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The use of hyperosmotic saline for chondroprotection: implications for orthopaedic surgery and cartilage repair

N.M. Eltawil †, S.E.M. Howie ‡, A.H.R.W. Simpson §, A.K. Amin §, A.C. Hall † *

† *Centre for Integrative Physiology, School of Biomedical Sciences, University of Edinburgh, United Kingdom*
‡ *MRC Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, United Kingdom*
§ *Department of Orthopaedic and Trauma Surgery, University of Edinburgh, United Kingdom*

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SUMMARY

Objective: Articular cartilage may experience iatrogenic injury during routine orthopaedic/arthroscopic procedures. This could cause chondrocyte death, leading to cartilage degeneration and posttraumatic osteoarthritis. In an *in vitro* cartilage injury model, chondrocyte death was reduced by increasing the osmolarity of normal saline (NS), the most commonly-used irrigation solution. Here, we studied the effect of hyperosmolar saline (HS) on chondrocyte viability and cartilage repair in an *in vivo* injury model.
Design: Cartilage injury was induced by a single scalpel cut along the patellar groove of 8 week old rats in the absence of irrigation or with either NS (300 mOsm) or HS (600 mOsm). The percentage of cell death (PCD) within the injured area was assessed using confocal microscopy. Repair from injury was evaluated by histology/immunostaining, and inflammatory response by histology, cytokine array analysis and ELISA (enzyme-linked immunosorbent assay).
Results: The PCD in saline-irrigated joints was increased compared to non-irrigated (NI) joints [PCD = 20.8% (95%CI; 14.5, 27.1); PCD = 9.14% (95%CI; 6.3, 11.9); $P = 0.0017$]. However, hyperosmotic saline reduced chondrocyte death compared to NS (PCD = 10.4% (95%CI; 8.5, 12.3) $P = 0.0024$). Repair score, type II collagen and aggrecan levels, and injury width, were significantly improved with hyperosmotic compared to NS. Mild synovitis and similar changes in serum cytokine profile occurred in all operated joints irrespective of experimental group.
Conclusions: Hyperosmotic saline significantly reduced the chondrocyte death associated with scalpel-induced injury and enhanced cartilage repair. This irrigation solution might be useful as a simple chondroprotective strategy and may also reduce unintentional cartilage injury during articular reconstructive surgery and promote integrative cartilage repair, thereby reducing the risk of posttraumatic osteoarthritis.

Introduction

Chondrocytes, the cells of articular cartilage, are exclusively responsible for the turnover of the extracellular matrix (ECM) and therefore their survival is crucial for maintaining the biological and biomechanical functions of the tissue throughout life[1]. During arthroscopic/orthopaedic procedures, articular cartilage may be subjected to mechanical injury either by accident or design. Cartilage damage has been described as, unquestionably the most common iatrogenic lesion in arthroscopic surgery[2]. Iatrogenic injury can occur when cartilage is probed, trimmed, drilled or cut by surgical instruments and this may lead to articular cartilage damage and chondrocyte death increasing the risk of posttraumatic osteoarthritis[3]. In addition, chondrocyte death may hinder the integration between the native and repair tissue in reconstructive surgery and therefore limit the full functional and structural restoration of the joint[4]. It is therefore vital to maintain chondrocyte viability to limit damage and promote integration and cartilage healing following injury.

Although cartilage repair has been reported in animal models of cartilage injury, the regenerated tissue usually has inferior biomechanical properties and less durability than normal hyaline

* Address correspondence and reprint requests to: A.C. Hall, Centre for Integrative Physiology, School of Biomedical Sciences, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh EH8 9XD, Scotland, United Kingdom. Tel: 44-(0)131-650-3263; Fax: 44-(0)131-650-2872.
E-mail address: a.hall@ed.ac.uk (A.C. Hall).

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cartilage[5]. Improved repair of a full thickness joint surface defect in a mouse model was associated with reduced chondrocyte death while persistence of cell death was associated with cartilage degeneration[6]. The potential role of chondrocyte death following injury in the pathogenesis of posttraumatic osteoarthritis has been investigated[3] and compounds (e.g., caspase inhibitors, antioxidants) have been tested to inhibit chondrocyte death and thus the extent/severity of the cartilage damage after injury[7,8].

During arthroscopic/articular surgery, the joint is normally irrigated with an artificial solution to improve visibility and provide a clear surgical field. Various fluids are currently used in clinical practice however there are concerns about the potential deleterious effect of these solutions on cartilage. Previous studies showed ultra-structural changes in the articular cartilage[9] and inhibition of proteoglycan metabolism after exposure to different irrigation solutions[10]. All the commonly-used solutions have an osmolarity considerably lower (~250–300 mOsm) than that of synovial fluid (~400 mOsm)[11]. *In situ* chondrocytes are osmotically sensitive and alterations in extracellular osmolarity may influence cell volume[12,13], cytoskeletal organization[14], gene expression[15], protein synthesis[16] and calcium signalling[13,14]. In particular, lower osmolarity rendered articular chondrocytes more susceptible to the damaging effect of mechanical trauma[12,17–19] whereas raising osmolarity markedly protected chondrocytes[12,17]. Moreover, *in situ* chondrocyte death following injury to human articular cartilage was significantly decreased after exposure to hyperosmotic saline (600 mOsm) compared to normal saline (NS) (300 mOsm)[18]. Thus, simply raising the osmolarity of NS could provide chondroprotection during routine orthopaedic surgery[17–19].

