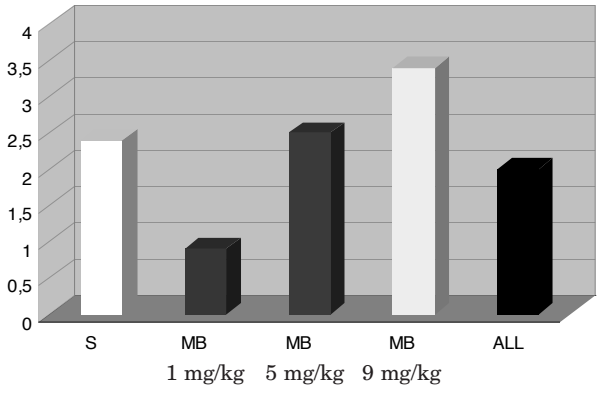

Figure 3. Comparison of hydroxyproline levels (mg/mg tissue). (*) Indicates significant difference (P<0.05) versus all groups (ANOVA). (**) Indicates significant difference (P<0.05) versus all groups (ANOVA). S: Saline; MB:Methylene Blue; ALL: Allopurinol.

demonstrates the dual effect of MB depending on the dose used. On the other hand, this study was able to enlighten its mechanism of action as mentioned in the first paragraph. The highly beneficial effect of a low dose might not be explained solely by the interference of the dye with free-radical generation since the effect of allopurinol; a xanthine oxidase inhibitor is significantly more different than methylene blue as it promotes adhesion formation. It is also reported that nitric oxide synthase inhibitors do not avoid peritoneal adhesion formation (8).

The results of this study demonstrate that MB has no impact on wound collagen synthesis as there was no significant difference when the hydroxyproline content of the incisions were compared with each other.

In conclusion, this data is thought to provide some evidence for a good candidate in the prophylaxis of surgery induced adhesions. However, additional research is needed to establish optimal dosages and a fuller understanding of its mechanism of action.

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