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원저

Suppressive effects of a water extract of *Ulmus davidiana Planch* (Ulmaceae) on collagen-induced arthritis in mice

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Abstract

Objective : Since Ulmus davidiana Planch (Ulmaceae) has been known to have anti-inflammatory and protective effects on damaged tissue, inflammation and bone among other functions, this study was undertaken to address whether the water extract of the bark of Ulmus davidiana Planch (Ulmaceae) (UD) could modulate the expression of inducible inflammatory cytokines in mice. The present study was also done in order to assess the therapeutic effects of UD in collagen-induced arthritis (CIA) in mice.

Methods : DBA/1 mice were immunized with bovine type II collagen. After a second collagen immunization, mice were treated with UD orally at 100 mg/kg once a day for 3 weeks. Paws were evaluated macroscopically for redness, swelling and deformities. The levels of TNF-α and IL-1ß in the ankle were examined. The severity of arthritis within the knee joints was evaluated by histological assessment of cartilage destruction and pannus formation.

Results : Administration of UD significantly suppressed the progression of CIA and inhibited the production of TNF-α and IL-1ß in the paws. The erosion of cartilage was dramatically reduced in mouse knees after treatment with UD. In the serum of UD-treated mice, the levels of IL-4 and IL-10, anti-inflammatory cytokines, were increased.

Conclusion : From the results, it was concluded that administration of UD has therapeutic effects on CIA including protection of cartilage and RA for a potential therapy.

Key words : Ulmus davidiana Planch (Ulmaceae), Collagen-induced arthritis, Cytokines (TNF-α, IL-1ß, IL-4, IL-10)

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I. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease involving multiple joints. The main pathology of the affected synovial tissue consists of hyperplasia and subintimal infiltration of T and B lymphocytes. Synovial tissue hyperplasia forms the pannus tissue that irreversibly destroys the cartilage and bone in the affected joint. RA progression is associated with elevated levels of tumour necrosis factor (TNF-α) and interleukin (IL)-1ß produced by macrophages and dendritic cells, an imbalance of Th1/Th2 and overproduction of antigen-specific immunoglobulins[1]. Most of the current treatments are directed to correction of the immune aberration that supposedly drives synovial cell proliferation and cartilage erosion. The first group of biological response modifiers approved for treatment of RA were the antagonists of TNF-α. They work by binding to the TNF-α receptor or binding directly to the TNF-α protein[2]. The action of all these drugs is based on suppression of the inflammatory reaction; to our knowledge no drugs have been developed for cartilage protection.

Abnormalities in the expression of cytokines or their receptors may result in various diseases including bacterial toxic shock, certain lymphoid and myeloid cancers, and Chagas' disease. Cytokine-relate therapies that either increase or decrease the immune response offer promise of reducing graft rejection, treating certain cancers and immunodeficiency diseases, and reducing allergic reactions[3].

Ulmus davidiana Planch (Ulmaceae) is a deciduous tree which is widely distributed in Korea. The barks of the stem and the root of this plant have been used in oriental traditional medicine for the treatment of oedema, mastitis, gastric cancer, and inflammation[4-5]. Recent results showed that UD aqua-acupuncture decreased WBC count, neutrophil, lymphocyte and monocyte in mice with Lipopolysaccharide(LPS) Induced Arthritis[6]. As a part of our search for new biologically active substances from traditional medicines, we evaluated whether extracts of U. davidiana stem barks (UD) could modulate the induction of RA in mice. UD water extract has been developed on the basis of the known function of the herb, as described in the literature of traditional Chinese and Korean medicine[7-8]. UD is known for their functions in maintaining or assisting blood circulation. UD has been used for protection against degeneration of cartilage and regeneration of damaged tissue[9]. UD has been used by oriental medicine physicians in Korea. Here, we show that UD (i) inhibited the development of CIA in mice, (ii) lowered the level of proinflammatory cytokines, such as TNF-α and IL-1ß, in the paws of mice with CIA, (iii) increased the concentration of the anti-inflammatory cytokine IL-4 and IL-10 in the serum, and (iv) had dramatic protective effects against cartilage destruction in the affected knee joint. UD is an outstanding candidate for use in general therapeutics and for use as a cartilage-protective medicine for use in RA.

