75682_Auto_Edited.docx

WORD COUNT 4335
TIME SUBMITTED 18-MAY-2022 10:26AM
PAPER ID 86062459

Name of Journal: *World Journal of Orthopedics*
Manuscript NO: 75682
Manuscript Type: REVIEW

Topical Use of Tranexamic Acid. Are There Concerns for Cytotoxicity?

Ioannis Gkiatas, Aristeidis-Panagiotis Kontokostopoulos, Spyridon E. Tsirigkakis, Ioannis Kostas-Agnantis, Ioannis Gelalis, Anastasios Korompilias, Emilios Pakos

Abstract

Tranexamic acid (TXA) has revolutionized modern blood management in orthopaedic surgery and especially in total joint arthroplasty by significantly reducing blood loss and transfusion rates. It is an antifibrinolytic agent and a synthetic derivative of the amino-acid lysine, which can inhibit the activation of plasminogen and the fibrin breakdown process. The administration of TXA can be intravenous (IV), topical and oral. In patients where the IV administration is contra-indicated, topical use is preferred. Topical administration of the drug theoretically increases concentration at the operative site with reduced systemic exposure, reduces cost, and gives the surgeon the control of the administration. According to recent studies topical administration of TXA is not inferior compared to IV administration, in terms of safety and efficacy. However, there are concerns regarding the possible toxicity in the cartilage tissue by the topical use of TXA mainly in hemiarthroplasty operations of the hip, unilateral knee arthroplasties, total knee arthroplasties where the patella is not resurfaced and other intraarticular procedures, like ACL reconstruction. The purpose of the present review is to present all the recent updates on the use of TXA focusing on the toxicity on chondrocytes and the articular cartilage that may or may not be provoked by the topical use of TXA.

INTRODUCTION

Tranexamic acid (TXA) is an antifibrinolytic agent and a synthetic derivative of the amino-acid lysine, which can inhibit the activation of plasminogen and the fibrin breakdown process [1]. Plasminogen is a glycoprotein pro-enzyme which is produced in the liver. Plasminogen molecules are folded into loops and lysine binding spots are located at the end of these loops. These lysine binding spots on plasminogen are connecting with lysine residues on fibrin. Tissue plasminogen activator is also released by endothelial cells and binds to fibrin, causing the conversion of plasminogen to plasmin. Plasmin severs fibrin into small fragments like d-dimers. This abruption causes the exposure of more lysine residues to plasminogen, therefore accelerating fibrinolysis [2]. The fact that TXA is a synthetic derivative of the amino-acid lysine causes the attachment of the lysine residues to the lysine binding points on plasminogen, thus preventing the binding of plasminogen to fibrin and blocking its activation to plasmin [2,3]. At higher concentrations, TXA is also a noncompetitive inhibitor of plasmin, therefore it stabilizes the clot formation [4]. Numerous studies conducted in animal tissue samples showed that TXA increases thrombus formation in a dose-dependent way [5]. Plasminogen receptors are also located on endothelial cells, monocytes, lymphocytes and platelets which indicates the role of plasminogen on inflammation. Thus, TXA may have another role, by inhibiting this procedure too [6].

TXA is associated with decreased blood loss in surgical procedures and inhibits the activation of plasminogen to a greater extent in comparison with another major antifibrinolytic agent, epsilon-aminocaproic acid (EACA) [6, 7]. TXA does not seem to be associated with a major difference in transfusion rates when compared with EACA [6].

After a mean TXA intravenous dose of 10 mg/kg on healthy individuals, peak concentration of the drug is observed at 1 h after administration and its half-life is approximately 2 h, with 90% excretion at 24 h. TXA remains in serum for approximately 8 h and in tissues for 17 h. Nevertheless, these pharmacokinetic factors may be different in patients that have been through major trauma and the efficacious TXA levels in these patients may differ with the effective levels in healthy individuals [7].

