대한침구학회지 제23권 제2호(2006년 4월)
The Journal of Korean Acupuncture & Moxibustion Society

Original Article

Effects of Deer Antler Water Extract(Pilose Antler of Cervus Korean TEMMINCK Var. Mantchuricus Sinhoe) on Chondrocytes

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Abstract

Objectives : Deer antler Water Extract(DAE), prepared from the pilose antler of *Cervus korean* TEMMINCK var. *mantchuricus Swinhoe* (Nokyong), a traditional immuno-suppressive and immuno-activating Korean herbal-acupuncture, is thought to play an important role in human bone remodeling.

Methods : To determine whether DAE can induce the differentiation of resting zone chondrocytes(RC) or not, confluent cell cultures were pretreated for 24, 36, 48, 72, and 120hrs with DAE. At the end of pretreatment, the media were replaced with new media containing 10^{-10}~10^{-8}M 1,25-$(OH)_2D_3$ and the cells incubated for an additional 24hrs.

Results : This second treatment was chosen because prior studies had shown that only the more mature growth zone chondrocytes(GC) respond to this vitamin D_3 metabolite. The effect of DAE pretreatment on cell maturation was confirmed by measuring alkaline phosphatase (ALPase)-specific activity. Changes in matrix protein synthesis were examined by measuring collagen synthesis, as well as $^{35}SO_4$ incorporation into proteoglycans. When RC cells were pretreated for 120h with DAE, treatment with 1,25-$(OH)_2D_3$ caused a dose-dependent increase in ALPase-specific activity and collagen synthesis, however, the proteoglycan production was not affected. RC cells pretreated with 1,25-$(OH)_2D_3$ responded like RC cells that had not received any pretreatment.

Conclusion : These results indicate that DAE directly regulates the maturation of RC chondrocytes into GC chondrocytes. Therefore it was indicated that DAE may play a significant role in regulating chondrocyte maturation during endochondral ossification.

· **Acceptance** : 2006. 3. 10. · **Adjustment** : 2006. 3. 18. · **Adoption** : 2006. 3. 18.
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Key words : Chondrocytes, Cartilage, Deer antler Water Extract (DAE), 1,25-dihydroxyvitamin D_3 ($1,25-(OH)_2D_3$)

I. Introduction

The structural integrity of the constituent cartilage and bone components, which is dependent upon an equilibrium between the processes of tissue synthesis and degradation during cartilage and bone remodelling, directs the normal joint function. Proteolytic enzymes are known to be involved in the catabolic aspect of normal tissue remodelling[1-2], and the altered activity of these enzymes is responsible for cartilage destruction and bone erosion associated with rheumatoid arthritis(RA). It is a systemic inflammatory disorder characterised by inflammatory cell infiltration of proliferated synovial linings, and subsequent tissue erosion. Although increased protease activity has been implicated in the pathogenesis of RA, differences in mechanism associated with these disorders remains not to be elucidated.

During endochondral bone formation, chondrocyte terminal differentiation is accompanied by an accumulation of calcium and phosphate ions. Prior to deposition of apatite in the extracellular matrix, there was a marked accumulation of phosphate ions[3]. Na-Pi transporters are expressed in chondrocytes[4]. At the mineralization front, cell death is linked directly to the elevation in environmental anion concentration and the concomitant rise in intracellular Pi levels.

The endochondral bone formation consists of a developmental cascade of chondrocyte differentiation that culminates in extracellular matrix mineralization before osteogenesis can occur. The process is required for normal growth and development of long bones and for certain kinds of bone repair. During the chondrogenic phase of the process, chondrocytes are responsible for the synthesis, maintenance, and maturation of a calcifiable extracellular matrix that is composed mainly of proteoglycan and collagen[5].

In the Liver, Vit. D_3 is converted into activity form by specific enzyme and then into 25-$OH-D_3$. This goes to the Kidney by specific transport protein through the blood and there, it is converted into $1,25-(OH)_2D_3$ by hydroxy reaction with another enzyme. $1,25-(OH)_2D_3$ facilitates absorption of Calcium and Phosphate and if in the blood concentration of Calcium and Phosphate increase, it will make skeletal normal calcification by linking Calcium and Phosphate and then depositing in the bone.

