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원 저

Modulation of Bone Mass, Strength and Turnover by a Cervi Pantotrichum Cornu Herbal Acupuncture in adjuvant-Induced Arthritic Rats

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국문초록

Adjuvant로 유발된 관절염 실험용쥐에서 골체, 골강도 및 골대체율의 변화에 대한 녹용 약침의 효과

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면역억제활성이 알려진 Cerves korean TEMMINCK var. manchur-icus Swinhoe(Nokyong) 약침(CPH)은 다모 뿔의 녹용을 열수추출한 용액이다. 본 연구에서는 녹용 약침의 효과를 adjuvant 유발 관절염 실험용쥐를 이용하여 골체, 골강도 및 골대체율의 감소를 평가하였다

위의 골 대사 관련 검정실험을 위하여 6주령의 암컷 실험용쥐에 20일간 약물투여를 실시하였다. 실험적인 관절염유발은 실험용쥐의 뒤쪽 다리에 Adjuvant를 주사하여 유발시킨 결과, 요부의 골 무기질함량과 밀도(BMC, BMD) 그리고 압축강도는 관절염 실험용쥐에서 감소되었다. 10일 경과 후, 골형성도(BFR/BS, BFR/BV)의 조직형태학적 기준척도인 혈청 osteocalcin 수가 정상대조군과 비교해 볼 때 현저하게 감소되

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었다. 그러나, 몸무게로 나눈 BMC치는 관절염 유발군과 정상군 사이에 큰 차이가 보이지 않았다. 그리고, 골무기질 함량은 정상군에 비해 감소하지 않았다. 20일 경과후, 몸무게로 나누거나 나누지 않은 BMC치 모두, 관절염 군에서 요부 몸체의 골무기질 함량과 강도가 정상군과 비교해 볼 때 현저하게 감소되었다. 잔존 소주(小柱)의 무기질 침착 표면은 현저하게 감소하였으며 파골 세포의 수는 증가하였다. 초기부터 매일 Shinsu(B23)에 CPH 약침 투여(10, 20, 50㎍/kg)는 20일 경과후 만성적인 다리 부종을 현저하게 방지하였으며, 골 무기질함량, 골강도 및 소주골 형성등의 감소와 파골세포수 증가도 완화하였다. Adjuvant주사로 장애를 받았던 연령대비 요부 길이 증가도 유지되었다.

이러한 결과는 Adjuvant에 의한 관절염 실험용쥐의 2차적인 골관절염에서 충분히 요추 몸체뼈와 강도 감소를 나타내기 위해서는 적어도 20일이 필요하다는 것을 제시하였다. 본 결과로부터 CPH가 실험용쥐의 관절염에 대한 골체, 골강도 및 골대체율을 조절하는데 유효하게 작용하고 있음을 알 수 있었다.

Key words : **Bone Mass, Strength, Turnover, Cervi Pantotrichum Cornu Herbal Acupuncture, Adjuvant-Induced Arthritic Rats**

I. Introduction

CPH is a new oriental medicine widely used in Korea.[1] CPH has immunomodulating activities, and was observed to inhibit the anti-sheep red blood cell antibody production and reduce the delayed-type hypersensitivity sensitized by methylated bovine serum albumin in mice[2]. CPH has been shown to reduce the chronic inflammation in adjuvant-induced arthritic (AA) rats. Against this background, the present study was performed to examine the changes in bone mass, strength, and turnover of the cancellous bone in AA rats and to assess the effects of CPH on these parameters in this model.

Arthritis is associated with systemic inflammatory disorders such as rheumatoid arthritis (RA)[3]. Persistently active disease and immobility in Arthritis were found to be factors that contribute to bone loss[4], but the control of these risk factors by nonsteroid antiinflammatory agents is frequently incomplete[5,6], and specific bone-sparing agents are sometimes required[7]. However, since many inflammatory factors including interleukin-1(IL-1), IL-6, tumor necrosis factor (TNF)-α, nitric oxide, and prostaglandins are known to affect bone formation and resorption[8,9,10,11], bone-sparing effects of inflammation-modulating agents may be expected when these agents are used for inflammatory diseases.