II. Materials and methods

1. Materials

1) Plant material

The stem barks of *U. davidiana* were collected from Mt. Phal-gong, Kyungbuk Province, South Korea in May 2002, and

identified by Professor Kap-Sung Kim, College of Oriental Medicine, Dongguk University, South Korea. Fresh stems were dried in a dark, well ventilated place. The voucher specimen (No. UD-W-57) is deposited in the Herbarium of this college.

2) Preparation of herb extract and fractions

UD was purchased from a market specializing in herbs (Kyungju herb market, Kyungju, Korea). The herb had a moisture content of <10% by weight, and was air-dried. Air-dried barks (totalling 70 g dry weight) were mixed, minced with a grinder, and extracted by storing in 1 ℓ of boiling water for 3 hours. The supernatant was filtered with 10 μm cartridge paper and ethanol was removed by rotary evaporation (Eyela, Tokyo, Japan), and concentrated extracts were freeze-dried. This process generally produced 15 g of brown powder.

2. Methods

1) MTT assays

Calvarial mouse bone cells[10-13] were seeded at 3000 and 5000 cells per well respectively in a 96-well plate. Cells were exposed to UD at concentrations in the range 5-5000 μg/ml at 37°C under an atmosphere containing 5% CO_2. After 96 hrs of incubation with UD, viable cells were stained with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (5 mg/ml; Sigma, St Louis, MO, USA) for 30 min. The medium was then removed and the formazan crystals produced were dissolved by the addition of 200 μl dimethylsulphoxide. Absorbance was determined at 540 nm using an ELISA (enzyme-linked immunosorbent assay) microplate reader. The median inhibitory concentration (IC50) was defined as the drug concentration that resulted in a 50% reduction in cell number compared with untreated controls. These values were derived from semi-log plots percent viability (% absorbance of treated sample/absorbance of untreated control x 100) vs drug concentration.

2) Acute oral toxicity study

To evaluate the acute toxicity of UD after a single oral dose, 8 male and 8 female Sprague-Dawley rats were assigned randomly to two experimental groups (five rats of each sex in each group) and were treated by gavage at doses of 0 and 20.0 g/kg body weight. Mortality, clinical signs, body weight changes and gross findings were monitored during the 21 days after treatment. This study was carried out in compliance with the Testing Guidelines for Safety Evaluation of Drugs (Notification No. 1999-61) issued by the Korea Food and Drug Administration, the Good Laboratory Practice Regulations for Non-clinical Laboratory Studies (Notification No. 2000-63) issued by the Korea Food and Drug Administration, and the Principles of Good Laboratory Practice issued by the Organization for Economic Cooperation and Development (1997)[14].

3) Induction of CIA and UD treatment

Inbred male DBA/1 mice (Jackson Laboratory, Bar Harbor, ME, USA), aged 9-10 weeks at the start of the experiments. were immunized intradermally at the base of the tail with 100 μg bovine type II collagen (Sigma, MO, USA) emulsified in Freund's complete adjuvant (Gibco BRL, Grand Island, NY, USA). On day 21, all mice were boosted with an intradermal injection of 100 μg type II collagen. The next day, mice that had no

macroscopic signs of arthritis were selected and divided into two groups, which each contained 10 mice. The control group were treated orally with 100 μl distilled water and the UD-treated group were treated orally with 100 μl UD at the concentration of 2 mg/ml for 18 days. The gradual onset of arthritis normally starts approximately 4 weeks after initial immunization. The progression of CIA was evaluated by macroscopic scoring of the paws every 3 days and histological analysis of the knees on day 18.

4) Macroscopic scoring of CIA

Erythema and swelling of the paws were scored on a scale of 0-4, with a maximum score of 4 for each paw, as described previously[15]. Arthritis was considered to be present if the score was >2. Two independent observers, without prior knowledge of the experimental groups, performed the scoring.