In vivo, resting zone chondrocytes enter a proliferative phase and undergo a period of maturation defined histologically. However, little is known about the mechanisms involved in the transition from a resting zone chondrocyte phenotype to a growth zone phenotype. Recently, it was found that $24,25-(OH)_2D_3$ causes resting zone cells to acquire responsiveness to $1,25-(OH)_2D_3$, a phenotype characteristic of growth zone chondrocytes[6].

In this culture model, $1,25-(OH)_2D_3$ inhibits [^{3}H]-thymidine incorporation by both cell types, but its effects on alkaline phosphatase(ALPase), collagen, and proteoglycan are cell-maturation-dependent. Growth zone cells exhibit increased alkaline phosphatase and proteoglycan sulfation, but $1,25-(OH)_2D_3$ has no effect on resting zone cells. In contrast, $1,25-(OH)_2D_3$ inhibits collagen production by resting zone chondrocytes.

Deer antler Water Extract(DAE), prepared from the pilose antler of *Cervus korean* TEMMINCK var. *mantchuricus Swinhoe* (Nokyong), a traditional immunosuppressive and immuno-activating Korean aqua-acupuncture, is a traditional Korean herb grown in south of Korea. DAE has been widely

used in the treatment of some immune-related diseases, especially rheumatoid arthritis (RA) and satisfactory results are obtained[7-10]. The deer antler has long been recognized as one of the most effective and powerful invigorants, as well as an enhancer of systemic resistance to infectious diseases. However, little is still known about the mode of action of this herbal medication on RA. Previously, it was confirmed that DAE protected chondrocytes from death at dose of 20 μg/kg concentration[10].

It was suggested that DAE might also play a role in inducing resting zone cells to progress down the endochondral pathway and acquire a growth zone-like phenotype. To confirm this, it was investigated whether resting zone cells acquired responsiveness to 1,25-$(OH)_2D_3$ following exposure to DAE.

Ⅱ. Materials and methods

1. Materials

A water extract of Deer antler were purchased from Kyungju Oriental Medical Hospital, Dongguk University, Kyungju city, Kyungpook, Korea as an i.p injection grade for human. For i.p. injection into rats, randomly selected tablets were ground and suspended in normal saline at a concentration of 50μg/10μℓ.

2. Methods

1) Chondrocyte cultures

Chondrocytes for this study were isolated from the resting zone and growth zone of the costochondral cartilage of 125g Sprague Dawley rats, as previously described[10], and cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 50 μg/m vitamin C, and 1% penicillin-streptomycin-amphotericin B in an atmosphere of 5% CO_2 and 100% humidity at 37°C. Fourth passage cells were used for the experiments because previous studies have demonstrated that these cells retain their differential phenotype, including response to the vitamin D metabolites at this passage[15]. At confluence, media were replaced with fresh media containing vehicle alone or DAE in phosphate-buffered saline (PBS). At appropriate time points, these media were replaced with fresh media containing vehicle alone or 10^{-10}~10^{-8}M 1,25-$(OH)_2D_3$.

2) Experimental design

Preliminary experiments were performed to determine the optimal dose and time of DAE addition on alkaline phosphatase activity in resting zone chondrocytes. Confluent fourth passage resting zone chondrocytes were treated for 1, 2, 4, 6, 8, or 10 days with control medium or medium containing 0, 5, or 10μℓ/mℓ DAE and then assayed for alkaline phosphatase (ALPase) specific activity. To verify that 1,25-$(OH)_2D_3$ did not affect the response of resting zone cells to DAE, resting zone chondrocyte cultures were treated for 24 h with DAE at concentrations of 0.1, 0.5, 1.0, 2.0, and 5.0μℓ/mℓ in the presence of either 10^{-9} or 10^{-8}M 1,25-$(OH)_2D_3$ prior to analysis of ALPase-specific activity.