Here we describe a reproducible *in vivo* animal model of scalpel-induced cartilage injury. Using this model, we demonstrate a chondroprotective effect of the hyperosmolar saline (HS) on chondrocyte viability and an improved repair outcome of articular cartilage following injury. We also studied the inflammatory response to the HS solution to assess its safe use.

Methods

Operative procedure

Eight-week-old male Sprague Dawley rats were anaesthetised using 3% isoflurane. After medial para-patellar arthrotomy, the patella was dislocated laterally to expose the patellar groove[20]. A partial thickness cartilage defect was then induced along the groove by a single gentle pass of a fresh No.11 scalpel blade in the absence of irrigation (no irrigation NI) or in the presence of normal (0.9%) saline (300 mOsm, (NS) Baxter Healthcare Ltd., UK) or hyperosmolar saline (HS) (600 mOsm, sucrose addition to saline)[19] (Fig. 1) with osmolarity measured by osmometer (Vitech Scientific Ltd., UK). Joints were lavaged for 5 min before and 5 min after the induction of the cartilage injury to allow chondrocytes to respond to the altered osmotic environment[19]. The patella was then relocated and the wound sutured in layers with coated vicryl 6-0 (polyglactin 910, Ethicon, UK). Sham operation was performed on the contralateral joint. Rats were given the analgesic buprenorphine (0.01 mg/kg) subcutaneously and allowed unrestricted activities in standard cages. All procedures were approved by the Local Ethics committee and UK Home Office.

For each group, ten rats were killed immediately after surgery (day 0) and five rats were killed at 1 day, 1, 2 and 8 weeks after surgery and serum obtained by cardiac puncture. Knee joints were dissected and the viability assay for *in situ* chondrocytes performed by incubating joints with 5-chloromethyl-fluorescein diacetate (CMFDA) and propidium iodide (PI) (1 h; both 10 μmol/L, Invitrogen, UK) to label live/dead cells respectively[18]. After confocal imaging, joints were decalcified and paraffin-embedded for histology and immunohistochemistry (Fig. 1).

Cartilage imaging

Consecutive axial optical sections of the fluorescently-labelled chondrocytes were acquired using confocal laser scanning microscopy (CLSM; Carl Zeiss Ltd., UK) at 10 μm intervals and combined to create a three dimensional image. For quantifying *in situ* chondrocyte death after injury, a region of interest (ROI) extending 200 μm on each side of the scalpel injury (x-axis) × 921 μm (y-axis) × 40 μm (z-axis) was created within the image using software [Volocity 4·0, UK; Fig. 2(A)]. Live and dead cells were identified by % voxel intensity and the percentage cell death (PCD = 100 × number of dead cells/number of dead and live cells) calculated in the ROI. Injury width was measured by LSM image software (Carl Zeiss Ltd, UK) at 50 μm interval in the y-axis using the CLSM images.

Histology and immunohistochemistry

Joints were sectioned at 5 μm intervals and stained with haematoxylin and eosin (H&E) and toluidine blue according to standard protocols. Cartilage thickness and injury depth were assessed by ImageJ software at day 0 using three non-consecutive sections (200 μm apart) and the percentage of injury depth to cartilage thickness calculated. The repair outcome was assessed using polarised light microscopy and the Wakitani scoring system[21] and synovitis evaluated using a well-established synovitis score[22]. Control non-operated, sham-operated and injured joints were assessed at the synovial insertion of medial femur, medial tibia, lateral femur and lateral tibia and the score of all four regions summed for a total joint synovitis score.

For immunostaining, sections were deparaffinised, digested with either pepsin (0.25 mg/ml, Sigma–Aldrich, UK) for type I and II collagen or chondroitinase ABC (0.25 unit/ml, Sigma–Aldrich, UK) for aggrecan, blocked in serum-free protein solution (Dako, UK) and incubated overnight with either anti-collagen I antibody (Abcam, UK), mouse anti-rat CD43 (AbD, Serotec, UK), anti-collagen II antibody (CIIC1) or anti-aggrecan antibody (12/21/1-C-6; both from the Developmental Studies Hybridoma Bank, Iowa). Sections were incubated with EnVision dual link system-HRP, and DAB substrate chromogen system used as peroxidase substrate (DakoCytomation, UK). Areas of immunostaining were measured using ImageJ software.

Cytokine array and ELISA

Relative changes in cytokine levels in serum samples from rats sacrificed 1 day and 1 week after surgery were screened and compared to control un-operated animals using the rat proteomic profiler array kit (R&D Systems, UK). Samples were pooled for each group, equal amounts of serum loaded on the blots in duplicate, and the average pixel density calculated using ImageJ. IL-1α levels were measured in serum samples from control and experimental rats using ELISA kits (R&D Systems, UK). The standard dilution series was extended to detect protein levels up to 15.6 pg/ml.

Statistical analysis

Data were analysed using SPSS v.21 (IBM, UK) and presented as means (95% CI lower limit, upper limit) where N indicated the number of animals in each group. Student's unpaired t-tests were used to compare between groups and Mann–Whitney U tests to compare histological scores. Differences were considered statistically significant at $P < 0.05$ and are indicated by asterisks with the actual P values given in text.