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HOW SHOULD TRANEXAMIC ACID BE ADMINISTERED IN HEMORRHAGIC SHOCK? CONTINUOUS SERUM CONCENTRATION MEASUREMENTS IN A SWINE MODEL

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ABSTRACT—**Background**: Tranexamic acid (TXA) reduces mortality in trauma patients. Intramuscular (IM) administration could be advantageous in low-resource and military settings. Achieving the same serum concentration as intravenous (IV) administration is important to achieve equal mortality reduction. Therefore, we aimed to investigate whether dividing an IM dose of TXA between two injection sites and whether an increase in dose would lead to serum concentrations comparable to those achieved by IV administration. **Methods**: Norwegian landrace pigs (n = 29) from a course in hemostatic emergency surgery were given TXA 1 h 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. The use of two injection sites was compared with distributing the dose on one injection site, and a dose of 15 mg/kg was compared with a dose of 30 mg/kg. All IM groups were compared with IV administration. **Results**: The groups were in a similar degree of shock. Increasing the IM 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 IV administration. Distributing the IM dose on two injection sites did not affect drug uptake, as shown by equal serum concentrations. **Conclusions**: For IM administration of TXA, 30 mg/kg should be the standard dose. With a short delay, IM administration will provide equal serum concentrations as IV administration, above what is considered necessary to inhibit fibrinolysis.

KEYWORDS—Injury; trauma; bleeding; coagulation

ABBREVIATIONS—EMS—emergency medical services; FELASA—Federation of European Laboratory Animal Science; IM—intramuscular; IV—intravenous; LC-MS/MS—liquid chromatography-tandem mass spectrometry; TXA—tranexamic acid

BACKGROUND

The antifibrinolytic agent tranexamic acid (TXA) prevents breakdown of blood clots and reduces mortality in trauma patients (1,2). For every 15 min passed when 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 (3,4). Tranexamic acid is normally administered intravenously (IV), but this may be time consuming and is not always possible within the critical initial phase after injury. Intramuscular (IM) administration would be advantageous in certain settings, such as in low-resource areas and military settings, where IV administration is difficult or qualified personnel is lacking. World Health Organization defined already in their 2017 guidelines that routes other than IV administration should be explored as a research priority (5).

Previous studies have shown that TXA can be given IM but achieves lower serum concentrations compared with IV administration at equal doses. These studies have demonstrated that IM administration of TXA can achieve serum concentrations exceeding the reported 10 to 17 μg/mL needed to inhibit fibrinolysis *in vitro* (6).

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Conflicts of interest: None of the authors have any conflicts of interest to declare.

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 was 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.

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However, there are compelling reasons to aim for a higher serum concentration: First, 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 IV-associated *in vivo* concentrations are what we must aim for. Second, 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 IM 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. We have previously demonstrated that IM uptake is reduced in traumatic shock.

Another option may be to increase the area of TXA available for IM 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 IM administration of TXA gives reason to believe that higher serum concentrations can be achieved through distributing the IM dose on different injection sites (7–9).

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

METHODS

Animal preparation, instrumentation, euthanasia, quantification of TXA, and statistical analysis were the same as in a previous study (8), but key elements are also described below. For details on animal preparation and instrumentation, please see the study by Bakke *et al.* (5).

Model

Specific pathogen-free 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 intra-abdominal, retroperitoneal, and intrathoracic injuries on the pig. The degree of shock was compared with a control group from a previous study that underwent anesthesia but no surgical procedure. For the current study, a normal dose of TXA was defined as 15 mg/kg bodyweight for the swine, as this is close to the standard dose of 1 g used in the CRASH-2 study if given to a 70-kg patient (1)

Instrumentation, anesthesia, and monitoring

Pigs were anesthetized directly at the farm, 10 min by car transport from the university laboratory. All pigs were anesthetized using IM azaperone 40 mg, ketamine 500 mg, and atropine 0.5 mg. Peripheral venous catheters were placed bilaterally in an ear vein. If the animals showed signs of inadequate anesthesia before intubation, they received a bolus of pentobarbital 2 mg/kg, followed by titration to maintain spontaneous breathing and sufficient anesthesia. We administered topical lidocaine in the airway and then intubated the pigs endotracheally in a supine position using a 7.0-mm outer diameter tube while the pigs were breathing spontaneously.

