How should tranexamic acid be administered in haemorrhagic shock? - continuous serum concentration measurements in a swine model

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Abstract

Background:
Tranexamic acid (TXA) reduces mortality in trauma patients. Intramuscular (i.m.) administration could be advantageous in low-resource and military settings. Achieving the same serum concentration as i.v. administration is important to achieve equal mortality reduction. Therefore, we aimed to investigate whether dividing an i.m. dose of TXA between two injection sites, and whether an increase in dose, would lead to serum concentrations comparable to those achieved by i.v. administration.

Methods:
Norwegian landrace pigs (n = 29) from a course in haemostatic emergency surgery were given tranexamic acid 1h after start of surgery. Blood samples were drawn at 0, 5, 10, 15, 20, 25, 35, 45, 60 and 85 min. The samples were centrifuged and serum TXA concentrations quantified with liquid chromatography–tandem mass spectrometry (LC–MS/MS). The use of two injection sites was compared to distributing the dose on one injection site, and a dose of 15 mg/kg was compared to a dose of 30 mg/kg. All i.m. groups were compared to i.v. administration.

Results:
The groups were in a similar degree of shock. Increasing the i.m. dose from the standard of 15 mg/kg to 30 mg/kg resulted in significantly higher serum concentrations of TXA, comparable to those achieved by i.v. administration. Distributing the i.m. dose on two injection sites did not affect drug-uptake, as shown by equal serum concentrations.

Conclusions:
For i.m. administration of TXA, 30 mg/kg should be the standard dose. With a short delay, i.m. administration will provide equal serum concentrations as i.v. administration, above what is considered necessary to inhibit fibrinolysis.

Background
The antifibrinolytic agent tranexamic acid (TXA) prevents breakdown of blood clots and reduces mortality in trauma patients. For every 15 minutes passed from a traumatic injury occurs until TXA is administered there is a 10% reduction of TXA-effect, advocating a need for rapid administration in injured patients. TXA is normally administered intravenously (i.v.) but this may be time consuming and is not always possible within the critical initial phase after injury. Intramuscular (i.m.) administration would be
advantageous in certain settings, such as in low-resource areas and military settings, where i.v. administration is difficult or qualified personnel is lacking. WHO defined already in their 2017-guidelines that other routes than i.v. administration should be explored as a research priority. Previous studies have shown that TXA can be given i.m. but achieves lower serum concentrations compared to i.v. administration at equal doses. These studies have demonstrated that intramuscular administration of TXA can achieve serum concentrations exceeding the reported 10–17µg/mL needed to inhibit fibrinolysis in vitro. However, there are compelling reasons to aim for a higher serum concentration: Firstly, the demonstrated TXA mortality reduction is based on iv. administration, providing higher serum concentrations. Until survival is shown also for the concentrations showing effect in vitro, these i.v.-associated in vivo-concentrations are what we must aim for. Secondly, the deviation around a median serum concentration of TXA, close to the threshold for fibrinolysis-inhibition, will result in a higher number of patients falling below the threshold and therefore not achieving the desired effect.

To increase the i.m. dose is the most obvious solution and is likely to lead to higher serum concentrations, but this needs to be investigated as other factors, such as reduced muscle perfusion and thereby reduced TXA-uptake into the circulation, may be limiting. Another option may be to increase the area of TXA available for i.m. uptake by distributing the dose on several injection sites. If reduced muscle perfusion from shock is a limiting factor distributing the increase in area for uptake would theoretically improve chances to increase serum concentrations. Also, variation in serum concentration among previous studies on i.m. administration of TXA give reason to believe that higher serum concentrations can be achieved through distributing the i.m. dose on different injection sites.

Therefore, to determine the appropriate TXA dose, we aimed to investigate whether dividing the standard and double i.v. dose between two intramuscular injection sites would result in serum concentrations comparable to those achieved by i.v. administration.

**Methods**

Animal preparation, instrumentation, euthanasia, quantification of TXA and statistical analysis were the same as in a previous study, but key elements are also described below. For details on animal preparation and instrumentation, please see Bakke et al (5).