Nevertheless, there are concerns that topical use of TXA may be toxic for chondrocytes and surrounding soft tissue, as stated in numerous studies [3,4,8–11]. The current manuscript is a narrative review and its purpose is to present the recent literature regarding the effect of the topical use of TXA, whether it can cause toxicity on chondrocytes and articular cartilage and if there is a standard safe level of the drug in which it can be harmless and efficacious.

USE OF TRANEXAMIC ACID (TXA) IN ORTHOPEDIC SURGERY

TXA can be effective to reduce blood loss in major surgical procedures and severe traumatic conditions. Thus, TXA can decrease the blood transfusion needs without increasing the risk of thromboembolic events. Many studies have supported its use in major orthopedic procedures [8,9,12–17]. Fillingham *et al* showed that TXA administration can reduce blood loss during total knee arthroplasty by approximately 100 mL, compared to a placebo [9]. Yi *et al* have also shown that a group of patients that received TXA after total hip arthroplasty had a lower blood transfusion rate in comparison with a placebo group [8]. These patients also had a lower mean total blood loss varying form approximately 200 mL to 350 mL in comparison with the placebo group [8]. Also, Li *et al* proved that a group of patients treated with TXA after spinal surgery had significantly lower blood loss and the number of patients that required blood transfusion was notably reduced, irrespective of using TXA in high or low dosage [10]. Generally, TXA has demonstrated efficacy in hip [8], knee [9] and shoulder [14] arthroplasties as well as in spinal surgery [12] and anterior cruciate ligament reconstruction [13]. Farrow *et al* found moderate quality evidence that TXA administration can reduce blood transfusion rate in patients undergoing hip fracture surgery, so the effectiveness of TXA in this surgical procedure is under investigation [15]. Numerous studies have also proved its efficacy during trauma operations based on data from the CRASH-2 trial and showed that TXA can reduce the mortality rates in trauma patients due to bleeding [16,17]. Few studies suggested the lack of potential benefit from the use of TXA in major orthopaedic procedures. For example, Lack *et al* concluded that there are no significant differences in

blood loss and transfusion rates between TXA and placebo [18]. However, the number of the studies supporting this conclusion is very limited and this heterogeneity can potentially be attributed to the variety of methods used for calculating the blood loss.

CONTRAINDICATIONS

Despite its wide use in major orthopaedic operations, TXA cannot be used in certain cases. More specifically, there are absolute and relative contraindications for TXA administration. Hypersensitivity and allergy to TXA as well as active thromboembolic disease and fibrinolytic conditions with consumption coagulopathy are the absolute contraindications for TXA [19].

The relative contraindications must consider the association between risk and benefit of TXA [19]. Relative contraindications include renal dysfunction, thrombosis disorders and preexisting coagulopathy or oral anticoagulants [19]. TXA is also contraindicated in patients with high-risk of seizures [19,20]. Pregnancy is another contraindication, but TXA can be administered when it is vitally indicated [20].

TXA ADMINISTRATION AND SAFE LEVELS (TABLE 1)

TXA can be administrated through the intravenous (IV) and oral route as well as topical. However, IV and oral TXA administration are associated with an increased risk of systematic complications, such as thromboembolic events [21]. Ker *et al* showed that although IV TXA can minimize blood loss during surgeries, it is arguable whether it can cause major thromboembolic events in the future [22]. In addition, since TXA is a derivative of the amino-acid lysine and goes through ionization in physiologic environment it is expected that its absorption through membranes may be inadequate, therefore its bioavailability will be moderate and we may need to administer higher doses of the drug in order to reach the desirable levels of TXA in articular cartilage, thus causing major systematic complications [23]. On the other hand, topical administration of the drug may have a lot of benefits like increased concentration at the operative site with reduced systemic exposure, cost reduction and surgeon control [24]. It has been also

shown that topical administration is not inferior compared to IV administration, in terms of safety and efficacy [25,26]. Abdel *et al* and Xu *et al* showed that IV administration of TXA has no advantage over topical administration of the drug as they have both caused lower rates of blood transfusion and these two groups had no clinically significant differences [26,27]. However, many authors investigated the combined route of administration of TXA, suggesting that combining both topical and IV administration of the drug could potentially have a beneficial effect on reducing blood loss during major orthopaedic procedures [28]. It is not clear yet, whether this combined route can lead to a better result, concerning the blood loss and the blood transfusion rates [29]. Dong *et al* suggested that combined topical and intravenous administration can further reduce blood loss while Li *et al* did not support this conclusion suggesting that this technique has no additional benefits [28,29].

