



Morphine Decreased Peritoneal Adhesion in Addicted Male Rats after Abdominal Surgery

Abbas Bakhtiari¹, Seyed Mohammad Hosseinipناه¹, Amaneh Mohammadi Roushandeh^{1*}

¹Anatomical Sciences Department, Medicine faculty, Hamadan University of Medical Sciences, Hamadan, Iran.

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ABSTRACT

Background: After abdominal surgery, postoperative adhesion is a serious problem that threatens the patients. The Present study evaluated the protective effects of morphine on abdominal adhesion in an experimental model in rat. **Materials:** An experimental intraabdominal adhesion model was created in 24 adult male rats by ischemic bottom method. The animals were divided into three groups. The control group was not treated by morphine. In the morphine group, the animals received morphine as 10 mg/kg once a day for three days after surgery. In group 3, the addicted animals to morphine received morphine as 10 mg/kg daily after surgery. Drug dependency was confirmed by naloxone. Adhesions were evaluated by nair and swollen scoring system 10 days after surgery. **Results:** There was a significant differences regard to the length ($P<.05$), thickness ($P<.05$), and severity of adhesions ($P<.05$) among the control, morphine, and morphine-addicted groups. **Conclusion:** Morphine reduced the severity of postoperative adhesions the present study. Morphine could affect postoperation adhesion through suppression of inflammation. It is suggested that opioid receptor(s) might involve in this process. Therefore, selective ligands could be used and offer a pharmacologic strategy in preventing adhesion formation.

Introduction

Peritoneal adhesions have been reported as an adverse side effect of surgery for more than a century and occur in 90-100% of cases.¹

Postoperative adhesion formation results from a series of local events at the trauma site. Peritoneal injury by surgery, infection, or irritation initiates a local inflammatory reaction, exudation, and fibrin deposition into which white blood cells; macrophages, fibroblasts, and mesothelial cells can migrate, proliferate, and/or differentiate. Within a few hours the lesion is filled by macrophages and other tissue repair cells whose exact precursors are still unclear.^{2,3} Postoperative adhesion formation is an important clinical problem after abdominal surgery.⁴ Chronic abdominal pain, feeding intolerance, bowel obstruction, need for reoperative surgery, and female infertility are the most common problems related to postoperative intraabdominal adhesions that threaten the patients health.^{5,6} Numerous agents such as phospholipase inhibitors, dextran, corticosteroids, phospholipids, methylene blue, anti-inflammatory drugs, polysaccharides, bioresorbable membranes, tissue plasminogen activator, and Ankaferd blood stopper have been studied to prevent adhesion formation.^{7,8}

The mechanisms related to peritoneal adhesion is not well studied but it is believed that inflammation has the major role. Inflammations caused by mechanical stimuli are the principal causes of intraperitoneal

adhesions.⁷ During infection, in contrast, the opioid concentration increases in the peripheral blood and at the sites of inflammatory reaction.^{9,10} In addition, opioid receptor is expressed in macrophages, indicating that opioid peptides produced during inflammation can stimulate opioid receptor.¹¹

Morphine is the main alkaloid of opium. Morphine has been shown to suppress several immune parameters, including lymphocyte proliferation¹² and natural killer cell activity.¹³ The administration of morphine mediates a histamine release. In addition to morphine's known effect on histamine release^{14,15} data suggest that it may also play a role in altering the immune response.^{16,17}

The number of people who addicted to morphine is high in Iran and epidemiologic studies showed less peritoneal adhesion occurred in those patients. Therefore, our study designed to investigate effects of morphine on reduction of abdominal adhesion after surgery in normal or addicted animals.

