대한침구학회지 제24권 제3호(2007년 6월)
The Journal of Korean Acupuncture & Moxibustion Society

원 저

Effects of *Cibotium Barometz* on RANKL from Collagen-induced Rheumatoid Arthritis Mice

Kim Young-ock*, Kim Geum-soog*, Lee Seung-eun*, Seong Nak-sul*, Cha Seon-woo*, Jekal Seung-joo**, Jang Hyoung-seok*** and Shin Joon-shik***

*Ginseng & Medicinal Corps Division National Institute of Corp Science Rural Development Administration
**Department of Clinical Laboratory Science, Health Science College, Wonkwang Unoversity
***Jaseng Hospital of Oriental Medicine

국문초록

류머티즘을 유발한 생쥐에서 RANKL에 대한 구척의 효과

김영옥* · 김금숙* · 이승은* · 성낙술* · 차선우* · 제갈승주** · 장형석*** · 신준식***

*작물과학원 인삼약초과 소재이용연구실
**원광대학교 보건과학대학 임상병리학과
***자생한방병원

목적 : 콜라겐으로 관절염을 유발한 생쥐에서 RANKL발현에 대한 구척의 효과를 연구하였다.

방법 : 면역조직화학법을 이용하여 관절의 염증과 골의 손실 정도를 증명하였다. 콜라겐으로 관절염을 유발한 DBA/1J종의 생쥐에 관절염 유발 3주후부터 8주까지 구척 (300mg/kg)을 투여한 후 발의 부종과 골 손실의 변화를 Safranin O염색으로 관찰하였다.

결과 : 5주간 구척의 투여로 관절의 부종과 무릎 관절의 염증을 막았다. 파골세포 분화인자인 RANKL은 대조군에 비해 구척 투여군의 관절에서 RANKL이 감소되었다.

결론 : 구척 투여의 결과로 골손실을 보호하였고, 뼈를 만드는 동화작용에 의해 RANKL의 발현을 개선할 수 있었다.

· Acceptance : 2007년 5월 30일 · Adjustment : 2007년 5월 30일 · Adoption : 2007년 6월 1일
· Corresponding author : Young-Ock Kim, Ginseng & Medicinal Corps Division National Institute of Corp Science Rural Development Administration, 209 Seodoondong Kwonsungu Suwonsi Kyeonggido Korea.
Tel. 82-31-290-6832 E-mail : monicaok@empal.com

핵심단어 : Rheumatoid Arthritis, Immunohistochemistry, DBA/1J, RANKL

Ⅰ. Introduction

Cibotium barometz has tonifies the liver and kidneys and strengthens the sinews and bones: used for deficient liver and kidneys with such symptoms as stiffiness, soreness or weakness in the lower back, spine, and lower extremities. Expels wind and dampness used for ind damp painful obstruction ith pain, soreness, or nmbness. Also used for swelling of the legs as a aftermath of illness.

Focal bone erosionis a hallmark of rheumatoid arthritis (RA), a severe immune-mediated disease that progresses over time to yield irreversible skeletal destruction. Bone erosions result from enhanced production of activated osteoclasts in affected joints[1,2]. A fact confirmed by the marked increase in osteoclasts that occurs in experimental animal models of polyarthritis[3,4]. Prevention of osteoclast has therefore become an important therapeutic principle of antirheumatic drug therapy. Formation of inflamed synovial tissue is a prerequisite for induction of bone loss. Several important clues suggest that increased bone resorption precipitates inflammation-mediated bone loss. First, osteoclasts, which are absent in normal synovial tissue, are abundantly present in inflamed synovial tissue and are attached to bone surfaces both in animal models of arthritis and in human RA[1,5]. The inflamed synovium expresses RANKL, that stimulate osteoclastogenesis and bone resorption[6]. RANKL is the primary mediator of osteoclast formation, function, and survival. RANKL is required for bone erosions in arthritic joints. The absence of osteoclasts and greatly reduced joint destruction observed in RANKL, knockout mice with arthritis[7]. Inhibition of RANKL may attenuate this negative effect on the osteoblast compartment and thus make these factors susceptible to anabolic stimuli.