For subsequent experiments, 2 and 5μℓ/mℓ DAE were used and cultures were pretreated for 24, 48, 72, and 120hrs. For those cells pretreated for 120hrs, fresh media containing the appropriate concentration of DAE were added at 72hrs. At the end of the pretreatment period, the media were again replaced with medium containing 1,25-$(OH)_2D_3$ at a concentration of 10^{-10}~10^{-8}M or vehicle alone and grown for an additional 24hrs. At that time, the cells were harvested and assayed as described in what follows.

3) AlPase-specific activity

Specific activity of ALPase (orthophosphoric

monoester phosphohydrolase alkaline [EC 3.1.3.1]) in Triton X-100 lysates of fourth passage resting zone chondrocytes[11] was measured as a function of *para*-nitrophenol release from *para*-nitrophenylphosphate, pH 10.2, as previously described[12].

4) Collagen and noncollagen protein synthesis

Incorporation of L-[^{3}H]-proline (New England Nuclear, Boston, MA) into collagenase-digestible protein (CDP) and collagenase-nondigestible protein (NCP) in the cell layer was used to estimate matrix protein synthesis by resting zone cells as previously described[13]. Percent collagen synthesis was calculated after multiplying the labeled proline in NCP by 5.4 to correct for its relative abundance in collagen14). This assay did not take into account any degradation that may have occurred.

5) [^{35}S]-sulfate incorporation

Proteoglycan synthesis was assessed by measuring [^{35}S]-sulfate incorporation in the cell layer according to the method of O'Keefe et al.[15], as described by us previously.

6) Statistical analysis

The data presented in the figures are from one experiment that was repeated three or more times with comparable results. For any given experiment, each data point represents the mean ± SEM for six individual cultures. The data were analyzed by analysis of variance, and statistical significance determined by comparing each data point to the control (containing ethanol vehicle) using Bonferroni's modification of the *t*-test.

III. Results

1. ALPase-specific activity

Optimal stimulation of alkaline phosphatase was seen in resting zone chondrocytes treated with 2 ㎕/㎖ DAE. The effect of DAE was biphasic, both in terms of dose response and time course. ALPase-specific activity of cultures treated with 10㎕/㎖ DAE was significantly elevated by 24hrs and remained at increased levels throughout the time course of the experiment, with comparable levels seen at 1, 2, 4, and 6 days of treatment. At 8 and 10days, 2㎕/㎖ DAE still stimulated ALPase activity above control levels; however, there was a noticeable decrease in activity on those days. Addition of 5㎕/㎖ DAE only stimulated ALPase activity when included in the culture medium for 2days, with no stimulatory effect seen at 1, 4, 6, 8, or 10 days. Addition of 10㎕/㎖ DAE had no effect on cell layer ALPase activity. Expansion of the dose response from 0.5 to 10㎕/㎖ DAE confirmed that maximal stimulation of ALPase took place at 0.5 to 2㎕/㎖. (Fig.1)

2. Effect of DAE pretreatment on ALPase-specific activity of resting zone chondrocytes treated with 1,25-$(OH)_2D_3$

DAE induced the resting zone cells to become responsive to 1,25-$(OH)_2D_3$, but the effect was dependent on DAE concentration and the time of

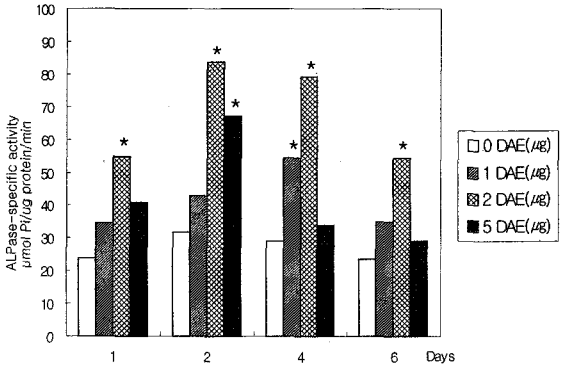

Fig. 1. Effect of DAE on ALPase-specific activity of the cell layer of resting zone chondrocytes
Cultures were treated for 1~10 days with 0, 1, 2, or 5㎕/㎖ of DAE, the cell layers isolated, and then assayed for ALPase-specific activity Each data point is the mean ± SEM of six cultures.