We obtained results of the serum osteocalcin levels, bone minerals and bone size of the lumbar bodies after CPH acupuncture to arthritis animals. To determine the effects of the disease and the BW gain on these

parameters, we have obtained the effective results in the AA rats.

Ⅱ. Materials and Methods

1. Animals and materials

Inbred, female Lewis rats (KCTC, Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea) were housed in metal cages under standard laboratory conditions of temperature(20~24℃) and humidity (45~65%). The rats had free access to tap water and to a commercial standard rat chow containing 0.74% calcium, 0.65% phosphorus, and 200IU/100g of vitamin D3 (F-2 ; Funabashi Farms, Chiba, Japan)through-out the experimental period. All experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Dongguk University.

2. Animal experiment for drug

Eighty rats, 6 weeks of age, were assigned to 4-8 groups of 8 animals each with reference to their BWs. One group was used as a baseline control. In the four AA groups, arthritis was induced by a single injection of 0.6mg of the adjuvant, heat-killed Mycobacterium butyricum (Difco Laboratories, Detroit, MI, USA) suspended in 0.1ml liquid paraffin into the left hind paw. The other two groups of rats were injected with paraffin vehicle alone and used for the normal controls. Two groups of rats, i.e., one adjuvant-injected group and one normal control group, were sacrificed 10 days after the injection. The other four groups (including the other control group) were maintained as usual for 20 days. In the four AA groups, CPH suspended in the vehicle (phospho-buffered saline) was administered by Shinsu(B23) acupuncture with a respective dose of 10, 20 or 50㎍/kg BW. Bone labeling with an intraperitoneal (I.P.) calcein injection (5mg/kg BW ; Wako Pure Chemicals, Osaka, Japan) was performed twice -6 and 2 days before the sacrifice. Blood samples were obtained from retroorbital vessels of these rats at the start and 10 and 20 post-injection days. Blood samples were centrifuged and the serum samples were stored at -80℃ until measured. Rats were sacrificed by exsanguination under ether anesthesia, and the 3rd, 4th, and 5th lumbar vertebrae (L3, L4, and L5) were harvested. The L3 specimens were fixed with 4% paraformaldehyde in 0.1 M phosphate-buffered saline containing 2% sucrose at 4℃. The L4 specimens were fixed with 70% ethanol. The L5 specimens were stored at -80℃ until the mechanical test.

3. Measurements of body weight and edema volume of the paw

The rats were weighted and the hind paw volume was measured with a volumetric apparatus (MK-550, Muromachi-kikai, Tokyo, Japan) at the start (baseline) and at 0, 5, 10,

15, and 20 days post-injection. The paw edema volume (ml) was used as the parameter of inflammation, and the edema value was obtained by dividing the paw volume at each time period by the value at the time of the adjuvant injection. The edema values were then expressed as percentages of the baseline values.

4. Measurement of serum osteocalcin levels of lumbar body

The serum osteocalcin levels were measured with a radioimmunoassay using anti-rat osteocalcin antibody (Biomedical Technologies Inc., Stoughton, MA, USA).

5. Measurements of bone mineral and size

The L4 vertebral body was isolated by removing the posterior elements and transverse processes. The bone mineral values of the L4 bodies were then measured by dual-energy X-ray absorptiometry (DCS-600, Aloka, Tokyo, Japan) using the small animal scan mode with irradiation applied anteroposteriorly to the specimen. The mineralization profile of the specimen was stored with the monitoring image. The BMC(mg) and BMD(mg/cm^2) values for the whole vertebral body were calculated. The coefficient values of variation of the BMC and BMD measurements were 0.94% and 1.27%, respectively. The volume of the L4 body was then measured by Archimedes' method with a volumetric apparatus. The BMC values were corrected for volume to obtain the values of three-dimensional (3-D) density (BMC/volume, mg/mm^3). Corrected BMC values for the BW were also calculated. The cranio-caudal length (i.e., height) of the L4 bodies were measured with vernier calipers on the soft X-ray radiograph taken after putting the specimens directly on the films. The mean values of the growth in the lumbar height during the experimental period were calculated by subtracting the mean values of the bone height of the baseline group from those in the treated groups.