We maintained anesthesia with an IV infusion of morphine 2 mg/kg/h, midazolam 0.15 mg/kg/h, and pentobarbital 4 mg/kg/h. The animals were mechanically ventilated with a tidal volume of 10 to 13 mL/kg, respiratory rate of 20 to 24/min, and positive end-expiratory pressure of 5 cm H_2O. Minute ventilation was titrated to maintain a pH of 7.4 $\pm$ 0.5, and inspired oxygen (FiO_2) was adjusted to maintain arterial pulse oximetry saturation (SpO_2) above 90%. Ringer acetate 8 to 10 mL/kg/h was infused to maintain euvolemia.

The depth of anesthesia was regularly controlled by the Federation of European Laboratory Animal Science (FELASA)–certified qualified anesthesia personnel. Pigs that were alive at the end of the experiment and emergency trauma course were euthanized by pentobarbital 300 mg, morphine 10 mg, and potassium chloride 50 mmol.

Experimental protocol

The animals were divided into four groups as shown in Figure 1. All four groups received TXA 1 h after the start of surgery, defined as the first incision.

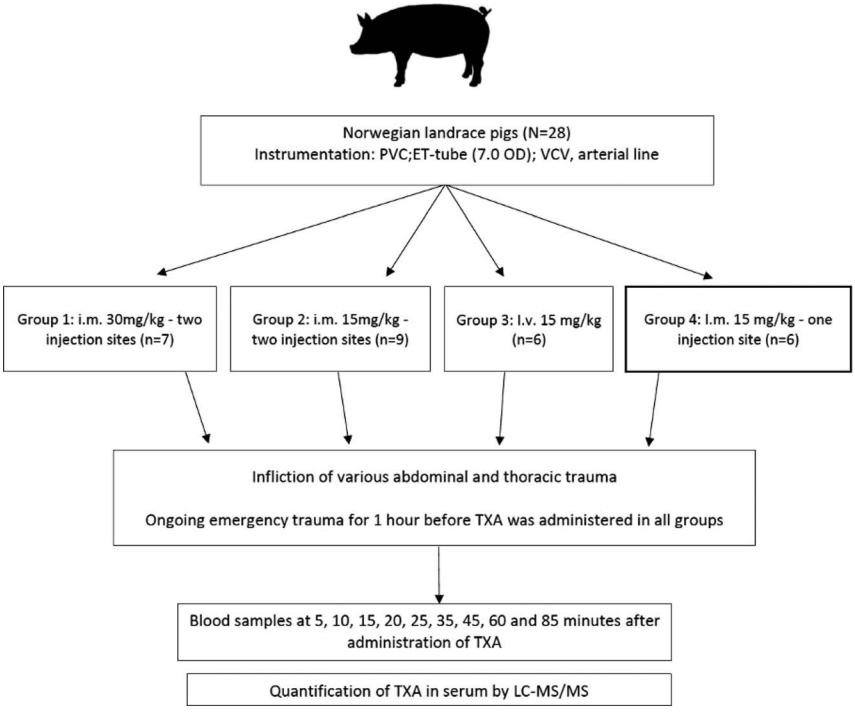

FIG. 1. **A general overview of the experimental protocol.** ET, endotracheal; LC-MS/MS, liquid chromatography-tandem mass spectrometry; OD, outer diameter; PVC, peripheral catheter; TXA, tranexamic acid; VCV, volume-controlled ventilation. Groups are compared with nonshocked pigs from Bakke et al. (8).