**Model**

Specific pathogen free (SPF) Norwegian landrace pigs (n = 29) were used in this present study, both male and female. All pigs were used as part of emergency trauma courses arranged by the Northern Norway Regional Health Authority. In these courses, surgical teams train in stabilization of trauma patients, and an instructor inflicts intraabdominal, retroperitoneal, and intrathoracic injuries on the pig. The degree of shock was compared to a control group from a previous study that underwent anesthesia but no surgical procedure. For the current study a normal dose of tranexamic acid was defined as 15 mg/kg bodyweight.
for the swine, as this is close to the standard dose of 1 gram used in the CRASH-2 study if given to a 70 kg patient ¹

**Experimental protocol**

The animals were divided in four groups as shown in Fig. 1. All four groups received TXA one hour after start of surgery, defined as the first incision. Using two i.m. injection sites Group 1 and 2 received 30 and 15 mg/kg TXA respectively. Using one i.m. injection site Group 4 received 15 mg/kg TXA. Group 3 received 15 mg/kg of TXA i.v. and was used as standard treatment control group. Blood samples were taken 5, 10, 15, 20, 25, 35, 45, 60 and 85 minutes after TXA administration. Samples were stored on Eppendorf tubes and set to coagulate for 1 hour, before being centrifuged at 7500 x g for 7.5 minutes. The obtained plasma was frozen at -20°C prior to LC-MS/MS analysis. A flowchart over the experimental process is provided in Fig. 1. FELASA (Federation of European Laboratory Animal Science) – certified personnel were present in the university lab to control the depth of anesthesia. Animals that were alive at the end of the experiment were euthanized by pentobarbital 300 mg, morphine 10 mg, and potassium chloride 50 mmol.

**Quantification of TXA concentrations**

Quantification of TXA in serum was performed with liquid chromatography tandem mass spectrometry (LC-MS/MS) as described in detail previously ⁶. TXA and TXA-13C₂,15N were purchased from Toronto Research Chemicals Inc. Ontario, Canada. Serum levels of TXA were analysed by using a Waters Acquity UPLC iClass FTN system with an autosampler and a binary solvent delivery system (Waters, Milford, MA) interfaced to Waters Xevo TQ-XS benchtop tandem quadrupole mass spectrometer (Waters, Manchester, UK). The following multiple reaction monitoring (MRM) transitions were used (bold transitions are qualifiers): m/z 158→123/95 and 161→125/96 (TXA and TXA-¹³C₂,¹⁵N). The method was found linear from 0.005 to at least 94 µg/mL (r² > 0.998). Lower limit of quantification (LLOQ) was found to be 0.0025 µg/mL (0.1 µL injection volume). Between-day coefficient of variation (CV) for TXA was < 10% on four consecutive days. CV for intraday precision value was < 6% and accuracy for recovery test was 94.2–106.2%

**Statistical analysis**

We used Shapiro-Wilks test to assess whether data were distributed normally, and One-way repeated measures ANOVA to compare changes from start of the experimental protocol in hemodynamic variables and from peak TXA serum concentrations (Cmax) for the serum concentration measurements. Occasional failure of hemodynamic measurements occurred in some animals. Hemodynamic average values are based on available data, on which statistical analysis was performed. When data were not normally distributed data, repeated measures ANOVA on ranks was used. When there were significant differences, we used Dunnett’s method to compare values within group vs. baseline. Differences in TXA serum concentrations and hemodynamic variables between groups were analysed by a One-Way Analysis of Variance test followed by an all-pairwise multiple comparisons procedure using Tukey’s test.
Repeated measures ANOVA on ranks and Dunn’s test was used when data were not normally distributed. Differences were considered significant at p < 0.05. Data are presented as mean ± standard deviation.

**Artificial intelligence (AI)**

AI was used for language support on the abstract, aims, first part of discussion and the conclusion. The authors first wrote the suggested paragraphs, these were then submitted to chatGPT (openai.com) with the prompt “rewrite into academic English”. The suggestions were then reviewed by the authors.

**Ethics**

The research animals were registered in the Norwegian Food Safety Authority’s audit and applications system (Forsøksdyrsforvaltningens tilsyns- og søknadsystem, FOTS), and their use approved by the Norwegian Food Safety Authority. The animal care and welfare officer (Person med særskilt kontrollansvar, PMSK) performed the local evaluation.

**Results**

All four groups had similar shock index and all groups had significantly higher shock index than animals undergoing anesthesia with no surgical procedure (see Fig. 2).

