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# Effects of Superoxide Dismutase 1 on cartilage chondrocyte metabolism: a protective mechanism against ankle osteoarthritis?

C4ME SUPPLEMENT

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### **Background**

Osteoarthritis (OA) is a ubiquitous degenerative disease that generates significant global disability and socio-economic burden, forcing more than 8.75 million people aged 45 or over to seek treatment in the UK/annum. (1) OA predominantly affects the lower limbs, however prevalence in the knee is 10-fold higher than in the ankle, with notably differing aetiology between the joints. Knee OA is characteristically a 'wear-and-tear' primary arthritis; in contrast, 90% of ankle OA cases are preceded by trauma-induced joint instability. (2) Hence, ankle cartilage is thought to be relatively protected from the catabolic phenotype that constitutes primary OA pathogenesis. Such protection is likely mediated through biochemical and biomechanical variations including lower concentrations of matrix metalloproteinases (MMPs) – cartilage degrading enzymes, and a reduced sensitivity to pro-inflammatory cytokines. These include Interleukin 1 $\beta$  (IL-1 $\beta$ ) and Tumour Necrosis Factor- $\alpha$  (TNF $\alpha$ ), which potentiate an OA phenotype in cartilage. (3, 4)

Prior RNA sequencing data identified a potential increased expression of the Superoxide Dismutase 1 (SOD1) gene in ankle cartilage relative to the knee; a gene encoding an intracellular antioxidant enzyme that reduces oxidative stress. Oxidative stress is elevated in OA cartilage and is associated with inducing chondrocyte dysfunction, partly through upregulation of OA-linked pro-inflammatory cytokines. (5) Following receipt of the Wolfson Intercalated Award (Royal College of Physicians), I used an *in vitro* model to investigate whether this relative increase in SOD1 was partly responsible for conferring protection against cytokine-induced chondrocyte catabolism.

## Methods

Immortalised ATDC5 mouse teratocarcinoma cells were utilised as a chondrocyte source. Cells were seeded within media suspension (100,000/well of a 48-well plate) and subsequently treated (n=6) with 0.31U or 0.63U of bovine erythrocyte SOD1 (25U/mg and 50U/mg of cellular protein respectively). This represented the physiological concentrations of SOD1 reported for knee and ankle articular cartilage in the literature (6). SOD1 treatment occurred in the presence or absence of 5ng/ml IL-1 $\alpha$  and 10ng/ml Oncostatin M treatment - used to mimic the pro-inflammatory catabolic response observed in OA; untreated cells served as controls. Cells were treated for 1, 2, 3 or 7 days, with media and cell lysates analysed for cytotoxicity, as well as markers of inflammation (nitric oxide (NO), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)) and chondrocyte metabolism (sulphated glycosaminoglycan (sGAG), type II collagen).

## Results

Following normalisation to ATDC5 protein content, as a marker of cell metabolic activity, it was observed that both 0.31U (p<0.001) and 0.63U (p<0.01) of SOD1 treatment blunted cytokine-induced NO synthesis at day 7. Furthermore, 0.31U of SOD1 treatment significantly lowered cytokine-induced PGE<sub>2</sub> media content by day 7 (p<0.05). Surprisingly, sGAG content was not affected by cytokine or SOD1 treatment.

## Discussion

SOD1 treatment significantly reduced cytokine-induced upregulation of NO and PGE<sub>2</sub> in an ATDC5 chondrocyte-like cell line. Limiting the synthesis of pro-inflammatory molecules, such as NO and PGE<sub>2</sub>, potentially reduces the cartilaginous tissue loss characteristic of OA, through decreasing MMP expression and the mitochondrial dysfunction that initiates a chondrocyte apoptotic response. (7) NO and PGE<sub>2</sub> additionally suppress neosynthesis of type II collagen and proteoglycans; (8) therefore, the demonstrated effects of SOD1 may facilitate cartilage regeneration plausibly perturbing OA pathogenesis. Restricting NO and PGE<sub>2</sub> production also disrupts positive feedback-mediated upregulation of pro-inflammatory cytokines, defining an additional mechanism by which SOD1 treatment may reduce joint inflammation. (8) With a potential protective role for SOD1 against a catabolic OA-like phenotype identified, it can be hypothesised that its elevated expression in ankle chondrocytes relative to knee may be partially responsible for the relative susceptibilities of each joint to osteoarthritic degeneration. Nevertheless, future studies are necessary to determine whether these SOD1-mediated reductions in catabolic activities correlate with measurable decreases in human cartilage matrix degradation.

Furthermore, our study may suggest applications for exogenous SOD1 in OA treatment, through attempting to recapitulate innate ankle SOD1 concentrations within OA afflicted joints. This concept is supported by literary evidence for the beneficial effects of Orgotein, a bovine non-specific SOD intra-articular injection, prior to its discontinuation due to adverse effects. (9) Tempol, a SOD mimetic, exhibits a more controlled attenuation of arthritic inflammation due to its biological membrane permeability but has further noxious systemic effects. (10) Therefore, further research into defining mechanisms of SOD1 endocytosis and its extracellular functionality may facilitate a future for recombinant SOD1 as a more effective treatment for OA.

## Lessons Learnt

This research project introduced skills such as statistical analysis, tissue culture, and laboratory techniques including pipetting, ELISAs and Western Blotting. With no prior experience of the techniques employed, I found the project a challenging and initially daunting experience, and often frustrating when meticulous time-consuming investigations revealed unusable or anomalous data. This included the data for 3 days of SOD1 treatment, yielding non-significant results and a low metabolic activity that implied compromised cell viability. This highlighted the uncertainty of research, the susceptibility of delicate techniques to error, and the importance of subsequent perseverance and investigation into possible explanations. Learning these challenging techniques and how to systematically approach and resolve problematic situations are skills I will now directly apply to similar future situations in medicine.

Moreover, conducting pioneering research was initially disconcerting, with no information to cross-reference findings. However, analysing novel results taught me a versatility with processing literature evidence, utilising current knowledge in the field to explain my own results. Upon presentation of my work, academic questioning surrounding the methods I employed made me aware of the importance of not only understanding what you are doing, but also why. Whether designing an experiment, explaining novel results or treating patients, I now have an increased appreciation for the importance of literature-based evidence and its skillful handling to guide decision-making. Not only are evidence-based decisions important, but so is an awareness of methodological reasoning and a pliability of your decisions to be in line with the latest evidence.

In conclusion, this project emphasised how pivotal research is to identify biological targets to optimise the treatment of patients. This philosophy has inspired me to integrate research into my future career, as I believe it paramount that doctors are involved in the furthering of medicinal knowledge.

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