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ORIGINAL ARTICLE

Influence of Injectable Platelet-Rich Fibrin on *ex vivo* Leak Pressure of Canine Enterotomy

Ali Heydari Azar Heris[1], Davoud Kazemi ✉[2]

[1] DVM Graduate, Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran. [2] Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran.

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ABSTRACT

Injectable platelet rich fibrin, the latest innovation among platelet concentrates is obtained by centrifugation of whole blood without any anticoagulants. The initially liquid product is later converted into gel form due to conversion of fibrinogen into fibrin. This feature allows it to be used in injectable form or as topical spray. Incisional dehiscence and leakage is a potentially fatal postoperative complication of enterotomy. This study was conducted to investigate whether injectable platelet rich fibrin could be used as suture line reinforcement method in canine enterotomy. It was hypothesized that topically applied injectable platelet rich fibrin could increase the leakage pressure of enterotomy incisions. A total of 28 enterotomy incisions measuring 3 cm in length were created on the antimesenteric border of jejunum in canine cadaveric samples. The incisions were sutured with simple interrupted or simple continuous patterns and the suture line was reinforced by topical injectable platelet rich fibrin in treatment groups. Leak testing was conducted immediately and 15 minutes after suture placement in control and treatment samples respectively and initial leakage pressure and maximum intraluminal pressure was recorded along with leakage location. Although statistically insignificant, application of injectable platelet rich fibrin in the simple continuous treatment group increased the mean initial leakage pressure in comparison to simple continuous control group (15 ± 7.228 vs. 11.67 ± 26.17). Mean maximum intraluminal pressure was increased in both simple interrupted (46.29 ± 30.53 vs. 39 ± 34.54) and simple continuous (47.78 ± 9.497 vs. 35.67 ± 31.46) treatment groups in comparison to their respective control groups. Leakage was observed from the incision line and from the needle holes outside the incision line in simple interrupted and simple continuous sutured incisions respectively. Although the results indicated the feasibility of using this method but in vivo investigations are required to determine whether it could be used in clinical cases.

Introduction

Enterotomy or surgical incision into the small intestine is usually performed to remove intestinal foreign bodies and obtain full thickness tissue biopsies.[1-4] Although enterotomy is a relatively common surgical procedure in small animal practice but it is not entirely devoid of potentially fatal consequences. Wound dehiscence and leakage are the most important post-operative complications of enterotomy. The resultant septic peritonitis significantly increases post-operative patient morbidity and mortality.[1,5,6] An incisional dehiscence and leakage rate of 2-12% with an associated maximum mortality rate of 12% has been reported for enterotomy in dogs.[7-10] Therefore, similar to other gastrointestinal

✉ Corresponding author. Email: dkazemi@iaut.ac.ir

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surgeries, prevention of dehiscence and leakage is an important aspect of enterotomy. Leak testing is a commonly performed intra-operative procedure to detect significant gaps between sutures which could initiate leaks.[11,12] Omentalization and serosal patching of the suture line are also advocated to provide support and prevent leakage of intestinal contents.[1,5,6] Various surgical adhesives or tissue sealants have also been investigated for leakage prevention in gastrointestinal surgeries. Fibrin glue is the earliest and the most extensively studied surgical sealant. It is a biological sealant derived from blood plasma with fibrinogen and thrombin acting as its main components. Fibrinogen is activated by thrombin in the presence of calcium ions and converted to fibrin clots. This procedure is similar to the latter stages of blood coagulation cascade. Commercially available fibrin based sealants are used as both tissue sealants and hemostatic agents during surgery.[13,14]

Injectable or liquid platelet rich fibrin is the latest innovation among platelet concentrates. Platelet rich fibrin (PRF), a second generation platelet concentrate, is prepared by centrifugation of whole blood without any anticoagulants in glass based test tubes. The process of blood coagulation is initiated during centrifugation which results in formation of a solid fibrin clot containing platelets and leukocytes termed PRF. Injectable platelet rich fibrin (i-PRF) is also prepared from whole blood without anticoagulant products but both the speed and time of centrifugation is reduced. Moreover, plastic test tubes are employed to prevent blood from coagulating. As a result, whole blood is separated after centrifugation into red blood cells at the bottom of the test tube and the liquid top portion containing platelets and leukocytes. The liquid top portion is termed i-PRF and can easily be aspirated and used in injectable form or as topical spray. After approximately 15 minutes, i-PRF solidifies and is converted into gel form due to conversion of fibrinogen into fibrin.[15,16]

The objective of this study was to investigate whether i-PRF could increase the resistance of intestinal tissue against wound dehiscence and leakage by recording the enterotomy leak pressure of canine cadaveric samples. The fact that i-PRF is converted from liquid into gel form similar to fibrin glue prompted us to hypothesize that it could be used as a method to reinforce enterotomy suture line and increase the recorded pressure at the time of leakage. To the best of our knowledge, this aspect of i-PRF use has not been previously investigated.