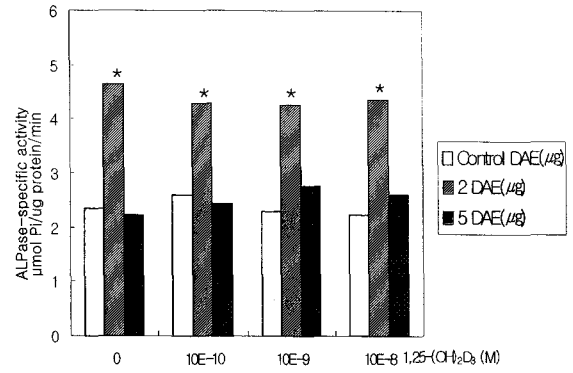

Fig. 2A. 24hrs pretreatment

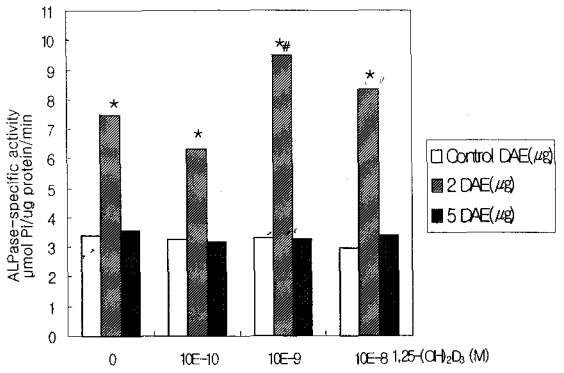

Fig. 2B. 5 days pretreatment

Fig. 2. Effect of DAE pretreatment on ALPase-specific activity of resting zone chondrocytes subsequently treated with $1,25\text{-}(OH)_2D_3$

Cultures were pretreated with DAE (2 or 5㎕/㎖) for 24hrs(Fig.2A) or 5days (Fig.2B) and then treated with 10^{-10}~10^{-8}M $1,25\text{-}(OH)_2D_3$ for an additional 24hrs. At harvest, cell layer APLase-specific activity was measured as described in Materials and Methods. The data are from one of three replicate experiments yielding similar results. Each data point is the mean ± SEM for six cultures.

*: $p<0.05$, treatment vs. control; #; $p<0.05$, vs. cells pretreated with DAE, but not subsequently treated with $1,25\text{-}(OH)_2D_3$.

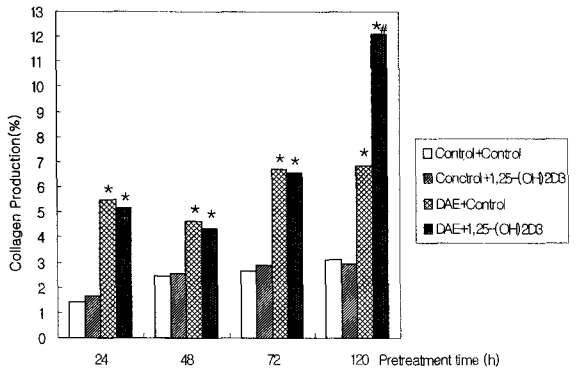

Fig. 3. Effect of DAE pretreatment time on ALPase activity of resting zone chondrocytes treated with $1,25\text{-}(OH)_2D_3$

Cultures were pretreated with 2㎕/㎖ DAE for 24, 48, 72, or 120hrs and then treated with 10^{-8} M $1,25\text{-}(OH)_2D_3$ for an additional 24hrs. At harvest, ALPase-specific activity in the cell layer was measured as described in Materials and Methods. The data are from one of three replicate experiments yielding similar results. Each data point is the mean ± SEM for six cultures.

