EFFECT OF LOCAL TRANEXAMIC ACID ON THE QUALITY OF BONE HEALING IN A RAT SPINAL FUSION MODEL

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Ethics approval

Dokuz Eylül University Multidisciplinary Laboratory Animal Experiments Local Ethics Committee approved this research with protocol number 19/2019 on May 8, 2019.

Informed Consent

Consent was not required because this study involved no human subject.

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ABSTRACT

Introduction: The use of the antifibrinolytic agent tranexamic acid has positive effects on bleeding control, but our knowledge is still limited regarding how fibrinolysis suppression changes the process of bone formation and the quality of bone. Because of the several side effects of systemic tranexamic acid, topical usage has been established in several procedures. This study aimed to investigate the effect of local tranexamic acid on vertebral fusion by using macroscopic, radiologic, and microscopic techniques. We also attempted to determine the safe dose range in case some doses had negative effects on fusion.

Methods: Twenty-eight Wistar albino rats underwent intertransverse fusion. All rats were randomized into four groups: groups treated with local tranexamic acid doses of 1 mg/kg (D1), 10 mg/kg (D10), and 100 mg/kg (D100) and the control group with no drug (D0). At the end of the eighth week, all rats were sacrificed for evaluation in terms of palpation, mammography, and histopathologic analysis.

Results: The manual palpation results presented with lower fusion rates in D10 and D100 groups than in the control group. Radiological examination results were significantly higher in the control group. The histopathologic examination revealed no significant differences between groups in the percent of new bone formation.

Conclusion: Our results showed that local administration of tranexamic acid reduced the quality and stability of fusion without a delay in bone formation. However, doses of 1 mg/kg did not reduce the stability in the palpation test. Our findings suggest that 1 mg/kg dose is a critical threshold above which tranexamic acid reduced the bone healing process of fusion and that surgeons should consider the doses of local tranexamic acid during surgery.

Keywords Rat; Bone healing; Spinal fusion; Tranexamic acid; Antifibrinolytic;

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INTRODUCTION

The use of an antifibrinolytic agent, tranexamic acid, has been shown to considerably reduce perioperative bleeding in several contexts, including cardiothoracic, orthopedic, and spinal surgeries¹⁻³. Tranexamic acid inhibits the interaction of plasminogen and the heavy chain of plasmin with lysine residues on the surface of fibrin because of its high affinity for the lysine-binding site of plasminogen, thereby resulting in the inhibition of the formation of fibrin degradation products⁴. There are two ways to use tranexamic acid: systemically and locally. The systemic administration of tranexamic acid has some potential adverse effects in patients with deep vein thrombosis, pulmonary embolism, myocardial infarction, and seizures⁵⁻⁷. These potential side effects have encouraged researchers to use local tranexamic acid for perioperative bleeding control.

Despite the positive effects of this drug on bleeding control, our knowledge is still limited regarding how fibrinolysis suppression changes the process of bone formation and the quality of bone. Fibrin matrices that form after fracture have essential factors for repair, and based on this information, most studies have reported the importance of fracture hematoma preservation in healing the bone⁸. Additionally, a few studies have shown that a decrease in fibrinolysis promotes healing^{9,10}. Conversely, the accumulation of fibrin has negative effects on tissue repair and results in increased local inflammation, which could cause osteoporosis¹¹.

Pseudoarthrosis remains one of the most common reasons for revision surgery following spine fusion operations¹². The most common factors associated with impaired fusion are smoking, infection, diabetes, vitamin D deficiency, impairment of bone formation, and advanced age¹³⁻¹⁶. Moreover, all these factors are related to impaired fibrinolysis activity^{17,18}.

The process of bone fusion is a kind of wound healing and is expected to be affected by fibrinolytic coagulation proteases and their inhibitors^{19,20}. Our investigations have directed us to determine the effect of local tranexamic acid on spine fusion. We also aimed to determine the safe dose range in case some doses have negative effects on fusion.

MATERIALS AND METHODS

Ethical Approval

We conducted this study in compliance with the principles of the Declaration of Helsinki. The study's protocol was reviewed and approved by the multidisciplinary laboratory animal experiment local ethics committee.

