Local Tranexamic Acid for Local Hemostasis in an Animal Liver Injury Model

Shahram Paydar, Mohammad Yasin Karami, Golinoush Sadat Mahmoudi Nezhad, Rouollah Rezaei, Ali reza Makarem, Ali Noorafshan, Shahin Mohseni

Trauma Research Center, Shahid Rajaee (Emtiaz) Trauma Hospital, Shiraz University of Medical Sciences, & Department of Urology, Shiraz University of Medical Sciences, 2 Department of Anatomy, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, 3 Department of Surgery, Division of Trauma and Emergency Surgery, Orebro University Hospital and Orebro University, Orebro, Sweden

Abstract

Background: Hyperfibrinolysis is a state of increased clot resolution often seen in trauma patients with ongoing hemorrhage. Tranexamic acid (TXA) inhibits fibrinolysis preventing clot resolution affecting hemorrhage continuation and is used by intravenous administration. Aims: The purpose of this study was to evaluate the local tranexamic acid application for hemostatic control in an experimental animal liver injury model. Settings and Design: This study was an experimental prospective treatment study to check the local TXA effects on liver injury. This study was approved by the Ethics Committee. Materials and Methods: Twenty adult male Sprague-Dawley white rats were equally randomized to two groups after a standardized liver injury was conducted under anesthesia. One group were “liver-packed” with gauze (TXA [−]) and the other group with gauze soaked in TXA (TXA [+]). Bleeding from the injured middle liver lobe was measured at 2 and 15 min, and at 48h second-look surgery, with euthanasia conducted at 14 days. The liver was sent for histopathological and stereological analysis. Statistical Analysis and Results: There was no difference in bleeding at 2 or 15 min after packing; however, larger amount of free blood at 48 h in the TXA (−) group was noticed. Five animals in the TXA (−) were alive at 14 days compared to eight animals in the TXA (+) group. Significantly larger volume density of fibrosis, granulation tissue, and amorphous tissue were seen in the TXA (+) group compared to the TXA (−) group at the stereological analysis. Conclusion: Local TXA application on the injured liver surface might offer better hemostatic control than packing alone. Further studies are mandated before the clinical application of our findings.

Keywords: Liver injury, local hemostasis, tranexamic acid

Introduction

Uncontrolled bleeding is still the most common cause of preventable deaths in trauma patients who survive the initial insult.¹⁻³ In the bleeding patient, the main priority is to get control of the source of bleeding. Not managing to do so, does not only increase the risk of mortality by exsanguination but also may lead to worsening of other concomitant injuries through reduced organ perfusion, most notably traumatic brain injuries.⁴⁻⁵

Massive uncontrolled hemorrhage contributes to and exacerbates the “lethal triad” consisting of coagulopathy, hypothermia, and acidosis.⁶⁻⁸ Studies have found that shock and resulting hypoperfusion activates hyperfibrinolysis, leading to clot resolution and continuation of bleeding at the source of injury. In addition, hyperfibrinolysis at the time of admission has been shown to be an independent risk factor for mortality.⁶⁻⁸⁻¹¹

In recent years, interest has increasingly focused on intravenous administration of tranexamic acid (TXA) for encountering the hyperfibrinolysis seen patient suffering major hemorrhage after severe trauma. The result has been positive with an increase in survival rates, which has been contributed to the downregulation of global fibrinolytic activity and thus decreased bleeding.¹²⁻¹³ However, the role of TXA in obtaining local hemostatic control is yet to be investigated.

Address for correspondence: Dr. Shahin Mohseni, Department of Surgery, Division of Trauma and Emergency Surgery, Orebro University Hospital and Orebro University, Orebro 701 85, Sweden. E-mail: paydarsh@gmail.com

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Gaining surgical control of ongoing hemorrhage from hepatic injuries may be difficult due to the hepatic parenchyma not being amenable for reapproximation with sutures or packing. This can be more challenging in severely multi-injured patients that have developed systemic coagulopathy. Several advanced local hemostatic agents have been developed during the last decade and are in use in the military and civilian sectors. However, these agents are expensive and not commercially available in many trauma centers in low-developed countries. In contrast, TXA is significantly less expensive and already in use at many of these centers for intravenous administration.