The effects of RANKL inhibition on systemic bone loss have not been studied in inflammatory arthritis models. Risk factors for systemic bone loss in RA patients include immobility and persistently active disease[8]. Unfortunately, there are no convenient biochemical markers that identify those RA patients at greatest risk for systemic bone loss. It was recently reported that serum RANKL levels are positively correlated with disease progression in RA patients[9]. RANKL might serve as a useful biomarker for local bone loss. Serum RANKL was also reported to correlate positively with bone resorption markers and with generalized bone loss in arthritis patients[10]. To explore these questions, we used well-characterized mice model of inflammatory arthritis (collagen-induced arthritisCIA), to examine the bone erosions by CB in arthritic joints.

Ⅱ. Material and methods

Animals and induction of arthritis. DBA/1J(80 males) weighing 25 - 32grams were received from Jung Ang companyand acclimated for at least 1 week prior to use. Animals were housed in filter-capped polycarbonate cages and maintained under constant environmental conditions(22±1°C, relative humidity 50%). Mices were kept on a 12 hour - 12 hour light - dark cycle and given bottled drinking water(purified by a reverse osmosis).

System; Edstrom Industries, Waterford, WI) and

pelleted chow(Jung Aang company) *ad libitum*. CIA was induced in male mices by a single intradermal injection into the tail base. The adjuvant consisted of 0.5mg of heat-killed mycobacteria H37Ra(Difco, Detroit, MI) suspended in paraffin oil. CIA was elicited in male mices by intradermal injection of porcine type II collagen (1mg total; Chondrex, Redmond, WA), emulsified 1:1 with Freund's incomplete adjuvant(Difco), at multiple sites.

1. Prepamiceion of CB extract

The roots of CB were from Jaseng Hospital of Oriental Medicine, Korea. The roots were air-dried avoiding sun-light and cut into small pieces for the experiment. The dried roots(500g) were soaked in 70% ethanol(4L) at room tempemiceure for 1 day and extracted for 1 hour three times with 70% EtOH in an ultrasonic appamiceus and filtered with filter paper(Advantec, Toyo Roshi Kaisha, Japan) to remove the debris. The EtOH extract was evapomiceed under reduced pressure by rotary evapomiceor(R-205, Büchi, Germany) and lyophilized with freezing dryer(Operon, Korea) to give 70% EtOH crude extract(180 g, yield 36 %). The EtOH crude extract(100g) was dissolved in distilled water(500㎖).

2. Treatment

Mices were randomly assigned 4 groups(n = 20/group): CB(300 mg/kg) and Ibuprofen(50 mg/kg) were administered orally after the onset of clinical disease.

3. Assessment of paw swelling

Swelling of hind paws was assessed daily from disease onset to day 3 weeks disease onset.

In CIA, paw swelling was measured by water plethysmography as previously described[11].

4. Immunohistochemistry

All mice were expired at 8weeks after CIA. Samples were fixed, decalcified, dehydmiceed, and embedded in paraffin. Four-micrometer sections were stained with Safranin O-Fast Green stain. RANKL was localised through immunohistochemical staining as previously described[12]. The area of the RANKL was traced using Image-Pro®Plus image analyzer(Media Cybernetics Inc.) at 100X magnification. The area of RANKL was expressed as the mean number of area per mm^2.

Statistical analysis

All results were expressed as the group mean ± SD. Clinical(continuous) data were assessed using a student's t-test. A p value of 0.05 was used to delineate significant differences between groups.

III. Results

1. Effects of CB extract on the mice hind-paw edema

We first evaluated the anti-inflammatory activity of CB(300mg/kg) on mice paw edema(Fig. 1.) during the whole experiment(8 weeks). CB and ibulopen(50mg/kg) inhibited swelling at 8 weeks after onset arthritis.

At 8 weeks after CIA, the edema of ankle joint (sham group; 0.197 ± 0.034, saline group; 1.132 ± 0.083, ibulopen group;0.386± 0.097, and CB group; 0.475 ± 0.066) were presented(Fig. 2).

2. Effects of CB extract on the area of RANKL

After induction of arthritis, CB treated mice with CIA gain 11.6% of control mice, respectively, as measured by the area of RANKL. Inhibition of RANKL with CB significantly reduced RANKL area (Fig. 2.).

