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The systemic effect and the absorption rate of aerosolized intra-peritoneal heparin with or without hyaluronic acid in the prevention of postoperative abdominal adhesions

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Abstract

Background Adhesions are a known cause of morbidity and mortality following abdominal surgery. Multiple approaches have been evaluated to prevent or minimize the occurrence of adhesions. Administration of aerosolized heparin and hyaluronic acid is an effective method to prevent postoperative adhesions whether they are used independently or in synergism. However, absorption rate and the systemic effect of heparin given intra-peritoneal have never been investigated. The purpose of this study was to evaluate the systemic effect and the absorption rate of heparin with or without hyaluronic acid in the prevention of postoperative abdominal adhesion.

Materials and methods This is a cross-sectional study comparing thirty rats ($n=30$) divided into 3 groups, each consisting of 10 rats. First group ($n=10$) received aerosolized intra-peritoneal heparin (IPH). Second group ($n=10$) received intra-peritoneal heparin with hyaluronic acid (IPHHA). Intravenous heparin (IVH) was given to the third group ($n=10$). Serum heparin levels were measured and compared between the groups over 120 min's period.

Results None of the rats had intra-operative bleeding. The level of serum heparin was significantly lower in the IPHHA group compared to IPH and the IVH at all points of measurements (30, 60, 90, and 120 min) ($p<0.0001$). The serum level of heparin of all groups peaked at 90 min. Area-under-the-curve 0–120 was significantly lower in the IPHHA group as compared to both IPH and IVH ($p<0.0001$).

Conclusion The aerosolized intra-peritoneal administration of heparin or heparin with hyaluronic acid resulted in minimal systemic absorption rendering it safe for the use as method to prevent intra-peritoneal adhesions. Human studies are planed next.

Keywords Heparin · Hyaluronic acid · Postoperative adhesions · Peritoneum · Laparoscopy · Laparotomy

Postoperative adhesions are associated with considerable morbidity, mortality, and have large financial and public health impact. These adhesions may occur after almost every abdominal surgery and are the leading cause of intestinal obstruction, infertility, chronic abdominal pain, and increases in surgical time and in the risk of bowel perforation during subsequent surgery [1, 2]. The significant burden of adhesions has led to the development of several anti-adhesion agents, although there is no consensus as to which is the most effective.

Peritoneal adhesion formation is the consequence of abnormal repair of the peritoneum following peritoneal injuries. Following injury, the inflammatory response activates the coagulation cascade. This leads to the formation of fibrin gel matrix over the damaged mesothelium, which is then degraded to reveal regenerated peritoneum. Normally, the fibrin mass is entirely removed from the peritoneal cavity by a fibrinolytic process. However, when the peritoneal fibrinolytic activity is suppressed, the fibrin persists, and the fibrin bands organize following the growth

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of macrophages, fibroblast, and endothelial cells leading to the formation of fibrous adhesions [3, 4].

Although intra-peritoneal adhesions can be the results of many pathological processes, such as cancer, peritoneal infections, or endometriosis, the trauma associated with surgery is the leading cause of peritoneal adhesion formation [5]. Adhesions can be surgically removed although, because of the high propensity for adhesions to re-form, the clinical effectiveness of adhesiolysis has been controversial [6]. Thus, the focus of adhesion management is now on prevention. Various measures can be taken to prevent adhesions from forming. Intra-peritoneal hyaluronan in variable form has been used widely as an anti-adhesion agent with various degrees of success. Intra-peritoneal heparin in different molecular weights has been successful in reducing postoperative adhesions in many animal studies and it is used regularly in ambulatory peritoneal dialysis to prevent fibrin formation. However, its success has been limited due to the lack of appropriate delivery mechanism. In our previous publication, we introduced a new safe and effective technique of aerosolized heparin and hyaluronic acid to prevent adhesions. The technique utilizes a novel aerosolization system to deliver the therapeutic dose [7]. However, absorption rate and the systemic effect of heparin given intra-peritoneal have never been investigated. The aim of this study was to evaluate the systemic effect and the absorption rate of heparin with or without hyaluronic acid in the prevention of postoperative abdominal adhesions.

Materials and methods

Animal module

All of the in vivo animal work was performed according to the University of Western Ontario Council on Animal Care, with the research protocol approved by the Animal Use Subcommittee. A non-survival rat model consisting of 30 male Wistar Albino Glaxo (WAG) rats ($n = 30$) weighing 250–300 g was chosen to simulate the absorption rate of intra-peritoneal heparin and to measure its level in the animal circulation. Sample size calculation showed that ten animals per group are required to demonstrate significant difference. The rats were divided into three groups, each consisting of 10. First group received 5 ml of intra-peritoneal aerosolized heparin at 100 IU/ml (IPH, $n = 10$). The second group received 5 ml of intra-peritoneal aerosolized mixture of 100 IU/ml heparin and 2% hyaluronic acid (IPHHA, $n = 10$). The third group had 5 ml of IV heparin at 100 IU/ml (IVH).