Materials and Methods

Sample Acquisition

The study protocol was approved by the ethics committee of our institution (Approval ID: IR.IAU.TABRIZ.REC.1401.227, Approval Date: 2022-11-01). This *ex vivo* study was conducted on healthy fresh small intestinal samples obtained from 3 mixed breed dogs (2 males and 1 female) which were euthanized for reasons unrelated to this study. Their approximate age was 1.5-4 years and they weighed 17.5-37.4 kg. Immediately after euthanasia, the jejunum of small intestine was identified and the mesentery was carefully separated to avoid plication. The jejunum of each dog was then sectioned into 17 cm long segments and their luminal contents were emptied by careful milking and flushing with tap water. Each segment was kept moist until use by means of saline soaked sponges. A total of 28 jejunal segments were obtained and enterotomy followed by leak testing was performed on all samples.

Surgical Procedure

Jejunal segments were occluded at both ends using doyen intestinal forceps and held by an assistant. A routine enterotomy incision measuring 3 cm was made at the center of the antimesenteric border of each segment using scalpel blade and metzenbaum scissors. The enterotomy incision was then sutured by simple interrupted or simple continuous suture patterns using 3/0 polydioxanone suture material (Pedesente, Dogsan, Trabzon, Turkey) (Figures 1A and B). The sutures were placed 2-3 mm apart and 2-3 mm from the wound edge. Following surgery, the jejunal segments were allocated into 4 groups consisting of simple interrupted control (n = 6), simple interrupted treatment (n = 7), simple continuous control (n = 6) and simple continuous treatment (n = 9). No further action was taken in the control groups while topical i-PRF was applied in the treatment groups to reinforce the suture line.

Preparation and Application of i-PRF

Preparation of i-PRF was done according to the method described by Wang *et al.*[15] Briefly, 50 ml of whole blood was collected from the cephalic or saphenous vein of a healthy 2 years old male mixed breed dog weighing 16 kg in 10 ml plastic test tubes without anticoagulant. The samples were immediately centrifuged at 700 rpm for 3 minutes using a table top centrifuge (Hermle Z 206 A, Germany). Approximately 1.5 ml of i-PRF was collected from the top layer of each test tube which was easily aspirated and topically applied onto the enterotomy suture line in the previously described treatment groups. Collection and topical application of i-PRF was done using a 5 ml syringe equipped with an 18 gauge needle and an approximate volume of 0.45 ml of i-PRF was poured onto each treatment sample. After 15 minutes, the liquid i-PRF was transformed into solid gel and its attachment to the suture line was clearly evident (Figures 1C and D).

Figure 1. Representative canine jejunal samples after enterotomy incision and closure using simple interrupted or simple continuous suture patterns without suture line reinforcement (A and B) and with i-PRF reinforcement (C and D). Note the presence of solidified i-PRF on and around the suture line in reinforced samples.

Leak Testing

Leak testing was performed immediately after suturing the enterotomy incision in the control groups while a minimum of 15 minutes was allowed for formation of i-PRF gel before leak testing in the treatment groups. The jejunal samples were occluded at both ends using doyen intestinal forceps and held by an assistant. The distance between each clamp and the enterotomy suture line was 6.5 cm therefore a 13 cm segment of intestine was used for leak testing. An 18 gauge intravenous catheter was inserted at each end from the anti-mesenteric border towards the suture line. The catheter inserted at the left side of each sample was attached via intravenous tubing to a 0.5 liter bag of dyed (methylene blue; dilution, 1:500) normal saline solution. The other catheter was attached to a pressure transducer (Medex MX960, Dublin, OH, USA) via a syringe pump infusion line (ET-1, JMS CO. LTD., Hiroshima, Japan) (Figure 2A). Dyed saline solution was injected into the lumen of jejunal segments at the rate of 450 ml/h[19, 30] by means of an infusion pump (OT-701, JMS CO., LTD., Hiroshima, Japan) and the pressures were recorded by a pressure monitoring device (EDAN iM70, Edan Instruments, Inc., Nanshan Shenzhen, China) attached to the pressure transducer. The intestinal segments were observed for leakage by 2 observers. Leakage was defined as the first observation of dyed solution leaking from the suture line or suture needle holes (Figure 2B). The pressure recorded at the time of leakage was termed initial leakage pressure (ILP). The infusion was continued until maximum intraluminal pressure (MIP) was recorded which was defined as the pressure recorded at the time of complete failure of the suture line or a stable drop in intraluminal pressure. Both ILP and MIP were recorded in mmHg. The leakage location was also noted for each segment. Leak testing of all specimens was completed within a 4 hour period after sample collection.