5) Histological processing and analysis of knee joints

Mice were killed by cervical dislocation. Thereafter, knee joints were dissected, fixed in 10% phosphate-buffered formalin for 2 days, decalcified in 10% EDTA (ethylene diamine tetraacetate) for 7 days, then embedded in paraffin. Standard frontal sections of 7 μm were prepared and stained with either haematoxylin-eosin or safranin O-fast green. Histopathological changes were scored using the following method, as described previously[14]. Cartilage depletion was indicated visually by diminished safranin O staining of proteoglycan matrix, and was scored arbitrarily as 0 when normal or as 1-3 according to the degree of depletion (loss of staining). A characteristic feature of CIA is the progressive loss of articular cartilage. The destruction was graded separately on a scale of 0-3, ranging from fully stained cartilage to destained cartilage or complete loss of articular cartilage. Pannus formation was scored arbitrarily as 0 when no pannus formed in the joint space or as 1-2 according to the degree of pannus formation. All these histological evaluation procedures were performed blind.

6) Measurement of cytokine levels in mice ankles and serum

TNF-α, IL-1ß, IL-4 and IL-10 were measured using commercially available ELISAs for TNF-α, IL-1ß, IL-4, IL-10 (R & D Systems, Minneapolis, MN, USA) according to the manufacturer's recommendations. Briefly, for TNF-α and IL-1ß, mice tarsi were snap-frozen in liquid nitrogen and ground into powder with a pestle, then lysed with lysis buffer (25mM Tris-HCl, 50mM NaCl, 0.5% sodium deoxycholate, 2% NP-40, 0.2% sodium dodecyl sulphate, 1mM phenyl methyl sulphonyl fluoride). The tissue lysates were used to measure the level of cytokines. For IL-4 and IL-10, mice were killed on the final day of experimentation and serum then was drawn to measure their levels. The levels of all these proteins were normalized to the total amount of cellular protein in prepared tissue lysates as measured by the DC protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA).

7) Statistical analysis

Data are expressed as mean±S.E.M. Differences in protein levels were compared by means of Student's t-test. Data on the incidence of arthritis were evaluated with the 2 test. Data on the progression of arthritis in the paws and the test of severity of pannus formation and cartilage erosion were evaluated with Student's t-test or the Wilcoxon rank sum test. P values <0.05 were considered

significant.

III. Results

1. Toxicity of UD

To examine the cytotoxicity of UD in the in vitro cell culture system, the MTT assay was performed. The results demonstrated that the concentration of UD required to inhibit growth by 50% (IC50) after 96 hrs was 350 μg/ml for mouse calvarial osteoblast cells.

To evaluate the acute oral toxicity of UD, we determined its single-dose toxicity in both sexes of rats at the dose of 15.2 g/kg body weight. This dose had no effect on mortality, clinical signs, body weight changes and gross findings in either sex. The results suggest that the lethal dose of UD is higher than 15.2 g/kg in rats of both sexes.

2. Inhibition of the progression of arthritis by UD

CIA in mice is an autoimmune type of arthritis which displays many characteristics in common with human RA. The onset of arthritis in DBA/1 mice occurs approximately 4 weeks after initial immunization with type II collagen. We first determined a therapeutically optimal concentration of UD by measuring the incidence of disease and the arthritis index by macroscopic examination of joint swelling and erythema at intervals of 5 days. Mice were treated orally with four concentrations of UD, ranging from 10 to 200 mg/kg body weight.

The incidence of arthritis was significantly less in UD-treated animals, and was lowest in animals receiving 100 mg/kg UD (Fig. 1A). Consistent with this result, the arthritis index was also lowest at this dose (Fig. 1B). On the basis of this result, all other experiments were performed using UD at 100 mg/kg.

