

# In vitro assessment of human chondrocyte viability after treatment with local anaesthetic, magnesium sulphate or normal saline

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## Abstract

**Purpose** Local anaesthetic agents are often used as an intra-articular analgesic following arthroscopic procedures. However, there is increasing evidence of a potential toxic effect to chondrocytes within the articular cartilage. The aim of this study was to compare the effect on human chondrocyte viability of treatment with bupivacaine, levobupivacaine and ropivacaine. The second aim was to compare the effect on chondrocyte viability of the local anaesthetics with magnesium, a potential alternative analgesic agent.

**Methods** Chondrocytes were exposed to one of the local anaesthetic agents (levobupivacaine 0.13, 0.25, 0.5%; bupivacaine 0.13, 0.25, 0.5%; ropivacaine 0.19, 0.38, 0.75%), normal saline or 10% magnesium sulphate for 15 min. Cells exposed to cell culture media served as controls. Twenty-four hours after exposure, cell viability was assessed using the CellTiter 96<sup>®</sup> Aqueous One Solution Cell Proliferation Assay.

**Results** There was no significant difference in chondrocyte viability after treatment with either normal saline or magnesium sulphate. With the exception of 0.13% levobupivacaine, all local anaesthetic treatment showed significantly greater toxic effects than either normal saline or magnesium sulphate. Statistically significant dose-dependent responses of decreasing cell viability were found with increasing local anaesthetic concentration.

**Conclusions** A dose-dependent reduction in chondrocyte viability after treatment with common local anaesthetic agents was confirmed. Local anaesthetic agents had a greater deleterious effect on chondrocytes than did 10% magnesium sulphate. These findings suggest the need for continuing caution with the use of intra-articular local anaesthetic. Magnesium sulphate is a potential alternative intra-articular analgesic agent.

**Keywords** Local anaesthetic · Magnesium · Arthroscopy

## Introduction

Intra-articular administration of local anaesthetic agents is a common practice in orthopaedic surgery, particularly after arthroscopic procedures. Their efficacy as an analgesic has been reported following wrist, hip, knee and ankle arthroscopy [1, 12, 18, 23].

Recent reports, however, have questioned the use of local anaesthetic via the intra-articular route. Initial reports focused on massive chondrolysis of the glenohumeral joint following the use of local anaesthetic infusions [15, 17, 25]. In vitro studies have focused on the time- and concentration-dependent effects of local anaesthetic agents on chondrocyte cultures [7, 8]. In an animal model, Chu et al. [6] have proven a lasting toxic effect of a single dose of intra-articular bupivacaine six-months after administration. Mitochondrial dysfunction with subsequent apoptosis is one possible explanation for the toxic effects seen in in vitro models, while an incompatibility of local anaesthetics with synovial fluid has been suggested as a potential cause for in vivo toxicity [4, 16].

While current opinion suggests a cautious approach to intra-articular use of local anaesthetic is warranted [30],

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Bondok et al. [5] have shown promising results using intra-articular magnesium sulphate as an alternative analgesic modality following knee arthroscopy. Magnesium also has reported chondroproliferative effects and this may represent an acceptable alternative analgesic [9].

The aim of this study therefore was to compare the respective toxic effects of commonly used local anaesthetic agents with those effects exerted by magnesium sulphate and normal saline.

## Materials and methods

Normal human chondrocytes (Promocell, Germany) were cultured under standard laboratory conditions (humidified atmosphere of 95% air and 5% CO<sub>2</sub>, 37°C). At passage, 4 cells were seeded into 96-well plates at a density of 10,000 cells/cm<sup>2</sup>.

Cells were exposed to either a local anaesthetic, magnesium sulphate or normal saline for 15 min. This time was chosen based on previous work [7, 8]. The treatments were then removed, and cells returned to incubation for a further 24 h. Bupivacaine, ropivacaine and levobupivacaine were selected as commonly used and studied local anaesthetic agents. Selected concentrations were based on commonly available commercial preparations. The magnesium sulphate concentration (10%) was chosen based on previous clinical work by Bondok et al. [5]. Normal saline (0.9%) was chosen as an arthroscopic fluid equivalent. Cells exposed to cell culture medium alone were used as controls.