At 8weeks after CIA, the area of RANK(sham

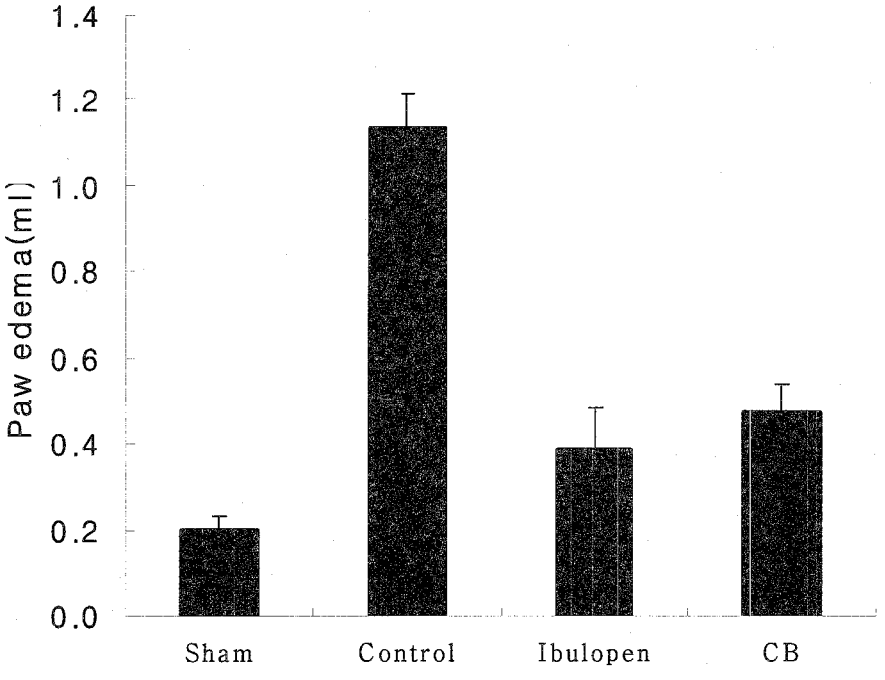

Fig. 1. Effects of 70% ethanol extract of CB on CIA mice paw edema. CB was administered during 5after onset arthritis. The change of footpad volume was determined at 5after onset arthritis. Each value represents the mean±SD of 20 mice per group. Sham; sham operated group, Control CIA group with saline, Iulopen CIA group with ibulopen(50mg/kg), CB; CIA group with CB(300mg/kg)

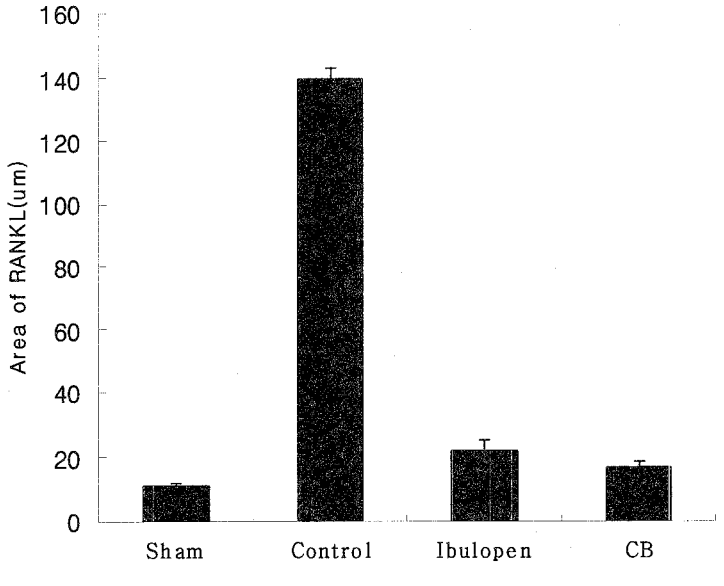

Fig. 2. Effects of 70% ethanol extract of CB on CIA mice RANKL area. CB was administered during 5after onset arthritis. The change of RANKL area was determined at 5weeks after onset arthritis. Each value represents the mean±SD of 20 mice per group. Sham; sham operated group, Control CIA group with saline, Iulopen CIA group with ibulopen(50mg/kg), CB; CIA group with CB(300mg/kg)