6. Histomorphometry

Following the bone mineral measurement, the L4 specimens were embedded in methyl methacrylate (MMA) after Villanueva bone staining. The L3 specimens were embedded in a mixture of MMA, hydroxyglycol methacrylate, and 2-hydroxyethylacrylate, and then polymerized at 4℃. Midsagittal sections 5μm thick were cut with a Reichert-Jung microtome (Model 2050 Supercut, Heidelberg, Germany). Sections of the L3 specimens were then stained for tartrate-resistant acid phosphatase (TRAP)[12]. Histomorphometry was performed with a semiautomatic image analyzing system linked to a light microscope (System Supply, Nagano, Japan). The entire region of the secondary spongiosa was measured. At the growth plate-metaphyseal junction, the region within 1mm from the growth plate was not measured to exclude the primary spongiosa. The region located within one cortical width from the endosteal surface was also excluded from the measurements.

7. Analysis of measured parameters

For bone structure, the trabecular bone volume (BV/TV, %) was measured in the L4 sections, and the values of trabecular number (Tb.N, mm^{-1}), thickness (Tb.Th, ㎛), and separation (Tb.Sp, ㎛) were calculated by assuming a constant geometry[13]. The BV/TV values were then corrected for the BW. For fluorescence labeling, the following parameters were measured in the L4 sections: double-labeled surface (dLS/BS, %); mineralizing surface (MS/BS, %); mineral apposition rate (MAR, ㎛/day), calculated as the distance between double labels divided by the labeling interval and multiplied by pi/4; the surface-referent bone formation rate (BFR/BS, $㎛^3/㎛^2$/day), calculated as (dLS + sLS/2) × MAR/BS; and the volume-referent bone formation rate (BFR/BV, %/day), calculated as (dLS + sLS/2) × MAR/BV. The BFR/BV values were then corrected for BW. The osteoclast surface (Oc.S/BS, %) and number (N.Oc/BS, mm^{-1}) were measured in the L3 sections: TRAP-positive cells that formed resorption lacunae at the surface of the trabeculae and contained one or more nuclei were identified as osteoclasts[14]. The histomorphometric parameters were named and defined according to the nomenclature proposed by the report from the American Society for Bone and Mineral Research (ASBMR) committee[15].

8. Statistical analysis

Values are shown as the mean and the SEM. The data of BW, paw edema, and serum osteocalcin levels were assessed by Dunnett's multiple test at each time period after a two-way analysis of variance (ANOVA). Student's t-test was performed to evaluate the differences in the parameters of bone minerals, histomorphometry, and mechanical tests between the AA and age-matched normal rats at 10 days. The effects of CPH dosing were evaluated by Dunnett's multiple range test after a one-way ANOVA. The changes in the parameters from those in the baseline controls were assessed by Dunnett's multiple range test.

Ⅲ. Results

1. Changes in body weight, paw edema in rats

The increases in rat's BW were significantly retarded after adjuvant injection in rats, and the differences in the values from the age-matched normal rats were significant from the 5th day onward. The values in the CPH-treated groups were higher than those in the AA control rats from the 10th day, but they did not reach the levels of the age-matched normal rats<Table Ⅰ-A>. In all CPH-treated groups, the percent values of the left paw edema were lower than those in the AA control rats from the 15th day (CPH). At20 days, the values of the right paw edema were significantly lower than those in the AA control rats <Table Ⅰ-B, Ⅰ-C>.

Table I. Changes in Body Weight (A), Left Paw Edema (B), and Right Paw Edema (C) in Adjuvant-Induced Arthritic (AA) Rats Treated with CPH Daily for 20 Days.

A) Body weight

	Days				
	0	5	10	15	20
Body weight(g)					
Normal	145.2±5.3	172.6±9.9*	184.7±7.9*	193.3±7.9*	204.7±12.0*
AA(control)	145.7±4.2	147.4±6.2#	132.7±8.6#	140.4±8.7#	151.4±8.3#
AA+CPH-1 (10μg/kg)	146.2±5.2	148.5±5.3#	150.3±5.6*#	152.2±7.3#	160.8±9.9#
AA+CPH-2 (20μg/kg)	145.7±5.3	157.8±7.6#	163.5±9.3*#	167.4±5.3*#	169.5±11.2*#
AA+CPH-3 (50μg/kg)	145.4±6.7	158.4±9.3#	167.4±8.9*#	168.4±7.5*#	174.4±8.9*#

