DOI: 10.1590/0100-69912016002007

The collagen, fibrinogen and thrombin biological adhesive is effective in treating experimental liver injuries

O adesivo biológico de colágeno, fibrinogênio e trombina é eficaz no tratamento de lesões hepáticas experimentais

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ABSTRACT

Objective: to evaluate the effectiveness of an collagen-based adhesive associated with fibrinogen and thrombin in experimental liver injuries in rats. **Methods:** we randomly divided 30 Wistar rats into three groups: A, B and C. All underwent a standard liver traumatic injury. In group A, the lesion was treated with the adhesive; in group B, with conventional, absorbable suture; group C received no treatment. We analyzed the time of hemostasis, mortality, occurrence of adhesions and any histological changes. **Results:** there was no statistical difference in relation to mortality (p=0.5820). The adhesive treated group showed the lowest hemostasis times (p=0.0573, odds ratio 13.5) and lower incidence of adhesions (p=0.0119). The histological alterations of the Groups A and B were similar, with foreign body granuloma formation separating the adhesive material and the hepatic stroma suture. **Conclusion:** the collagen adhesive associated with fibrinogen and thrombin was effective in treating experimental hepatic injury, providing a lower incidence of adhesions between the liver and surrounding structures.

Keywords: Wounds and Injuries. Liver. Hemostatics. Thrombin. Tissue Adhesives.

INTRODUCTION

The surgical techniques to approach liver bleeding include local compression, cauterization, bandages, sutures, resections and drainage[1,2]. In complex liver lesions accompanied by hemodynamic instability, laparotomy is indicated for bleeding control with eventual Pringle maneuver[2-4], ligation of affected vessels and ducts, as described by Patcher[2], and even damage control surgery[5].

The development of a wide variety of hemostatic agents and tissue adhesives that occurred in recent years[6] offers surgeons the opportunity to use these products in order to achieve quicker and easier bleeding control. The seriousness and the difficulty in managing certain cases of liver trauma motivate the search for new therapeutic alternatives, especially for bleeding control. The efficiency of the new hemostatic lead to the hypothesis to test the efficacy of collagen adhesives associated with fibrinogen and thrombin, compared with the conventional suture in the treatment of experimental traumatic liver injury.

METHODS

This experimental study was conducted in the Surgical Technique Laboratory of the Faculdade de Medicina de Jundiaí, Jundiaí-SP, and was approved by the Ethics Committee for Animal Use with number 81/110.

We included 30 adult, male, Wistar rats, with a mean age of 3.55 months, weighing on average 442,80g (342g-527g). The animals were randomly divided into three groups, A, B and C, ten subjects in each.

All rats received premedication with atropine at a dose of 0.05 mg/kg subcutaneously in the dorsal region and acepromazine (Acepran® 1% – Univet, São Paulo) 1mg/kg by the same route. After 15 minutes of application of premedication, they received an association of tiletamine and zolazepan (Zoletil® 50 – Virbac, São Paulo) 20mg/kg intramuscularly. We initiated the operative procedure after full action of the anesthetic drugs, monitored by loss of corneal and eyelid reflexes and limbs flexion.

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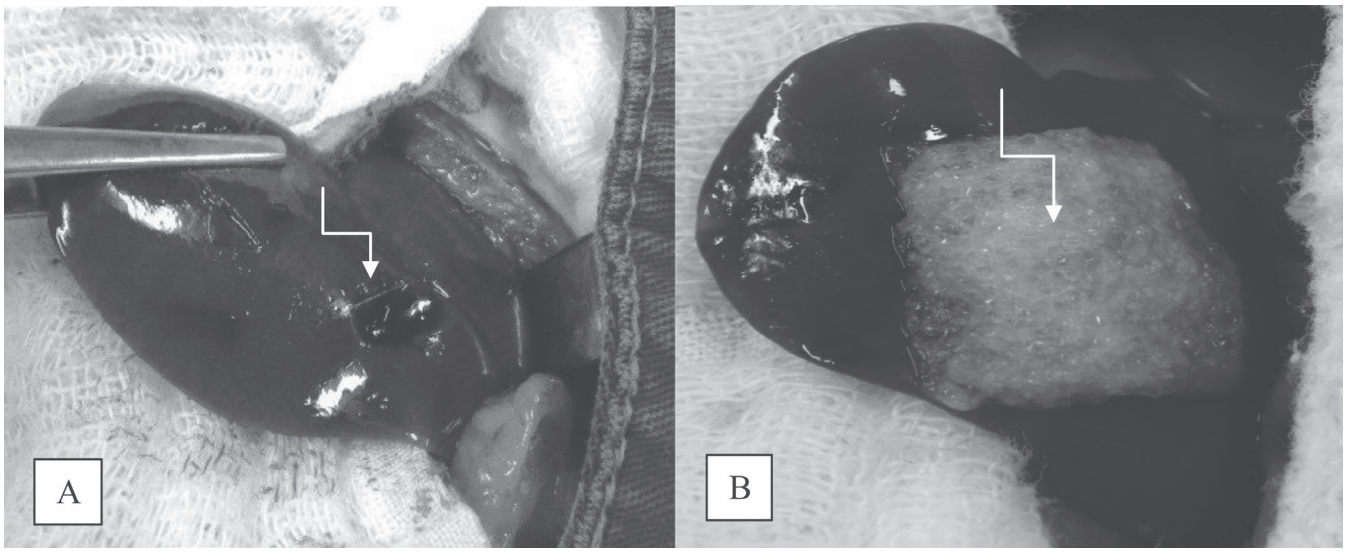