Animals

Healthy adult male Wistar albino rats weighing approximately 400 g and with normal motor activity were selected. All experiments were conducted at a temperature of approximately 20°C under a 12 h dark/light cycle. No feeding restrictions or changes were implemented.

Experimental Design

An ARRIVE checklist was used to ensure that the correct protocol had been applied. All rats were randomized into four groups by using sealed envelopes. Local tranexamic acid (Transamin 100 mg/ml, Teva İlaç, İstanbul) were determined on the basis of previously conducted studies: 1 mg/kg (D1 group), 10 mg/kg (D10 group), 100 mg/kg (D100 group), and no tranexamic acid (D0 group)²¹. Two rats in the D0 group died after the radiographic examination.

At 8 weeks postoperatively, plain radiographs were obtained. Then, specimens were prepared for manual palpation and histopathologic analysis. Palpation tests were performed immediately after sacrifice to reduce the effect of rigor mortis.

Surgical Procedure

After the rats were weighed, ketamine (Ketalar, Pfizer) 50 mg/kg and xylazine (Alfazyne, Egevet, İstanbul) 5 mg/kg were administered intraperitoneally for anesthesia. Preoperative ceftriaxone 50 mg/kg (Rocephin, Roche, İstanbul) was administered as prophylaxis 30 min before the incision was made²². The rats were positioned prone, and a dorsal midline skin incision between iliac crests was made. The transverse processes of the L4–L5 vertebrae were decorticated. Approximately 250 mg of morselized autogenous bone grafts were harvested from the posterior parts of the iliac wings and placed into the fusion area (Figure 1). Tranexamic acid solution (1 g in 10 ml of normal saline, prepared before the surgical procedure) was poured directly via injector to the fusion site according to dose ranges, given above.

81 Evaluation of Fusion

82 At the end of the eighth week, sedated rats were positioned supine under mammography (Selenia®
83 Dimensions® Mammography System, Hologic, Massachusetts, USA) (Figure 2). A previously described
84 radiological scoring system was used to assess the radiographs (Table 1)²³. All radiographs were evaluated
85 blindly and scored by four researchers (two radiologists and two spine surgeons) twice 1 month apart on the
86 Sectra Uniview digital imaging platform (version 21.2.11.6289, Linköping, Sweden). The first round scores were
87 not planned to investigate for evaluation because we thought that the quality of images from mammography was
88 not as high as micro-CT and there would be some difficulties to interpret them better. The second-round scores
89 of each researcher were categorized. Radiographs were evaluated on the basis of the frequency of categorized
90 scores.

91 All spines underwent palpation tests by three orthopedic surgeons immediately after sacrifice. Blinded
92 evaluation of motion at the fusion level was performed utilizing a previously described fusion mass scoring
93 system: “0” points, indicating the same range of motion as a nonfused level one below or one above the fusion;
94 “1” point, indicating a decrease in the range of motion; and “2” points, indicating no motion²⁴. All values were
95 categorized for each group and evaluated as the frequency of scores.

96 Each specimen underwent en bloc resection after manual palpation examination, and the specimens
97 were then fixed in 10% formalin solution for 24 h and decalcified for 48–72 h in 20% formic acid. Four to eight
98 consecutive sections with a thickness of 5–6 microns were generated by microtome and stained with
99 hematoxylin and eosin. Heiple’s modified Lane and Sandhu scoring²⁵) was used to determine histopathological
100 changes in the cortical and trabecular bones and marrow areas (Table 2).

101 The distributions of the data were checked with the Kolmogorov–Smirnov normality test. Continuous
102 variables are presented as the mean $\pm$ standard deviation (SD). The differences were compared using one-way
103 analysis of variance with Tukey’s post hoc analysis for normally distributed data and the Kruskal–Wallis test for
104 nonnormally distributed data. The Mann–Whitney U test was used for post hoc analysis with pairwise
105 comparisons. Categorical data are presented as numbers and percentages (%). Pearson’s chi-square test was used
106 to compare the fusion rates of each group. Spearman correlation coefficient analysis was used for the assessment

of the intraobserver and interobserver correlation of the observer in radiological and palpation examinations. All analyses were performed using IBM SPSS Statistics 22 software. A p- value below 0.05 was accepted as statistically significant.

