Effects of Oxygen Tension on Proteoglycan Synthesis and Gene Expression in Bovine Primary Chondrocytes in Response to Glucosamine Sulfate

Chengjuan Qu1, Marjo Jauhiainen2, Seppo Auriola2, Mikko J. Lammi2
1Institute of Biomedicine, Anatomy, University of Kuopio, Kuopio, Finland; 2Department of Pharmaceutical Chemistry, University of Kuopio, Kuopio, Finland
chengjuan.qu@uku.fi

Introduction: Chondrocytes live in low level oxygen (O2) (5%) environment in vivo and are well adapted to low O2 environment (1,2). The O2 tension in vivo in cartilage has been estimated to be in the range of 1-7.5% (3). Glucosamine sulfate (GS) has been used to treat the patients with osteoarthritis as a disease-modifying agent. Our previous studies indicated that GS under normal O2 tension did not increase GAG synthesis, aggrecan mRNA expression, and UDP-sugars level (4,5). The objective of this study was to investigate whether low O2 tension could affect PG synthesis, gene expression and UDP-sugars level in response to GS and sulfate treatments.

Materials and Methods: Bovine primary chondrocytes were cultivated in high or low glucose DMEM in low or normal O2 incubator, the cells were also treated with GS or sulfate for 30 min or 24 h in low or normal O2 incubator 2 or 8 days after isolation. The intracellular UDP-sugars levels were analyzed by reversed-phase HPLC electrospray ionization mass spectrometry (5). Aggrecan, type II collagen mRNA expression and GAG synthesis were quantitated using quantitative RT-PCR and 35S-sulfate incorporation, aggrecan promoter activity was investigated using dual-luciferase reporter gene assay. Statistically significant differences (p<0.05) between control and treated groups were analyzed with two-related-samples tests.

Results: Effect of O2 tension on GAG synthesis in response to GS or sulfate(n=9)
Low O2 tension significantly increased GAG synth (Fig. 1A,B), however, 1 mM GS (Fig. 1C) under low and normal O2 tension significantly decreased GAG synthesis.
Effect of O2 tension on aggrecan and type II collagen mRNA expression in response to GS and sulfate(n=9)
Low O2 tension significantly increased aggrecan (Fig. 2A,B) and type II collagen (Fig. 2C,D) mRNA expression. Type II collagen mRNA expression was significantly decreased by 100 μM GS under low O2 tension (Fig. 2C) and by 100 μM sulfate under normal O2 tension (Fig. 2D).
Effects of O2 tension on aggrecan promoter activity in response to GS and sulfate(n=5)
In line with sulfate incorporation and aggrecan mRNA results, aggrecan promoter activity was higher after the cells were incubated in low O2 tension than in normal O2 tension. However, GS and sulfate treatment under low O2 tension did not affect aggrecan promoter activity.
Effect of O2 tension on intracellular UDP-hexosamines (UDP-HexN) and UDP-glucuronic acid in response to GS and sulfate(n=5)
Two-day-culture in low O2 tension did not markedly change the levels of UDP-sugars, while in 8-day-cultures in low O2 tension there was a trend to increased level of UDP-HexN. In normal O2 tension, 1 mM GS increased UDP-HexN at 30 min in 2- and especially in 8-day-cultures, while in low O2 tension, UDP-HexN level did not markedly differ from control cultures. In general, the levels of UDP-sugars in 8-day-culture were higher than those in 2-day-cultures in low and normal O2 tension.
Effect of glucose concentration on GAGs, and aggrecan and type II collagen mRNA in low O2 tension incubation (n=6)
The GAG synthesis in low-glucose DMEM was significantly higher than that in high-glucose DMEM in low O2 tension. However, there were no changes found in the aggrecan or type II collagen mRNA, or aggrecan promoter activity in low or high glucose DMEM in low O2 incubation.

Discussion: In this study, we show that low O2 tension significantly increased GAG synthesis, and aggrecan and type II collagen mRNA expression in bovine primary chondrocytes. This is in line with the previous data indicating an increased GAG synthesis in bovine chondrocytes in bioreactor (6) and meniscus cells (7) at low O2. Our results suggest that cultured chondrocytes in low O2 produce more aggrecan and type II collagen. In normoxia, supplemental 1 mM GS increased UDP-HexN level, which confirms the results of our previous study (5). An 8-day-culture in low O2 caused a marked increase in UDP-HexN level already in control cultures, suggesting a switch in the metabolic pathway for carbohydrate utilization due to low oxygen. Under these conditions, supplemental GS had only a minor effect on intracellular UDP-HexN level. Importantly, we noticed no increase on the GAG synthesis when the cells were treated with GS or sulfate under low and normal O2 tension. Previously, high concentration of GlcN has been shown to inhibit CS synthesis (8). The results of this study further confirmed our previous results showing that exogenous GS under normal O2 tension did not stimulate PGs synthesis (4,5).

In conclusion, the low oxygen tension stimulated PG synthesis, aggrecan and type II collagen mRNA expression in bovine primary chondrocytes. In fact, high concentration of GS under low or normal oxygen tension could inhibit PGs synthesis. A longer culture period under low O2 tension changed the UDP-sugar balance.