*: $p < 0.05$, treatment vs. control; #: $p<0.05$, vs. cells pretreated with DAE, but not treated with $1,25\text{-}(OH)_2D_3$.

exposure. Treatment with DAE for 24 hrs did sightly increase the responsiveness of the cultures to either 10^{-10}, 10^{-9}, or 10^{-8}M $1,25\text{-}(OH)_2D_3$, regardless of the concentration of growth factor used. (Fig. 2A) However, after pretreatment for 5 days with 2㎕/㎖ DAE, there was a significant increase in ALPase-specific activity in cultures challenged with 10^{-9} or 10^{-8}M $1,25\text{-}(OH)_2D_3$. (Fig. 2B)

3. Time-dependent effect of DAE pretreatment on ALPase activity of resting zone chondrocytes treated with $1,25\text{-}(OH)_2D_3$

Whereas stimulation of ALPase by 2㎕/㎖ DAE was seen at 24, 48, 72, and 120 hrs, the increase in $1,25\text{-}(OH)_2D_3$ responsiveness was only seen following a 120 h exposure to the growth factor. (Fig.3)

4. Effect of DAE pretreatment on collagen production by resting zone chondrocytes treated with $1,25\text{-}(OH)_2D_3$

Collagen synthesis was inhibited by 10^{-10}~10^{-8}M $1,25\text{-}(OH)_2D_3$ in control cultures, and by 10^{-9}~10^{-8}M $1,25\text{-}(OH)_2D_3$ in DAE-pretreated cultures at 24hrs. However, when the cultures were pretreated with 2㎕/㎖ DAE for 5days, there was a significant increase in collagen synthesis after

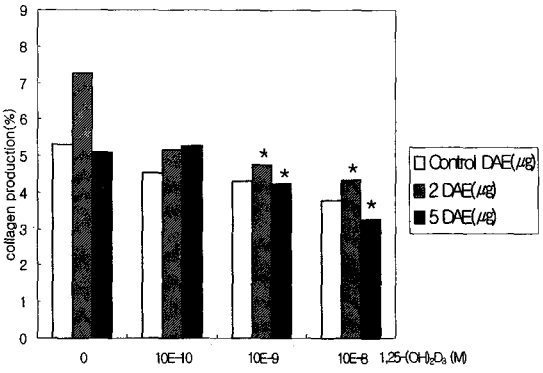

Fig. 4A. 24 hrs pretreatment

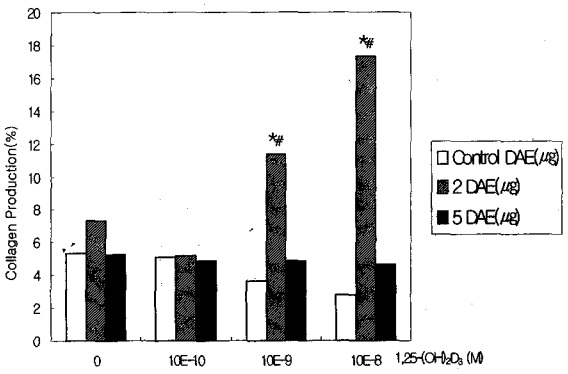

Fig. 4B. 5 days pretreatment

Fig. 4. Effect of DAE pretreatment on collagen production by resting zone chondrocytes treated with $1,25\text{-}(OH)_2D_3$

Cultures were pretreated with DAE (2 or 5㎕/㎖) for 24hrs(Fig.4A) or 5days (Fig.4B) and then treated with 10^{-10}~10^{-8}M $1,25\text{-}(OH)_2D_3$ for an additional 24h. Each data point is the mean ± SEM for six cultures.

*: $p < 0.05$, treatment vs. control; #: $p<0.05$, vs. cells pretreated with DAE, but not subsequently treated with $1,25\text{-}(OH)_2D_3$.