Figure 1. *A) Hepatic Injury (2x magnification); B) Final aspect of the adhesive, indicated by the arrow on the liver injury (2x magnification)*

All rats underwent laparotomy under aseptic technique, started from the xiphoid, approximately 3cm long. After opening the abdominal wall, we positioned a small orthostatic retractor and identified the liver, the organ chosen to perform the standardized injury with a biopsy surgical instrument (Punch Keyes® – ABC Surgical Instruments, Brazil) 5mm in diameter, introduced 5mm in depth into the parenchyma (Figure 1A).

From then on, we treated the animals according to the group to which they belonged. In Group A, after one minute of bleeding we performed treatment of injury using the surgical collagen adhesive associated to fibrinogen and thrombin (Tachosil® – Nycomed, Austria), previously activated in 0.9% saline (Figure 1B), with subsequent cleaning of the cavity and abdominal wall closure. In Group B, one minute after bleeding, we performed treatment of the injury with parenchymal liver suture using 3-0 polyglactin-910 (Vicryl® – Ethicon, USA) and subsequent cleaning of the cavity and the abdominal wall closure. In Group C, control group, we did not carry out any treatment of the hepatic injury, and only closed the abdominal wall.

In the experiments in groups A and B were recorded the hemostasis times for further analysis. Postoperatively, all rats received analgesia with dipyrone drops added to water and diet with appropriate chow at will. After eight weeks, the surviving rats were euthanized in a carbon dioxide chamber, with immediate necropsy for observation of intra-abdominal conditions and removal of the liver for histological analysis.

The study variables were the time to hemostasis, the occurrence of deaths, the occurrence of adhesions and any histological changes.

The hemostasis time was the time required to control bleeding are noted in the groups A and B. In group C, we did not record the time to hemostasis, immediately closing the abdominal wall after the liver injury. In the study design, we opted not to interfere in any way in the hemostasis of the induced injuries of the control group. We feared that, during the bleeding observation to note the time of hemostasis, if the bleeding was heavy the researcher might fell motivated to interfere with gauze compression or absorbing the blood with gauze. Attitudes like these would interfere with the results, with a tendency to decrease adhesions.

We classified adhesions into five grades, adapting the classification described in 1964 by Mazuji *et al*.[7]: Grade zero – absence of adhesion; Grade I – adhesion in the liver injury site to the abdominal wall, small and irregular; Grade II – in the liver injury site to the abdominal wall and to the omentum, of medium intensity and easy separation; Grade III – adhesion in the liver injury site to the abdominal, to the omentum and to the intestinal loops, intense and of difficult separation; Grade IV – adhesion in the injury site to any other region, very intense, homogeneous and difficult to separate. After analysis of

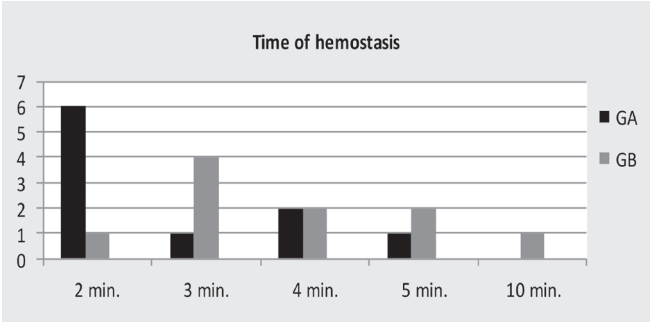

Figure 2. *Distribution of the lesions repair times between groups A and B. Vertically, the number of rats, and horizontally, the time of hemostasis*