TXA can be administered topically with various technics [30]. Intra-articular or peri-articular application is the most common technic of TXA administration [30]. Some surgeons perform a peri-operative wash of the drug in order to avoid its topical side effects [30]. Clamping drainage is another proposed technique which creates an intra-articular tamponade, thus producing temporary haemostasis and increasing the tissue contact time with the drug [31].

TXA was supported as a safe drug for topical use in a few studies. In the study of Degirmenci *et al*, an in-vivo study on experimental animals, TXA seemed to have a significant contribution on the healing of osteochondral defects [1]. This was observed not only macroscopically but also histopathologically, where samples treated with TXA had clearly better recovery than the control groups. More specifically, the macroscopic examination of the animal samples was performed by observing any abnormality of the location, color, shape and size of the tissue in situ, four and eight weeks after TXA administration. The group that received TXA seemed to have better recovery, after both four and eight weeks, in comparison with a control group. Histopathologically, the group that received TXA had higher regeneration rate of subchondral tissue and cartilage tissue repair rate than the control group. No problems were mentioned in

terms of safety of the drug. Ambra *et al*, in a study carried out on experimental animal samples, also supported the efficacy and safety of the drug [3]. Images of full-thickness cartilage sections showed a similar number of live cells, as well as a minimal presence of dead cells, no matter the duration of incubation and the concentrations of TXA that were administered. Moreover, this study suggests that TXA doses up to 4 mg/mL for up to 6h don't seem to have any detrimental effect on cell viability. This study was carried out on experimental animal tissue, however samples of the animals that were chosen are the closest to the human samples. Also, TXA doses were adapted depending on the thickness of each tissue sample.

Marmotti *et al* conducted another study, in which human tissue samples were harvested [4]. This study also supported the hypothesis that TXA is a safe drug. Low doses of TXA (7 mg/mL) were administered for 48h and the samples were assessed after short time (immediately after exposure) and long time of exposure (1, 2 and 6 wk).. The drug was completely safe and did not have any effect on cells ability to migrate. Also, cells treated with TXA did not seem to have any effects in terms of morphology, no matter the time of exposure, in comparison with a control group. In this study, there was also conducted a cell phenotype analysis between the group that received TXA and the control group. Results showed that TXA exposure had no significant effects on cells phenotype after both long-term and brief time of exposure. A further biochemical analysis investigated any possible alteration in glycosaminoglycan (GAG) concentration but no significant difference was noted between the TXA-treated group and the control group. Bolam *et al*, supported in their scoping review that TXA is a safe drug, when administered topically [30]. More specifically, they excluded 15 studies that were considered eligible for their review. Some of these studies used human tissue samples while others used animal tissue samples. The authors concluded that there is a dose-dependent effect of TXA on chondrocytes, tenocytes, synoviocytes and periosteum-derived cells. Both human and animal tissue samples were exposed to varying doses of the drug (1, 2, 4, 20, 25, 50 and 100 mg/mL) for different times of exposure (from 10 mins to 48h), The cultures that were exposed to TXA doses higher than 20 mg/mL had

reduced cell viability and had effects on cell morphology. Thus, samples that were treated with doses lower than 20 mg/mL had no significant detrimental effects. This conclusion supports the safety of the drug in a dose-dependent way.

Wang *et al* studied the effects of topical administration of TXA on human fibroblast cells [32]. These cells where cultured for brief time (10 min) and long time of exposure (6, 12, 24 and 48h) to different drug concentrations (12,5 ,25, 50 and 100 mg/mL). The authors suggested that there is a time and dose-dependent way that TXA affects the fibroblast cells. More specifically, the samples had no detrimental effects when exposed to a high concentration of TXA (100 mg/mL) but for a limited exposure (10 min) or when exposed to a lower concentration of TXA (12,5 mg/mL) for a more chronic exposure (from 6 to 48h). However, when fibroblasts where exposed to high concentrations of the drug for a long period of time they had clear effects concerning the cell viability.