Statistical Analysis

The normality of data was assessed by Shapiro-Wilk test. Comparison of ILP and MIP between groups was performed using one way analysis of variance followed by the Tukey multiple comparisons test. All statistical analysis was performed using GraphPad Prism version 8 (GraphPad Software Inc., Boston, MA, USA) and significance was defined as $p < 0.05$. Data are presented as mean ± standard deviation.

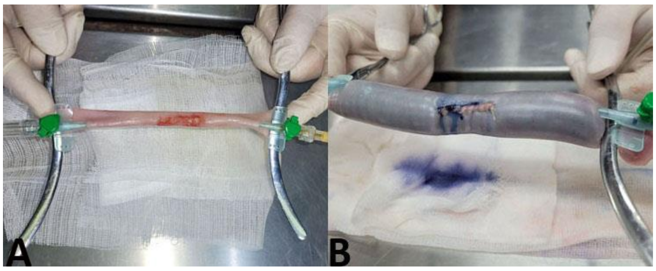

Figure 2. Setup for leak testing on a canine jejunal sample after enterotomy and i-PRF reinforcement (A) and visible leakage of dyed saline solution after start of leak testing (B).

Results

Statistically significant differences in ILP and MIP were not observed between the four treatment groups (p = 0.762 and p = 0.8073, respectively) (Figures 3 and 4). Application of i-PRF in the simple continuous treatment group increased the mean ILP in comparison to simple continuous control group (15.00 ± 7.228 vs. 11.67 ± 26.17). Mean MIP was increased in both simple interrupted and simple continuous treatment groups in comparison to their corresponding control groups (46.29 ± 30.53 vs. 39 ± 34.54 and 47.78 ± 9.497 vs. 35.67 ± 31.46). With respect to leakage location, all samples sutured with simple interrupted sutures leaked from the incision line between the sutures while leakage occurred from the needle holes outside the incision line in simple continuous sutured samples and the use of i-PRF had no influence on this pattern of leakage location.

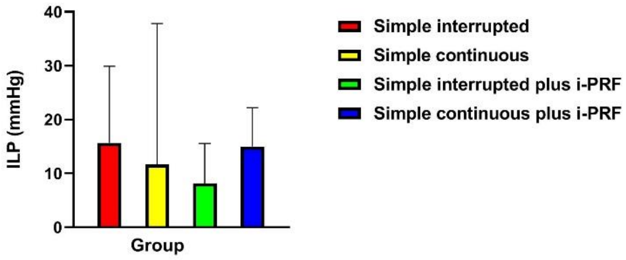

Figure 3. Mean initial leakage pressure of the experimental groups, error bars indicate standard deviation.

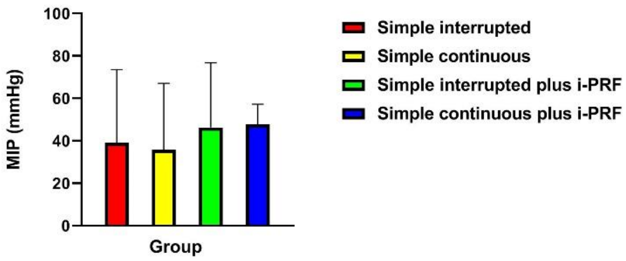

Figure 4. Mean maximum intraluminal pressure of the experimental groups, error bars indicate standard deviation.