RESULTS

Twenty-eight rats were used in this study. The average weight of bone grafts for all groups was 249.08 ± 11.9 mg with no statistical difference among all groups ($p = 0.22$). In the control group, two rats died after radiographic examination, likely because of anesthesia. The remaining 25 rats underwent all tests with no complications.

Spearman's rho correlation coefficient was high in the palpation scoring ($r = 0.83$). On the palpation test, the total scores of all observers were used to categorize spinal fusion as complete, partial, or absent and the distribution of fusion rates was shown in Figure 3. The rate of partially fused spines in the D0 group was 3 (20%), that in the D1 group was 8 (38.1%), that in the D10 group was 3 (14.3%), and that in the D100 group was 3 (14.3%). The rate of completely fused spines in the D0 group was 9 (60%), that in the D1 group was 13 (61.9%), that in the D10 group was 11 (52.4%), and that in the D100 group was 7 (33.3%). Statistically significant differences in fusion rates were detected in all groups ($p = 0.002$). The rates of completely and partially fused spines were similar between the control and D1 groups ($p = 0.32$). Evaluation of fusion rates in tranexamic-treated groups showed a decreasing trend with increasing dosage, and these differences were significant ($p = 0.009$).

Based on the radiological examination, Spearman's rho correlation coefficient was moderate in the first evaluation ($r = 0.46$) and high in the second evaluation ($r = 0.61$). All results of the second rounds were categorized as complete, partial, or absent spinal fusion distribution of fusion rates, which are shown in Figure 4. The rate of partially and completely fused spines in the D0 group was 96.4%, that in the D1 group was 67.9%, that in the D10 group was 85.7%, and that in the D100 group was 82.1%. There was a significant difference between groups ($p = 0.007$). However, the evaluation of fusion rates in tranexamic-treated groups showed no difference with changing dosages ($p = 0.27$).

Histopathological examination of each specimen was performed under a light microscope (Figure 5).

Heiple's scoring system was applied, and all results are shown in Table 3. The total score was the highest in the D1 group, followed by the D100, D0, and D10 groups. However, there was no statistically significant difference in the mean values of the union, spongy, compact, or bone marrow subgroups or in the total scores ($p = 0.13$). The mean values of all subgroups in the tranexamic acid-treated groups have been evaluated; only the union subgroups showed a difference ($p = 0.04$). The rate of union in the D1 group was higher than that in the D10 group (Dunn's multiple comparison test, $p = 0.03$).

DISCUSSION

In this study, our results indicated that the addition of tranexamic acid adversely affected the quality of fusion. The palpation test shows that this adverse effect was dependent on dosage but independent of radiographic examination. Histopathologic changes were similar in all groups.

To our knowledge, almost all studies on this topic have been about the effectiveness of tranexamic acid for bleeding control in spinal surgeries⁵⁾. However, the effect of local tranexamic acid on fusion quality has not yet been investigated. In the literature, there were a few studies that investigated the effects of the fibrinolysis mechanism on bone union. Heather et al.¹¹⁾ found that fibrinogen was the exogenous activator in bone remodeling and caused bone resorption leading to osteoporosis. Schoenecker et al.²⁶⁾ found that although aminocaproic acid and tranexamic acid did not show any impact on mineralization with the treatment dosage, they decreased osteoblastic proliferation depending on the dosage. Yuasa et al.⁸⁾ found that in cases where fibrinolysis deteriorated, the accumulation of fibrin deposits prevented the soft callus from transforming into a vascularized rigid callus. On the basis of these studies, we hypothesized that tranexamic acid would have a negative effect on vertebral fusion.

The evaluation time of fusion with manual palpation varies between 2 and 12 weeks, with an average of 6 weeks. Fusion ranges were increased significantly in the eighth week²⁷⁾. Hence, we evaluated each specimen 8 weeks after the surgical procedure.