Using two IM injection sites, groups 1 and 2 received 30 mg/kg and 15 mg/kg of TXA, respectively. Using one IM injection site, group 4 received 15 mg/kg of TXA. Group 3 received 15 mg/kg of TXA IV and was used as the standard treatment control group. Blood samples were taken 5, 10, 15, 20, 25, 35, 45, 60, and 85 min after TXA administration. Samples were stored on Eppendorf tubes and set to coagulate for 1 h, before being centrifuged at 7500 × *g* for 7.5 min. The obtained serum was frozen at −20°C before LC-MS/MS analysis. A flowchart over the experimental process is provided in Figure 1. FELASA-certified personnel were present in the university laboratory 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 (8). Tranexamic acid and TXA-13C2,15N were purchased from Toronto Research Chemicals Inc, Ontario, Canada. Serum levels of TXA were analyzed by using a Waters Acquity UPLC *I*-Class flow-through needle 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 transitions were used (bold transitions are qualifiers): m/z 158 → 123/**95** and 161 → 125/**96** (TXA and TXA-$^{13}C_2$,^{15}N). The method was found linear from 0.005 to at least 94 μg/mL ($r^2 > 0.998$). Lower limit of quantification was found to be 0.0025 μg/mL (0.1-μL injection volume). Between-day coefficient of variation for TXA was <10% on four consecutive days. Coefficient of variation for intraday precision value was <6%, and accuracy for recovery test was 94.2% to 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 method to compare values within group versus baseline. Differences in TXA serum concentrations and hemodynamic variables between groups were analyzed by a one-way ANOVA test followed by an all-pairwise multiple comparisons procedure using Tukey test. Repeated measures ANOVA on ranks and Dunn 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

Artificial intelligence was used for language support on the abstract, aims, the 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 was 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 (Fig. 2).

One versus two IM injection sites

Figure 3 compares the serum concentration between TXA administered IM with one versus two injection sites and a dose of 15 mg/kg. There was a significant difference between the IV group and the IM groups until 35 min after receiving TXA, with IV administration achieving an initial Cmax of 54 μg/mL before declining to a similar level as the IM groups that held a steady

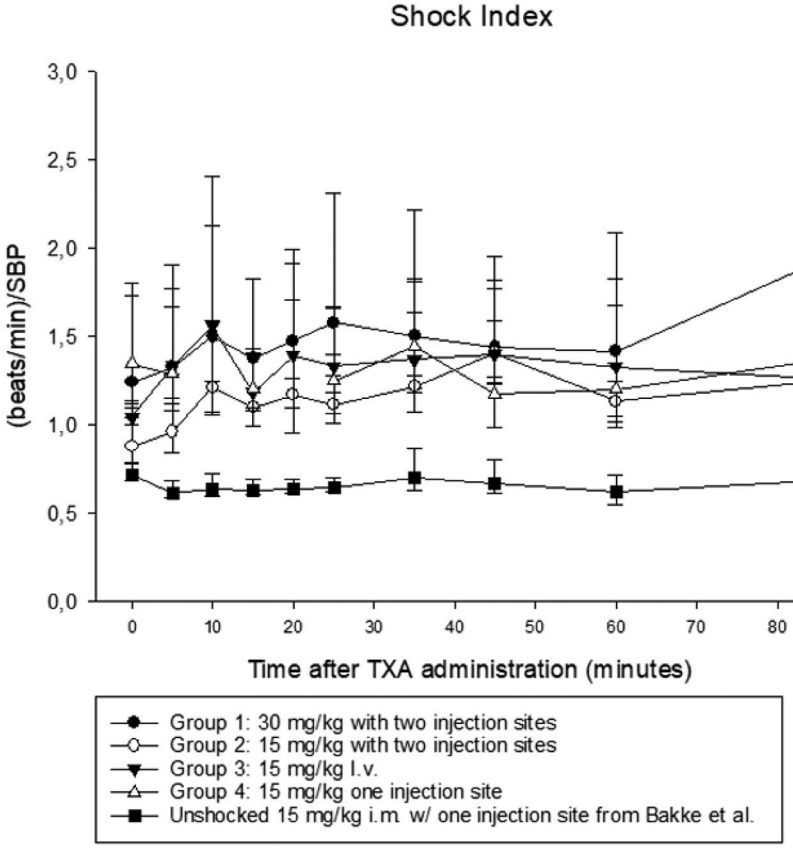

FIG. 2. **Shock index.** Shock index was calculated from heart rate and systolic blood pressure. SI = HR/SBP. HR, heart rate; SBP; systolic blood pressure; SI, shock index.