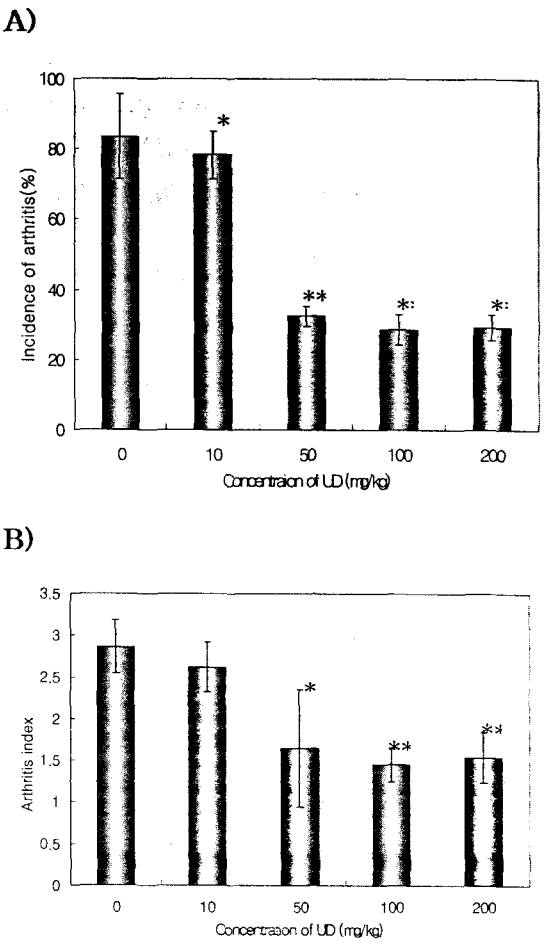


Fig. 1. Dose-response experiment on the treatment of arthritis with UD in CIA mice.

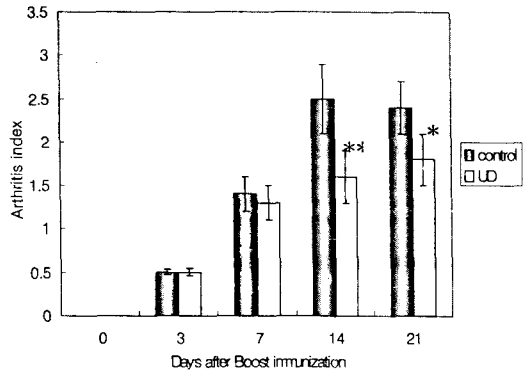
From the day after the booster immunization, UD was given orally once a day. Inhibition of arthritis (A) and the arthritis index (B) were measured on day 21 after UD treatment. Data are mean±S.E.M. (10 mice in each group). *P<0.05; **P<0.01 vs control (dose=0 mg/kg) (2 test in A and Wilcoxon rank sum test in B).

3. Time course of the suppressive effect of UD on arthritis in CIA mice

The time course of the disease status of

animals is shown in Fig. 2A. When animals were treated with 100 mg/kg of UD daily, the progression of arthritis was dramatically inhibited in mice treated with UD compared with control mice treated with vehicle (distilled water). The increase in paw thickness was significantly less in mice treated with UD than in control mice (Fig. 2B). These data showed that administration of UD could suppress the course of collagen-induced arthritis in mice.

A)



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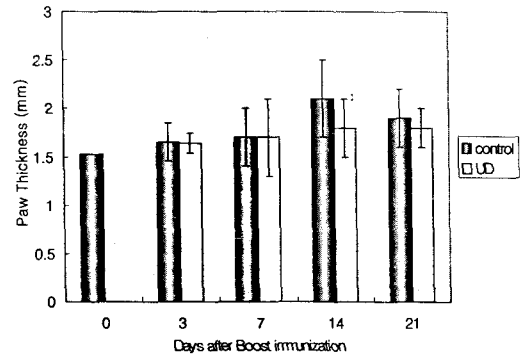


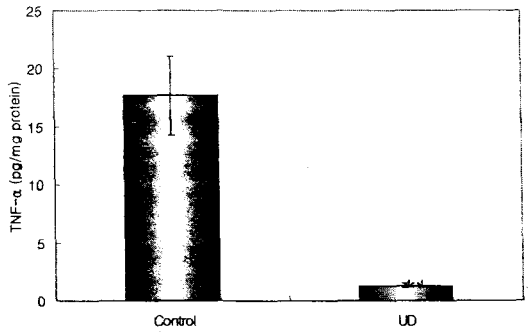
Fig. 2. Time course of the suppressive effect of UD on arthritis in CIA mice.