B) Left paw edema

	Days				
	0	5	10	15	20
Edema (% of baseline)					
Normal		100	100	100	100
AA (control)	ND	316.8±9.4#	389.6±13.2#	403.7±12.5#	367.6±8.6#
AA+CPH-1 (10μg/kg)	ND	289.4±4.3#	355.4±4.3#	303.5±3.6*#	267.5±5.6*#
AA+CPH-2 (20μg/kg)	ND	287.5±8.3#	302.6±7.8*#	297.4±7.5*#	264.4±9.7*#
AA+CPH-3 (50μg/kg)	ND	256.7±6.9*#	264.3±7.3*#	267.7±6.5*#	249.5±6.6*#

C) Right paw edema

	Days				
	0	5	10	15	20
Edema (% of baseline)					
Normal			100	100	100
AA (control)	ND	100	225.6±16.4#	238.6±14.2#	239.6±7.9#
AA+CPH-1 (10μg/kg)	ND	100	210.5±21.6#	220.4±13.7#	195.6±6.2*#
AA+CPH-2 (20μg/kg)	ND	100	202.4±14.4#	219.5±8.4#	198.6±7.6*#
AA+CPH-3 (50μg/kg)	ND	100	185.7±17.8#	198.6±16.3#	178.4±9.7*#

Each value represents the mean and standard error of 10 rats. *P<0.01; significantly different from the age-matched AA control groups; # P<0.01; significantly different from the age-matched normal rats (Dunnett's test after ANOVA). ND : not determined

control rats from the 15th day (CPH). At 20 days, the values of the right paw edema were significantly lower than those in the AA control rats <Table I-B, I-C>.

2. Changes in serum osteocalcin levels

In the normal rats, the serum osteocalcin levels did not change during the first 10 days, but they significantly decreased from 10 to 20 days post-adjuvant injection. In the AA control rats, the values were lower than those in the age-matched normal rats and were significantly reduced compared with the baseline values at 10 days. The values in the CPH-treated rats with the doses of 20(middle-dose) and 50 (high-dose)㎍/kg were higher than those in the AA control rats at 10 days. The values in the high-dose group were maintained at the level of the age-matched normal rats at 10 days<Table II>.

3. Effects of CPH on the bone minerals and sizes of lumbar body

The parameters of L4 BMC and BMD increased age dependently in the normal rats. At 10 days, these parameters (except for the volume and the BMC corrected for BW, Height) in the AA control rats were lower than those in the age-matched normal rats. Although values of BW and BMC, 3-D density were slightly decreased than that of baseline control. At 20 days, all of these parameters in the AA rats were lower than those in the age-matched normal rats. The values of BMD, 3-D density, and BMC corrected for BW were reduced compared with the baseline control. These bone mineral values in the CPH-treated groups with the middle and high doses were higher than those in the AA control rats, but

Table II. Changes in Serum Osteocalcin Levels in Adjuvant-Induced Arthritic Rats Treated with CPH Daily for 20 Days.

	Days		
	0	10	20
Serum osteocalcin (ng/mℓ)			
Normal	55.4±8.3	53.5±5.5**	30.2±1.4
AA (control)	62.7±11.2	24.5±3.4##	31.2±4.5
AA+CPH-1(10㎍/kg)	54.6±8.4	33.54±6.4##	28.5±3.4
AA+CPH-2(20㎍/kg)	53.7±11.2	40.4±7.6*##	29.7±3.5
AA+CPH-3(50㎍/kg)	52.3±9.4	48.7±9.5**	29.4±4.2

Each value represents the mean and standard error of 10 rats. *P < 0.05; **P < 0.01; significantly different from the age-matched AA control groups; ##P < 0.01; significantly different from the age-matched normal rats (Dunnett's test after ANOVA).

Table Ⅲ. Effects of CPH on The BMC, BMD, Volume, and Longitudinal Height of The 4th Lumbar Vertebral Body in Adjuvant-Induced Arthritic Rats.