The purpose of the current study was to evaluate the short- and long-term efficacy of local TXA in a high-grade liver injury animal model. Previous studies, including from our center, have used Sprague-Dawley rat models of high-grade liver injuries. A comparative study of the anatomy of rat and human livers showed that the fundamental structures of rat and human livers were similar. The hypothesis was that local TXA would decrease the rate of local fibrinolysis and thereby lead to stronger clot formation, leading to better bleeding control at the injury site. We hypothesized that damage control packing with gauze soaked in TXA would have a better effect than standard gauze packing alone as primary local bleeding control, second-look laparotomy at 48 h and overall survival at 14 days in a rodent liver injury model.

**Materials and Methods**

**Ethical statement**

The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (No: IR.SUMS.MED.REC.1396), in accordance with the International Helsinki convention’s animal experimentation agreement. The present study was in accordance with the standards of the National Society of Medical Research (“Principles of Laboratory Animal Care”) and “The Guide for the Care and Use of Laboratory Animals” (NIH publication No. 86–23, revised 1985) and checklist as per the ARRIVE guideline for reporting animal research was followed. The animals’ health status was monitored throughout the experiments by a health surveillance program according to the Federation of European Laboratory Animal Science Associations (FELASA) guidelines. The rats were free of all pathogens listed in the FELASA recommendations.

**Study design**

Random allocation was performed at the time of surgery with closed envelopes into two groups of 10 adult male Sprague-Dawley white rats in each; those who had their liver laceration packed with gauze (TXA [−]) and those where the gauze had been impregnated with tranexamic acid 500 mg/5 ml (TXA [+]).

No power calculation was carried out due to the lack of previous studies in the field.

**Housing and husbandry**

Standard food and water were supplied in separate cages with a controlled temperature of 22°C (humidity of 55%) with 12 h light and dark photo cycles. They had free access to equal amounts of standard rodent chow and water. All cages contained wood shavings, bedding, and a cardboard tube for environmental enrichment. All animals were given 1 week to adapt to this environment before the start of the experiment.

**Experimental procedures**

The animals were anesthetized with an intramuscular injection of ketamine (50 mg/kg; Alfasan International, Woerden, the Netherlands) and xylazine (10 mg/kg; Alfasan International). After the completion of anesthesia and sterilization of the surgical site, a midline abdominal laparotomy was performed. After gaining access to the intra-abdominal cavity, a standardized liver laceration, consisting of a 20 mm in length and 5 mm in depth and width to the middle lobe of the liver was carried out with a scalpel [Figure 1]. Buprenorphine at a dose of 0.01 to 0.05 mg/kg SC was used as an analgesic agent during the operation. If signs of pain were observed in the animals postoperatively, i.e., staggering and/or arching of the back, an additional dose of buprenorphine was administered. All animals showed sign of pain in the first 300 min postoperative period and received buprenorphine at a dose of 0.01–0.05 mg/kg SC.

In an attempt to control for procedural bias, all surgeries were carried out by a single surgeon.

After 2 min of uncontrolled bleeding, the liver was packed, and the free blood in the peritoneal cavity was collected by suction and measured using a syringe. A reassessment of the hemostatic status of the abdominal cavity was made following an additional 15 min. Subsequently to the reassessment, a second packing was carried out, and the gauzes were left in place and the fascia was closed. The animals were returned to their cages where they were cared for by a veterinarian. A second-look laparotomy was performed after 48 h for the evaluation of the injured liver. After removal of the gauze, cautery was used in cases where remaining oozing bleeding was noticed. Any free blood was collected, and once again the fascia was closed before the animals were returned to their cages. Euthanasia was performed after 14 days from the initial surgery. All rodent livers were collected and sent to the pathology department in formaldehyde for histopathological assessment.

Two primary clinical outcomes of interest were intraperitoneal bleeding at 2 and 15 min and after 48 h and the incidence of
mortality. In addition, one secondary outcome measure was evaluated: histological changes assessed using the stereological method in the liver tissue (day 14).

**Histopathological evaluation of liver tissue by the stereological method**

Stereology method was used to quantify the injured liver parenchyma and to compare the TXA (+) and TXA (−) packed cohorts. Stereology is the three-dimensional interpretation and quantification of a two-dimensional cross-section of tissue by microscopic evaluation. The stereological technique requires only a few “representative” plane sections from which a three-dimensional interpretation can be extrapolated statistically. The major middle lobe which had been fixed in neutral-buffered formaldehyde was assessed for weight and volume by immersion method.[21,22] After completion of this step, the liver parenchyma was sliced in sections of 25 μm after it had been processed in blocked cylindrical paraffin. Then, 10–12 sections for each part of major lobe were sampled through systemic uniform random sampling. The sampled sections of the major lobe were then processed and embedded in the same paraffin block. Overall, 4 μm sections were prepared and stained using Heidenhain’s azan trichrome and hematoxylin and eosin and were analyzed.