To avoid bias, we did not allocate rats into groups before the surgical procedure. Grouping of rats was performed only after the bone graft was taken from the iliac wing, followed by the decortication of transverse processes and placement of grafts into the fusion area. Afterward, the group of animals that underwent the fusion operation was decided according to a prerandomized sealed envelope.

Mechanical test types have higher specificity and sensitivity than radiological examinations. Manual palpation is the most frequently used mechanical test. The absence of movement by palpation is the gold standard for the determination of fusion^{27,28}). Intervertebral movement of the spine with palpation is analogous to manual testing in humans during surgical exploration²⁹). In our study, manual palpation was performed by three independent orthopedic surgeons who were blinded. We showed that the use of local 1 mg/kg tranexamic acid had no effect on fusion; however, increasing doses of tranexamic acid had a negative effect on fusion.

It might not be sufficient to detect fusion in clinical trials and animal experiments²⁸). Thus, radiographic examination for the evaluation of fusion has been used for a long time. In our study, radiological examination was performed only by direct graphics. Scoring was performed by two radiologists and two spinal surgeons. In our radiological imaging protocol, only the AP view was used because, in the lateral view, the vertebral body was projected on the intertransverse area and did not allow the assessment. In the first round, the interrater reliability was found to be moderate. In the second round of radiographic scoring, the control group had the best outcomes, and local tranexamic acid adversely affected fusion regardless of the dose. Furthermore, interrater correlation was found to be high. We attributed the difference between both scores to the image quality. Evaluating radiographies twice provided the assessment of the intraobserver and interobserver differences. Additionally, the increased correlation between researchers in the second round of evaluation led us to think that the researchers adapted to the criteria of the scoring system and learned to interpret them better, although images were presented in shuffled order with no definitive signs on them. Thus, we did not include the data of the first round examination. There is always a possibility of inconsistency between manual palpation and other radiographic evaluations, and it will be necessary to define the fusion criteria to overcome this problem^{28,30}). Consequently, radiological examination revealed that the control group had the best in fusion, and tranexamic acid adversely affected fusion, independent of dosage. Changing the dose did not show any significant effect.

Histopathological investigation is an objective way to evaluate new bone production. Thus, a fusion decision is made by evaluating bone bridge formation. However, there is always a possibility of missing this bone bridge in the slice taken. Bone healing and bone fusion are also dynamic and continuous processes, so the histopathologic results are strongly dependent on the timing of sampling. Histopathologic scoring can only be performed according to the stage of sampling time. Among the groups, no significant difference was found ($p = 0.13$). The results showed that tranexamic acid did not delay or accelerate fusion regardless of dose.

The limitations of this study include its nature as an experimental study in a rat model. There are anatomical differences between humans and rats, and the speed of bone healing is faster in rats, which leads to less susceptibility to environmental factors. Because of these differences, tranexamic acid may affect the quality of vertebral fusion differently in rats and humans. Another limitation of our study was the use of mammography instead of micro-CT, considering its advantages. Radiographs were evaluated twice in 1 month by four researchers to strengthen the results and increase confidence. It should be kept in mind that this error may occur in all fusion studies that utilize manual examination. Future studies are needed to determine the impact of tranexamic acid on the quality of spine fusion by researching the molecular changes of bone graft substitutes through the fusion process. Additionally, clinical and radiographic follow-ups may be performed to investigate the effect of local tranexamic acid with different doses on vertebra fusion in humans.

Local administration of tranexamic acid reduced the quality and stability of fusion without a delay in bone formation. There was no statistically significant difference in histopathological results between the groups. However, 1 mg/kg dose did not reduce the stability in the palpation examination. Our findings suggest that 1 mg/kg dose might be a critical threshold above which tranexamic acid reduced the bone healing process of vertebra fusion and surgeons may consider the administration doses of local tranexamic acid during fusion surgery. Additionally, this study will be the reference for further studies on how tranexamic acid affects bleeding control at a dosage that does not affect fusion in the future.

