Tranexamic Acid toxicity in human tenocytes: caution in clinical practice

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INTRODUCTION
Tranexamic acid (TXA) is an anti-fibrinolytic that is commonly used to reduce perioperative bleeding. However, in view of the potential safety concerns (thromboembolism and Acute Kidney Injury) related to intravenous administration of TXA its use as a topical agent including intra-articular injection or peri-operative wash has been increasingly common in orthopaedic practice. Several studies have investigated the efficacy of topical administration demonstrating comparable homeostasis with no apparent significant side effects¹. Currently topical tranexamic acid is administered at concentrations of between 1–100mg/ml, much higher than that used for intravenous administration². Adult soft tissues have poor regenerative capacity and therefore damage to these tissues are devastating to the patient. We therefore sought to investigate the effects of TXA on primary human tenocytes in vitro using clinically relevant concentrations.

METHODS
All procedures and protocols were approved by the local NHS research ethics (REC) committee. Primary human tenocytes were cultured from hamstring tendon tissue obtained during hamstring tendon ACL reconstruction. The in vitro effect of TXA at concentrations of 0, 1, 50 & 100mg/ml over various time points, on primary human tenocytes was measured using MTT assays and fluorescent microscopy. Multi-apoptotic protein detection arrays were used to determine a likely mechanism and this was subsequently confirmed through further Caspase 3 ELISA’s and MTT assays in the presence/absence of a pan-caspase inhibitor. Statistical analysis was conducted by use of paired and 2-sample t-tests and ANOVA.

RESULTS
MTT Assays revealed a significant fall in cell viability when exposed to TXA at 100mg/ml for 4 hours. Additionally it showed that concentrations of 1, 50 or 100mg/ml of TXA for 24 hours also caused a significant fall in cell viability. There was no statistically statistical difference seen at 1 hour or with lower doses. There was a significant increase in cell apoptosis seen upon fluorescent microscopy when cells were exposed to 50 or 100mg/ml TXA for 4 hours. Subsequent Analysis of the apoptosis related proteins revealed an increase in Pro-Caspase-3, Cleaved Caspase-3, Catalaize, HSP27 & Fas/TNFRSF6/CD95. This indicated that the TXA induced apoptosis via caspase-3 dependent mechanism.

DISCUSSION
Our study provides evidence that TXA is toxic to human tenocytes when exposed at high concentrations (100mg/ml) over a short duration, but also even at the lowest concentrations (1mg/ml) over a longer duration (24 hours). This shows clear evidence of toxicity at clinically relevant concentrations. Fluorescent microscopy confirmed increased apoptosis at doses of 100mg/ml over 1 hour and50mg/ml or higher at 4 hours. Led by the results of an apoptotic multi-protein array we have illustrated a likely Caspase-3 mediated mechanism. Increasingly, high dose TXA is being used as a peri-operative wash or intra-articular injection to combat post-surgical bleeding. This has been shown to result in a significantly reduced post-operative blood-transfusion requirement. There is a paucity of published literature regarding its potential toxicity profile with regards to intra-articular soft tissues. This study highlights the need for caution when considering this route of administration of TXA.

SIGNIFICANCE
Increasingly, high dose TXA is being used as a peri-operative wash or intra-articular injection to combat post-surgical bleeding. This study highlights the need for caution when considering this route of administration of TXA in clinical practice due to increased tendon cell death.

REFERENCES

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Figure 1

Figure 1. The effect of TXA concentration (0, 1, 50, 100mg/ml) on Tenocyte viability. MTT assays performed at 1, 4 and 24 hours. * indicates significant difference from control.

Figure 2

Figure 2. The effect of TXA concentration (0, 1, 50, 100mg/ml) on Tenocyte apoptosis. DrPepHab™ assays performed at 1 and 4 hours, expressed as percentage of apoptotic/live cells. * indicates significant difference from control.

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