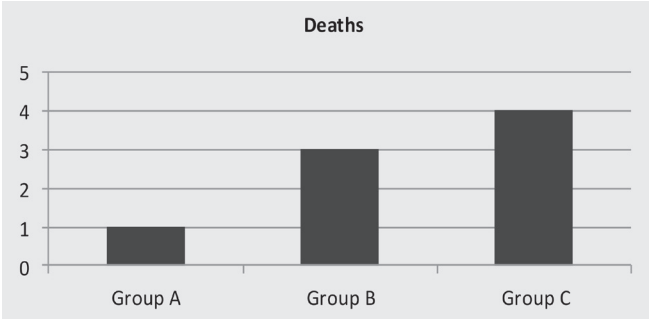

Figure 3. *Distribution of deaths between groups A, B and C. Vertically the number of deaths, and horizontally, the Groups.*

adhesions, we removed the rats livers and placed them in 10% formalin with subsequent preparation of slides with hematoxylin-eosin and picrosirius for microscopic analysis.

Statistical analysis was performed with the presentation of absolute (n) and relative (%) frequency distribution tables for all variables.

We analyzed the variables death, hemostasis time and the occurrence of adhesions with the Fisher's exact test. For the qualitative death variable, we made the comparison using the Fisher's exact test because the conditions of application of the chi-square test were not met. For the variable time of hemostasis, we compared the occurrence of the shorter time, which was two minutes between the two groups (adhesive and suture), using the Fisher's exact test, because it is a qualitative variable; we also calculated the odds ratio with its respective confidence interval. The significance level for the statistical tests was 5%.

RESULTS

Hemostasis Time

The overall average was 3.5 minutes, ranging between two and ten minutes. In Group A, the average time was 2.4 minutes, with the shortest time two, and the longest, five. In Group B, the average time was 4.2 minutes, ranging from two to ten minutes.

The distribution of the occurrence of hemostasis time of each group is shown in Figure 2.

When we grouped and analyzed the results with time equal to two minutes and longer than two minutes, in groups A and B (Table 1) we obtained a borderline significance between them by the Fisher exact test (p=0.0573). The *odds ratio* was 13.5 (range 1.20 to 15.2), which means that the animals of group B are 13.5 times more likely to have greater hemostasis time than two minutes. Therefore, this data shows statistical significance.

Death

Group A showed mortality of 10% (1/10 animals), group B had mortality of 33.3% (3/10 animals) and group C, 40% (4/10 animals). Overall mortality was 26.67% (8/30 animals). Table 2 and Figure 3 show the distribution of the number of deaths in each group.

The Fisher's exact test did not identify difference with statistical significance when comparing Group A with Group B (p=0.5820), Group A with Group C (p=0.3034) and Group B with Group C (p=1.0000).

Table 1. *Distribution of hemostasis time equal to two minutes and greater than two minutes in groups A and B in absolute numbers and percentages (in parentheses)*

	2 minutes	> 2 minutes	Total
Group A	6 (60%)	4 (40%)	10 (100%)
Group B	1 (10%)	9 (90%)	10 (100%)
Total	7 (35%)	13 (65%)	20 (100%)

Hemostasis time – animals of Group A versus Group B – Fisher exact test, p = 0.0573, Odds Ratio = 13.5.

Table 2. *Distribution of deaths in each group in absolute numbers and percentages.*

	Group A	Group B	Group C	Total
Death (n)	1	3	4	8
Death (%)	10	33.3	40	26.67

Adhesions

Group A had three rats with Grade 0 adhesions and six with Grade I. Group B had two rats with Grade I adhesions, three with Grade II and two with Grade III. The C group had one mouse with Grade I adhesions, four with Grade II and one with Grade III. No rat showed Grade IV adhesions.

Table 3 shows the distribution of the degree of adhesions in each study group.

When analysed the adhesions variable, we found that Group A had a lower incidence than Group B, with statistical significance (p=0.0119 – Fisher's exact test). A similar result occurred when comparing group A with group C (p=0.0069). When comparing Groups B and C, we found no statistically significant difference (p=1.0000).

Histological Changes

Histological changes found in the slides of the rats' livers of Group A were reaction to the foreign body with formation of histiocytes palisades, separating amorphous material (adhesive) from stromal liver cells (Figure 4) and plasma cell infiltrate and bilirubin extravasation due to ductal injury. We also observed intense collagen deposition (Figure 5), with dense fibrosis. Histological changes found in the slides of Group B rats' livers were foreign body, granuloma-type inflammatory reaction around the suture fragments, with giant cells and absent fibrosis. The slides of the rats in Group C showed extravasation of red blood cells, without formation of inflammatory tissue.