HOW TXA AFFECTS CELL CYCLE

In eukaryotic cells, cell cycle has five different phases, G0, G1, S, G2 and M in order. In G0 phase there is a cell cycle arrest, while in G1 phase cells are gaining mass and activating growth signals. G2 is also a phase where cells are growing and organizing their genome. In S-phase (or Synthesis) takes part the duplication of the chromosomes and in M-phase (or Mitosis) the duplicated chromosomes and the cells are divided [33]. In order to investigate the way that TXA affects the biological functions of cells, Goderecci *et al* assessed the effects of the drug on the cell cycle [34]. Cells incubated on TXA for only 10 minutes did not seem to have any significant alterations on cell cycle profile. However, when cells were treated with TXA for 24h or 48h there were significant changes. The number of cells in the G0/G1 phase were decreased while there was an increase of cells in the G2/M phase and a significant increase of cells in the S phase, when compared to the control group and the group incubated for 10 minutes. As for the cells treated with TXA for 10 minutes and after incubated with fresh medium for 24h, there was an increased amount of cells in the G0/G1 phase and a considerable decrease of cells in the G2/M phase, approaching the cell cycle profile of cells on the

control group. Another study that investigated the potential mechanism through which TXA could cause cytotoxicity, suggests that a caspase-3-dependent apoptotic mechanism could be responsible for the detrimental effects of TXA in human cells [10].

TXA CYTOTOXICITY (TABLE 2)

However, intraarticular TXA administration leads to exposure of intact articular cartilage to the drug. Consequently, there have been many concerns that TXA may cause detrimental effects on articular cartilage and have cytotoxic effects on chondrocytes, especially since chondrocyte death is related with the development of further cartilage degeneration and osteoarthritis [3].

TXA can cause massive cytotoxicity. McLean *et al* conducted a study where human tendon tissue, synovial tissue and cartilage tissue were obtained during major orthopaedic surgeries and TXA seemed to have significant effects on each kind of tissue [26]. All samples were exposed to a wide range of TXA concentrations (1, 50 and 100 mg/mL) for 4h, 16h or 24h [10]. Significant reduction on cell viability as well as high rates of apoptosis were observed in all types of tissue in a time and dose dependent way. McLean *et al* tried to identify potential mechanisms that may cause these high rates of apoptosis and they came to the conclusion that increased amount of apoptotic proteins occurred in tenocytes and chondrocytes, in comparison with the control groups, thus suggesting that TXA-mediated cell death may be caused by apoptotic proteins like caspase-3. Lower doses had lower impact on cells but still influenced cell function. Moreover, the authors reported that high percentage of cell death occurred in the control groups, most likely due to tissue drying between surgical harvesting and completion of graft preparation and also due to age-related changes, as most of the samples were harvested from elderly patients. However, that did not seem to have any major impact on the study outcome as these factors affected every sample group.

Parker *et al* also proved the drug's cytotoxic effects by exposing human chondrocytes on 2D and 3D cultures to TXA (10, 20, 40 mg/mL for 3h and 6h) [11]. On 2D cultures, chondrocytes seeded onto numerous well-plate tissue cultures plastic trays while on 3D