Surgical technique

Pre-operatively, all rats were allowed food and water ad libitum. At the start of the procedure, the rats were placed into an anesthetic inhalation chamber attached to an anesthetic machine breathing in 1% oxygen and 2% isoflurane until they were sedated. Then the rat was transferred from the chamber onto an operating table and placed on a face mask attached to an anesthetic machine breathing in a continuous flow of 1% oxygen and 2% isoflurane until an IV line was established. The IV catheter was placed in the tail vein to administer injectable anesthetic. Once the IV catheter has been placed, the rats were then removed from the face mask and anesthetic machine and placed on a warm heating blanket. Through the IV line, the rats were kept on a CRI of 50 mg/kg/h propofol and 50 mg/kg/h ketamine for the duration of the study in addition to oxygen supplied via face mask. The rats were carefully monitored throughout the procedure. The laparoscopic surgery was performed via a single port placed on the left side of the animal mid-line. A 5-mm disposable port was inserted via open method for the creation of CO_2-pneumoperitoneum with a maximum intra-peritoneal pressure of 4 mmHg that was maintained throughout the operative period, and also used for the delivery of the therapeutics. Intra-operatively, the animals underwent a laparoscopic aerosolization of heparin with or without hyaluronic acid using the Intramyst system (Northgate Technologies Incorporated, Elgin, Illinois, USA) for both the IPH and IPHHA groups. At the end of the aerosolization, the port was removed and the wound was close with 3/0 vicryl. The total pneumoperitoneum time in all animals was 7 min with the same intra-peritoneal pressure of 4 mmHg during the operative period. There was no fluctuation in the intra-abdominal pressure in all animals. The IVH group received the IV heparin through the established IV line. Utilizing the second tail vein, 0.5 ml of blood was withdrawn from the animals to perform Serum Heparin Assay at the following intervals: 30 min, 60 min, 90 min, 120 min. The samples were prepared and stored as per our laboratory instruction before shipping them to the laboratory.

This study was a non-survival model, so after the withdrawal of the last blood sample, all rats were euthanized with sodium pentobarbital through IV line.

A postmortem examination was carried out to look for signs of internal bleeding, hematoma, and collections in the head, thorax, abdominal and pelvic cavities. Due to the small blood volume in our rat model, we did not perform any coagulation studies and we elected to depend on postmortem examination.

Statistical analysis

Statistical analysis was carried out using STATA 12 software (StataCorp LP, College Station, TX). Continuous data were presented as means ± SD. Biochemical variables were compared by analyzing the changes of means over time and by examining the areas-under-the-curve (AUCs). Continuous variable data were tested for normal distribution and statistically significant differences were determined using Analysis of Variance (ANOVA). All data were presented as the mean ± standard error of the mean (SEM). A p value of less than 0.05 will be considered to be significant.

Results

All of the 30 rats survived the operative procedure with no mortality. There was no distress to the animals and no intra-operative bleeding. Comparing the three groups, the level of serum heparin was significantly lower in the IPHHA group compared to IPH and the IVH at all points of measurements (30, 60, 90, and 120 min), $p < 0.0001$. The IPHHA serum level of heparin was also significantly reduced when compared to IPH ($p < 0.001$), Fig. 1. The AUC 0–120 was significantly lower in the IPHHA group as compared to both IPH and IVH ($p < 0.0001$), Fig. 2. Postmortem examination on all the rats ($n = 30$) showed no signs of collection, hematoma, or internal bleeding in the head, thorax or the abdominal cavity which demonstrated the lack of systemic effect on the aerosolized heparin in this study.

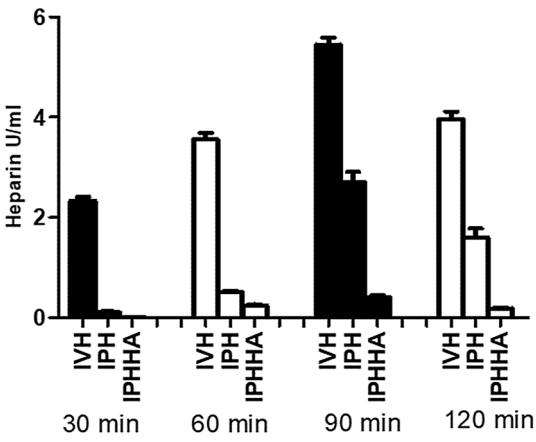

Fig. 1 Level of serum heparin was significantly lower in the intra-peritoneal heparin and hyaluronic acid mixture (IPHHA) group compared to intra-peritoneal heparin (IPH) and the intravenous heparin (IVH) at all points of measurements ($p < 0.0001$)