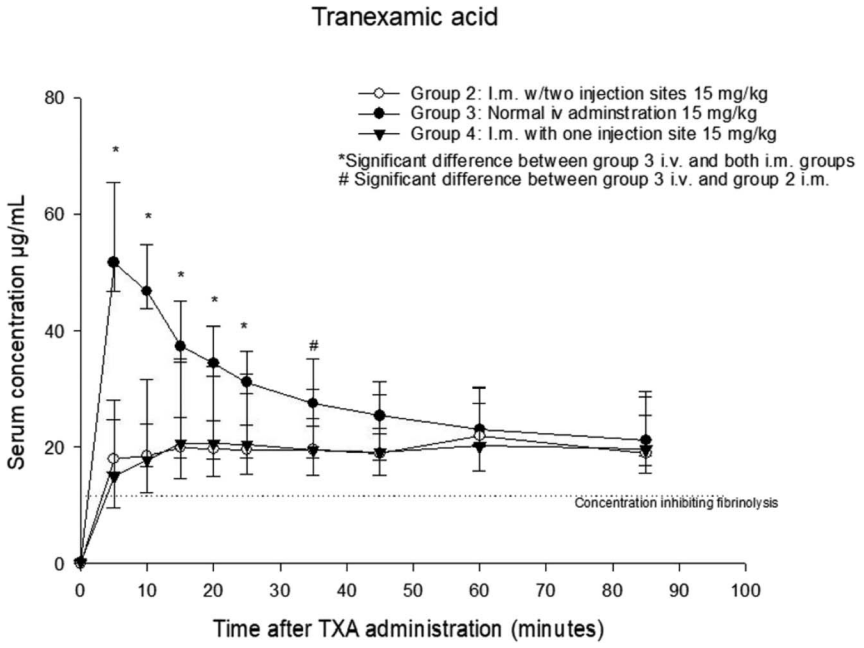

FIG. 3. **One versus two IM injection sites.** This figure shows the two IM groups with standard and double dose TXA compared with the IV control group with standard dose TXA. IM, intramuscular; IV, intravenous; TXA, tranexamic acid.

serum concentration around 20 µg/mL throughout the protocol. From 35 min, the groups were quite similar in serum concentration, as shown in Figure 3. But there was no difference in concentration between the IM groups with one versus two injection sites. Both methods of IM administration gave serum concentrations above the *in vitro* threshold for inhibiting fibrinolysis within 5 min (6,10).

Normal dose IM versus double dose IM

Increasing the IM dose from 15 mg/kg to 30 mg/kg resulted in significantly higher serum concentrations of TXA (Fig. 4). Group 3, the IV group, reached its mean maximum serum concentration quickest at 54 µg/mL after 5 min. However, after approximately 10 min, group 1, the 30 mg/kg IM group, had serum concentrations at comparable levels to IV administration.

After IV administration, serum concentration had a gradual decline, whereas the 30 mg/kg IM group peaked with maximum serum concentration at 25 min and kept a relatively steady serum concentration throughout the rest of the protocol. Compared with the group receiving IM 15 mg/kg, the group receiving 30 mg/kg IM had significantly higher serum concentration of TXA at all