cultures the authors tried to simulate the 3D nature of articular cartilage by using chondrocyte-laden gelatine-methacryloyl (GelMA) hydrogels. However, this study also suggests that there may be a safe dose for the drug. More specific, on the 2D cell-culture model the drug had significant effects on cell viability on doses between 20 mg/mL and 40 mg/mL. On the contrary, cells treated with doses lower than 20 mg/mL did not seem to be affected by the drug. In 3D cultures cell viability was decreased in a time and dose-dependent way. Furthermore, Parker *et al* inspected the effect of TXA on glycosaminoglycan GAG concentration and found no significant differences between the control group and the groups exposed to the drug, thus suggesting that high doses of TXA can cause cell death but they do not affect the quality of the cartilage tissue. Another study, carried out by Tuttle *et al* on murine and bovine chondrocytes, also reached out the conclusion that TXA can be cytotoxic [24]. Chondrocytes treated with high doses of TXA (50 and 100 mg/mL) had clear detrimental effects on cell viability in a time-dependent way. However, cells treated with 25 mg/mL had no considerable decrease on cell viability. Moreover, in this study it was observed that there was a small but significant loss of GAG in groups that were treated with TXA in comparison with the control groups, in contrary to the conclusion of Parker *et al* suggesting that TXA does not affect GAG concentrations. These studies did not take into consideration all the pharmacological factors that normally take place inside the human joint. The time of cell exposure to TXA in an in-vitro study is way longer than the time of exposure that theoretically occurs in-vivo where other kinds of cells (inflammatory cells, platelets) could have a potential role in the drug's clearance. As a result, Mclean *et al* suggested that the in-vitro nature of these studies might have an influence on the results [10].

Another interesting finding is that washing the drug out of the cultures after a few minutes of exposure seems to decrease TXA's cytotoxicity [34]. According to Goderecci *et al*, when the drug was washed out of the cultures, only the highest dose of TXA (100 mg/mL) affected cell cycle profile and cell viability [34]. However, 20, 50 and 70 mg/mL of TXA did not have any detrimental effect on cell function when the drug was washed out after 10 minutes of incubation. These samples were compared with other samples

that were exposed to same TXA concentration for 24h and 48h without washing the drug out, in which TXA had significant effects in doses higher than 50 mg/mL. Tuttle *et al* observed the same outcome when animal tissue samples were incubated in TXA for different amount of time [24]. Samples were incubated in various TXA concentrations for three different time points, 8h, 24h and 48h. Samples incubated in high TXA levels presented complete cell death, no matter the time of exposure. However, in low levels of TXA, cell viability was notably lower on samples incubated for 24h when compared with samples incubated for 8h. Complete cell death was observed on the 48h sample, thus supporting the conclusion that the amount of time cells are exposed to TXA is a major factor concerning the cytotoxicity of the drug.

A few studies observed the effects of TXA in different kind of tissue samples. Marmotti *et al* carried out a study, in which human cartilage and synovial biopsies were obtained from patients during major orthopedic procedures [4]. Each kind of tissue was incubated in the same concentration of TXA for the same amount of time. TXA had the same effects on those groups, it showed no cytotoxicity on both samples and was completely safe for all types of cells. Other investigators harvested synovial and cartilage from patients which were also treated with the same TXA doses for the same amount of time [10]. Apoptosis rate was approximately the same for all three types of tissue. Viability was decreased in all types of cells in a different time point for each dose level, however the detrimental result was the same in all tissue samples. Thus, TXA seems to interact the same way with all types of tissue and it has the same effects in all types of cells.

TXA EFFECTS ON GAG CONCENTRATION

Marmotti *et al* proved in their study that TXA did not cause any significant change on GAG concentration by conducting a biochemical analysis [4]. Moreover, Parker *et al* supported this conclusion as they noted no significant difference on GAG concentration between the TXA-treated cells and the placebo-treated cells and thus suggesting that TXA had no effect on the quality of cartilage tissue irrespective of the drug's toxicity on chondrocytes [27]. However, Tuttle *et al* observed in their study that TXA had a small but

significant effect on GAG concentration, in contrary to the conclusion of Marmotti *et al* and Parker *et al* [21]. This differentiation may be due to the nonidentical period that cells where exposed to TXA, thus causing a different effect on GAG concentration in each study. Parker *et al* used a shorter and more clinically appropriate length of exposure to the drug to come to a more accurate conclusion [27].