Day	Treatment	BW (g)	BMC (mg)	BMD (mg/cm²)	Volume (mm³)	Height (mm)	3-D density (mg/cm³)	BMC /BW (mg/g)
Day 0	Normal	159.3	21.2	48.4	45.1	5.87	456.6	0.131
Day 10	Normal	$180.3^{b,h}$	$25.5^{a,h}$	$52.6^{a,h}$	46.3	$6.23^{b,g}$	$522.4^{b,h}$	0.132
	AA(control)	150.5	19.7	50.6	47.7	6.54	436.4	0.135
Day 20	Normal	$198.3^{d,h}$	$27.7^{d,h}$	$61.2^{d,h}$	$53.2^{d,g}$	$6.76^{d,h}$	$548.4^{d,h}$	0.151^{d}
	AA(control)	156.6^{f}	19.4^{f}	$45.3^{f,h}$	43.6^{f}	6.34^{f}	$421.2^{f,h}$	$0.118^{f,g}$
	AA+CPH-1 (10μg/kg)	$162.3^{e,f}$	21.1^{f}	$45.4^{c,f,h}$	44.5^{f}	$6.43^{c,g}$	$445.4^{d,f}$	0.120^{f}
	AA+CPH-2 (20μg/kg)	$169.4^{d,f,h}$	$22.1^{d,f}$	$49.5^{d,f}$	45.8	$6.72^{c,h}$	$454.4^{d,f}$	0.123^{f}
	AA+CPH-3(50μg/kg)	$173.4^{d,f,h}$	$23.2^{d,f,g}$	$50.5^{d,f,g}$	48.6	$6.92^{d,h}$	$456.6^{d,}$	$0.127^{c,e}$

Data are the mean value (standard error) of 10 rats per group

AA: adjuvant-induced arthritis, BMC: bone mineral content, BMD: bone mineral density, BW: body weight, 3-D density: three-dimensional density (BMC/volume)

$^{a}P<0.05$, $^{b}P<0.01$; significantly different from AA control at 10 days (Student's t-test)

$^{c}P<0.05$, $^{d}P<0.01$; significantly different from AA control at 20 days (Dunnett's test after ANOVA)

$^{e}P<0.05$, $^{f}P<0.01$; significantly different from age-matched normal rats at 20 days (Dunnett's test after ANOVA)

$^{g}P<0.05$, $^{h}P<0.01$; significantly different from normal rats at 0 day (Dunnett's test after ANOVA)

the values did not reach the levels of theage-matched normal rats. The lumbar height values in the middle- and high-dosegroups were maintained at the level of the age-matched normal rats<Table Ⅲ>.

4. Effects of CPH on the histo mor-phometry of lumbar trabecular bone

At 10 days, the values of BV/TV, Tb.Th, and Tb.N in the AA rats were smaller than those in the age-matched normal rats. The values of BV/TV (before and after correction for BW) and Tb.Th were reduced compared with the baseline controls. At 20 days, these values were further reduced. In CPH-treated groups, the BV/TV and Tb.N values were greater and the Tb.Sp values were less than those in the AA control rats. In the middle- and high-dose groups, the BV/TV values were maintained at the level of the baseline controls, but did not reach the levels of the age-matched normal rats. The Tb.N values were larger than the age-matched normal rats whereas the Tb.Th values remained smaller than the age-matched normal rats<Table Ⅳ>.

Table IV. Effects of CPH on The Trabecular Bone Volume and Microstructural Indices of The 4th Lumbar Vertebral Body in Adjuvant-Induced Arthritic Rats

Day	Treatment	BV/TV (%)	BV/TV/BW (%/g)	Tb.Th (μm)	Tb.N (mm^{-1})	Tb.Sp (μm)
Day 0	Normal	27.6	0.176	83.2	3.29	216.4
Day 10	Normal	31.2^{a}	0.158	82.7^{a}	3.57^{a}	187.4^{a}
	AA (control)	23.5^{g}	0.143^{g}	59.7^{h}	3.27	235.4
Day 20	Normal	30.4^{d}	0.173^{d}	90.4^{d}	3.45	178.4^{d}
	AA (control)	$17.4^{f,h}$	$0.121^{f,h}$	$55.4^{f,h}$	3.21	$254.4^{f,g}$
	AA+CPH-1 (10μg/kg)	$22.1^{c,f}$	$0.152^{c,}$	$65.4^{f,g}$	3.65^{c}	211.2^{d}
	AA+CPH-2 (20μg/kg)	$25.4^{d,c}$	0.155^{d}	$73.3^{c,f,g}$	$3.78^{d,g}$	201.2^{d}
	AA+CPH-3 (50μg/kg)	28.4^{d}	0.163^{d}	$78.3^{d,e}$	$3.80^{d,h}$	193.2^{d}

