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HIP BONE MARROW LESIONS IN ASYMPTOMATIC AND OSTEOARTHRITIC ADULTS: PREVALENCE, RISK FACTORS AND SIGNIFICANCE

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Purpose: Bone marrow lesions (BMLs) at the knee have an important role in the pathogenesis of osteoarthritis (OA), being associated with increased pain, accelerated cartilage loss, and increased risk of total knee replacement. However, data is limited for the role of BMLs at the hip. Therefore, this study aimed to determine the prevalence and associations of BMLs at the hip in an asymptomatic and an osteoarthritic population.
Methods: 142 asymptomatic and 19 participants with hip OA were recruited from existing cohorts. Hip magnetic resonance imaging was performed and used to assess femoral head BMLs, cartilage volume and bone area.
Results: The demographic characteristics of the asymptomatic versus the OA population were as follows: age 66.8 +/- 7.4 vs. 59.5 +/- 7.6 years ($p<0.001$), female 55.6% vs. 57.9% ($p=0.85$), body mass index 27.6 +/- 4.8 vs. 27.2.+/-.4.8 kg/m2 ($p=0.73$). The prevalence of BMLs was 17.6% in the asymptomatic population and 63.2% in the OA population ($p<0.001$). BMLs were strongly associated with OA after adjusting for age, gender and body mass index (odds ratio 5.32, 95% CI 1.78, 15.9, $p=0.003$). BMLs were associated with lower femoral head cartilage volume in the whole population (regression coefficient -245.7 mm3, 95% CI -455.5, -36.0, $p=0.02$). In the OA population, BMLs were also associated with lower femoral head cartilage volume (regression coefficient -426.6 mm3, 95% CI -855.2, 2.14, $p=0.05$) after adjusting for age, gender, body mass index, femoral head bone area and hip OA (for analysis of the total population).
Conclusion: Femoral head BMLs are common in those with OA, but are also present in asymptomatic individuals with no clinical hip OA. They are associated with reduced hip cartilage volume. These findings suggest that BMLs at the hip may provide a novel target for the treatment and prevention of hip OA.

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HYDROGEN SULFIDE DONORS ALLEVIATE IL-1β INDUCED INFLAMMATION-LIKE EFFECTS IN HUMAN ARTICULAR OSTEOARTHRITIC CHONDROCYTES

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Purpose: Hydrogen sulfide, H2S, has been recently recognized as an important signaling molecule. Once exclusively thought of as a toxic gas, it is now being considered as the third gas transmitter together with NO and CO. H2S has been found to be implicated in many inflammatory pathologies and there are studies that suggest that it can act as an anti-inflammatory and anti-oxidant agent. In the present study, we analyzed the influence of two H2S donors (NaHS and GYY4137) on several aspects that characterize the inflammatory process in osteoarthritis (OA). Specifically we looked at: 1) Nitric oxide (NO) production and inducible NO synthase (iNOS) levels; 2) Production of reactive oxygen species (ROS) and antioxidant enzyme superoxide dismutase 2 (SOD2); 3) The production of prostaglandin E-2 (PGE-2); and 4) Levels of matrix metalloproteinase 3 (MMP3).
Methods: Primary human chondrocytes were isolated from OA tissue. Cells were stimulated with a pro-inflammatory cytokine (interleukin-1β, IL-1β, 5ng/ml) and we used different concentrations (ranging from 50 μM to 1000 μM) of the two H2S donors to investigate their ability to ameliorate the effects of IL-1β on the cells. NO production was quantified through the Griess reaction. Protein levels were visualized through immunocytochemistry and quantified with appropriate software; mRNA expression levels were detected with qRT-PCR. ROS levels were quantified with a fluorescence microscope after DCFH or dihydrorhodamine 1,2,3 treatment. PGE-2 levels were measured with a specific EIA.
Results: Treatment with IL-1β caused an increase in NO and PGE-2 production, iNOS protein levels, and SOD2 and MMP3 mRNA and protein levels. GYY4137 and NaHS treatment was effective in reducing NO production down to 25% and 50% of the stimulated values (respectively), although none were able to recover the non stimulated state (Figure 1A). Even though H2S might react directly with it, NO reduction was probably the result of a decline in iNOS stimulation, because iNOS protein levels were also reduced by both GYY4137 and NaHS treatment. H2S released by GYY4137 and NaHS probably reacts directly with the ROS present in the OA cells and we saw a reduction in both mRNA and protein levels of SOD2 in the stimulated cells. These effects were also accompanied by a reduction in PGE-2 levels: all GYY4137 concentrations led to about a 60% reduction in PGE-2 in the stimulated cells and low NaHS concentrations (50-200μM) to about a 75% reduction (Figure 1B). We also saw reductions in both MMP3 mRNA and protein levels after treatment with the H2S donors.
Conclusions: Results obtained so far suggest that there might be a therapeutic window for H2S donors that show anti-inflammatory and antioxidant properties. This might be of interest in the alleviation of OA-induced inflammation processes and it should be further explored.