TXA EFFECTS DEPENDING ON PATIENT'S AGE

TXA may have different effects on chondrocytes depending on the age of patients. Marmotti *et al* obtained human biopsies from young donors (patients between 14 and 40 years-of-age) in order to inspect the effects of TXA (7mg/mL) on these samples, both short term and long term [4]. Biopsies from young donors did not seem to develop any kind of damage in terms of morphologic and immunophenotypic characteristics no matter the time of exposure. However, McLean *et al* harvested tissue samples from older patients (specific age not provided) undergoing total knee and hip arthroplasties [10]. In this study, just like previously, biopsies were checked for any effect of TXA after a short and a long period of incubation. Cell viability was significantly reduced after 24h of exposure to TXA, regardless the TXA dose. This suggests that TXA may be safer for younger patients and cause more serious detrimental effects on older patients.

FUTURE

While many studies have been carried out on tissue samples, it is important to carry out in-vivo studies in order to support the data that were excluded from in-vitro studies, considering all the pharmacological interactions inside the human joint such as drug clearance and tissue distribution and observe the development of any side effects. Additionally, more studies are needed to confirm the possibility that there is a safe TXA dose for topical use.

Safety of the drug is quite important as well as effectiveness of the drug. Studies should not only suggest the safe doses of TXA but also inspect the healing process in each dose level and if TXA contributes in the recovery process. Moreover, more data is needed to

clarify the molecular mechanism of TXA cytotoxicity. This could help us understand the process through which TXA causes detrimental effects in human cells and make clear its molecular correlation with cell apoptosis.

Effects of TXA on GAG concentration are only discussed in a limited number of studies. This is an interesting topic that should be furtherly discussed. Many studies suggest that TXA is cytotoxic, but we should also clarify whether TXA causes any significant differences on GAG concentration. This will help us to conclude whether the drug affects the quality of cartilage tissue, irrespective of any cytotoxic effects that the drug may have.

A topic that is not greatly discussed and should be furtherly investigated is the cost *vs* benefit of a potential therapeutic intervention with topical TXA. Clinical studies should be carried out to investigate whether the efficacy of topical use of TXA is as great as the cost of the drug and if there is a point to use the drug topically instead of IV.

CONCLUSION

Given the low evidence of available literature and the lack of clinical data there seems to be no clear outcome considering the effects of the drug on chondrocytes, as some studies suggest that TXA is clearly cytotoxic [10,11,24,34] while others support the safety of the drug [1,3,4]. Most of these studies came to the conclusion that TXA can have effects on chondrocytes in a time and dose-dependent way [10,11,24,34]. Higher doses can cause detrimental effects on cells especially when they are exposed to the drug for a long period of time [24,34]. Other studies suggest that TXA seems to have the same effects on every type of tissue inside the human joint and each type of cell interacts the same way with the drug [4,10]. Nevertheless, some studies proved that there seems to be a safe TXA level where the healing process can continue without any effects on the joint function [1,3,4,11,24]. There are indications that high doses of TXA can affect the cell cycle profile, but this suggestion needs further investigation [34]. The suggestion that TXA's effects depend on the age group of the patients also needs further investigation [4,10].

75682_Auto_Edited.docx

ORIGINALITY REPORT

1 %

SIMILARITY INDEX

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet	19 words — < 1%
2	Luiz Felipe Ambra, Laura de Girolamo, Wanting Niu, Amy Phan, Myron Spector, Andreas H. Gomoll. "No effect of topical application of tranexamic acid on articular cartilage", Knee Surgery, Sports Traumatology, Arthroscopy, 2017 Crossref	16 words — < 1%
3	J. D. Parker, K. S. Lim, D. C. Kieser, T. B. F. Woodfield, G. J. Hooper. "Is tranexamic acid toxic to articular cartilage when administered topically?", The Bone & Joint Journal, 2018 Crossref	13 words — < 1%
4	Turgut Karlidag, Mucahit Yildiz, Sinasi Yalcin, Neriman Colakoglu, Irfan Kaygusuz, Emrah Sapmaz. "Evaluation of the effect of methylprednisolone and N-acetylcystein on anastomotic degeneration and regeneraton of the facial nerve", Auris Nasus Larynx, 2012 Crossref	13 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	OFF
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 12 WORDS