From the day after the booster immunization, UD and control vehicle (distilled water) were given orally once a day. The arthritis index (A) and paw thickness (B) were measured at day intervals. Data are mean±S.E.M. (6 mice in each group). *P<0.05; **P<0.01 vs control (Wilcoxon rank sum test in A and Student's t-test in B).

4. Effects of UD on levels of TNF-α and IL-1ß in the paws

TNF-α and IL-1ß are potent proinflammatory proteins and are important in the pathogenesis of RA[11]. TNF-α and IL-1ß are known to be present in large quantities in affected synovial fluid. Effects of UD on the levels of TNF-α and IL-1ß in the paws were examined. The paws were excised and total cellular proteins were extracted. The levels of the two cytokines were measured after standardization of the protein concentration. Consistent with the joint swelling result, marked decreases in the levels of TNF-α and IL-1ß were observed in the paws of mice treated with UD compared with the control mice (P<0.01) (Fig. 3A and 3B). These results suggest that UD inhibits the production of proinflammatory cytokines in the affected paws of CIA mice.

A)



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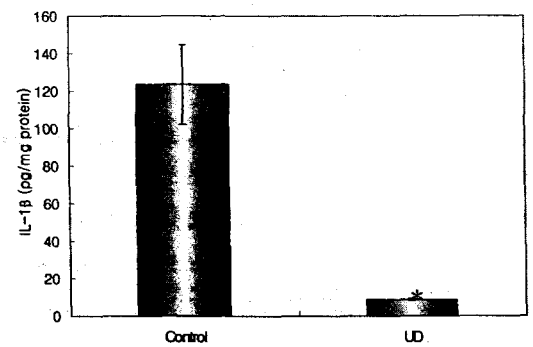

Fig. 3. Effects of UD on levels of TNF-α and IL-1ß in ankle joints.

Ankle joint extracts were prepared from joint tissues and analysed for TNF-α (A) and IL-1β (B). Data for TNF-α and IL-1β are mean±S.E.M. of 20 joints. **P<0.01 vs control (Students' t-test).

5. Inhibition of cartilage destruction

Effects of UD were also examined by histological examination in synovial tissues. Safranin O staining of proteoglycan in the cartilage showed that the proteoglycan was well preserved in joints treated with UD but not in joints treated with control vehicle (not shown). A statistically significant difference (37.9% inhibition) in the severity of cartilage erosion was found between the UD-treated group and the control group (Fig. 4). Sections stained with haematoxylin and eosin showed that pannus in the knee joint was decreased in mice treated with UD compared with those treated with control vehicle. Thinning and hyalinization of the cartilage were also inhibited (not shown). Comparison of the histological grades of pannus formation between the experimental group and the control group showed that the difference was about 30%, representing mild inhibition of pannus formation (Fig. 5). These results indicate that UD might have suppressive effects on CIA through cartilage protection rather than inhibition of pannus formation.

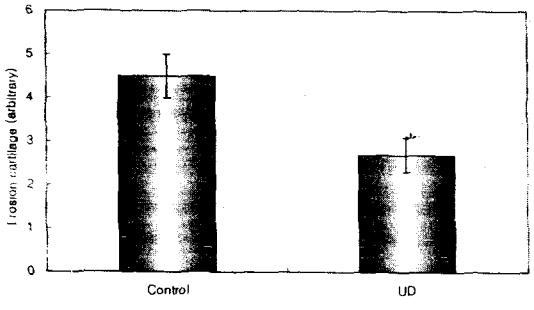

Fig. 4. Effects of UD on cartilage erosion in CIA.

Data represent 20 samples for each group. Erosion of cartilage was markedly inhibited in the knees of mice treated with UD. **P<0.01 vs control (Wilcoxon rank sum test).