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FIGURE CAPTIONS

Figure 1. Placement of the bone grafts harvested from the posterior iliac wing to the intertransverse interval (the grafts are shown by arrows).

Figure 2. Radiological examination of the rats: (A) control group, (B) D1 group, (C) D10 group, and (D) D100 group. Arrows indicate a fusion mass in the intertransverse field.

Figure 3. Fusion rates in manual palpation examination for all groups.

Figure 4. Fusion rates in the radiographic examination for all groups.

Figure 5. Specimens showing the formation of bone, cartilage, and marrow tissue in the intertrabecular fields (H&E, 40X). (A) Specimen with 4 points for union. The blue arrow demonstrates the field of chondrogenic healing with the prominence of chondrocyte cells. Green arrows demonstrate trabecular bones, and hematopoietic cells become reorganized by gathering together. (B) Specimen with 3 points for union. The blue arrow demonstrates the field of chondrogenic healing with the prominence of chondrocyte cells. Black arrows indicate the new woven bone fields. The green arrow displays the fibrosis generated by the loose connective tissues in the irregular trabecular bone fields that have gathered together. (C) Specimen with 1 point for union. Irregular bone formation (black arrows) and connective tissue containing dense fibrosis in intertrabecular fields (this area is shown by the blue arrow) (H&E, 20X). Normal bone marrow containing hematopoietic cells (green arrow). (D) Specimen with 2 points for union. Blue arrows show dense cartilage tissue with chondrocytes. The area shown by the black arrow shows endochondral ossification, which is the beginning of bone formation.

88x117mm (96 x 96 DPI)

209x100mm (96 x 96 DPI)

396x225mm (47 x 47 DPI)

395x228mm (47 x 47 DPI)

328x245mm (96 x 96 DPI)

TABLES

Table 1. Radiographic scoring

Score	Criteria
0	No bone mass
1	Only unilateral bone mass
2	Bilateral radiolucent bone mass
3	Bilateral bone mass with unilateral radiolucency
4	Non-radiolucent bilateral bone mass

Table 2. Heiple's Modified Lane and Sandhu Histological Classification

Union		
	None	0
	Fibrous union	1
	Osteochondral union	2
	Bone union	3
	All reorganized	4
Spongiuous		
	Complete resorbed, replaced by connective tissue	0
	Most of resorbed	1
	Partial resorbed	2
	No osseous cellular activity	3
	Early apposition of new bone	4
	Active apposition of new bone	5
	Mostly reorganized spongiuous	6
	Totally reorganized spongiuous	7
Bone marrow		
	Dead	1
	Replaced by fibrous material	2

New fibrous tissue	3
Two-thirds of marrow, replaced by new tissue	4
Less than one-quarter of adult marrow	5
Fatty marrow	6
Compact (cortical)	
None	0
Begin to appear	1
Formation	2
Totally formed	3
Total points	24

Table 3. Distribution of histopathologic examination results for each group

Group	Union	Spongious	Compact	Bone marrow	Total
(n)	Mean $\pm$ standard deviation	Mean $\pm$ standard deviation	Mean $\pm$ standard deviation	Mean $\pm$ standard deviation	Mean $\pm$ standard deviation
D0	2.2 $\pm$ 0.8	6.6 $\pm$ 0.5	2.6 $\pm$ 0.5	5.8 $\pm$ 0.4	17.2 $\pm$ 1.6
D1	3.1 $\pm$ 0.9	6.9 $\pm$ 0.4	2.7 $\pm$ 0.5	6 $\pm$ 0	18.7 $\pm$ 1.4
D10	1.9 $\pm$ 0.7	6.4 $\pm$ 0.8	2.4 $\pm$ 0.5	5.6 $\pm$ 0.5	16.3 $\pm$ 2.1
D100	2.3 $\pm$ 0.8	7.0 $\pm$ 0.0	2.6 $\pm$ 0.5	5.9 $\pm$ 0.4	17.6 $\pm$ 1.4
p*	0.75	0.19	0.79	0.25	0.13

* One-way analysis of variance test.