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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-1β (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-1β (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 *u*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-1β 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-1β + 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.