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Title: Tranexamic acid -Finding a balance between functionality and chondrotoxicity in a clinical setting
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Introduction:
Little is known about the impacts of tranexamic acid (TXA) on human chondrocytes. It is now used extensively in surgery where blood loss could be expected such as total joint replacement surgery, and in trauma settings. TXA has been shown to be beneficial in terms of functional post-operative outcome in joint replacement and therefore it may be desirable to extend its use to include procedures which retain native joint cartilage.

However, some studies on animal cells have indicated that TXA may be damaging to chondrocytes. It is therefore of importance to quantify the concentration range at which this damage occurs and the extent of the damage that is caused.

TXA is an anti-fibrinolytic agent that binds to plasminogen, preventing formation of plasmin. This inhibits the breakdown of fibrin clots, as well as fibrinogen, thus increasing clotting and decreasing bleeding and blood loss. There are several ways to deliver TXA to a patient – oral, intravenous, intra-articular and peri-articular. Local routes of administration such as intra-articular or peri-articular are attractive because they increase the dosage control of the surgeon at the site of operation and do not expose the patient to a clotting agent systemically. Dosage of tranexamic acid is variable both within and between these routes, with no apparent consensus on the optimum therapeutic dosage being released – this ranges between 10-100 mg/ml.

TXA is excreted unchanged from the body in the urine, with 90% of the original dosage being excreted after 24 hours. At a concentration of 10 ug/ml, TXA has been shown in vitro to produce 80% coagulation. The half-life of TXA in joint fluid is at 3 hours. This means it is only active in the body for a short period.

Aim:
To determine if there is a point at which TXA is toxic to chondrocytes.
To determine if there is a safe dosage range in which TXA can be used in procedures where native cartilage is retained.
To determine if toxic and safe use ranges are clinically relevant.

Impact:
This project, at the time of writing, is the first to explore toxicity of TXA to human chondrocytes and at clinically relevant time points in mammalian cells. This project will give an indication of the concentration of TXA that is non-toxic to chondrocytes in procedures where native cartilage is retained, such as uni-compartment knee replacement and arthroscopic procedures.

Method:
We used 3 methods to test the effect of TXA on chondrocytes:
A 2D culture of human articular chondrocytes (HACs) was exposed to TXA at concentration ranges that could be expected in both intravenous (25-200 ug/ml) and intra-articular administration (5-40 mg/ml), for a duration of 3, 6 and 12 hours. Outcomes measured were the proportion of cells caused to detach from well plates.

HACs were seeded into gelatin methacryloyl (GelMA) hydrogels and then incubated for 4 weeks to allow differentiation of the cells and formation of cellular matrix. The gels were then exposed to 10-40 mg/ml TXA for 3 hours. Outcomes measured were Live/Dead staining and cell count and glycosaminoglycan (GAG) quantification.
Human articular cartilage explants were gained from donor patients undergoing total knee replacement. 4mm punch biopsies of this cartilage was then taken and exposed to TXA for 3 hours at concentrations of 10-40 mg/ml. Outcomes measured were Live/Dead staining and cell count, glycosaminoglycan (GAG) quantification.

Results:
In the 2D intravenous range study no change in cell attachment (cytotoxicity effect) was seen between the control group and the groups exposed to TXA over 12 hours.
In the 2D intra-articular range study, there was a decreased cell attachment with both increasing TXA concentration and duration of exposure. After 3 hours 81% of cells exposed to 0 mg/ml were attached, 72% at 5 mg/ml, 67% at 10 mg/ml, 54% at 20 mg/ml, and 23% at 40 mg/ml. After 6 hours, 82% of cells at 0 mg/ml were attached, 77% at 5 mg/ml, 69% at 10 mg/ml, 45% at 20 mg/ml and 8% at 40 mg/ml. After 12 hours, 70% of cells at 0 mg/ml were attached, 63% at 5 mg/ml, 59% at 10 mg/ml, 48% at 20 mg/ml and 4% at 40 mg/ml.

In our 3D hydrogel study, there was a decrease in percentage of live cells with increasing concentration of TXA. After 3 hours treatment, 73% of cells exposed to 0 mg/ml TXA were live, 53% at 10 mg/ml, 41% at 20 mg/ml and 41 % at 40 mg/ml. A Dimethylmethylene blue assay (DMMB) to quantify the effect of TXA on GAG showed that there was no effect at any concentration measured following 3 hour exposure.

In the 3D tissue explant model Live/Dead analysis, when comparing to location matched controls after 3 hours; at 10 mg/ml exposure 74% of cells were live compared to 72% in the controls, at 20 mg/ml 65% of cells were live compared to 75% in the controls and at 40 mg/ml 48% of cells were live compared to 68% of controls. A DMMB assay showed no effect on GAG content after 3 hours exposure.

Conclusion:
This study shows that TXA is not chondrotoxic at concentrations expected following intravenous exposure. It also shows that TXA is chondrotoxic at high concentrations, in a clinically relevant timeframe. There was no effect seen on extracellular matrix (GAG) due to TXA.