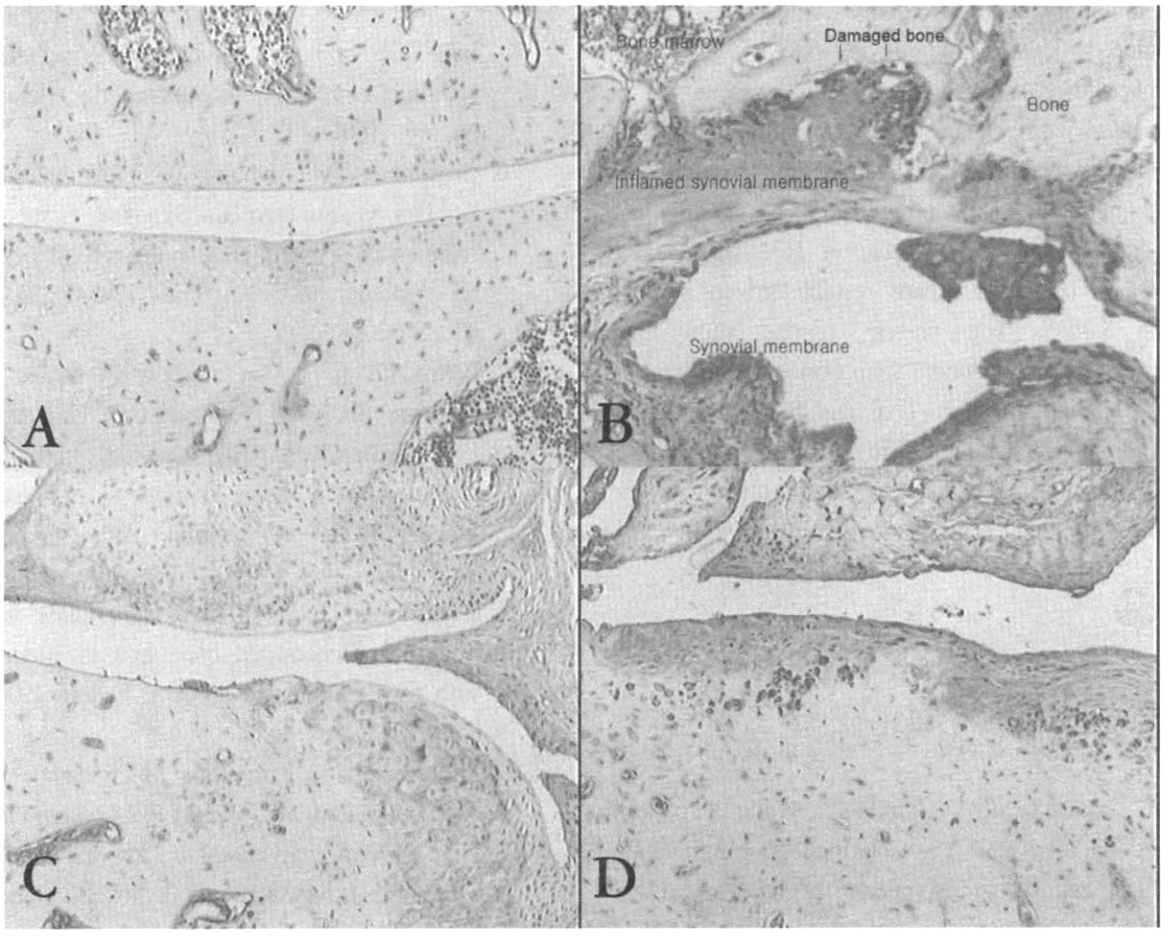

Fig. 3. Distribution of RANKL expression during disease progression in the cartilage of mice with CIA. Darkfield demonstmiceion of RANKL. Sham (A) didn't show localization of RANKL.(original magnification x 400), Control (B) showed large localization of RANKL. It shows marked mononuclear cell infiltration with cartilage-bone destruction (original magnification x 400), Ibulopen (C) showed small localization of RANKL. only limited synovial lining cell hyperplasia was detected (original magnification x 400), CB (D) showed small localization of RANKL almost like panel C. still limited synovial lining cell hyperplasia was detected.(original magnification x 400)

group; 10.93 ± 1.059, saline group; 139.44 ± 3.547, ibulopen group; 21.182± 3..818, and CB group; 16.178 ± 2.250) were presented(Fig. 2).

Upon treatment with CB, RANKL wasfound predominantly at sites, such as the junction between cartilage, bone, osteoclasts and invasion of inflamed tissue at sites of bone damage were most prominent(Fig. 3B). CB treated mice showed only few sites of RANKL expression(Fig. 3D), while in the untreated arthritic mice(Fig. 3A).