One versus two intramuscular injection sites

Figure 3 compares serum concentration between TXA administered i.m. with one versus two injection sites and a dose of 15 mg/kg. There was significant difference between the i.v. group and the i.m. groups until 35 minutes after receiving TXA, with i.v. administration achieving an initial Cmax of 54 µg/mL before declining to a similar level as the i.m. groups that held a steady serum concentration around 20 ug/mL throughout the protocol. From 35 minutes the groups were quite similar in serum concentration, as shown in Fig. 3. But there was no difference in concentration between the i.m. groups with one versus two injection sites. Both methods of i.m. administration gave serum concentrations above the in vitro-threshold for inhibiting fibrinolysis within 5 minutes.

Normal dose i.m. vs double dose i.m.

Increasing the i.m. dose from 15 mg/kg to 30 mg/kg resulted in significantly higher serum concentrations of TXA (Fig. 4). Group 3, the i.v. group, reached its mean maximum serum concentration quickest at 54 µg/mL after five minutes. However, after approximately ten minutes group 1, 30mg/kg i.m. group had serum concentrations at comparable levels to iv. administration.

After i.v. administration, serum concentration had a gradual decline, whereas the 30 mg/kg i.m. group peaked with maximum serum concentration at 25 minutes and kept a relatively steady serum concentration throughout the rest of the protocol. Compared to the group receiving i.m. 15 mg/kg the group receiving 30 mg/kg i.m. had significantly higher serum concentration of TXA at all times. However,
all groups were above the serum concentration needed to inhibit the fibrinolysis in vitro at all measuring points.

**Discussion**

Dividing the i.m. dose of TXA between two injection sites did not result in higher serum concentrations compared to a single injection site. However, increasing the administered i.m. dose from the normal 15 mg/kg to 30 mg/kg did lead to TXA serum concentrations comparable to i.v. administration, and while the serum concentrations did not rise quite as rapidly as i.v administration it was double the reported threshold required to inhibit fibrinolysis within 5 minutes of administration \(^8,10\). Furthermore, the TXA serum concentration remained stable throughout the 85-minute experimental period following i.m. injection. Thus, our study suggests that administering 30 mg/kg TXA i.m. is equally effective as standard iv. administration, ensuring a consistent serum concentration of TXA during patient transport to advanced care. Consequently i.m. administration is not only a viable alternative when i.v. administration is unavailable but may also be a valuable option for maintaining elevated serum concentrations over a prolonged time, when bleeding control is essential and human resources are scarce.

Furthermore, administering TXA i.m. can be preferable over i.v. administration, as it does not require i.v. access. Studies have shown that i.m. administration of adrenaline for out-of-hospital cardiac arrest was faster than iv. administration by EMS personnel \(^11\). Combat medicine and low-resource settings have been suggested as settings in which i.m-administration may be desirable \(^12\). If our data are applied in such clinical setting, it is likely that the theoretical benefit of a quicker rise in serum concentration with i.v. administration is offset by the potential time-consuming nature of establishing peripheral vein catheterisation, and therefore even superior to i.v. administration. Also, in high-resource settings i.v. access can occasionally be difficult, particularly in shocked patients, where i.m. administration is a useful alternative. Intraosseous administration is another option but may not always be available and require relatively expensive equipment.

The sustained high serum concentrations observed after administration of 30 mg/kg TXA also suggests that this may be preferable to repeated TXA boluses and that it may be an alternative to a continuous i.v. infusion of TXA. This needs to be explored further.

Also, the group given a normal i.m. dose of TXA (15 mg/kg) achieved an average serum concentration above the reported in vitro limit needed to fully inhibit fibrinolysis. It is possible that this dose will be sufficient for most patients, but this must be further explored in survival studies as current survival data are based on iv. use \(^1\). While the average value for every measuring point was above the needed limit concentration by using the standard dose in the present study, the use of 30 mg/kg TXA will ensure that also patients in the lower reference range will reach this limit.