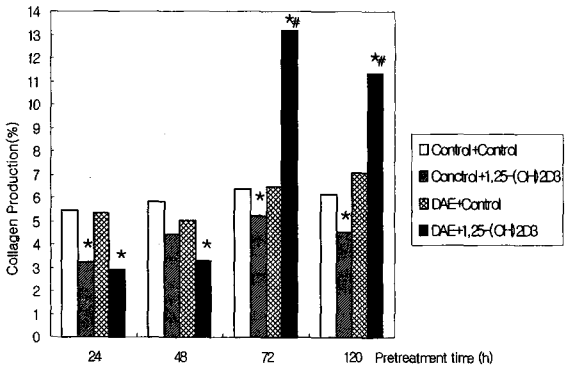

Fig. 5. Effect of DAE pretreatment time on collagen production by resting zone chondrocytes treated with $1,25\text{-}(OH)_2D_3$

Cultures were pretreated with 2㎕/㎖ DAE for 24, 48, 72, or 120hrs and then treated with 10^{-8}M $1,25\text{-}(OH)_2D_3$ for an additional 24h. At harvest, percent collagen production was measured as described in *Materials and Methods*. The data are from one of three replicate experiments yielding similar results. Each data point is the mean ± SEM for six cultures.

*: $p < 0.05$, treatment vs. control; #: $p < 0.05$, vs. cells pretreated with DAE, but not subsequently treated with $1,25\text{-}(OH)_2D_3$.

challenge with 10^{-9} and 10^{-8}M $1,25\text{-}(OH)_2D_3$. In contrast, control cultures still exhibited $1,25\text{-}(OH)_2D_3$ -dependent inhibition of collagen production. (Fig.4)

5. Time-dependent effect of DAE pretreatment on collagen production by resting zone chondrocytes treated with $1,25\text{-}(OH)_2D_3$

The time course of the effect of DAE pretreatment on $1,25\text{-}(OH)_2D_3$ -stimulated collagen production showed that responsiveness to $1,25\text{-}(OH)_2D_3$ was not present at 24 or 48hrs, but was present at 72 and 120hrs. Proteoglycan sulfation by resting zone cells was unaffected by treatment with DAE, or by challenge with $1,25\text{-}(OH)_2D_3$, whether or not the cells were treated with DAE for up to 120hrs. (Fig.5)

Ⅳ. Discussion

The present results showed that DAE can induce resting zone cells to acquire a $1,25\text{-}(OH)_2D_3$-responsive phenotype characteristic of more mature growth zone chondrocytes with a dose-dependent manner. Although 2㎕/㎖ DAE was effective, higher concentrations of the growth factor did not elicit this response. This suggests that resting zone cells are sensitive to a limited

range of DAE concentrations. The effects of DAE on the phenotypic shift in maturation state were time-dependent. Responsiveness to 1,25-$(OH)_2D_3$ required at least 5 days of exposure to the DAE, indicating that DAE initiates a cascade of responses leading to upregulation of receptors to the vitamin D metabolite.

When cells like chondrocytes or osteoblasts are isolated from tissue, they retain a memory of their maturation state in vivo, and the cells continue their proliferation and differentiation. Thus, in the absence of DAE, resting zone chondrocytes continue to express increased ALPase activity as a function of time. In control cultures at 5 days, collagen synthesis was still inhibited in control cultures challenged with 10^{-10}~10^{-8}M 1,25-$(OH)_2D_3$. However, DAE treatment for 5 days induced dose-dependent responsiveness to 1,25-$(OH)_2D_3$ in the resting zone chondrocyte cultures. These data are consistent with the resting zone cultures acquiring a growth zone-like phenotype, because 1,25-$(OH)_2D_3$ stimulates collagen synthesis in growth zone chondrocyte cultures[16]. Unlike the results obtained for collagen synthesis, proteoglycan production was not affected by DAE pretreatment, suggesting that this marker of chondrocyte differentiation may either require additional culture time with DAE in the acquisition of this phenotypic marker.

These cells also have been shown to respond to 1,25-$(OH)_2D_3$. However, the molecular mechanism(s) for these effects is not understood. In future study, therefore, it will be interesting to examine whether 1) 1,25-$(OH)_2D_3$ influences growth factor and cytokine signaling via the JAK/STAT pathway in mice bone cells; 2) whether 1,25-$(OH)_2D_3$ prolonged cytokine signaling via the JAK2/STAT5 pathway; 3) whether pretreatment of cells with 1,25-$(OH)_2D_3$ is also necessary in order to detect the IL-1β and TGF-β-induced transcriptional response by the activation of the STAT5-responsive reporter gene; and 4) whether the treatment of 1,25-$(OH)_2D_3$ affects on IL-1β and TGF-β -signaling via the JAK/STAT pathway in mice bone cells[17].