DISCUSSION

The induced liver injuries tried to reproduce intermediate lesions that correspond to grade III lesions when compared to liver trauma classification of the American Association for the Surgery of Trauma (AAST)[1,3,8].

For the choice of tissue adhesive, we looked for a product that could take advantage of the properties of bleeding, barrier offered by mechanical hemostatic agents, associated with direct action on blood clotting, offered by active hemostatic agents. Thus, the choice fell on a combination of products already on the market, represented by the combination of collagen associated to fibrinogen and thrombin[9-13]. This is a totally biological product, without synthetic components. This adhesive was evaluated in clinical studies as support to hemostasis in different kinds of surgery, most often in elective situations, especially on parenchymatous organs, showing effectiveness in controlling bleeding[9-13].

Frilling, in 2005, reported the adhesive superiority compared with the argon beam during liver resection with respect to homeostasis time[12]. We obtained similar

Table 3. *Distribution of Adhesions in Groups A, B and C.*

	ADHESIONS		
	Group A	Group B	Group C
Grade zero	3	0	0
Grade I	6	2	1
Grade II	0	3	4
Grade III	0	2	1
Grade IV	0	0	0

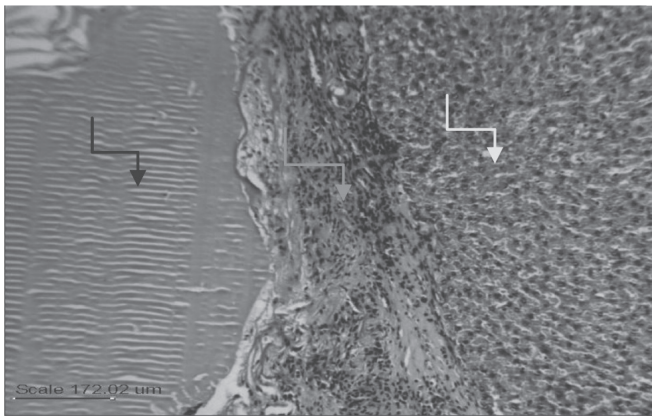

Figure 4. *Photomicrograph of histological section stained with hematoxylin-eosin, showing a Group A rat liver. The black arrow points to the adhesive amorphous material; the green arrow points to the foreign body, granuloma-type inflammatory process area, with histiocytes distributed in palisade, separating the adhesive material from the liver stroma.*

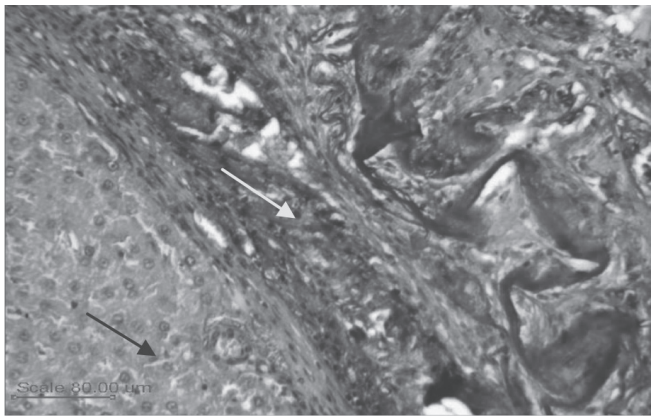

Figure 5. *Photomicrograph of histological section of the Masson's trichrome biochemical reaction, showing a Group A rat liver. The yellow arrow points to collagen fibers stained in red permeating inflammatory lymphonuclear and giant cells; the black arrow points to the liver parenchyma.*

findings when evaluating the injury repair time with the use of adhesive compared with conventional suturing. The shorter hemostasis time obtained reflects the easy handling and effectiveness of the material in controlling bleeding, a fact already identified with the use of collagen alone, as demonstrated by Mantovani *et al*.[14], or when combined with fibrinogen and thrombin, as shown by experimental studies using dogs[9] and pigs[10]. It is noteworthy that in some rats treated with injury suture, the extended time to achieve hemostasis was due to the difficulty of manipulation of the liver tissue, which was very frail.

Like the collagen, fibrinogen and thrombin adhesive, other hemostatic agents are also cited as effective in the control of various types of bleeding. In 1990, De la Garza and Rumsey showed effectiveness in controlling bleeding with the use of fibrin glue in two patients suffering from liver trauma[15]. In the same year, Ochsner *et al*. used this product in 26 patients suffering from liver and splenic injuries, also with effective bleeding control[16].