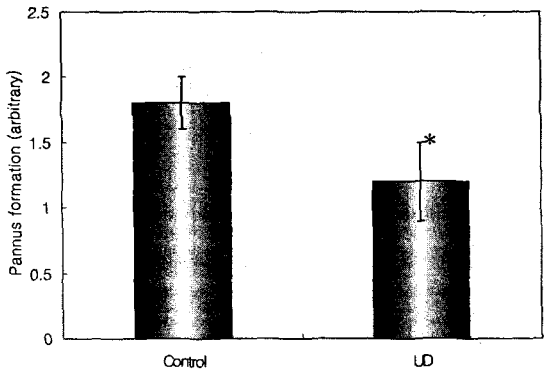

Fig. 5. Effects of UD on pannus formation in CIA.

Data represent 15 samples for each group. The grade of pannus formation was markedly lower in the knees of mice treated with UD. *P<0.05 vs control (Wilcoxon rank sum test).

6. Effects of UD on serum levels of IL-4 and IL-10

It has been reported that systematic treatment with IL-4 ameliorates disease progression and protects against cartilage destruction[16]. IL-10 has also been reported to exert a protective effect in CIA at high doses[17]. Levels of IL-4 and IL-10 in serum were compared between the experimental and control groups. The serum level of IL-4 in UD-treated mice was significantly higher than that in the control group (Fig. 6A). In addition, there was a significant difference in the level of IL-10 (Fig. 6B). This suggests that UD might have cartilage protection effects in the CIA model by regulating anti-inflammatory cytokines such as IL-4.

Sera were obtained on the last day of the experiments and diluted appropriately; levels of IL-4 (A) and IL-10 (B) were determined with

ELISAs. Data are mean±S.E.M. of 10 mice. *P<0.05 vs control (Students' t-test).

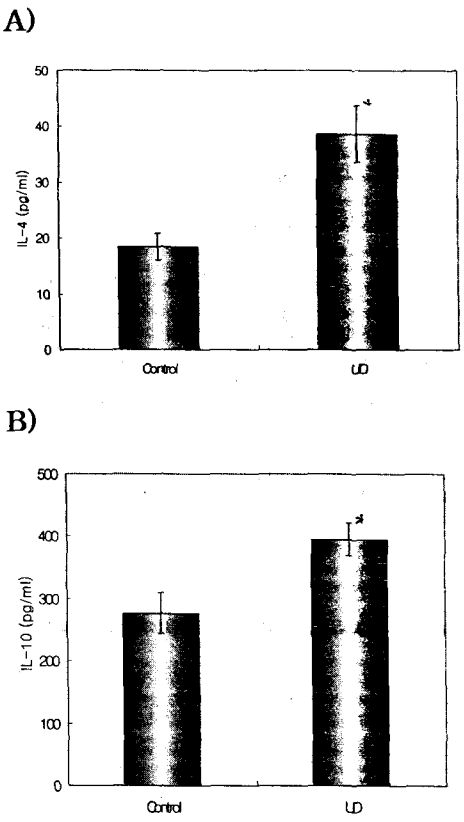

Fig. 6. Effects of UD on serum levels of IL-4 and IL-10.

Ⅳ. Discussion

UD is an extract developed to have therapeutic effects in inflammatory diseases involving cartilage destruction, such as RA. UD is a deciduous tree which is widely distributed in Korea. The barks of the stem and the root of this plant have been used in oriental traditional medicine. According to published work that is well accepted by the oriental traditional medicine community, UD was formulated to facilitate blood circulation as well as to reduce anti-inflammatory activity. The UD have been used for hundreds of years in this oriental region, for the treatment of oedema, mastitis, gastric cancer, and inflammation, and their safety and efficacy are well established through a long history of human use, but their use still lacks scientific support[9,18]. Although the barks of *U. davidiana* stem and root have been used in oriental traditional medicine for inflammatory diseases, the action mechanisms of this species are not nearly understood. It may be important to understand how this plant extract performs anti-inflammatory action in vivo. To evaluate the role of *U. davidiana* on inflammatory diseases, we studied the effect of the water extract of *U. davidiana* on the production of collagen-induced RA in rats in vivo. Our results showed that the *U. davidiana* clearly reduced this inflammatory disease in a dose-dependent manner.