The effect of TXA was measured comparing normal liver parenchyma to injured liver parenchyma by the amount of granulation tissue, amorphous tissue, and fibrosis with the proportion of the lacerated area to the total area of the liver using the stereological method, i.e., measuring volume density.[22,25]

**Estimation of the volume of normal liver tissue, granulation tissue, amorphous tissue, and fibrosis**

The microscopic evaluation was done using computerized video-microscopy systems. The stereological counting equipment consisted of a Nikon E-200 microscope (Nikon, Japan) with a motorized stage linked to a computer and a flat monitor. For doing stereological counting, the stereological grid of points was generated using designed software, and stereological probes (point grids and counting frames) were superimposed onto the live images of the sections. Volume density (Vv) refers to the fraction of the unit volume of the tissue occupied by a structure.[3,22-25] The volume density of the normal liver tissue, granulation tissue, amorphous tissue, and fibrosis (the fraction of unit volume of the liver occupied by normal liver tissue, granulation tissue, amorphous tissue, and fibrosis) were estimated using the point counting method.[3,22-25] Briefly, a grid of points was superimposed on the images of the liver sections viewed on the monitor at final ×400 times. The density was computed according to the following formula:

\[ Vv = \frac{P}{P + (structure)} \]

where \( Vv \) (structure, reference) = \( P \) (structure)/\( P \) (reference), where “\( P \) (structure)” and “\( P \) (reference)” represented the total number of points hitting the structures of interest (normal liver tissue, granulation, amorphous, and fibrotic tissue) and the total number of points laid on the profiles of the liver sections, respectively.[25,26]

**Statistical analysis**

The results are shown as mean ± standard deviation. Statistical comparisons were performed using the application of the Mann–Whitney U-test (SPSS Statistics software, version 22, Chicago, IL, USA). Values of \( P < 0.05 \) were considered as statistically significant.

**RESULTS**

**Bleeding**

The rodents had a mean weight of 300 ± 50 g. TXA (+) and TXA (−) rodents had mean weight of 310 ± 50 g and 295 ± 30 g preoperatively \( (P = 0.43) \), and the mean weight at the time of euthanasia was 275 ± 35 g and 260 ± 40 g \( (P = 0.49) \), respectively.

No statistical difference could be measured with regard to intraperitoneal bleeding between the groups at 2 min \( (\text{TXA (−)} \text{ vs. } \text{TXA (+)}: 1.2 ± 0.18 \text{ ml vs. } 0.99 ± 0.15 \text{ ml, } P = 0.69) \) or at 15 min \( (\text{TXA (−)} \text{ vs. } \text{TXA (+)}: 0.14 ± 0.05 \text{ ml vs. } 0.18 ± 0.07 \text{ ml, } P = 0.196) \) after liver injury. However, there was a significant difference between the groups measuring intra-abdominal free blood at the second look laparotomy \( (\text{TXA (−)} \text{ vs. } \text{TXA (+)}: 0.82 ± 0.16 \text{ ml vs. } 0.65 ± 0.04 \text{ ml, } P < 0.001) \). In the TXA (−) animals, 30% (3/10) needed cauterization and repacking at the 48-h second look surgery, whereas no such treatment was necessary in the TXA (+) group.

**Deaths and adverse events**

Although not reaching statistical significance \( (P = 0.159) \), a higher rate of mortality was seen in the TXA (−) compared to TXA (+) cohort (50% vs. 20%) at 14 days. All deaths occurred following the second look laparotomy. Surgical site infection developed in four surviving animals and was effectively treated with local antibiotic ointment (bacitracin, and Polymyxin B) and simple dressing.

**Stereological results**

No significant differences in weight or volume of the major liver lobe were detected between two groups [Figure 1]. A significantly larger volume density of fibrosis, granulation tissue, and amorphous tissue were [Figure 2] seen in the TXA (+) group compared to the TXA (−) group. Normal liver parenchyma was to a larger extent seen in the TX (−) group compared to the TX (+) group [Table 1].

