**Alendronate Chondroprotective Effects in vitro. Effect of Alendronate on Glycosaminoglycan Production and Cell Metabolism under Low Osmotic As Seen in Disc Degeneration**

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**Introduction:** The bisphosphonates have been known to chemists since the 19th century, when the first synthesis occurred in 1865 in Germany [1]. Bisphosphonates are small molecular size (<300 Da) stable analogs of pyrophosphste, widely used for the treatment of osteoporosis and other bone disease [2]. Aggrecan concentration falls markedly during osteoarthritis with unfortunate biomechanical and physiological consequences. However, many questions on the effect of alendronate to disc cells remain unanswered. In this study, we examined how alendronate influence the rate of GAG accumulation in nucleus pulposus (NP) cells in a three-dimensional culture system cultured under conditions seen in normal disc tissue and also under the low-osmotic conditions as seen in disc degeneration.

**Methods:** NP from the coccyx of 50 bovine feet was used for the experiments described here. Cells were isolated from the disc, encapsulated in alginate beads. They were cultured for 5 days in alginate beads in DMEM containing 6% FBS under 21% O₂ at cell densities of 4 million cells/ml. Medium osmolality was altered by addition of 5 mol/l sodium chloride (NaCl) and was monitored using a freezing point osmometer (Semi-micro osmometer, Knauer, Germany). Chondrocytes were cultured in culture fluid with a normal osmotic pressure (400 mOsm) as seen the healthy joint or a low osmotic pressure (270 mOsm) as seen the osteoarthritis. In previous study, the potentiated effect of alendronate was maximal at 10⁻⁸ mol/l [3]. The medium was changed every day and alendronate (10⁻⁸ mol/l) was added to both groups every day; cell cultured without alendronate served as control and the effects of osmolality were compared. The cell viability profile was determined by manual counting using trypan blue staining. Lactate production was measured enzymatically as a marker of energy metabolism. Rate of sulfate GAG synthesis was measured using a standard ³⁵S-sulfate radioactive method. GAG accumulation (as a measure of proteoglycan) was measured using a DMB assay. Data were entered into a database and analyzed by using SPSS statistical soft-ware, version 14.0J (SPSS Inc, Chicago, IL). A probability of 5% was considered statistically significant.

**Results:** GAG production at 400 mOsm was about 0.055 mg/ml/day, while it was only about 0.036 mg/ml/day at 270 mOsm. During culture at 270 and 400 mOsm, GAG production was increased about 1.29 and 1.21 times by addition of alendronate, respectively (Fig.1). Lactate production was decreased by about 20% under 270 mOsm compared with that at 400 mOsm. The rate of lactate production per live cell was significantly higher for cells cultured at 400 mOsm with alendronate than those at 270 mOsm after 5 days (Fig.2). Sulphate incorporation rates was similarly increased by addition of alendronate (Fig.3).

**Discussion:** The disc tissue is avascular, and the metabolic activity of its cells is regulated by various factors in the extracellular matrix, such as oxygen, osmolality, and pH. The osmolality of the extracellular matrix is regulated by negatively charging the GAG chains of proteoglycans which adjust ionic composition. Takeno et al [4]. demonstrated that GAG production was largest in the 400mOsm, and the capacity for GAG production and cell metabolism (lactate production) was low under hypo-osmolality, and cell deaths were observed on electron microscopy. Thus, it may be said that osmotic pressure gradient disturbance associated with reduced proteoglycans is an important factor contributing to the development of osteoarthritis. The goals of our study were to determine the role of extracellular osmolality in regulating production of GAG by alendronate and its effects on matrix turnover. Our data showed that pathologic conditions under low osmolality (270 mOsmol) apparently decreased GAG production, cell metabolism (lactate production) and GAG synthesis. In vitro alendronate-induced increases are prevented by inhibition of cell metabolism under pathologic conditions as seen in the disc degeneration, but alendronate increased GAG production, cell metabolism (lactate production) and GAG synthesis. The alendronate acts extracellularly and so exerts disc-protective effects on NP cells in healthy and pathological conditions.

**Significance:** Alendronate have been demonstrated to have disc-protective effects, to reduce the incidence and progression of disc degeneration.

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**References:**
Fig. 1. Time course of GAG accumulated.
(*: P < 0.05 by Scheffe’s test for control group vs. alendronate group)
Fig. 2. Time course of lactate production rate.

(*: P<0.05 by Scheffe's test for control group vs. alendronate group)
Fig. 3. Time course of sulphate incorporation rate.
(*: P<0.05 by Scheffe’s test for control group vs. alendronate group)

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