Data are the mean value (standard error) of 10 rats per group

AA: adjuvant-induced arthritis, BW: body weight, BV/TV: bone volume, Tb.Th: trabecular thickness, Tb.N: trabecular number. Tb.Sp: trabecular separation

$^{a}P<0.05$, $^{b}P<0.01$; significantly different from AA control at 10 days (Student's t-test)

$^{c}P<0.05$, $^{d}P<0.01$; significantly different from AA control at 20 days (Dunnett's test after ANOVA)

$^{e}P<0.05$, $^{f}P<0.01$; significantly different from age-matched normal rats at 20 days (Dunnett's test after ANOVA)

$^{g}P<0.05$, $^{h}P<0.01$; significantly different from normal rats at 0 day (Dunnett's test after ANOVA)

5. Effects of CPH on bone formation and resorption of lumbar body

The values of MAR, BFR/BS, and BFR/BV (before and after correction for BW) decreased age dependently in the normal rats. At 10 days, these parameters of bone formation in the AA rats were lower than those in the age-matched normal rats. At 20 days, the dLS/BS, MS/BS and BFR/BS values in the AA groups remained lower, but the BFR/BV values did not differ significantly from the age-matched normal rats. The values of N.Oc/BS in the AA rats were larger than those in the age-matched normal rats, and increased compared with the baseline control.

In the CPH-treated groups with the middle and high doses, all of the parameters of bone formation were larger than those in the AA rats. The dLS/BS and MS/BS values were maintained at the normal levels. The values of BFR/BS and BFR/BV corrected for the BW were larger than those of the age-matched normals, but the values were still significantly reduced compared with the baseline controls. The values of Oc.S/BS and N.Oc/BS in the high-dose group were lower than those in the age-matched normals, and reduced compared with the baseline controls<Table V>.

Table V. Effects of CPH on Bone Formation and Resorption of Lumbar Vertebral Bodies in Adjuvant-Arthritic Rats

Day	Treatment	dLS/BS (%)	MS/BS (%)	MAR (㎛/day)	BFR/BS (㎛3/㎛2 /day)	BFR/BS /BW (㎛3/㎛2 /day/g)	Oc.S/BS (%)	N.Oc/BS (㎜-1)
Day 0	Normal	23.4	30.5	1.47	45.4	0.321	11.7	4.70
Day 10	Normal	24.5^{b}	35.3^{b}	1.27	43.4^{b}	0.234^{b}	12.0	4.48
	AA (control)	0.58^{h}	8.4^{h}	1.01	9.6^{h}	0.071^{h}	12.9	5.10
Day 20	Normal	$13.6^{d,h}$	26.4^{d}	0.93^{h}	$22.3^{c,h}$	0.098^{h}	11.1	4.49^{c}
	AA (control)	$1.7^{f,h}$	$9.3^{f,h}$	1.14^{g}	$8.9^{e,h}$	0.060^{h}	13.0	$6.21^{e,g}$
	AA +CPH-1 (10㎍/kg)	$7.2^{f,h}$	$14.6^{f,h}$	1.03^{g}	14.6^{h}	0.079^{h}	8.5^{c}	4.13^{d}
	AA +CPH-2 (20㎍/kg)	$15.2^{d,h}$	24.6^{d}	1.22	$30.4^{d,h}$	$0.162^{d,e,h}$	8.1^{c}	3.71^{d}
	AA +CPH-3 (50㎍/kg)	$17.3^{d,g}$	28.3^{d}	1.22	$34.5^{d,g}$,	$0.210^{d,g,f}$	$7.4^{d,e,g}$	$3.23^{d,g}$

Data are the mean value (standard error) of 10 rats per group

AA: adjuvant-induced arthritis, BW: body weight, dLS/BS: double-labeled surface, MS/BS: mineralizing surface, MAR: mineral apposition rate, BFR/BS: bone formation rate, Oc.S/BS: osteoclastic surface, N.Oc/BS: number of osteoclasts