At 24 h, cell viability was assessed using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, UK). This is a colorimetric assay containing a tetrazolium compound. When the tetrazolium is reduced, it produces a coloured formazan product that is soluble in cell culture medium and maximally absorbed at 490 nm on

light spectroscopy. The amount of formazan that is produced is directly proportional to the number of viable cells. A 20-µl aliquot of CellTiter 96® AQueous One Solution was added to the well and the absorbance was measured using light spectrometry 4 h later. This method of assessing cell viability has previously been used successfully [13, 20].

We measured the absorbance of formazan in each sample well to measure cell viability. The control well values were assumed to be equal to one, and treatment wells are all reported as a proportion of this.

## Statistical analysis

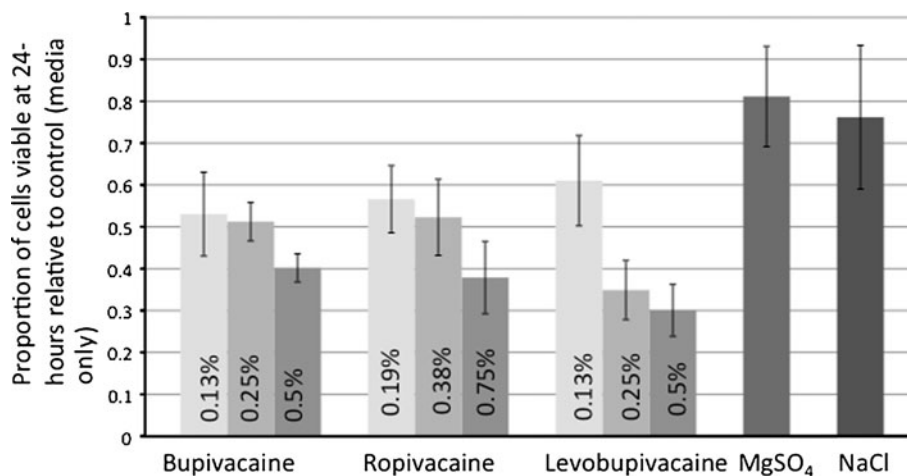
One-way ANOVA was used to assess for trends within groups and paired *t*-tests for testing statistical significance between individual treatments. Statistical significance was accepted as  $P < 0.05$ .

## Results

Results and values are demonstrated graphically in Fig. 1. There was no significant difference in chondrocyte viability after treatment with either normal saline or magnesium sulphate (n.s.). When comparing wells treated with local anaesthetic agents to those treated with magnesium sulphate, all wells demonstrated a significant reduction in cell viability (all  $P < 0.05$ ) with the exception of levobupivacaine 0.13% (n.s.). Compared with the wells treated with normal saline, those treated with local anaesthetic agents all showed significant reductions in viability (all  $P < 0.05$ ) with the exception of levobupivacaine 0.13% (n.s.).

Assessment of the effect of individual local anaesthetic agents with one-way ANOVA showed a statistically significant decrease in cell viability with increasing

**Fig. 1** Bar chart demonstrating the cell viability (y) after respective treatments (x). Viability is shown as a proportion of control (cells treated with cell culture media only)



concentration of bupivacaine ( $P = 0.01$ ), ropivacaine ( $P < 0.01$ ) and levobupivacaine ( $P < 0.01$ ).

## Discussion

The key finding from this study was confirmation of a dose-dependent response to local anaesthetic concentration, a finding consistent with previous reports using both human and bovine cell lines [7, 8, 19, 22].