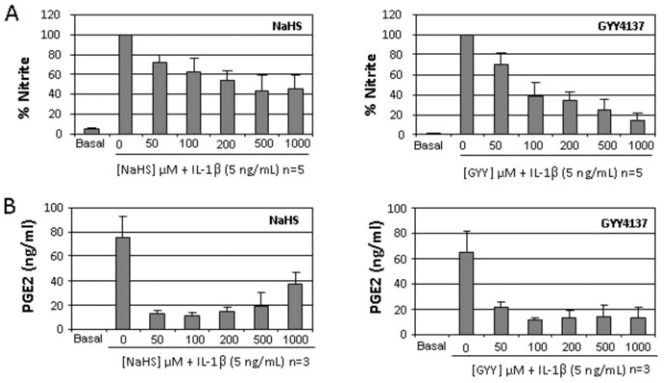

Figure 1. Effect of different concentrations of H_2S donors NaHS and GY4137 on (A) NO and (B) PGE-2 production in IL-1β stimulated human articular osteoarthritic chondrocytes.

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DOWNREGULATION OF WNT SIGNALING PATHWAY AND MIR335 THROUGH MIR335 IN MSCS FROM OSTEOARTHRITIS PATIENTS

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Purpose: Wnt signalling pathway regulates mesenchymal stem cell (MSC) self-renewal and differentiation. In osteoarthritis (OA) the Wnt signalling is altered in MSCs isolated from the bone marrow (BM-MSC) and likely contributing to OA pathogenesis. miRNAs are critical regulators of mesenchymal stem biology and overexpression of specific miRNA (mir335), which in addition is upregulated by the Wnt/β-catenin signalling pathway, has been shown to inhibit osteogenic differentiation. According to these evidences, the aim of this study was to delineate the effect over Wnt genes after mir335 overexpression in BM-MSCs from OA patients.
Methods: RNA samples from BM-MSC of three OA patients and three controls were analyzed by quantitative PCR to determine the expression of mir335 and MEST gene (which controls mir335 expression). The effect of mir335 overexpression in Wnt signaling was determined using a GFP gene reporter assay of BM-MSC transduced with a lentiviral vector containing mir335 and GFP in one OA patient. Transducted (GFP+) cells were purified 48h after transduction by fluorescence-activated cell sorting (FACS). Transducted GFP cells as well as non-transducted cells were used as control. Cells were further induced to osteogenic differentiation and studied at 0, 10 and 21 days. Simultaneous expression of 84 Wnt signalling pathway related genes were analyzed by a PCR Array profiling.
Results: miR335 expression levels was reduced by about 50% in OA patients compared to expression levels found in controls. As expected, MEST gene was also clearly downregulated. A different behaviour was observed during differentiation to osteoblast lineage. At initial stages (0-10 days) BM-MSC transducted with mir335 showed an up-regulation of 16 Wnt signalling pathway related genes. Interestingly, seven out of the 16 were downregulated in the non-transduced cells. These genes includes the PITX2, SFRP1, SFRP4,WIF1,WNT16, WNT2 and WNT6. Towards the terminal stage of bone forming cells (10-21) days, a marked reduction of gene expression was detected in comparison to control