Methods and Materials

Animals and surgery

All procedures were approved by the Ethic committee of Hamadan University of Medical Sciences. 24 male wistar rats weighing 225-275g were obtained from the animal house of Hamadan University of Medical Sciences. The rats were housed in standard plastic

*Corresponding Author: Amaneh Mohammadi Roushandeh, Medicine faculty, Hamadan University of Medical Sciences, Hamadan, Iran. Tel: 09143078216, Email: a.mohammadiroshandeh@umsha.ac.ir

cages (3 rats per cage) in an environmental room at 22°C and 58–65% relative humidity with a controlled 12-hour light/dark cycle for at least 10 days before experimentation. The animals were divided into three groups (n=8). Adhesion was generated in control group without morphine administration. Animals in group 2 received morphine as 10 mg/kg once a day for three days after surgery. In group 3, at first, the animals addicted to morphine by Marschal method, then, they received morphine as 10 mg/kg daily after surgery. To induce addiction in rats, they received morphine for three days as below:

15 mg/kg at 8 o'clock, 15 mg/kg at 12 and 30 mg/kg at 16 o'clock at first day

30, 30 and 45 mg/kg at same time at second day

45, 45 and 60 mg/kg at the same time at third day

Drug dependency was proved with naloxone injection at the day 4. Two rats were randomly selected among animals in group 3 and injected with 2 mg/kg naloxone (Daroo-Pakhsh Co., Tehran, Iran) subcutaneously in medial part of thigh¹⁷. Naloxone-injected rats were all kept in a transparent cage for 20 minutes and were controlled for morphine withdrawal signs, like wet dog shakes (jumping), headshakes, diarrhea, paw tremor, ptosis, writhing, or teeth chattering. A rat demonstrating at least 4 of these signs during the observation period was considered to be addicted^{17,18}.

Surgical procedure

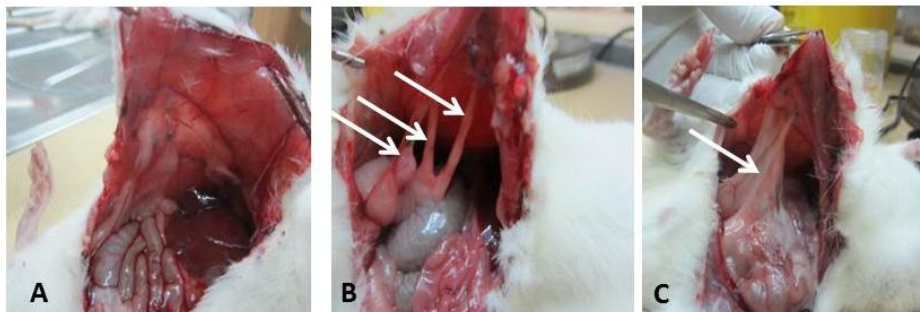


Figure 1. peritoneal adhesion induced with ischemic button method. (A) Laparotomy without ischemic button method and no peritoneal adhesion occurred. (B) Peritoneal adhesion after ischemic button method. Notice to adhesion bands (Arrows). (C) Increase in thickness of peritoneal adhesion in control group without morphine administration.

Statistical analysis

All results were presented as the mean \pm standard deviation of mean. Statistical analyses were performed by one-way ANOVA of variance using SPSS version 20 and Tukey-Kramer Test as a post test. The differences between means was considered statistically significant when P -value was less than 0.05 ($P < 0.05$).

Results

There were no intraoperative complications. In the subsequent postoperative period, all rats in control group survived but there was a loss of 1 and 2 rats in morphine and addicted groups respectively, due to infection postoperatively. No sign of pain was observed in the rats. All animals recovered quickly after

The surgical procedures were performed under aseptic conditions. The operations were performed under 50 mg/kg ketamine hydrochloride (Ketalar; Eczacibasi, Istanbul, Turkey) with the animals breathing spontaneously. The animals were placed on a heating mat warmed to 38° C. After shaving with electrical clippers, the surgical field was disinfected. Sterile covers were fenestrated and applied to the surgical field. The abdominal wall was incised longitudinally in the midline. On right abdominal side wall, 3 ischemic buttons were created. To this effect, the musculoperitoneal tissue was lifted with a hemostat, which resulted in the formation of a tissue button. The ischemic buttons were created using a novel technique in two steps. First, the ligature was fixed to the tissue button by stitching with the suture through the base of the button. This was followed by ligature on one side of the button. Second, the ischemic button was completed by ligature of the complete base of the button. In both groups, the buttons had a diameter of 0.5 cm, were spaced 1 cm apart, and aligned parallel to the midline along an imaginary line through the mammary glands. Following the creation of the experimental lesions, the midline laparotomy was closed in two layers. The musculoperitoneal layer was closed with a running suture (Vicryl 3-0, Ethicon). Ten days after surgery rats were sacrificed and adhesion were evaluated by Nail and Swolin standard methods.¹⁹

anesthesia and were active and able to drink and eat, and moved seemingly without discomfort. According to the Nair scale, there was significant difference with regard to the severity of adhesions between control and Morphine and control and addicted groups respectively ($P < 0.05$).