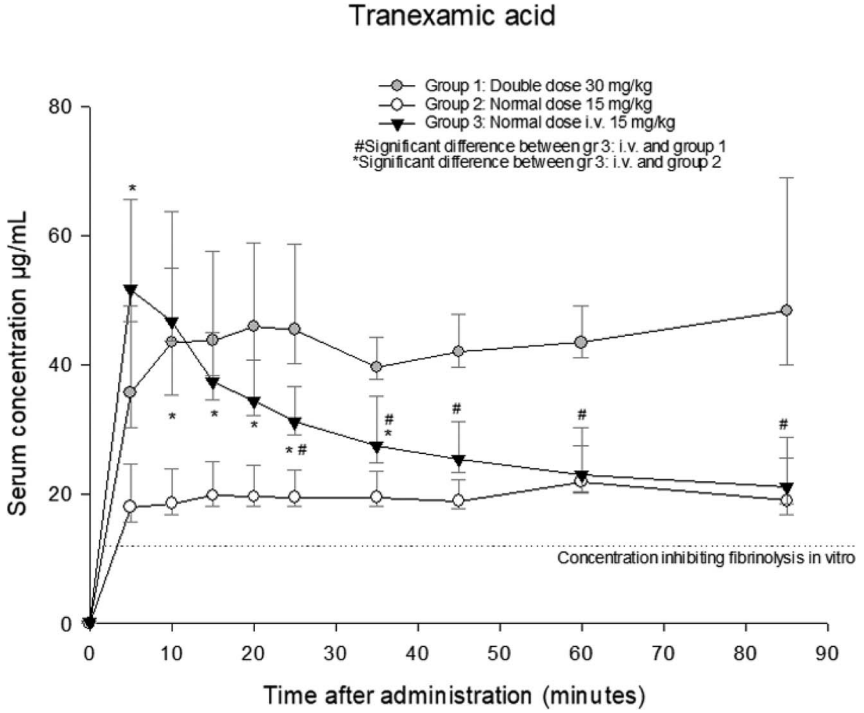

FIG. 4. **Standard dose IM versus double dose IM.** Group 1 received double dose TXA in two injection sites, 5 mL in each thigh. Group 2 received standard dose TXA 15 mg/kg in two injection sites. Group 3 received standard dose TXA 15 mg/kg IV. IM, intramuscular; IV, intravenous; TXA, tranexamic acid.

times. However, all groups were above the serum concentration needed to inhibit the fibrinolysis *in vitro* at all measuring points.

DISCUSSION

Dividing the IM dose of TXA between two injection sites did not result in higher serum concentrations compared with a single injection site. However, increasing the administered IM dose from the normal 15 mg/kg to 30 mg/kg did lead to TXA serum concentrations comparable to IV administration, and although the serum concentrations did not rise quite as rapidly as IV administration, it was double the reported threshold required to inhibit fibrinolysis within 5 min of administration (8,10). Furthermore, the TXA serum concentration remained stable throughout the 85-min experimental period following IM injection. This was found in a model with shocked animals, and our study therefore suggests that administering 30 mg/kg TXA IM is equally effective as standard IV administration in trauma, ensuring a consistent serum concentration of TXA during patient transport to advanced care. Consequently, IM administration is not only a viable alternative when IV 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 IM can be preferable over IV administration, as it does not require IV access. Studies have shown that IM 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 IM administration may be desirable (12). If our data are applied at such clinical setting, it is likely that the theoretical benefit of a quicker rise in serum concentration with IV administration is offset by the potential time-consuming nature of establishing peripheral vein catheterization and is therefore even superior to IV administration. Also, in high-resource settings, IV access can occasionally be difficult, particularly in shocked patients, where IM administration is a useful alternative. Intraosseous administration is another option but may not always be available and require relatively expensive equipment, and it also requires more training than IM administration.

The sustained high serum concentrations observed after administration of 30 mg/kg TXA also suggest that this may be preferable to repeated TXA boluses and that it may be an alternative to a continuous IV infusion of TXA.

Also, the group given a normal IM 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). Although 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. Although 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 IM with current available concentrations will exceed 5 mL, the maximum recommended volume for IM injections. It would be beneficial to produce TXA in a more concentrated version to avoid such big IM volumes. For rapid IM 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 (13).

Limitations

There were some missing data points in the study: For each pig, we had 10 measuring points for hemodynamic and concentration measurements. For hemodynamic measurements, we used systolic blood pressure and heart rate to calculate shock index. For concentrations measurements, in group 4, we did not have the measurements for 10 and 20 min, because we elaborated the protocol since this group was collected. Pigs with missing values >50% was excluded (n = 1). None of the pigs had more than two missing measurements, so each of the n = 29 pigs had less than 20% missing values, and the data gave us a trend despite this. Twenty-two of 29 pigs had complete measurements regarding concentrations. In all, these missing data are unlikely to affect the general results.

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.

Furthermore, all pigs were under general anesthesia unlike most patients who will receive TXA, which can affect muscular uptake of TXA (14). General anesthesia may contribute to decrease the negative effects that shock have on muscle perfusion through relaxed sympathic tone. Therefore, this study can overestimate the uptake compared with patients who are not under general anesthesia. We also know that general anesthesia in itself can impair microcirculation of skeletal muscle and may impair IM uptake (14). Lastly, there is a possibility that muscle uptake in shock is different between pigs and humans. Although IM uptake of TXA in human trauma patients have been demonstrated, care must be taken and IM administration must be studied further in human subjects (15). Not least one must take into account the possibility that TXA administration may affect subsets of trauma patients differently (16).

CONCLUSION

It is possible to achieve serum concentrations of TXA equivalent to IV administration by increasing the IM dose to 30 mg/kg. Dividing the IM dose between 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 IM use. In a clinical context, it is likely that IM administration of 30 mg/kg TXA would be faster, simpler, and therefore a superior alternative to IV administration in certain settings.

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