$^{a}P < 0.05$, $^{b}P < 0.01$; significantly different from AA control at 10 days(Student's t-test)

$^{c}P < 0.05$, $^{d}P < 0.01$; significantly different from AA control at 20 days (Dunnett's test after ANOVA)

$^{e}P < 0.05$, $^{f}P < 0.01$; significantly different from age-matched normal rats at 20 days (Dunnett's test after ANOVA)

$^{g}P < 0.05$, $^{h}P < 0.01$; significantly different from normal rats at 0 day (Dunnett's test after ANOVA)

6. Effects of CPH on the mechanical tests of lumbar bone

The parameters of the ultimate compressive strength were age-dependently increased in the normal rats. At 10 days, the strength values in the AA group, both before and aftercorrection for volume, were smaller than thosein the age-matched normals. However, the strength values corrected for BW did not significantly differ from those of the age-matched normal rats and the baseline controls. At 20 days, all of the parametervalues in the AA group were smaller than those in the age-matched normal rats and significantly reduced compared with the baseline controls. In the CPH-treated groups, all of the strength values were significantly higher than those in the AA rats. All of the values except the ultimate strength corrected for volume were maintained at the levels of the baseline controls, but they did not reach the levels of the age-matched normal rats<Table VI>.

Table VI. Effects of CPH on The Compressive Bone Strength of The 5th Lumbar Vertebral Body in Adjuvant-Arthritic Rats

Day	Treatment	Ultimate Strength (N)	Structural stiffness (N/㎜)	Ultimate strength/volume (N/㎟)	Ultimate strength/BW (N/G)
Day 0	Normal	165.4	2321.2	8.76	1.23
Day 10	Normal	204.3[b,h]	2512.2	8.54[b]	1.11
	AA(control)	152.2	1445.4	6.09[h]	0.98
Day 20	Normal	258.5[d,g]	2985.4[d]	8.93[d]	1.34[d]
	AA(control)	94.5f,[h]	965.5[f,g]	3.54[f,h]	0.66[f,h]
	AA+CPH-1(10㎍/kg)	144.3[d,]	1897.5[c,e]	5.21[c,f,h]	0.87[d,f]
	AA+CPH-2 (20㎍/kg)	162.3[d,f]	1823.3[c,f]	5.67[d,f,h]	0.94[d,f]
	AA+CPH-3 (50㎍/kg)	179.5[d,f]	1997.5[c,c]	6.54[d,f,h]	1.04[d,f]

Data are the mean value (standard error) of 10 rats per group
AA: adjuvant-induced arthritis, BW: body weight
[a]P<0.05, [b]P < 0.01; significantly different from AA control at 10 days (Student's t-test)
[c]P<0.05, [d]P < 0.01; significantly different from AA control at 20 days (Dunnett's test after ANOVA)
[e]P<0.05, [f]P < 0.01; significantly different from age-matched normal rats at 20 days (Dunnett's test after ANOVA)
[g]P<0.05, [h]P < 0.01; significantly different from normal rats at 0 day (Dunnett's test after ANOVA)

IV. Discussion

Effect of water extract of CPH, prepared from the pilose antler of Cervus korean TEMMINCK var. mantchuricus Swinhoe (Nokyong), a traditional immuno-suppressive and immuno-activating Korean aqua-acupuncture, was investigated to examine its effects on the reductions in bone mass, strength, and turnover in adjuvant-induced arthritic rats. A 20-day administration experiment was designed and performed using 6-week-old female Lewis rats. Arthritis was induced by injecting the adjuvant into the hind paw of the Lewis rats. The results demonstrated that the age-dependent increases in the lumbar bone minerals and size were inhibited in AA rats. The CPH administration reduced the development of the chronic inflammation, maintaining the increases in the size of the lumbar body in this animal model. The increases in the bone minerals as maintained in the animals treated with CPH, but the normal growth rates could not be attained, even with the high dose.

The lumbar BMD and 3-D density values

were time-dependently decreased in this animal model. The decreases in the BV/TV and Tb.Th suggested the deterioration of bone structure. The bone strength was also decreased after adjuvant injection. Though the increases in the lumbar height differed between the normal and arthritic rats, the differences were less than 1 mm for a 20-day period, warranting the comparison of the histomorphometry data of the trabecular bone when the appropriate fields were defined for measurement.

