Effect of Alendronate on Proteoglycan Production and Cell Metabolism in Bovine Disc Cells in Alginate Culture
Dose-Response Study

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Financial Disclosure: None
Background

- The bisphosphonates have been known to chemists since the middle of the 19th century, when the first synthesis occurred in 1865 in Germany. Bisphosphonates are stable analogs of pyrophosphate, widely used for the treatment of osteoporosis and other bone disease. They are small molecules (<400MW) and easily able to diffuse into cartilaginous tissues from the circulation.

Alendronate Sodium Trihydrate

\[
\begin{align*}
\text{OH} & \quad \text{OH} & \quad \text{OH} \\
\text{O} = \text{P} & \quad \text{C} & \quad \text{P} = \\
\text{O} & \quad \text{OH} & \quad \text{CH}_2 & \quad \text{OH} \\
\text{CH}_2 & & \text{CH}_2 & \\
\text{NH}_2 &
\end{align*}
\]

Molecular weight: 325.12
**Chondroprotective effects of Bisphosphonates**

- Bisphosphonates have been reported to reduce cartilage degradation in animal model of arthritis.
  

- In controlled clinical trials, knee osteoarthritis patients showed benefit from bisphosphonates in terms of pain, function, and radiographic joint space narrowing.
  

- Lehmann et al. demonstrated that the urinary excretion of collagen type II C-telopeptide degradation products (CTX-II), a new marker of cartilage degradation, decreased significantly in response to bisphosphonate treatment.
  

All these observations suggest that bisphosphonates may have chondro-protective effects in humans.
Purpose

- In this study, we examine how whether alendronate has a chondroprotective effect on the intervertebral disc.

- Proteoglycan loss is one of the first signs of disc degeneration; however, there is little information regarding the effect of alendronate on the GAG accumulation in disc tissue. We examined whether different concentrations of alendronate influence the rate of GAG accumulation in nucleus pulposus (NP) cells in a three-dimensional culture system cultured under conditions seen in normal discs and also under the low-osmotic and hypoxic conditions as seen in degenerated disc.
Materials and Methods

Alginate culture system

• Sterile removal of NP cells of bovine caudal discs.
• Enzymatic digestion of matrix - 1mg ml\(^{-1}\) collagenase for 18 hours in 400 mOsm medium.
• Wash cells. Resuspend in alginate to concentration of 4 million cells ml\(^{-1}\).

Materials and Methods

102mM \(-\) CaCl\(_2\)

Chondrocytes + alginate

0.9% NaCl (x3)

DMEM with 6% FCS

• Incubate at 37 °C/5% CO\(_2\)

• 400 mOsmol
• pH 7.4
• 21% O\(_2\)

Dose-response studies determining the ability of alendronate to increase GAG production and cell metabolism: The goals were to examine how alendronate concentration influences the metabolism of disc cells. They were then cultured for 5 days under 21% oxygen with 10\(^{-1}\) - 10\(^{-12}\) mol/l alendronate; cell cultured without alendronate served as control.
• The cell viability profile across intact beads was determined by manual counting using fluorescent probes (LIVE/DEAD Viability/Cytotoxicity Kit, Molecular Probes).

• Glycosaminoglycan (GAG) accumulation (as a measure of proteoglycan) and lactate production were measured using a DMB assay (Enobakhare et al, 1996) and a standard enzymatic method respectively. Rate of sulfate GAG synthesis was measured using a standard $^{35}$S-sulfate radioactive method (Maroudas, 1980)
The results of dose-response study indicated that alendronate *in vitro* affected chondrocytes viability at concentrations \( \geq 10^{-4} \text{ mol/l} \); with no viable cells observed in 5 days cultures at concentrations \( \geq 10^{-3} \text{ mol/l} \).

In the significant differences by Scheffé test are represented as: +, *P<0.05* (compared with control).
Effect of different alendronate concentrations on GAG concentration (A) and GAG accumulated per million cells (B) by NP cells after 2 days and 5 days in culture.

- Cells were encapsulated in alginate beads, cultured in 400 mOsmol-DMEM with 6% serum under air.
- This results give pooled data from 5 separate experiments.
- Amount of GAG concentration (A) and GAG accumulated/million cells (B) increased with time in culture.
- At day 5, more GAG at $10^{-8}$-$10^{-7}$ mol/L of alendronate than control (0 mol/L).

In the significant differences by Scheffé test are represented as: +,* P<0.05 (compared with control). Values are expressed as average ± standard error.
Effect of different alendronate concentrations on lactate production rate (A) and 35S-sulfate incorporation rate (B).

At day 5, cell metabolism decreased with time in culture and was higher in the presence of alendronate of $10^{-9}$-$10^{-7}$ mol/l than control ($\ast\ast$: $P<0.05$, Scheffé test).

Sulfate incorporation rates significantly increased at $10^{-8}$ mol/l-alendronate compared with control ($\ast\ast$: $P<0.05$, Scheffé test). Sulfate incorporation rate fall with time in culture ($P<0.05$, 2 way ANOVA with repeated measures between 2 and 5 days).
Bisphosphonates can enhance the differentiation and bone forming activities of osteoblasts (Reinholz GG, et al., Cancer Res 2000)


Cartilaginous cells originate from the same stem cell lineage as osteoblasts, and thus, possibly, bisphosphonates affect this cell types by common mechanisms.

To our best knowledge, in vitro cytotoxicity and cell metabolism of this compound on disc cells has not been previously examined.
Effect of alendronate in beads on GAG production per million cells

Data normalized to results at 0 mol/L (control)

- GAG per cell higher at $10^{-9}$-$10^{-7}$ mol/L.
Neogi et al. [Ann Rheum Dis 67:1427, 2008.] evaluated the effect of alendronate on the progression of radiographic spinal osteophytes and disc-space narrowing in vivo. They demonstrated that alendronate was associated with less spinal osteophytes and disc-space narrowing progression than placebo.

In this study, we focused mainly on the effect of alendronate on NP cells, although alendronate is generally thought to inhibit osteoclastic bone resorption. The results of dose-response study indicated that alendronate in vitro affected NP cell viability at concentrations ≥ 10^{-4} mol/l; with no viable cells observed in 5 days cultures at concentrations ≥ 10^{-3} mol/l. The present results showed that alendronate does not affect NP cell viability in vitro or their GAG production at concentration ≤ 10^{-5} mol/l. At lower concentrations, no effect on cell viability was seen and indeed at some concentrations alendronate stimulated production of GAG and appeared chondroprotective. The largest stimulation of GAG production rate, GAG accumulation and energy metabolism was seen after 5 days of culture in cultures containing 10^{-8} mol/l alendronate. It has been suggested that alendronate might have either direct or indirect effects on disc cells.
Conclusions

• This study was performed to determine the effects of varying concentrations and exposure times of alendronate on the viability and proteoglycan metabolism of bovine disc cells in vitro.

• The potentiating effect of alendronate was maximal at around $10^{-8}$ mol/l.

➢ **Alendronate have been demonstrated to have chondroprotective effects, to reduce the incidence and progression of disc degeneration.**