

Osteoarthritis and Cartilage. 2018; 26 Suppl 1: S69

Impairment of hydrogen sulfide synthesis in chondrocytes under high glucose environment: a link between type 2 diabetes and osteoarthritis

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Purpose: Epidemiological and experimental findings support the hypothesis that type 2 diabetes is an independent risk factor of osteoarthritis (OA). However, the mechanisms underlying the connection between both diseases remain unclear. Changes in the balance of hydrogen sulfide (H₂S) play an important role in the pathogenesis of diabetes and its complications. Likewise, we and other authors have observed a protective impact of H₂S induction on activation of pathological pathways in the chondrocyte. In this study we examined the modulation of H₂S levels in chondrocytes subjected to glucose stress, central feature of diabetes, to elucidate whether impairment in H₂S-mediated signaling could participate in the establishment of diabetes-related OA.

Methods: Chondrocytes were isolated from OA cartilage of diabetic (DB) or non diabetic (non-DB) patients. T/C28a2 and primary human chondrocytes were stimulated at 24h and 72h w/o IL-1 β (5 ng/mL) under a normal (5.5 mM; NG) or a high (25 mM; HG) glucose environment. Gene and protein expression of enzymes involved in H₂S synthesis (cystationine γ -ligase [CSE], cystationine β -synthase [CBS], and 3-mercaptopyruvate sulfurtransferase [3-MT]) and HO-1 were assessed by RT-qPCR and WB, respectively. Mitochondrial reactive oxygen species (ROS) production and mitochondrial membrane potential were measured using MitoSOX and Tetramethylrhodamine, Methyl Ester, Perchlorate (TMRM), respectively. To determine the involvement of H₂S in catabolic pathways activated by HG in chondrocytes, NaSH and GYY 4137 (500 Chondrocytes were isolated from OA cartilage of diabetic (DB) or non diabetic (non-DB) patients. T/C28a2 and primary human chondrocytes were stimulated at 24h and 72h w/o IL-1 β (5 ng/mL) under a normal (5.5 mM; NG) or a high (25 mM; HG) glucose environment. Gene and protein expression of enzymes involved in H₂S synthesis (cystationine γ -liase [CSE], cystationine β -synthase [CBS], and 3-mercaptopyruvate sulfurtransferase [3-MT]) and HO-1 were assessed by RT-qPCR and WB, respectively. Mitochondrial reactive oxygen species (ROS) production and mitochondrial membrane potential were measured using MitoSOX and Tetramethylrhodamine, Methyl Ester, Perchlorate (TMRM), respectively. To determine the involvement of H₂S in catabolic pathways activated by HG in chondrocytes, NaSH and GYY 4137 (500 μ M), a fast- and slow-releasing H₂S donor respectively, were employed.

Results: Freshly isolated chondrocytes from OA cartilage of diabetic patients showed lower levels of H₂S synthesizing enzymes than those of non-DB patients. Likewise, chondrocytes T/C-28a2 exposed to HG stress expressed lower mRNA levels of CSE, CBS and 3-MT after 3 days of incubation compared to those incubated in NG conditions (0.41-fold [CSE], 0.42-fold [CBS] and 0.52-fold [3-MT]; n = 6, p < 0.05). Whereas no effect was observed at shorter time of treatment (1 day). At protein level, we detected a similar modulation in the 3 enzyme involved in H₂S synthesis (0.83-fold [CSE], 0.66-fold [CBS] and 0.79-fold [3-MT]; n = 6, p < 0.05). Additionally, IL-1 β attenuated the gene and protein expression of CBS elicited by chondrocytes incubated in NG (0.47-fold and 0.86-fold, respectively; n = 6, p < 0.05). Besides, we registered a slight decrease of mitochondrial membrane potential (TMRM) in those chondrocyte incubated for 24h in HG (0.94 fold compared with NG; n = 6, p < 0.05). However, we failed to detect any modulation in mitochondrial ROS production. Interestingly, the expression of pro-inflammatory chemokine IL-8 was significantly higher in chondrocytes under HG than NG condition (6-fold; n = 5, p < 0.05); whereas protein levels of heme oxygenase 1, an anti-inflammatory enzyme, were reduced in HG exposed chondrocytes (0.77-fold; n = 6, p < 0.05). GYY 4137 and NaSH co-treatment recovered HO-1 expression and reduced IL-8 levels in chondrocytes under IL-1 β + HG conditions. In addition, similar results were registered in primary human chondrocytes from OA cartilage.

Conclusions: We have identified a reduction of H₂S synthesis as a critical feature involved in hyperglucidic-mediated dysregulation of articular chondrocytes. The impairment of H₂S signaling could participate in the mechanisms underlying the predisposition to OA development in diabetic individuals and may open new opportunities for treating patients with a diabetes-related OA phenotype.