Considering above-mentioned cytokines, The complex cellular interactions involving cells of the immune, inflammatory, and hematopoietic systems are mediated by a group of secreted low-molecular-weight proteins collectively called cytokines. Most cytokines act on nearby target cells, although in some cases a cytokine can act on the cell that secretes it or on a distant cell. The biological activities of cytokines exhibit pleiotropy, redundancy, synergy, and antagonism, which contribute to the complexity of cytokine network.

Biochemical purification of cytokines and subsequently the cloning of cytokine genes revealed that many activities previously attributes to different cytokines in fact were mediated by a relatively small number of multifunctional proteins. These discoveries brought some simplification to a field that was plagued an overwhelming array of supposedly single-function factors. Today, the most important recognized cytokines

include IFN-ν, interleukins 1-13, TNF-α, TNF-ß, and TGF-ß.

Development of an effective inflammatory response depends on the action of numerous cytokines. These include IL-1, IL-8, and IFN-ν, which aid in the movement of leukocytes to tissue sites where antigen is located. The phagocytic activity of macrophages and neutrophils is promotes by IFN-ν and TNF-α. The inflammatory response is, in part. limited by TGF-ß, which also promotes wound healing[3].

We demonstrated the marked effectiveness of oral administration of UD in protecting CIA mice against joint destruction. Histological analysis revealed pronounced protection against cartilage and bone erosion. This protective effect of UD appears to result from its control of key components in RA pathogenesis, including the down-regulation of TNF-α and IL-1ß. Therefore, UD is an important negative regulator of both inflammatory and destructive proteins related to RA. According to our results, UD also appears to act as positive regulator of IL-4. IL-4 and IL-10 have been thought to be upstream regulators that control the progression of RA negatively [16,17]. IL-4 was not detected in the synovium of patients with RA, while IL-10 was reported to be produced in substantial amounts in the RA joint[19]. This lack of IL-4 may contribute to the uneven balance between destructive and regulatory mediators in the synovium of RA. IL-4 appears to be involved in the CIA model, especially in the protection of cartilage and bones[30]. Consistent with these findings, IL-4 has been shown to enhance the synthesis of type I procollagen in human mononuclear phagocytes and cartilage explants[20]. In the serum of mice treated with UD, the level of IL-4 and IL-10 were maintained consistently at a level two-fold higher than that in control mice, which may lead to the protection of cartilage. Therefore, our data suggest that the cartilage-protective effects of UD might result from the up-regulation of IL-4 and IL-10.

Overall, our results suggest that the effect of *U. davidiana* Planch in the inhibition of inflammatory diseases may be partially associated with the down-regulation of TNF-α and IL-1ß. Our results indicate that UD has great potential as an alternative to these treatments, and has no adverse effects. UD can be given orally, and it inhibits disease progression by both controlling inflammatory proteins and protecting cartilage. Its cost is also estimated to be substantially lower than that of recombinant proteins. The data presented in this study show that UD warrants further investigation, including preclinical and clinical studies. We are now in progress to isolate active molecules, as have tried[21].

V. References

1. Panayi GS. T-cell-dependent pathways in rheumatoid arthritis. Curr Opin Rheumatol. 1997;9(3):236-40.
2. Fleischmann RM. Early diagnosis and treatment of rheumatoid arthritis for improved outcomes: focus on etanercept, a new biologic response modifier. Clin Ther. 1999;21(9):1429-42.
3. Kuby, Janis. Immunology. New York: W.H. Freeman and Company. 1994;321.
4. Lee SJ. Korean Folk Medicine, Monographs Series No. 3. Publishing Center of Seoul National University, Seoul, South Korea. 1996;39.