Also, based on the Swolin scale, there were significant differences about the length, thickness and number of adhesion bands between control and addicted groups ($P < 0.05$).

However, no significant differences were detected between three groups about location of adhesion.

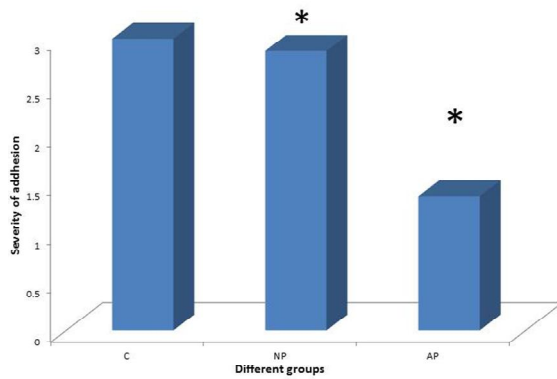


Figure 2. severity of peritoneal adhesion evaluated by Nair scoring. C=control – NM: normal rat that received morphine – AM: addicted rat that received morphine.

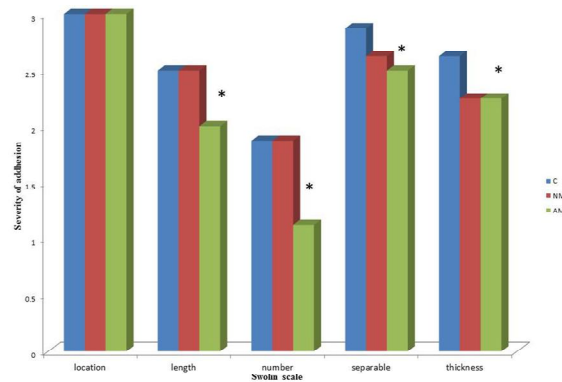


Figure 3. Peritoneal adhesion scores based on Swolin scale in different groups. C=control – NM: normal rat that received morphine – AM: addicted rat that received morphine.

DISCUSSION

In the current study, we investigated whether morphine could influence postoperative adhesion formation in the rats. Drug dependency confirmed by Marschal method in which morphine injected instead of another methods in which morphine solved in animals drink water. Although Gellert et al¹⁷ found that 10% of their animals did not readily accept the drinking solution. However, we did not observe any failure of the treatment.

As it is presented in our findings, morphine decreased peritoneal adhesion in normal (Non addicted) group compared to control and addicted groups. Also, peritoneal adhesion decreased in addicted group compared to control. Badawy et al used morphine at 0.1 mg/mL, reported that this concentration resulted in an estimated daily intake of 50 mg/kg.¹⁸ However, because the effect of morphine intake in higher concentrations is unknown, the relationship between opioid intake and adhesion characteristics remains speculative.¹⁸

Khorram-manesh et al showed that opioids could decrease the abdominal adhesion in addicted animals. Their findings about severity of adhesion are similar to

our study. But our surgery method and adhesion evaluation in addition morphine concentration were a little different. They induced adhesion with caecum abration instead of ischemic button method in our study. Location of adhesion in all groups in our study was similar because of the surgery method instead of variable places of adhesion found in their study.²⁰

The mechanism of peritoneal adhesion is under research and not defined completely. An imbalance between fibrin deposition and fibrinolysis during the peritoneal healing process may result in adhesion formation.^{21,22} It is proved that opioid such as morphine and their receptors could have an important role on adhesion formation. They could have an impact on adhesion formation through release of biologically active molecules, macrophages, mesothelial cells, platelets and inflammation.

In conclusion, we found that morphine could improve peritoneal repair in addicted and non addicted in standard experimental model of adhesion. In our ongoing project we investigate effects of other opioids, pethidine, on abdominal adhesion. However more studies about another family of opioids and their mechanisms need to be clarified.

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