Discussion

This ex vivo study aimed to investigate the effect of i-PRF on leak pressure of canine enterotomy. In experimental animal studies, the progress of intestinal wound healing following surgery can be evaluated using biomechanical, biochemical and histological methods.[17] However, in cadaveric studies only the biomechanical methods are available. Bursting pressure or leak pressure which reflects the resistance of the intestinal wall to increasing intra-luminal pressures resulting from continuous instillation of fluid into the intestinal lumen is the most frequently employed biomechanical parameter.[17] Our methodology was similar to previous cadaveric studies investigating the effect of surgical sealants/adhesives or suture line reinforcement methods on small intestinal anastomosis and enterotomy.[18-21] Simple interrupted and simple continuous suture patterns were used in this study for closure of incisions as they are the most frequently employed and recommended suture patterns for closure of canine enterotomy in clinical cases.[1,2,6,22] Therefore, this study aimed to determine whether there were any differences in leak pressure of enterotomy incisions sutured with simple interrupted or simple continuous patterns and reinforced with i-PRF.

Platelet concentrates are broadly categorized into first and second generation products or platelet rich plasma (PRP) and PRF, respectively. The differences between the two types are mainly related to their preparation methods, the final form of the obtained product and whether it requires activation or not during use.[23,24] However, all platelet concentrates including i-PRF are considered as rich sources of growth factors and therefore have been used in wound healing and regenerative medicine involving human and various animal models. But, as previously mentioned, this is the first study to investigate the use of i-PRF as a suture line reinforcement method therefore there are no similar studies for comparison.

The results of the present study indicated that it was feasible to use i-PRF as a suture line reinforcement method in canine enterotomy. The process of i-PRF preparation and topical application on suture line was easily accomplished. However, statistically significant increase in ILP and MIP was not observed with the use of i-PRF. In the present study, the mean ILP and MIP of enterotomy incisions sutured with simple interrupted pattern were higher than those sutured with simple continuous pattern in control specimens. This is in accordance with the findings of another study investigating the effect of four different closure patterns on ex vivo leak pressure of canine enterotomy.[22] Use of i-PRF as a suture line reinforcement method increased the mean ILP in simple continuous sutured group and mean MIP in both simple interrupted and simple continuous sutured groups. It has been reported that intraluminal pressure in the jejunum of healthy non-anesthetized dogs during peristalsis reaches a maximum of 25 mmHg.[6,22,25,26,28-30] Based on this finding, it has been recommended that sutured enterotomy incisions should withstand ILP of above 25 mmHg to prevent leakage in clinical settings.[6,12,27,29] In the present study, the mean ILP of all treatment groups was lower than 25 mmHg while the mean MIP was higher than this value. Postmortem changes associated with tissue autolysis could have an adverse effect on leakage pressure of cadaveric tissue samples. In order to better mimic the in vivo conditions, it has been suggested that freshly collected intestinal samples should be used for leakage testing within a 4 hour period.[25,30] A significant finding of this study was the difference in leakage location between the treatment groups. Leakage occurred from within or outside the incision line in simple interrupted and simple continuous sutured enterotomies respectively even when i-PRF was used. It has been suggested that leakage at needle holes outside the incision line has little clinical effect on development of catastrophic enterotomy failure leading to septic peritonitis.[22]

The major limitation of this study was its ex vivo nature and the use of cadaveric specimens instead of living tissue. In clinical cases, incision of the gastrointestinal tract is immediately followed by hemorrhage, platelet aggregation and formation of fibrin clots. Apart from controlling further hemorrhage, the fibrin clot also has adhesive properties.[31] Furthermore various growth factors such as platelet derived growth factor, transforming growth factor and vascular endothelial growth factor are released from activated platelets which have an essential role in intestinal wound healing.[32] This sequence of events are not observed in ex vivo studies. Considering the aforementioned points and the fact that i-PRF is a rich source of growth factors, it is postulated that the use of i-PRF as a suture line reinforcement method in an in vivo setting could significantly improve the biomechanical and other aspects of intestinal wound healing. Indeed, a significant increase in bursting pressure of jejunal and colonic anastomosis has been reported utilizing in vivo rat and rabbit models where PRF membrane was wrapped around the anastomosis.[33,34] A further limitation of this study was small sample size due to financial constraints and the unavailability of canine cadavers. Another unavoidable reason for small sample size was that all enterotomies and leak tests were performed in a limited time period of 4 hours.

In conclusion, this was the first study to utilize i-PRF as a suture line reinforcement method in canine enterotomy. The results indicated the feasibility of using this method in an ex vivo setting. The biomechanical properties of enterotomy incisions measured in the form of leak pressure were non-significantly increased with the use of i-PRF. Further studies using larger sample size and in particular in vivo animal models are required to investigate the effect of this method on various aspects of small intestinal wound healing and to determine whether this method could be used in clinical cases.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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