IV. Discussion and conclusion

Our results provide evidence that a 70% ethanol extract of Cibotium barometz stems possesses anti-inflammatory activity. The extract was tested with the standard models for chronic inflammation. The present study shows that the 70% ethanol extract from the stem of Cibotium barometz, especially at the high dose (300orally treated), has

an inhibitory effect on edema formation in arthritis -induced mice paw model.RANKL has been implicated as a pathogenetic factor for bone loss in patients with rheumatoid[13] arthritis. In animal models of arthritis, RANKL plays a causal role in the initiation and progression of local bone erosions. The essential role of RANKL in bone erosions has been clearly established in animals with CIA[14]. The present results show that stimulation of bone formation enhances the bone-sparing effect of TNF and RANKL blockade in immune-mediated arthritis. In consequence, CB is a promising new class of therapeutics with the potential to reduce in rheumatoid arthritis.

V. Reference

1. Gravallese EM, Harada Y, Wang JT, Gorn AH, Thornhill TS, Goldring SR. Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. Am J Pathol. 1998 152 : 943 - 51.
2. Takayanagi H, Oda H, Yamamoto S, Kawaguchi H, Tanaka S, Nishikawa T, Koshihara Y. A new mechanism of bone destruction in rheumatoid arthritis: synovial fibroblasts induce osteoclastogenesis. Biochem Biophys Res Commun. 1997 ; 240 : 279 - 86
3. Bolon B, Morony S, Capparelli C, Hu YL, Campagnuolo G, Duryea D, Feige U. IL-1ra and PEG sTNF-R1 reduce osteoclast numbers in mices with adjuvant arthritis. Arthritis Rheum. 2000 ; 43 : S233.
4. Romas E, Bakharevski O, Hards DK, Karsogiannis V, Quinn J, Ryan P, Martin TJ, Gillespie MT. Expression of osteoclast differentiation factor at sites of bone erosion in collageninduced arthritis. Arthritis Rheum. 2000 ; 43 : 821 - 6.
5. Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature. 1999 ; 402 : 304 - 9
6. Gravallese EM, Manning C, Tsay A, Naito A, Pan C, Amento E. Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. Arthritis Rheum. 2000 ; 43 : 250 - 8.
7. Pettit AR, Ji H, von Stechow D, Muller R, Goldring SR, Choi Y, Benoist C, Gravellese EM. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol. 2001 ; 159 : 689 - 99.
8. Gough AK, Liley J, Eyre S, Holder RL, Emery P. Generalised bone loss in patients with early rheumatoid arthritis. Lancet. 1994 ; 344 : 23 - 7.
9. Ziolkowska M, Kurowska M, Radzikowska A, Luszczykiewicz G, Wiland P, Dziewczopolski W, Filipowicz-Sosnowska A, Pazdur J, Szechinski J, Kowalczewski J, Rell-Bakalarska M, Maslinski W. High levels of osteoprotegerin and soluble receptor activator of nuclear factor B ligand in serum of rheumatoid arthritis patients and their normalization after antitumor necrosis factor treatment. Arthritis Rheum. 2002 ; 46 : 1744 - 53.
10. Geusens P, Landewe R, Chen D, Dunstan C, Garnero P, van der Linden S. Receptor activator of the nuclear factorkappa-B ligand (RANKL) in serum is related to bone density, cartilage breakdown and inflammation in early active rheumatoid arthritis. J Bone Miner Res. 2002 ; 17 : S1 ; S386.
11. Campagnuolo G, Bolon B, Feige U. Kinetics of bone protection by recombinant osteoproteger in therapy in Lewis mices with adjuvant arthritis. Arthritis Rheum. 2002 ; 46 : 1926 - 36.
12. Vu TH, Shipley JM, Bergers G.MMP-9 /gelatinase B is a key regulator of growth

plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell. 1998 ; 93 : 411 - 22

13. Ritchlin CT, Haas-Smith SA, Li P, Hicks DG, Schwarz EM.Mechanisms of TNF-a and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis. J Clin Invest. 2003 ; 111 : 821 - 31.
14. Romas E, Sims NA, Hards D, Lindsay M, Quinn J, Ryan P, Dunstan CR, Martin TJ, Gillespie MT.Osteoprotegerin reduces osteoclast numbers and prevents bone erosions in collagen- induced arthritis. Am J Pathol. 2002 ; 161 : 1419 - 27.