**DISCUSSION**

Hemorrhagic shock continues to be the most common cause of preventable deaths in trauma patients.[27] Despite advancements in trauma care and subsequent increases in overall survival, the mortality in injured patients who present in a state of hemodynamic shock is high and have not changed much during the last decades.[5,28]

In a recent study, Marsden et al. detected a 48% mortality rate in trauma patients in need of trauma laparotomy presenting with hypotension.[5] Thus, early bleeding control remains one
of the most important steps in the treatment of the multi-injured patient. However, this task is complicated by the nature of the parenchyma of some organs making them less amenable for approximation by suturing or simple coagulation by surgical devices.

The associated hypothermia, acidosis, and systemic coagulopathy seen in up to a quarter of trauma patients on arrival to the emergency department further increases the bleeding disorder. Further, hyperfibrinolysis activated by the hemorrhagic shock adds to the hemostatic disorder and have been identified as an independent predictor of mortality in hemorrhagic trauma patients.

Hyperfibrinolysis is a state of increased clot resolution by fibrin or fibrinogen cleavage due to excessive plasmin formation or a reduction of plasmin decomposition by a reduction in α2-antiplasmin. Hyperfibrinolysis is associated with severe and potentially life-threatening hemorrhage and subsequent tissue hypoxia. TXA is a synthetic lysine analog that inhibits the conversion of plasminogen to plasmin by preventing the binding of plasminogen to the fibrin molecule. In higher concentration, TXA can inhibit plasmin activity directly.

The reduced plasmin concentration and subsequent activity lead to a decreased fibrin cleavage and thus stronger clot formation reducing the risk of ongoing hemorrhage at the site of injury.

In recent years, there has been an increased interest in the intravenous administration of TXA after severe trauma since several studies have shown significant increase in survival rates in the severely injured patient group.

These positive findings have been attributed to the decrease in fibrinolysis activity, leading to better hemorrhage control. However, there are still many unknown factors regarding the intravenous administration of TXA. For example, recently, there has been some questions raised to the safety of administration of systemic TXA with an increased risk of thrombosis. It is also debated whether intravenous administration can achieve a high enough concentration at the local bleeding site required to be able to downregulate hyperfibrinolysis.

In view of this, studies investigating the local application of TXA for bleeding control have been carried out in the setting of elective total knee arthroplasty and dental surgery showing promising results. However, there are no studies investigating the role of local TXA application in obtaining bleeding control in trauma patients. In the current study, using an animal model of severe liver injury, we could detect a decrease in bleeding in the cohort packed with TXA soaked gauze. More importantly, the stereological study showed a higher density of fibrosis and granulation tissue at the site of liver injury in the TXA (+) group. These findings do strengthen the theory that decreased hyperfibrinolysis leads to better coagulation and hemorrhage control at the site of injury.

The current study is, however, limited by the low number of rodents in each group. Both control and treatment groups had fewer than ten participants surviving to 14 days for having a stereological analysis done. Also this study outlines a rodent model of improved bleeding control through the use of TXA soaked gauze but may not achieve the same result in humans. Before considering applying local TXA treatment in clinical practice, the authors recommend a repeated study design with higher statistical power.

**Conclusion**

Packing with local TXA application on the injured liver surface might offer better hemostatic control than packing alone. Further studies are mandated before the clinical application of our findings.

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**Conflicts of interest**

There are no conflicts of interest.

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**Table 1: Mean (standard deviation) of the stereological data between control and tranexamic acid impregnated gauze packed groups**

| Group                  | TXA (−) (n=5)       | TXA (+) (n=8) | P     |
|------------------------|---------------------|---------------|-------|
| Mean±SD of Vv NT       | 0.995±0.057         | 0.896±0.082   | 0.024 |
| Mean±SD of Vv fibrosis | 0.005±0.005         | 0.052±0.036   | 0.024 |
| Mean±SD of Vv GT       | 0.0001±0.0001       | 0.024±0.023   | 0.032 |
| Mean±SD of Vv AM       | 0.0001±0.0001       | 0.03±0.021    | 0.031 |

SD: Standard deviation, Vv: Volume density, TXA: Tranexamic acid, NT: Normal liver tissue, GT: Granulation tissue, AM: Amorphous
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