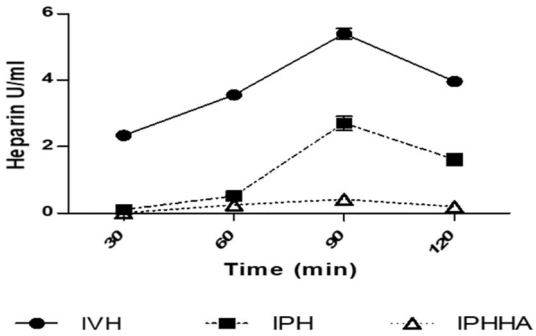

Fig. 2 Area-under-the-curve (AUC) 0–120 was significantly lower in the intra-peritoneal heparin and hyaluronic acid mixture (IPHHA) group compared to intra-peritoneal heparin (IPH) and the intravenous heparin (IVH) ($p < 0.0001$)

Discussion

Development of postoperative adhesions is a widespread consequence of surgical trauma and healing following open or laparoscopic abdominal or pelvic surgery and is associated with significant complications. Their most important morbidity is small-bowel obstruction, but other morbidities include female infertility and dyspareunia, chronic pain, and increased risk of visceral injury at subsequent laparotomy or laparoscopy [8, 9]. Besides the medical comorbidity associated with adhesion, there is an economic burden on the healthcare system, individual patients, and society. Although minimally invasive surgery contributed to a significant reduction in adhesion rate, the adhesiolysis-related costs continue to rise and the estimated expenditure of care for those patients is approximately $2.3 billion [10].

The generally accepted method for preventing adhesion formation during surgery is to minimize surgical trauma. More traumatic types of surgery, namely laparotomy, lead to increased risk of damage. This leads to an increased risk of adhesion formation compared to less invasive procedures such as laparoscopy [11]. Despite the extensive efforts undertaken to prevent peritoneal adhesion formation, no single method has thus far been successful. A large number of therapeutic modalities have been studied clinically and in animal models in an attempt to decrease the frequency and severity of adhesion formation after peritoneal injury. The two methods mainly used are either to use agents to modify the fibrinolytic process or create an inert barrier that allows peritoneal healing to occur.

Hyaluronic acid is a linear polysaccharide with repeating disaccharide units that are composed of sodium d-glucuronate and *N*-acetyl-D-glucosamine. It is a naturally occurring component of peritoneal fluid that aids in tissue lubrication and structural integrity. However, its low viscosity and high rate of peritoneal reabsorption make hyaluronic acid unsuitable for adhesion prevention [12]. Anticoagulants such as

heparin can reduce adhesion formation by inhibition of the coagulation cascade and promotion of fibrinolysis [13]. The use of heparin for intra-peritoneal irrigation in a dose that can reduce adhesion formation was associated with hemorrhage and delayed wound healing, but low-dose heparin irrigation showed no benefit in adhesion reduction [14, 15]. In our previous publication, we introduced a novel technique of aerosolized intra-peritoneal heparin and hyaluronic acid in the prevention of postoperative abdominal adhesions [7]. The combination of fibrinolytic modulator (heparin) and a physical barrier (hyaluronic acid) at a maximum dosage with a very small volume that can be introduced using the aerosolization technique proved to be an effective method to prevent adhesions in animal module. The next stage was to confirm the success of this novel method in a human-based study. However, there were lack of data regarding the systemic absorption and hemorrhagic effect of heparin given intra-peritoneally. The anticipated side effects expected from the use of aerosolized heparin are mainly local ones. However, the knowledge that there is absorption of small amounts of heparin across the peritoneal membrane requires one to be alert to the possibility of systemic side effects. In this study, using the rat module, we showed that both IPH and IPHHA groups had lower serum level than the IVH group. Moreover, the IPHHA heparin serum level was significantly lower than the IPH level; this could be due to the barrier effect that hyaluronic acid exerts which decreases heparin peritoneal absorption and its low blood level. The absorption level of heparin was so small that we do not believe it will have any noticeable or significant impact on the clinical picture and due to the small blood volume in our rats' model, no coagulation studies were performed and we used physical examination during the procedure and postmortem examination after the operation to look for signs of bleeding. None of the rats had bleeding during the surgical intervention and there were none found during the postmortem examination. All the rats survived the procedure and there physiological parameters did not change during the study period.

Using this animal module, we concluded that the aerosolized intra-peritoneal administration of heparin with or without hyaluronic acid resulted in minimal systemic absorption rendering it safe for the use as method to prevent intra-peritoneal adhesions. Human studies are planed next.

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Compliance with ethical standards

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