Several experimental studies show the effectiveness of fibrin adhesive in controlling hepatic hemorrhage in dogs[17], pigs[18,19], rats[20] and rabbits[21], with good adhesion to the injured liver, little local inflammatory reaction and few complications. In our study we obtained similar findings to those of the cited works.

The occurrence of adhesions, which can be classified as a complication of surgical treatment, was statistically lower in the group treated with the adhesive compared with the group treated with suture (p=0.0119). This may be due to the animals treated with suture presenting major bleeding and bruising at the site of injury, resulting in greater inflammatory reaction and consequent adhesion.

Frena and Martin[13], in 2006, found the absence of biliary fistulas with the use of this product in elective hepatectomies in humans, which also occurred in our study, even when dealing with liver trauma, which increases the chance of this complication.

The mortality of the group treated with the adhesive (10%) showed no statistically significant difference from the group treated with suture (33.3% / p = 0.5820) and the control group (40% / p=0.3034). In a study of 1,000 patients suffering from liver trauma led by Feliciano *et al*. between 1979 and 1984, the mortality rate found was 10%[22], and in another study, conducted by Saaiq *et al*. in Islamabad, Pakistan, between 2003 and 2010, mortality was 9.73%[23]. Thus, mortality with the adhesive experimental use is similar to those found in liver trauma treatments conventionally performed in humans.

The presence of foreign body inflammatory reaction found in the histological analysis of the rats' livers treated with the collagen adhesive associated with fibrinogen and thrombin was similar to changes found in studies using fibrin glue in rats[24], fibrin glue in rabbits[21] and polyglycolic acid mesh in pigs[25]. We did not observe histological findings suggestive of liver tissue necrosis or vacuolar degeneration, as described with the use of cyanoacrylate[26], or the presence of

abscesses near the adhesive application areas. The intense collagen deposition identified close to the adhesive application areas (Figure 5) is an important fact, if we consider that collagen is essential for the injured tissue repair process[24].

Conservative treatment of isolated liver trauma has been increasing in recent decades, reaching levels of 80% in the present day[27]. This fact, associated with the development of less invasive therapies such as angiography with embolization[28,29], decreases the need for surgery to control liver bleeding. However, in situations of hemodynamic instability or with associated trauma to other organs, particularly in hollow viscus, surgical treatment is often mandatory[1,3,8,22,27,28]. The liver operative approach can be a complex procedure, requiring great skill and experience of the surgeon[2]. This study showed that a collagen adhesive associated with fibrinogen and thrombin was effective in the treatment of traumatic liver injury in rats and has the potential to be used by surgeons during the same approach in humans. Its ease of handling when compared with liver tissue suturing, leading to diminished bleeding control time and low complications rates, are the main points favorable for this material.

We conclude that the treatment with collagen adhesive associated with fibrinogen and thrombin was effective in experimental hepatic injury, opening new perspectives for use in liver injuries in humans.

ACKNOWLEDGEMENT

We thank Professor Siani Sirlei Morais for the statistical analysis of this study.

R E S U M O

Objetivo: *avaliar a eficácia de um adesivo a base de colágeno associado ao fibrinogênio e trombina, no trauma hepático experimental em ratos.* **Métodos:** *toram incluídos no estudo 30 ratos Wistar, igualmente divididos aleatoriamente em três grupos: A, B e C. Todos foram submetidos à lesão traumática hepática padronizada. No grupo A, a lesão foi tratada com o adesivo, no grupo B, com sutura convencional com fio absorvível, e no grupo C, não houve tratamento da lesão. Foram analisados o tempo de hemostasia, mortalidade, ocorrência de aderências e eventuais alterações histológicas.* **Resultados:** *os resultados mostraram que não houve diferença estatística em relação à mortalidade (p=0,5820). O grupo tratado com adesivo apresentou os menores tempos de hemostasia (p=0,0573 e odds ratio 13,5) e menor ocorrência de aderências (p=0,0119). Microscopicamente as alterações histológicas dos grupos A e B foram semelhantes, com a formação de granuloma de corpo estranho separando o material do adesivo e do fio de sutura do estroma hepático.* **Conclusão:** *o adesivo de colágeno associado ao fibrinogênio e trombina foi eficaz no tratamento do trauma hepático experimental, proporcionado menor ocorrência de aderências entre o fígado e as estruturas vizinhas.*

Descritores: *Ferimentos e Lesões. Fígado. Hemostáticos. Trombina. Adesivos Teciduais.*

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Received: 10/10/2015
Accepted for publication: 17/03/2016
Conflict of interest: none.
Source of funding: none.

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