DAE which is pilose antler of deer has been used invigorate the shen-yang, replenish vital essence and blood and strengthen muscle and bones in Korean traditional medicine for a long time. Also DAE has been widely used in the treatment of osteoporósis and satisfactory results are obtained.

The bioactivity of the deer antler may act as an immune enhancer[18-19]. Previously, antifungal activity, and the suppressing activity of the hyphal transition from yeast in corpus albicans, was found from deer antler extract[20]. By using biochemical and chemical methods, we identified the compound from deer antler extract that is responsible for the suppression of hyphal transition as 2-lysophosphatidylcholine (lyso-PC), and this suppression may be mediated through the MAP kinase pathway[21].

The mechanism by which the DAE extract modify the clinical status of RA are not well understood. DAE administered orally into the patients inhibited proinflammatory cytokine production from both T cells and macrophages and potent effects on RA[9]. The degenerative joint disorder RA is characterised by cartilage destruction and bone erosion, resulting from mechanical wear or following joint tissue inflammation, respectively. The precise mechanisms responsible for the differential pathogenesis of RA remain unknown. DAE extract might be a useful tool for the treatment of RA. It would be incredible if the drugs as powerful as this did not have seriuos toxicity, but further studies will be necessary to answer this question. However, biochemical and metabolic analysis of the constituents of DAE extract have to be analysed in further delineating its mechanisms of action in arthritis.

In summary, the study demonstrates that resting zone chondrocytes in culture can be induced to differentiate into the more mature phenotype characteristic of growth zone chondrocytes, showing that DAE plays a significant role in growth plate cartilage development. The exact mechanism utilized

by DAE in this process is not clear, although it is likely to be mediated by its effects upon vitamin D3 metabolism. Resolution of these issues is presently underway.