Arthritis is defined as a systemic skeletal disease characterized by low bone mass and the microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. However, a 10-day period may not be long enough in this model to discriminate between the effects of minimum weight gain by adjuvant injection and the activity of the disease on the skeleton.

This study confirmed the early reduction of active bone surface for bone formation and the subsequent increase in bone resorption in the AA rats[16,17]. The decrease in the bone surface for bone formation indicated that the number of osteoblasts engaged in the mineralization of bone matrix was acutely suppressed by the adjuvant injection[17,18]. Thus, the adjuvant injection reduced the parameters of bone turnover, both surface- and volume-referent (BFR/BS and BFR/BV), by the cessation of the differentiation of osteoblasts for the mineralization of bone. The apparent improvement of BFR/BV relative to the age-matched normals at 20 days does not indicate the recovery of the active surface for bone formation. The BFR/BS and BFR/BV values corrected for BW in the AA rats were significantly smaller than those in the normal age-matched rats at 10 days.

Prevention of the inflammatory paw edema by CPH administration at middle and high doses was apparently only partial in this animal model. The CPH administration was not able to substantially inhibit the development of acute inflammation in the adjuvant-injected paw. However, since the paw edema values at 20 days post-injection were significantly reduced by all three doses, CPH could be potent to regulate the inflammation at the chronic phase of the disease of rodent adjuvant-induced arthritis.

The effects of CPH administration on the bone mass and strength were associated with the prevention of both a decrease in the bone formation surface and an increase in the number of osteoclasts. The parameters of bone tissue turnover such as BFR/BV in the middle- and high-dose groups were maintained at levels higher than that of the age-matched normal controls. However, since the values of BV/TV and Tb.Th were still lower, the focal balance of the amounts of bone formed and resorbed for the experimental period were not maintained even with the high-dose CPH. The increase in the Tb.N values seemed to be related to the reduced bone resorption by the agent.

The cellular mechanism whereby CPH prevents osteopenia in adjuvant-induced arthritic rats is not known. It is obvious that the effects of the agent were not due to the maintenance of BW. Rather, since the effect of CPH on the parameters of the labeled surfaces appeared to be associated with the reduction of the chronic-phase inflammatory edema, it may be that the immuno-modulating activities of this compound are related to the protection of the osteoblast recruitment induced by the adjuvant injection in rats.

The results of the present study confirmed that the reductions in bone mass and strength of the lumbar bone in adjuvant-induced arthritic rats were due to the activity of the AA disease. The active surface for bone formation was reduced in the early period after the adjuvant injection. However, a 10-day period was not long enough to assess the preventive effects of the therapeutic agent used on the bone mass and strength. Although its cellular mechanism is not certain, CPH, a new oriental medicine, was found to be effective in preventing the decreases in bone mass and strength when administered for 20-days, by improving the bone turnover in the AA rats. However, since the dose levels used in the clinical setting are not similar to the doses used here, the results of this study do not imply a similar effect of this agent on bone turnover in humans. Further investigations are necessary before any conclusions can be drawn about the bone mass-sparing effect of CPH in human inflammatory disorders.

V. Conclusion

1. The values of BW in the CPH-treated groups were higher than those in the AA control rats from the 10th day, although they did not reach the levels of the age-matched normal rats.

2. CPH treatment showed prevention and protection left hind paw edema. At 20 days, the right hind paw edema were significantly decreased than those in the AA control rats.

3. The serum osteocalcin levels was increased in the CPH-treated rats

4. In the CPH-treated groups with the middle and high doses, the bone mineral values were higher than those in the AA control rats, but the values did not reach the levels of the age-matched normal rats.

5. In the CPH-treated groups, the BV/TV and Tb.N values were greater and the Tb.Sp values were less than those in the AA control rats. The Tb.N values were larger than the normal values whereas the Tb.Th values remained smaller than the normal values.

6. In the CPH-treated groups with the middle and high doses, all of the parameters of bone formation were larger than those in

the AA rats.

7. In the CPH-treated groups, all of the strength values were significantly higher than those in the AA rats. All of the values except the ultimate strength corrected for volume were maintained at the levels of the baseline controls.

VI. References

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