INTRODUCTION: Bisphosphonates are well known potent inhibitors of osteoclast activity and are widely used in the clinical treatment of osteoporosis and various systemic metabolic bone diseases.\(^{1}\) Alendronate, a nitrogen-containing bisphosphonate, is a potent inhibitor of bone resorption widely used for the treatment and prevention of osteoporosis. Recently, bisphosphonates have been demonstrated to have chondroprotective effects, to reduce the incidence and progression of osteophytes in animal models of osteoarthritis, and to modify osteoblast function in vitro. In an observational epidemiological study, compared to non-users, women on alendronate had less severe knee pain and less subchondral bone attrition and bone marrow lesions.\(^{2}\) Proteoglycan loss is one of the first signs of osteoarthritis. There is little information however on the effect of alendronate on the accumulation of glycosaminoglycan (GAG) in cartilaginous tissue. In this study, we examine how concentrations of alendronate influence the rate of GAG accumulation in cartilaginous cells in a three-dimensional culture system.

METHODS: Cartilage was obtained from metacarpal phalangeal joints of 18-24 month bovine. Chondrocytes were isolated by collagenase digestion (1 mg/ml for 18 hours), and encapsulated in alginate beads [3]. Chondrocytes in alginate beads were cultured in DMEM containing 6% foetal calf serum (FCS) at 370 mOsmol at cell densities of 4 million cells/ml. They were then cultured for 5 days under 21% oxygen with 1*10\(^{-4}\), 1*10\(^{-5}\), 1*10\(^{-6}\), 1*10\(^{-7}\) and 1*10\(^{-2}\) mol/L alendronate, and, without alendronate as control. These were analyzed in real time, after 2 and 5 days. The cell viability profile was determined by manual counting using trypan blue staining. Lactate production was measured enzymatically and glycosaminoglycan (GAG) accumulation was measured using a DMB assay. Rate of sulfate GAG synthesis was measured using a standard \(^{35}\)S-sulfate radioactive method. 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: The amount of GAG accumulated in the culture of articular chondrocytes increased with time in culture (Fig.1). After 5 days, a bimodal response was evident with the concentration of GAG accumulated rising as alendronate concentration was increased from 0 to 1*10\(^{-1}\) mol/L, through it appeared to diminish at very high concentration of alendronate. At 0 (control) and 1*10\(^{-4}\) mol/L of alendronate, the concentration of GAG in the beads reached 175.8 ± 45.6 and 305.9 ± 49.2 μg/mL, respectively, in 5 days. Evidence of greater cellular activity for cells cultured at 1*10\(^{-6}\) to 1*10\(^{-1}\) mol/L was seen from measurement of lactate production per live cell (Fig.2). The rate of sulfate incorporation per live cell was greater at 1*10\(^{-3}\)mol/L than control (Fig.3). Lactate production and sulfate incorporation decreased with time in culture.

DISCUSSION: The introduction of bisphosphonates for prevention and treatment of osteoporosis has been very important for patients. Numerous in vitro and in vivo studies have considered the very potent inhibitory effect of alendronate on osteoclast activity. Alendronate have been shown to interact directly with osteoblast and regulate their interactions with the osteoclasts.\(^{3}\) Chondrocytes originate from the same stem cell lineage as osteoblast. Therefore, alendronate will affect the metabolism of cartilaginous cells by common mechanisms. Another possible mode of action of alendronate on cartilage metabolism might involve the inhibitory action of these compounds on osteoclasts in the subchondral bone.\(^{4}\) Thus, animal models of osteoarthritis have also suggested that treatment with alendronate is partially chondroprotective. The present study demonstrated that alendronate could induce significant increase GAG production of chondrocytes at 1*10\(^{-4}\) to 1*10\(^{-1}\) mol/L of alendronate after 5 days in culture. This data suggest that alendronate may have chondroprotective effects in humans.


SIGNIFICANCE: Alendronate have been demonstrated to have chondroprotective effects, to reduce the incidence and progression of osteoarthritis.

ACKNOWLEDGEMENTS: This work was supported by a grant-in-aid from the Ministry of Education, Science and Culture of Japan (grant no.21591896).

Fig.1. Time course of GAG accumulated. (#[after 2 days], *[after 5 days]: P<0.05 by Scheffe’s test for control group vs. each group)

Fig.2. Time course of lactate production rate. (#[after 2 days], *[after 5 days]: P<0.05 by Scheffe’s test for control group vs. each group)

Fig.3. Time course of sulphate incorporation rates. (#[after 2 days], *[after 5 days]: P<0.05 by Scheffe’s test for control group vs. each group)