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SORBITOL-MODIFIED HYALURONIC ACID REDUCES OXIDATIVE STRESS, APOPTOSIS AND MEDIATORS OF INFLAMMATION AND CATABOLISM IN HUMAN OSTEOARTHRITIC CHONDROCYTES

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Purpose: We design a study to elucidate the precise molecular mechanisms by which Sorbitol-modified hyaluronic acid (sorbitol/HA) exerts beneficial effects in osteoarthritis (OA).
Methods: Human OA chondrocytes were treated with increasing doses of sorbitol/HA and thereafter with or without interleukin-1beta (IL-1β) or hydrogen peroxide (H_2O_2). Signal transduction pathways and parameters related to oxidative stress, apoptosis, inflammation, and catabolism were investigated.
Results: Sorbitol/HA prevented IL-1 β-induced oxidative stress, as measured by reactive oxygen species (ROS), p47-NADPH oxidase (p47-NOX) phosphorylation, 4-hydroxynonenal (HNE) production and HNE-metabolizing glutathione-s-transferase A4-4 (Gsta4-4) expression. Moreover, sorbitol/HA stifled IL-1β-induced metalloproteinase 13 (MMP-13), nitric oxide (NO) and prostaglandin E2 (PGE_2) release as well as inducible NO synthase expression. Investigation of the apoptosis process revealed that this gel significantly attenuated cell death, caspase-3 activation and DNA fragmentation elicited by exposure to a cytotoxic H_2O_2 dose. Examination of signalling pathway components revealed that sorbitol/HA prevented IL-1β-induced p38 mitogen-activated protein kinase and nuclear factor-kappa B activation, but not that of extracellular signal-regulated kinases 1 and 2.
Conclusions: Altogether, our findings support a beneficial effect of sorbitol/HA in OA through restoring redox status and reducing markers of apoptosis, inflammation and catabolism involved in cartilage damage.

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LOCAL RAS AND ROS IN THE HYPERTROPHIC DIFFERENTIATION OF CHONDROCYTES

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Purpose: In 2013, we reported that the local renin-angiotensin system (RAS) can modulate the hypertrophic differentiation of chondrocytes activating angiotensin II type 1 receptor (AT1R) or angiotensin II type 2 receptor (AT2R). However, the details of the modulation have not been revealed. On the other hand, many researchers have already reported that the local RAS can modulate hypertrophic changes modulating the generations of reactive oxygen species (ROS) in vascular endothelial cells. The purpose of our study is to reveal whether or not the local RAS modulates the hypertrophic differentiation of chondrocytes modulating the generations of ROS.
Methods: We cultured ATDC5 cell line under the inducing of 10 μg/ml bovine insulin for 14 days after they had reached confluence. Then, we added 1.0 μg/ml angiotensin II, 1.0 μg/ml Olmesartan and 1.0 μg/ml PD123319 to activate AT1R or AT2R separately. 6 times after the activation of the receptors, we measured the concentrations of ROS. We also administered vitamin C, vitamin E and hyaluronic acid (HA) and measured the concentrations of ROS and the expressions of type X collagen (Col.X), matrix metalloproteinase 13 (MMP13) and runt-related transcription factor (Runx2).
Results: The generations of ROS were downregulated activating AT1R and were conversely upregulated activating AT2R. In the upregulated hypertrophic differentiation caused by activating AT2R, the expressions of Col.x, MMP13 and Runx2 and the generations of ROS were downregulated administering vitamin C, vitamin E and HA. However, in the downregulated hypertrophic differentiation caused by activating AT1R, we did not find significant changes in the the expressions of Col.x, MMP13 and Runx2 and the generations of ROS administering vitamin C, vitamin E and HA .
Conclusions: The local RAS could modulate the hypertrophic differentiation of chondrocytes modulating the generations of ROS. Upregulation of hypertrophic differentiation of chondrocytes cased by activating AT2R could be suppressed by administering vitamin C, vitamin E and HA.

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PANNEXIN 3: A NEW CHANNEL INTO THE MECHANISMS OF OSTEOARTHRITIS

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Purpose: With the increasing burden of osteoarthritis (OA), there is a tremendous need to understand the molecular pathways driving cartilage catabolism in OA. Because these mechanisms are incompletely understood, we have no effective interventions to slow or halt disease progression, therefore limiting our ability to treat patients to symptomatic management and ultimately joint replacement. Ectopic hypertrophic differentiation of articular chondrocytes has been observed during various stages of the disease, and is an important pathway causing cartilage loss and cell death. During endochondral ossification, increasing cell size, catabolic gene expression, reductions in type II collagen and aggrecan and ultimately apoptosis lead to cartilage resorption in order make way for new bone, and some of these processes are recapitulated to various degrees in OA. One possible key factor in this process is Pannexin 3(Panx3), an ATP releasing channel forming glycoprotein that we have shown to be upregulated 5-fold in OA cartilage (rat) and in the hypertrophic zone of the growth plate. Overexpression of Panx3 in ATDC5 cells leads to accelerated differentiation while knockdown leads to delayed differentiation (Iwamoto et al 2010). Therefore we hypothesize that Panx3 is an important driver of chondrocyte hypertrophy and will investigate its role further using mice deficient for Panx3 both globally and specifically in the cartilage.
Methods: Using the Cre-LoxP system we have generated mice with global deletion of Panx3 (CMV-cre) and with cartilage specific deletion (Col2Cre). Development of OA is assessed after surgically inducing OA. Surgical destabilization of the medial meniscus (DMM) has been shown to produce reliable OA like pathologies and this technique has been performed on both mutant and control mice aged 20 weeks. 8 weeks post-surgery, these mice are compared to assess the severity of OA using the OARSI histopathological scoring system and immunohistochemistry for markers of OA and hypertrophy (MMP13, ColX, matrix breakdown products). Serum biomarkers of matrix breakdown will be detected using ELISA. Additionally, to determine whether activity levels change in parallel to OA severity, spontaneous activity is measured.
In parallel to our in vivo studies, using primary articular chondrocytes isolated from both mutant and control mice, we further investigate molecular pathways affected by Panx3. By stimulating these cells with pro-inflammatory or pro-hypertrophic factors (IL-1b, IL8, TNFa) we can measure differential responses using qPCR and western blot to identify changes in the expression of OA-relevant genes and markers of hypertrophy while also measuring ATP release.
Results: Mice with whole body deletion of Panx3 develop normally, with no growth-plate, joint or skeletal abnormalities, making them suitable models to study OA progression. In control mice that have undergone DMM surgery, we see strong increases in Panx3 protein staining as detected by IHC, localized primarily around lesions consistent with early cartilage degeneration. We expect to see that the loss of Panx3, both in cartilage, and in the whole animal will lead to reduced OA development following surgery in addition to reduced catabolic and hypertrophic markers. We also expect that primary chondrocytes from Panx3 deficient mice will be less responsive to pro-inflammatory and pro-hypertrophic stimuli, and that ATP release will also be impaired compared to controls.
Conclusion: Our in vivo study will identify whether Panx3 plays a role in OA progression using outcome measures ranging from histology to functional deficiency's while our in vitro studies will provide insight into some of the downstream mechanisms responsible. This will explore a novel target for the development of OA therapy.

Cartilage Repair

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M2-MACROPHAGES MODULATE THE CARTILAGE-FORMING CAPACITY OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM/PROGENITOR CELL

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