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RESEARCH PAPER

Effects of xylazine and dexmedetomidine on equine articular chondrocytes *in vitro*

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Abstract

Objective To assess the effects of xylazine and dexmedetomidine on equine chondrocytes, *in vitro*.

Study design Prospective, experimental study.

Study material Equine articular chondrocytes from five male horses.

Methods Chondrocytes were isolated from healthy equine articular cartilage of the metacarpo/metatarsophalangeal joints. Cell viability was assessed using the WST-8 assay by exposing chondrocytes to xylazine (0.5, 1, 2, 4, 8, 16.6, 25, 50 mg mL^{-1}) or dexmedetomidine (0.001, 0.005, 0.01, 0.05, 0.175, 0.25 mg mL^{-1}) for 15, 30 and 60 minutes. Based on the results of these tests, cells were treated with xylazine (1, 4, 25 mg mL^{-1}) or dexmedetomidine (0.05, 0.175, 0.25 mg mL^{-1}) for 15 minutes to further evaluate: cell viability by neutral red uptake; cell membrane integrity by lactate dehydrogenase release and by fluorescence microscopy with Hoechst 33342 and propidium iodide (PI), and apoptosis by flow cytometry using double staining with annexin V-fluorescein isothiocyanate/PI and by cell morphology.

Results Both drugs reduced cell viability in a dose-dependent manner. Specifically, all xylazine concentrations, except 0.5 mg mL^{-1} and 1 mg mL^{-1}, significantly reduced cell viability, whereas the effects of dexmedetomidine were evident only at 0.175 mg mL^{-1} and 0.25 mg mL^{-1}. The highest concentrations of xylazine (25 mg mL^{-1}) and dexmedetomidine (0.25 mg mL^{-1}) caused loss of membrane integrity. Cell morphology and flow cytometry analyses demonstrated signs of late apoptosis in xylazine-treated cells, and signs of late apoptosis and necrosis in dexmedetomidine-treated cells.

Conclusions and clinical relevance This study offers new insights into the potential chondrotoxicity induced by dexmedetomidine and xylazine. Therefore, the intra-articular administration of α_2-agonists should be conducted with care, especially for doses of $\geq$ 4 mg mL^{-1} of xylazine and 0.175 mg mL^{-1} and 0.25 mg mL^{-1} of dexmedetomidine.

Keywords α_2-agonists, apoptosis, chondrotoxicity, dexmedetomidine, xylazine.

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Introduction

The drive to develop effective and safe analgesic treatment for joint pain, and to limit the complications associated with systemically administered drugs, has resulted in an increased focus upon the role of infiltration analgesia. Intra-articular (IA) injection of analgesics such as local anaesthetics, opiates, non-steroidal anti-inflammatory drugs, NMDA-receptor antagonists and α_2-adrenoceptor agonists has been found to be effective in providing pain relief in osteoarthritic joints and during arthroscopic procedures (Alagol et al. 2005; Unlu et al. 2006; Lavelle et al. 2007; Ballieul et al. 2009). However, knowledge of the short- and longterm effects of these compounds on articular cartilage is incomplete. Some adverse effects of the most commonly used local anaesthetics (e.g. bupivacaine, lidocaine) have been reported in the chondrocytes of humans and animals *in vivo* and *in vitro* (Ballieul et al. 2009; Chu et al. 2010; Park et al. 2011; Baker & Mulhall 2012).

Recently, IA administration of α_2-agonists such as dexmedetomidine in humans (Gomez-Vazquez et al. 2007; Al-Metwalli et al. 2008; Paul et al. 2010; Alipour et al. 2014) and dogs (Soto et al. 2014) and xylazine in horses (Di Salvo et al. 2014) has been shown to ameliorate postoperative outcomes after arthroscopy. Similarly, IA detomidine provided analgesia in horses after experimentally induced lameness (Sardari et al. 2005). The IA administration of α_2-agonists appears to be devoid of systemic effects such as hypotension and bradycardia because a relatively small dose is required to be effective and there is limited systematic absorption as the articular surface is vascularized poorly (Al-Metwalli et al. 2008; Soto et al. 2014). Regardless of the species treated, the analgesic effects of IA dexmedetomidine (Paul et al. 2010; Soto et al. 2014) and xylazine (Di Salvo et al. 2014) appear to result from direct local action, although systemic absorption cannot be excluded. Additionally, constitutive expression of α_2-adrenergic receptors in chondrocytes (Opolka et al. 2012) suggests possible specific effects of these drugs on cellular signalling pathways.

Although IA injection of these α_2-agonists may provide analgesia, research into their possible consequences in the articular environment is lacking. Xylazine is known to have a proapoptotic effect on several cell types, including splenocytes (Cupić et al. 2001), thymocytes (Cupić et al. 2003), myocardiocytes (Feng et al. 2013) and endothelial cells (Silva-Torres et al. 2014). The effects of dexmedetomidine on the death or survival of cells remain controversial. Dexmedetomidine showed protective effects against apoptosis induced by isoflurane or ketamine (Sanders et al. 2009; Duan et al. 2014) and by ischaemia–reperfusion injury (Luo et al. 2015). Conversely, high doses of dexmedetomidine induced apoptosis in human neutrophils *in vitro* (Kishikawa et al. 2008).

The purpose of this study was to assess the possible chondrotoxic effects of α_2-agonists on equine chondrocytes *in vitro* using specific assays to test cellular viability, mitochondrial and lysosomal activities, membrane damage, apoptosis and necrosis.

The study was based on the hypothesis that xylazine and dexmedetomidine might be cytotoxic to chondrocytes in a dose- and time-dependent manner, possibly as a result of the involvement of different cellular pathways in apoptosis and necrosis.

Materials and methods

Primary culture of equine chondrocytes

Chondrocytes were isolated from healthy articular cartilage of the metacarpo/metatarsophalangeal joints of five horses (Thoroughbred males, aged 3–7 years). Joints were obtained from a local slaughterhouse within 1–2 hours of slaughter and were disarticulated after aseptic skin preparation using chlorhexidine gluconate. Equine tissues were used in accordance with the guidelines of the Animal Care and Use Committee of Perugia University.

Cartilage slices were harvested under sterile conditions, using sterile scalpels, rinsed twice in Dulbecco's phosphate-buffered saline (DPBS) (Sigma-Aldrich Corp., MO, USA), without Ca^{2+} and Mg^{2+}, containing 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 250 μg mL^{-1} amphotericin B as an antimycotic, and then minced. Slices were then digested primarily with 0.25% trypsin for 10 minutes at 37 °C and subsequently with 2 mg mL^{-1} collagenase type Ia (Sigma-Aldrich Corp.) at 37 °C for 8–10 hours.

Undigested tissue was separated from cells using a 70 μm cell strainer (BD Biosciences, Inc., CA, USA). Cells were collected by centrifugation (10 minutes at 700 ***g***), washed in DPBS and re-suspended in low-glucose Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich Corp.) and supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich Corp.), 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. Viable cells (> 95% by trypan blue exclusion) were then placed into culture flasks (10×10^3 cells/cm^2) and expanded in monolayer

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