The effectiveness of heparin, platelet-rich plasma (PRP), and silver nanoparticles on prevention of postoperative peritoneal adhesion formation in rats[1]

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Abstract

Purpose: To assess the effectiveness of heparin, platelet-rich plasma (PRP), and silver nanoparticles on prevention of postoperative adhesion in animal models.

Methods: Sixty males Albino Wistar rats aged 5 to 6 weeks were classified into five groups receiving none, heparin, PRP, silver nanoparticles, PRP plus silver nanoparticles intraperitoneally. After 2 weeks, the animals underwent laparotomy and the damaged site was assessed for peritoneal adhesions severity.

Results: The mean severity scores were 2.5 ± 0.9, 2.16 ± 0.7, 1.5 ± 0.5, 2.66 ± 0.88, and 2.25 ± 0.62 in the control, heparin, PRP, silver and PRP plus silver groups, respectively with significant intergroup difference (p = 0.004). The highest effective material for preventing adhesion formation was PRP followed by heparin and PRP plus silver. Moreover, compared to the controls, only use of PRP was significantly effective, in terms of adhesion severity (p = 0.01).

Conclusion: Platelet-rich plasma alone may have the highest efficacy for preventing postoperative peritoneal adhesions in comparison with heparin, silver nanoparticles and PRP plus silver nanoparticles.

Key words: Heparin. Platelet-Rich Plasma. Metal Nanoparticles. Siver. Tissue Adhesions. Rats.

■ Introduction

The incidence of postoperative adhesions has been estimated at 67%-93% remained almost constant despite several attempts to prevent their formation[1]. Peritoneal adhesions can frequently cause complications such as bowel obstruction, ileus, chronic abdominopelvic pain, infertility and abdominal or even systemic infections[2]. Hence, patients are at risk of repetitive surgical interventions, which increases cost, morbidity, and mortality.

These complications may even lead to death, based on the reports by the National Hospital Discharge Survey, about approximately 2,000 people die every year in the United States from intestinal obstruction due to adhesions[3].

Therefore, applying proper strategies to prevent adhesions formation should be considered in all clinical and surgical settings. Several preventive methods and potential preventive agents have been studied, including lavage of abdominal cavity, lytic agents, macrophage promoting factors, nonsteroidal anti-nflammatory drugs, heparin, dextran 70, ringer lactate, antihistamine, prostaglandin synthesis inhibitors, calcium channel blockers, rofecoxib, and green tea extract[4-8]. These agents may indirectly prevent the creation of adhesions by inhibiting inflammatory processes leading to adhesion formation, or may act as physical barriers in separating injured surfaces[9].

Reportedly, intraperitoneal injection of heparin can prevent creation of adhesion bands by inhibiting fibrin formation[10]. Besides, some described the role of platelet-rich plasma (PRP) in improving adhesions by better wound healing and enhancing the physiological processes at the site of the injury or surgery by clotting or via its anti-inflammatory effects[11,12]. Silver nanoparticles-loaded physical barriers were also reported to be effective as anti-adhesion, due to their anti-infection quality[13,14].

We conducted the present study to assess the effectiveness of heparin, PRP, and silver nanoparticles on prevention of postsurgical intraabdominal adhesions in animal models.

■ Methods

We conducted this experimental animal study after obtaining the approval of the Ethics committee of Hamadan University of Medical Sciences**.**

In this prospective experimental animal study, 60 male Albino Wistar rats aged 5 to 6 weeks were employed. They were kept in 12 hours of light and 12 hours of dark at 21°C and humidity of 60 ± 5%, for five weeks, to be adapted with laboratory environment, mature in age and gain weight between 200 and 220 grams. Animals were fed in same conditions, with free access to food and water. After reaching the age of puberty and spending adaptation stage, the animals were classified to five subgroups including 1) deperitonalization at anterior wall of caecum without administration of any agent (control group), 2) deperitonalization at anterior wall of caecum with administration of PRP (PRP group), 3) deperitonalization at anterior wall of caecum with administration of silver nanoparticles (silver group), 4) deperitonalization at anterior wall of caecum with administration of PRP in combination with silver nanoparticles (PRP plus silver group), and 5) deperitonalization at anterior wall of caecum with administration of heparin (heparin group). All groups were anesthetized using intravenous administration of Ketamin 40 mg/kg in absolutely sterile condition, without using any antibiotic. Each rat was laid in dorsal recumbency position, the abdominal skin was disinfected with Betadine 10%, and then the hair of the given area of the skin was completely shaved with a razor. A 3 cm incision was made on the caecum area of the abdomen to access the peritoneal surfaces.

After opening the anterior abdominal wall in the region of the caecum, a 1 × 1 cm^2 piece was deperitonalized and then in the control group, the anterior abdominal wall was closed without using any agent. Likewise, the abdominal wall in the region of the caecum was closed after the use of PRP, 40 ppm of silver nanoparticles, PRP with 40 ppm silver nanoparticles, or 500 IU of heparin in 2 ml of saline solution, intraperitoneally in other study groups. After 2 weeks, the animals were re-anesthetized with ketamine, underwent laparotomy and the damaged site was assessed for severity of peritoneal adhesions, according to the Nair classification as follows: score 0: complete absence of adhesion, score 1 or mild adhesion: single band of adhesion between viscera or from one viscus to abdominal wall, score 2 or moderate adhesion: two bands either between viscera or from viscera to abdominal wall, score 3 or severe adhesion: more than two bands between viscera or viscera to abdominal adhesions wall, and score 4 or very severe adhesion: viscera directly adherent to abdominal wall[15].