In particular, the results are consistent with those of Chu et al. [7] who reported a reduction in human chondrocyte viability to 41% of saline control at 7 days after exposure of cells to 0.25% bupivacaine for only 15 min. The findings of Piper et al. [26] who reported that ropivacaine was significantly less toxic than bupivacaine in a monolayer culture model were not replicated. However, they did report again that both agents exerted a significantly greater toxic effect than saline alone—a finding also noted in this study. These results suggest that local anaesthetics alone exert a toxic effect on human chondrocytes above and beyond any toxic effect that is exerted by arthroscopic fluid. This is in contrast to an early report that suggested that there was no contraindication to using intra-articular bupivacaine as it has similar effects to saline [24].

The second key finding of this study was that magnesium sulphate exerts a less toxic effect on monolayer chondrocyte culture than local anaesthetic. There was no significant difference between the magnesium sulphate and normal saline treatments suggesting that magnesium sulphate is no more detrimental to chondrocyte viability than arthroscopic fluid. Furthermore, there were significant differences between the local anaesthetic agents and the magnesium sulphate. This supports the approach of Bondok et al. [5] who found 10% magnesium sulphate solution to be superior to placebo as an intra-articular analgesic agent following knee arthroscopy. El-Sharnouby et al. [10] also found that intra-articular magnesium sulphate conferred similar analgesic control to intra-articular bupivacaine following knee arthroscopy. Their basis for using magnesium sulphate was its NMDA-receptor blocking ability [21]. Magnesium sulphate has reported chondroproliferative properties and these findings may represent an acceptable less toxic, future intra-articular agent [9].

It is worth noting that a number of other agents have been trialled as intra-articular anaesthetic agents following knee arthroscopy, and the effect of these agents on chondrocyte viability has not been assessed in this study. However, of these other agents, morphine and midazolam have both been shown to have little or limited analgesic benefit [3, 27, 28]. Steroid administration is sometimes considered at knee arthroscopy, particularly if there is an

inflammatory component to the intra-articular pathology. However, Seshadri et al. [29] have shown that the methylprednisolone has an additive toxic effect with lidocaine and caution is warranted.

Although this study is perhaps limited by its *in vitro* nature as it is unknown how closely *in vitro* research mimics the *in vivo* state, monolayer culture of chondrocytes has been well used as a method of assessing *in vitro* cell response to various treatments [14]. Nevertheless, more complex models incorporating three-dimensional scaffolds may offer a more realistic representation of *in vivo* chondrocyte behaviour and these models may be the future gold standard for *in vitro* analysis. Cell flow cytometry, although more expensive, is a more often used mode of assessing for viability in cell culture models [29]. Although the ideal mode of cell viability testing is unknown, use of a common model would allow for better comparison of individual studies.

Despite numerous reports of the toxic effects of local anaesthetic agents on chondrocytes, it must also be acknowledged that some authors have shown in animal models that an infusion of bupivacaine has no lasting deleterious effect [11]. Some authors feel that a single dose of intra-articular bupivacaine is safe and that the real threat lies only with the use of continuous infusion of high concentrations of local anaesthetics [31].

In the study by El-Sharnouby et al. [10], a combination of bupivacaine and magnesium sulphate was more effective than either agent alone at providing post-operative analgesic control. Furthermore, a recent *in vitro* study assessing the additive effect of magnesium sulphate to local anaesthetic agents found that magnesium sulphate attenuated the toxic effects of local anaesthetic [2]. Arthroscopic surgeons need to be aware that most commonly available local anaesthetic carries a dose-dependent toxic effect, of which the long-term consequences remain uncertain. The findings of this study and of others' suggest that arthroscopists may consider the addition of magnesium sulphate to their chosen local anaesthetic, if not a combination of ropivacaine and magnesium sulphate, when selecting an intra-articular agent following knee arthroscopy [2, 10].

## Conclusion

In summary, three commonly used local anaesthetic agents all conferred a dose-dependent toxic effect on human chondrocytes and continued caution is warranted regarding their use as an intra-articular analgesic. This toxic effect is significantly different to that exerted by magnesium sulphate or normal saline. Magnesium sulphate solution may represent an acceptable intra-articular analgesic agent and warrants further attention.

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