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Original article

In vitro toxicity of local anaesthetics and corticosteroids on supraspinatus tenocyte viability and metabolism

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Summary *Background/Objective:* The purpose of this study was to evaluate supraspinatus tenocyte viability and metabolism in explants exposed to various local anaesthetics and corticosteroids. Our hypothesis was that the tendons exposed to these common injectates would have significantly decreased cell viability and metabolism compared with controls.
Methods: Supraspinatus tendon explants were obtained from dogs, placed in a culture media, and randomly assigned to one of the following groups: culture media only (control), 1% lidocaine, 0.5% lidocaine, 0.25% bupivacaine, 0.125% bupivacaine, 0.0625% bupivacaine, betamethasone acetate (5 mg), methylprednisolone acetate (40 mg), or triamcinolone acetonide (40 mg). Cell viability was determined on Days 1 and 7 after culture treatment using calcein AM (live cell) and Sytox Blue (dead cell) stains. Tissue metabolism was assessed on Days 1 and 7 using the resazurin blue metabolic assay. Significant differences were evaluated using a one-way analysis of variance with Tukey *post hoc* analysis.
Results: Compared with the controls, there were significant decreases in cell viability noted at Days 1 and 7 in tenocytes exposed to 1% lidocaine, betamethasone, and methylprednisolone. Significant decreases in cell metabolism were also noted at Days 1 and 7 in those groups. Treatment with 0.125% bupivacaine, 0.0625% bupivacaine, and triamcinolone demonstrated no decrease in cell viability or metabolism when compared with controls at any time point.
Conclusion: This data confirms that peritendinous injection of commonly used local anaesthetics and corticosteroids results in significant supraspinatus tenotoxicity *in vitro*. Further *in vivo* studies are required before making definitive clinical recommendations.

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Introduction

Local anaesthetic and corticosteroid injections have been widely used as a treatment option for many musculoskeletal conditions. Intra-articular injections are often used to treat arthritic joints whereas extra-articular or peritendinous injections are often used to treat inflammatory conditions [1–3]. Recent studies, however, have suggested potential toxicity at the cellular level after routine use of certain injection agents [4–7]. While the acute clinical result is often relief of symptoms, the potential for toxicity at the cellular level and associated long term morbidity has not yet been fully elucidated.

Numerous studies have been conducted which have demonstrated chondrotoxic properties of local anaesthetic and corticosteroid agents [4–13]. Some of these studies have shown that even a single exposure to anaesthetics or corticosteroids may result in loss of chondrocyte viability. These studies have demonstrated damaging effects of anaesthetic agents alone or in combination with corticosteroids. While there have been many recent investigations documenting chondrotoxicity of local anaesthetics and cortisone derivatives, there have been less studies demonstrating similar toxic effects on tenocytes and tendons [14–18]. Rotator cuff pathology, in particular, is frequently treated clinically with steroid injections, and the deleterious effects of various steroid derivatives on rotator cuff tendons have been shown in previous animal models [1,3,19,20]. Considering the current usage frequency of steroid and combination steroid and anaesthetic injections, it is valuable to further evaluate the effects of multiple commonly used agents.

The purpose of this *in vitro* study was to evaluate the toxicity of commonly used clinical doses of both local anaesthetics and corticosteroids on tenocytes in a canine supraspinatus tendon explant model. This explant model preserves the extracellular matrix and cell heterogeneity of the tissues in an effort to optimally mimic *in vivo* conditions. Our hypothesis was that the tendons exposed to routinely used local anaesthetics and corticosteroids at clinically relevant concentrations would have significantly decreased cell viability and metabolism compared with controls.

Methods

Tissue culture and harvest

All procedures were approved under the Institutional Animal Care and Use Committee policies and procedures for the use of canine cadaveric tissues. Seven adult (age = 2–4 years and mean weight = 28.6 kg), purpose-bred, intact female mongrel canine cadavers were obtained immediately after euthanasia that was performed for reasons unrelated to this study. All shoulder joints used were free of intra- and extra-articular pathology based on complete gross examination. Supraspinatus tendon samples were harvested from the shoulder under sterile conditions. Tissue explants, 4-mm thick, were prepared using a dermal biopsy punch (Fray Products, Buffalo, NY, USA) and were sliced in half to observe the viability of tenocytes across the thickness of the specimen.

The tendon tissue explants were cultured in 24-well plates (Becton Dickinson Labware, Franklin Lakes, NJ, USA) in media containing Dulbecco's modified Eagle's medium with high glucose (Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with 1% insulin–transferrin–selenium, penicillin, streptomycin, amphotericin B, L-ascorbic acid, L-glutamine, and nonessential amino acids. Explants ($n = 7$/group) were cultured for 24 hours prior to assignment to one of the following treatment groups: 1% lidocaine, 0.5% lidocaine, 0.25% bupivacaine, 0.125% bupivacaine, 0.0625% bupivacaine, betamethasone acetate (5 mg), methylprednisolone acetate (40 mg), and triamcinolone (40 mg). The concentration for each treatment group, shown in Table 1, was based on the average volume of injectate used for treatment of equivalent human pathology and the volume of drug required to obtain the desired concentration for explant culture [21]. Explants were cultured in 1 mL of treatment or control media and incubated at 37 °C with 5% CO_2 at 95% humidity for either 24 hours or 7 days.

Cell viability

Cell viability in tendon explants was assessed after 1 day and 7 days of culture by fluorescent microscopy using the fluorescent stains calcein AM (excitation = 495 nm; emission = 515 nm) to stain live cells and Sytox Blue (excitation = 633 nm, 635 nm; emission = 658 nm) to stain nonviable cells (Life Technologies, Carlsbad, CA, USA). At the time of tissue collection on each day, the explants were incubated in the stain for 30 minutes at room temperature. Tissue images were recorded at 4× magnification using an

Table 1 Amount of medication used for each treatment subset.

Group	Media (mL)	Drug (mL)
Negative control	7	
0.25% Bupivacaine	7 +	5
0.125% Bupivacaine	7 +	2.5
0.0625% Bupivacaine	7 +	1.25
1.0% Lidocaine	7 +	2
0.5% Lidocaine	7 +	1
Betamethasone 5 mg	7 +	1.25
Methylprednisolone (depo) 40mg	7 +	1
Triamcinolone (kenalog) 40mg	7 +	1

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