V. References

1. V. Testa, F. Capasso, N. Muffulli, A. Sgambato and P.R.J. Ames, Proteases and antiproteases in cartilage homeostasis. *Clin Orthop Relat Res* 308 (1994), 79-84.
2. T.A. Einhorn and R.J. Majeska, Neutral proteases in regenerating bone. *Clin Orthop* 262 (1991), 286-297.
3. I.M. Shapiro and A. Boyde, Microdissection: Elemental analysis of the mineralizing growth cartilage of the normal and rachitic chick. *Metabol Bone Dis Rel Res 5* (1984), 317-326.
4. Masfield, K., C.C. Teixeria, C.S. Adams and I.M. Shapiro. Phosphate ions mediate condrocyte apoptosis through a plasma membrane transporter mechanism. *Bone* 28, 1-8 (2001).
5. D.S. Howell and D.D. Dean, Biology, chemistry and biochemistry of the mammalian growth plate. In: F.L. Coe and M.J. Favus Editors, *Disorders of Bone and Mineral Metabolism* Raven, New York (1992), 313-353.
6. Z. Schwartz, D.D. Dean, J.K. Walton, B.P. Brooks and B.D. Boyan, Treatment of resting zone chondrocytes with 24,25-dihydroxyvitamin D3 [24,25-$(OH)_2D_3$] induces differentiation into a 1,25-$(OH)_2D_3$-responsive phenotype characteristic of growth zone chondrocytes. *Endocrinology* 136 (1995), 402-411.
7. Wang B-X, Zhao X-H, Yang X-W, Kaneko S., Hattori M., Namba T. and Nomura Y. Inhibition of lipid peroxidation ny deer antler (Rokujo) extract in vivo and in vitro. Journal of Medical and Pharmaceutical Society for WAKAN-YAKU 5, 123-128 (1988).
8. Wang B-X, Zhao X-H, Qi S-B, Kaneko S., Hattori M., Namba T. and Nomura Y. Effects of repeated administration of deer antler (Rokujo) extract on biochemical changes related to aging in senescence-accelerated mice. Chem. Pharm. Bull. 36, 2587-2592 (1988).
9. J.K. Kim, Inhibitory effects of Cervi Pantotrichum Cornu herbal acupuncture on type II collagen-induced arthritis, Thesis for Mr' D. Dongguk University, graduate school, Kyungju, Korea (2000).
10. Y. H. Choi, Protective and Anti-arthritic Effects of Cervi Pantotrichum Cornu herbal acupuncture, Inhibiting Dihydroorotate Dehydrogenase, on Phosphate Ions-mediated Chondrocyte Apoptosis and Rat Collagen-induced Arthritis, Thesis for Dr' D. Dongguk University, graduate school, Kyungju, Korea (2001).
11. B.D. Boyan, Z. Schwartz, L.D. Swain, D.L. Carnes, Jr and T. Zislis, Differential expression of phenotype by resting zone and growth region costochondral chondrocytes in vitro. *Bone* 9 (1988), 185-194.
12. J.P. Bretaudiere and T. Spillman, Alkaline phosphatases. In: H.U. Bergmeyer Editor, *Methods of Enzymatic Analysis* Vol. 4 Chemica, Weinheim (1984), 75-92.
13. Z. Schwartz, VL Sylvia, Y. Liu, DD Dean and BD Boyan. Treatment of resting zone chondrocytes with TGF-b1 induces differentiation into a phenotype of growth zone chondrocytes by downregulating responsiveness to 24,25-$(OH)_2D_3$ and upregulating responsiveness to 1,25-$(OH)_2D_3$. *Bone* 23, 465-470 (1998).
14. J.N. Beresford, J.A. Gallagher and R.G.G. Russell, 1,25-dihydroxyvitamin D3 and human bone-derived cells in vitro: Effects on alkaline phosphatase, type I collagen and

proliferation. *Endocrinology* 119 (1986), 1776-1785.

15. R.J. O'Keefe, J.E. Puzas, J.S. Brand and R.N. Rosier, Effects of transforming growth factor-b on matrix synthesis by chick growth plate chondrocytes. *Endocrinology* 122 (1988), 2953-2961.
16. Z. Schwartz, D.L. Schlader, V. Ramirez, M.B. Kennedy and B.D. Boyan, Effects of vitamin D metabolites on collagen production and cell proliferation of growth zone and resting zone cartilage cells in vitro. *J Bone Miner Res* 4 (1989), 199-207.
17. Forrest, S. M., Ng, K. W., Findlay, D. M., Michelangeli, V. P., Livesey, S. A., Partridge, N. C., Zajac, J. D., Martin, T. J.(1985). Characterization of an osteoblast-like clonal cell line which responds to both parathyroid hormone and calcitonin. *Calcif. Tissue Int.* 37;51-56.
18. J.S. Suh, J.S. Eun, J.N. So, J.T. Seo and G.J. Jhon. 1999. Phagocytic activity of ethyl alcohol fraction of deer antler in murine peritoneal macrophage *Biol. Pharm. Bull.* 22, 932-935.
19. S.-Y. Han, S.-H. Cho, S.-Y. Kim, J.-T. Seo, S.-J. Moon and G.-J. Jhon. 1999. Monoacetyldiglycerides as New Ca^{2+} Mobilizing Agents in Rat Pancreatic Acinar Cells. Bioorg. *Med. Chem. Lett.* 9, 59-64.
20. H.S. Park, G.J. Jhon and W.J. Choi. 1998. Deer antler extract selectively suppresses hyphal growth in dimorphic fungus, *Candida albicans*. J. *Microbiol. Biotechnol.* 8, 291-294.
21. Min J, Lee YJ, Kim YA, Park HS, Han SY, Jhon GJ, Choi W. 2001. Lysophosphatidylcholine derived from deer antler extract suppresses hyphal transition in *Candida albicans* through MAP kinase pathway. *Biochim Biophys Acta.* 1531, 77-89.