5. Jun CD, Pae HO, Kim YC, Jeong SJ, Yoo JC, Lee EJ, Choi BM, Chae SW, Park RK, Chung HT. Inhibition of nitric oxide synthesis by butanol fraction of the methanol extract of Ulmus davidiana in murine macrophages. J Ethnopharmacol. 1998;62(2):129-35.
6. Do WS, Kim KH, Kim KS. Effect of Aqua-acupuncture(Cortex Ulmi Pumilase, Ramulus Cinnamomum, Radix Achyranthis, Apitoxin, Calculus Bovis·Fel Ursi·Moschus compound) in Mice with Lipopolysaccharide Induced Arthritis. The Journal of Korean Acupuncture & Moxibustion Society. 2001; 18(1):157-69.
7. Shizhen L. (1590 A.D.). Bon Cho Gang Mok Compendium of materia medica.. Peking: Hwa Ha. 1998;1369-70.
8. Shin MK. Clinical Traditional Herbalogy. Seoul: Young Lim Sa. 1984;614-5.
9. Heo J. (1613 A.D.). Dong Ui Bo Gam The precious mirror of oriental medicine.. Seoul: Bub In. 2002;1968.
10. Kim YK, Kim KS, Chung KH, Kim JG, Kim KS, Lee YC, Chang YC, Kim CH. Inhibitory effects of deer antler aqua-acupuncture, the pilose antler of Cervus Korean TEMMINCK var mantchuricus Swinhoe, on type II collagen-induced arthritis in rats. Int Immunopharmacol. 2003;3(7):1001-10.
11. Kim CH, Kim YH, Kim YK, Kang BS, Lee TK, Moon SH, Park YG. IL-1ß induces and TGF-ß reduces vitamin D3-induced bone resorption in mouse calvarial bone cells. Immunol Invest. 2003;32(3): 171-86.
12. Kim CH, Kang BS, Lee TK, Park WH, Kim JK, Park YG, Kim HM, Lee YC. IL-1ß regulates cellular proliferation, prostaglandin E2 synthesis, plasminogen activator activity, osteocalcin production and bone resorptive activity of the mouse calvarial bone cells. Immunopharmacol Immunotoxicol. 2002;24(3):395-407.
13. Hong HT, Kim HJ, Lee TK, Kim DW, Kim HM, Choo YK, Park YG, Lee YC, Kim CH. Inhibitory effect of a Korean traditional medicine, Honghwain-Jahage (water extracts of *Carthamus tinctorius* L. seed and Hominis placenta) on interleukin-1-mediated bone resorption. J Ethnopharmacol. 2002;79(2):143-48.
14. Shin SS, Jin M, Jung HJ, Kim B, Jeon H, Choi JJ, Kim JM, Cho Chung SH, Lee YW, Song YW, Kim S. Suppressive effects of PG201, an ethanol extract from herbs, on collagen-induced arthritis in mice. Rheumatology (Oxford). 2003;42(5):665-72.
15. Kim JM, Ho SH, Park EJ. Angiostatin gene transfer as an effective treatment strategy in murine collagen-induced arthritis. Arthritis Rheum. 2002;46(3): 793-801.
16. Joosten LA, Lubberts E, Helsen MM. Protection against cartilage and bone destruction by systemic interleukin-4 treatment in established murine type II collagen-induced arthritis. Arthritis Res. 1999;1(1):81-91.
17. Apparailly F, Verwaerde C, Jacquet C, Auriault C, Sany J, Jorgensen C. Adenovirus-mediated transfer of viral IL-10 gene inhibits murine collagen-induced arthritis. J Immunol. 1998;160(1): 5213-20.
18. Kim CM, Shin MK, An DK, Lee KS. Chinese medicine encyclopedia. Seoul: Jung Dam. 1998;4319-21.
19. Katsikis PD, Chu CQ, Brennan FM, Maini RN, Feldmann M. Immunoregulatory role of interleukin-10 in rheumatoid arthritis. J Exp Med. 1994;179(5):1517-27.
20. Jorgensen C, Apparailly F, Couret I, Canovas F, Jacquet C, Sany J.

Interleukin-4 and interleukin-10 are chondroprotective and decrease mononuclear cell recruitment in human rheumatoid synovium in vivo. Immunology. 1998;93(4):518-23.

21. Kim JP, Kim WG, Koshino H, Jung J, Yoo ID. Sesquiterpene O-naphthoquinones from the root bark of Ulmus davidiana. Phytochemistry. 1996;43(2):425-30.