In theory, dividing the TXA dose between two injection sites could lead to a greater amount of TXA available for uptake if peripheral circulation is impaired. However, in our study we found that dividing the
normal dose between two injection sites did not give significant difference in serum concentration from using one injection site. While the serum concentration was not affected by dividing the dose between injection sites, there are other reasons for dividing the dose. The volume needed to inject both 15 mg/kg and 30 mg/kg i.m. with current available concentrations will exceed 5 mL, the maximum recommended volume for i.m. injections. It would be beneficial to produce tranexamic acid in a more concentrated version to avoid such big intramuscular volumes. For rapid i.m. administration it would be advantageous to have TXA in prefilled syringes, especial in areas with big distances to advanced health care and lack of healthcare workers.\textsuperscript{13}

\section*{Limitations}

One of the main limitations for this study is the model that was used, with animals used for a hemostatic emergency trauma course. Injuries, surgical intervention, blood loss and fluid resuscitation were therefore not standardized. Even so, the trends in our data are consistent, and all animals were in a similar state of hemorrhagic shock.

Further, all pigs were under general anesthesia unlike most patients that will receive TXA, which can affect muscular uptake of TXA.\textsuperscript{14} General anesthesia may contribute to decrease the negative effects that shock have on muscle perfusion through relaxed sympathetic tone. Therefore, this study can overestimate the uptake compared to patients that are not under general anesthesia. We also know that general anesthesia can impair microcirculation of skeletal muscle, and may impair i.m. uptake.\textsuperscript{14}

\section*{Conclusion}

It is possible to achieve serum concentrations of TXA equivalent to intravenous administration by increasing the intramuscular dose to 30 mg/kg. Dividing the intramuscular dose on two injection sites did not affect serum concentrations. These results are expected to be applicable to humans and 30 mg/kg, should accordingly be the standard dose for i.m use. In a clinical context, it is likely that intramuscular administration of 30 mg/kg TXA would be faster, simpler, and therefore a superior alternative to iv. administration in certain settings.

\section*{Abbreviations}

AI – Artificial intelligence

EMS – Emergency medical services

FELASA (Federation of European Laboratory Animal Science

FTN – Flow through needle

I.m. - Intramuscular
I.v.- Intravenous

LC–MS/MS - liquid chromatography–tandem mass spectrometry

TXA- Tranexamic acid

**Declarations**

**Ethics approval and consent to participate**

The research animals were registered in the Norwegian Food Safety Authority’s audit and applications system (Forsøksdyrsforvaltningens tilsyns- og søknadsystem, FOTS), and their use approved by the Norwegian Food Safety Authority (FOTS application number 15492) and the named animal care and welfare officer (Person med særskilt kontrollansvar, PMSK).

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets for the current study are available from the corresponding author on request.

**Competing interests**

None of the authors have any conflicting interests to report.

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**Authors’ contributions**

TL participated in design, collected data, performed the statistical analyses, and wrote the manuscript. HKB conceived of the study, participated in design, and co-wrote the manuscript. OMF performed laboratory analyses and co-wrote the manuscript. EWN provided the lab facilities, and co-wrote the manuscript. ESD participated in design, participated in statistical analyses, and co-wrote the manuscript. All authors read and approved the final manuscript.

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None of the authors have any conflicts of interest to declare

References


**Figures**
Norwegian landrace pigs (N=28)  
Instrumentation: PVC;ET-tube (7.0 OD); VCV, arterial line

Group 1: i.m. 30mg/kg - two injection sites (n=7)  
Group 2: i.m. 15mg/kg - two injection sites (n=9)  
Group 3: i.v. 15 mg/kg (n=6)  
Group 4: i.m. 15 mg/kg - one injection site (n=6)

Infliction of various abdominal and thoracic trauma  
Ongoing emergency trauma for 1 hour before TXA was administered in all groups

Blood samples at 5, 10, 15, 20, 25, 35, 45, 60 and 85 minutes after administration of TXA

Quantification of TXA in serum by LC-MS/MS

**Figure 1**

A general overview of the experimental protocol. PVC: peripheral catheter, ET: endotracheal, OD: outer diameter, VCV: volume- controlled ventilation, TXA: Tranexamic acid, LC-MS/MS: liquid chromatography- tandem mass spectrometry. Groups are compared to non-shocked pigs from Bakke et al.

**Figure 1**

Experimental protocol
Figure 2

Shock Index

Time after TXA administration (minutes)

- Group 1: 30 mg/kg with two injection sites
- Group 2: 15 mg/kg with two injection sites
- Group 3: 15 mg/kg i.v.
- Group 4: 15 mg/kg one injection site
- Unshocked 15 mg/kg i.m. w/ one injection site from Bakke et al.
Figure 3

One vs two intramuscular injection sites
Figure 4

Standard dose i.m. vs double dose i.m.