The British Student Doctor, 2019;3(3):35-37
doi: 10.18573/bsdj.125
C4ME Supplement

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

C4ME SUPPLEMENT

AUTHOR INFORMATION

Jorge Adam Carter
Cardiff University School of Medicine

Dr Emma Blain
Cardiff University School of Biosciences

Address for Correspondence:

Jorge Adam Carter
Cardiff University School of Medicine
The Cochrane Building
Heath Park, Cardiff
CF14 4YU
United Kingdom

Email: CarterJA@cardiff.ac.uk

No conflicts of interest to declare

Accepted for publication: 17.10.19

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 1ß (IL-1ß) and Tumour Necrosis Factor-∝ (TNF∝), 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∝ 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_2 (PGE_2)) 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 ($p \leq 0.001$) and 0.63U ($p \leq 0.01$) of SOD1 treatment blunted cytokine-induced NO synthesis at day 7. Furthermore, 0.31U of SOD1 treatment significantly lowered cytokine-induced PGE_2 media content by day 7 ($p \leq 0.05$). Surprisingly, sGAG content was not affected by cytokine or SOD1 treatment.

Discussion

SOD1 treatment significantly reduced cytokine-induced upregulation of NO and PGE_2 in an ATDC5 chondrocyte-like cell line. Limiting the synthesis of pro-inflammatory molecules, such as NO and PGE_2, 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_2 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_2 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.

References

1. Versus Arthritis. The state of MSK health report 2018. 2018 [accessed 27 July 2019].
Available from:
https://www.arthritisresearchuk.org/arthritis-information/data-and-statistics/state-of-musculoskeletal-health/what-is-the-scale-of-the-problem.aspx.

2. Valderrabano V, Horisberger M, Russell I, Dougall H, Hintermann B. Etiology of Ankle Osteoarthritis. Clinical Orthopaedics and Related Research. 2009;467:1800.

https://doi.org/10.1007/s11999-008-0543-6
PMid:18830791 PMCid:PMC2690733

3. Eger W, Schumacher B, Mollenhauer J, Kuettner K, Cole A. Human knee and ankle cartilage explants: catabolic differences. Journal of Orthopaedic Research. 2002;20:526-34.

https://doi.org/10.1016/S0736-0266(01)00125-5

4. Hendren L, Beeson P. A review of the differences between normal and osteoarthritis articular cartilage in human knee and ankle joints. The Foot. 2009;19:171-76.

https://doi.org/10.1016/j.foot.2009.03.003
PMid:20307471

5. Scott J, Gabrielides C, Davidson R, Swingler T, Clark I, Wallis G, et al. Superoxide dismutase down regulation in osteoarthritis progression and end-stage disease. Annals of Rheumatic Diseases. 2010;69:1502-1510.

https://doi.org/10.1136/ard.2009.119966
PMid:20511611 PMCid:PMC3789136

6. Koikes M, Nojiri H, Kanazawa H, Yamaguchi H, Miyagawa K, Nagura N, et al. Superoxide dismutase activity is significantly lower in end-stage osteoarthritic cartilage than non-osteoarthritic cartilage. PLoS One. 2018 [accessed 17 Sept 2019].
Available from:
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0203944.

https://doi.org/10.1371/journal.pone.0203944
PMid:30222787 PMCid:PMC6141073

7. Mathiessen A, Conaghan P. Synovitis in osteoarthritis: current understanding with therapeutic implications. Arthritis Research & Therapy. 2017;19:18.

https://doi.org/10.1186/s13075-017-1229-9
PMid:28148295 PMCid:PMC5289060

8. Mastbergen S, Bijlsma J, Lafeber F. Synthesis and release of human cartilage matrix proteoglycans are differently regulated by nitric oxide and prostaglandin-E2. Annals of the Rheumatic Diseases. 2008;67:52-58.

https://doi.org/10.1136/ard.2006.065946
PMid:17485421

9. Afonso V, Champy R, Mitrovic D, Collin P, Lomri A. Reactive oxygen species and superoxide dismutases: Role in joint diseases. Joint Bone Spine. 2007;74:324-329.

https://doi.org/10.1016/j.jbspin.2007.02.002
PMid:17590367

10. Cuzzocrea S, McDonald M, Mota-Filipe H, Mazzon E, Costantino G, Britti D, et al. Beneficial effects of tempol, a membrane-permeable radical scavenger, in a rodent model of collagen-induced arthritis. Arthritis and Rheumatism. 2000;43:320-328.

https://doi.org/10.1002/1529-0131(200002)43:2<320::AID-ANR11>3.0.CO;2-9

bsdj.org.uk


/thebsdj


@thebsdj


@thebsdj

Journal DOI
10.18573/issn.2514-3174

Issue DOI
10.18573/bsdj.v3i3

Cardiff University Press
Gwasg Prifysgol Caerdydd

The British Student Doctor is an imprint of Cardiff University Press, an innovative open-access publisher of academic research, where 'open-access' means free for both readers and writers.

cardiffuniversitypress.org