Quantitative and qualitative variables were compared using one-way ANOVA and Fisher's exact tests, respectively. Statistical significance was determined as a p value of ≤ 0.05. All statistical analysis was performed using SPSS software (version 19.0, SPSS Inc., Chicago, Illinois).

■ Results

The severity of peritoneal adhesions was significantly different across the study groups. In this regard, severity scores of peritoneal adhesions of 3 and 4 were observed in 50% and 8.3% in control, 33% and none in heparin, none and none in PRP, 41.7% and 16.7% in silver, and 33.3% and none in PRP plus silver groups, respectively ($p < 0.001$). The frequency of the different severity scores of peritoneal adhesions in each group is shown in Figure 1. The mean severity scores were 2.5 ± 0.9, 2.16 ± 0.7, 1.5 ± 0.5, 2.66 ± 0.88, and 2.25 ± 0.62 in the control, heparin, PRP, silver and PRP plus silver groups, respectively with significant intergroup difference ($p = 0.004$). The highest effective material for preventing adhesion formation was PRP followed by heparin and PRP plus silver. Moreover, compared to the controls, only use of PRP was significantly effective, in terms of adhesion severity ($p = 0.01$).

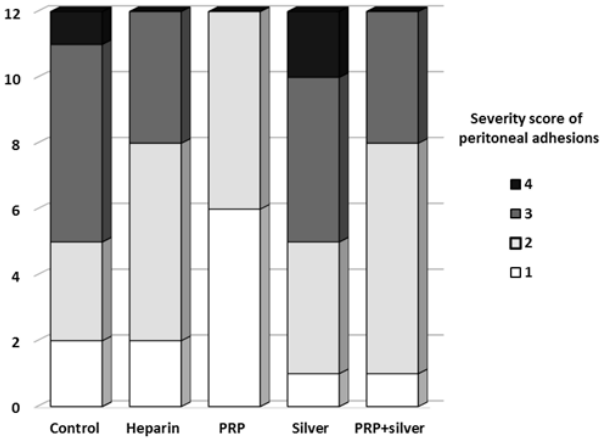

Figure 1 - The frequency of the different severity scores of peritoneal adhesions in each group.

■ Discussion

Postsurgical adhesions may develop as a result of inflammatory reactions. Trauma, inflammatory or fibroproliferative reaction on the peritoneal surface or the intraabdominal organs, mediates tissue healing and adhesion formation[5,16,17]. According to our findings, the most applicable agent for preventing peritoneal adhesions may be PRP. PRP is an autologous product that concentrates a high number of platelets in a small volume of plasma[18]. This product mimics the last step of the coagulation cascade, leading to the formation of a fibrin clot, which consolidates

and adheres to the application site in a short period of time. Evidencing hemostatic and healing properties, PRP is able to hold tissues or materials in a required configuration[19]. PRP has been used in humans in different kinds of transplant procedures such as dentistry, maxillofacial surgery, and ophthalmology[20-23]. In addition, PRP may be considered as a carrier for biologically active agents and a healing substance causing less post-surgical pain[24,25]. Besides its beneficial effects on wound healing, it seems that PRP-related anti-inflammatory role may inhibit distribution of some pathways predisposing to adhesions.

In our study, along with PRP, heparin has also been shown to be effective on healing peritoneal adhesion. In a study by Kement *et al*.[26] on similar animal models, the use of heparin significantly reduced the severity score of adhesion and the number of animals with adhesions in different locations of the abdominal cavity compared to the control group. It was also observed that, higher dosages of heparin seemed to be more effective. Vela *et al*.[27] showed that rats treated with a subcutaneous minidose heparin and low molecular weight heparin showed significantly lower adhesion and abscess formation, compared to the control rats. Peritoneal inflammation caused by a peritoneal injury leads to the formation of an inflammatory exudate which contains strands of fibrin. The fibrinogenic mechanism after this step is very similar to that which happens during coagulation[28]. Thus, using anticoagulant products such as low molecular weight heparin is a bright idea for adhesion prevention, and it has been shown in experimental settings, that heparin may stop or slow down the adhesiogenesis. Besides, heparin has also well-known anti-inflammatory effects including anti-complement activity and inhibition of histamine, serotonin and endothelin-1 release from mast cells[29]. Since heparin theoretically reduces fibrin formation and inflammation, it can be extensively monitored for adhesion prevention.

In our study, despite anti-inflammatory and antimicrobial effects of silver nanoparticles, they had little effect on preventing adhesions. Also, it has been shown that the combination of silver nanoparticles and PRP was more effective against peritoneal adhesion formation, however the difference was not significant. In a study by Wong *et al*.[14], it was shown that silver nanoparticles are effective in decreasing inflammation and peritoneal adhesions without significant toxic effects. In another study by Tian *et al*.[30], silver nanoparticles exerted positive effects through their antimicrobial properties including reduction in wound inflammation, and modulation of fibrogenic cytokines, describing its action as a novel therapeutic direction for wound treatment.

■ Conclusion

Platelet-rich plasma PRP alone may have the highest efficacy for preventing postoperative peritoneal adhesions in comparison with heparin, silver nanoparticles and PRP plus silver nanoparticles.

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