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Chondroprotective Effects of *Cinnamomum cassia* Blume in a Rat Model of Osteoarthritis

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Abstract : The present study was conducted to evaluate the efficacy *of Cinnamomum cassia* Blume (CC) extract on the repair of damaged cartilage in a rat model of osteoarthritis (OA) by anterior cruciate ligament transection (ACLT) and medial meniscus resection (MMx). Forty-eight rats were assigned to six groups (n = 8 per group): sham as negative control (NC), positive control (PC), diclofenac sodium (DS, 2 mg/kg), CC 25 mg/kg, CC 50 mg/kg and CC 100 mg/kg groups. Treatments were 12 weeks from 7 days after ACLT + MMx. Loss of cartilage and joint instability were significantly reduced in response to treatment with CC or DS compared to the PC ($p < 0.05$). CC significantly ameliorated cartilage degradation in a dose-dependent manner as assessed by histological findings ($p < 0.01$). A reduction in the severity of structural changes and a dose-dependent increase in Safranin-O staining intensity were observed in CC treatments, indicating that cartilage degradation was inhibited. Although DS did not affect the increase in active caspase-3 and cleaved poly(ADP-ribose) polymerase-induced apoptosis during the progression of OA, cells reactive to these apoptotic markers were decreased significantly by CC ($p < 0.05$). However, treatments with CC or DS did not influence the uptake of 5-bromo-2'-deoxyuridine. The findings suggest that CC can exert a chondroprotective action on OA through anti-inflammatory and anti-apoptotic properties.

Key words **:** *Cinnamomum cassia* Blume, osteoarthritis, apoptosis, caspase-3.

Introduction

Osteoarthritis (OA) is a joint disease characterized by changes in the structure and function of the articulation, mainly due to a degenerative process the takes place in the articular cartilage (27). Among the many animal models of OA, anterior cruciate ligament transection (ACLT) in combination with resection of medial menisci (MMx) results in joint instability and induces cartilage degeneration, subchondral bone sclerosis, and osteophyte formation, which mimics the pathological changes detected in human OA (11). In a rat ACLT model, there is an increase in the number of cells exhibiting signs of degeneration or even death, often related to chondrocyte apoptosis (31), as previously shown in dogs (4) and in the rabbit (10).

Cartilage hypocellularity also contributes to the development of clinical and experimental OA due to chondrocyte death by either apoptosis or necrosis (1). So critical are chondrocytes to the OA process that disease progression can be judged by the viability of chondrocytes and their ability to resist apoptosis (23). Expression of caspase-3, a member of the interleukin-1β-converting enzyme family responsible for the cleavage of poly-(ADP-ribose) polymerase (PARP) during cell death (20), has also been found to be higher in rats OA cartilage and to be correlated with chondrocyte apoptosis as determined by the terminal deoxynucleotidyl transferase dUTP nick end labeling method, leading to a reduced density of living cells (32).

Cinnamomum cassia Blume (CC) has been reported to have significant anti-allergic, anti-ulcerogenic, anti-pyretic, anesthetic, and analgesic activities (14,19). Moreover, CC extract has anti-inflammatory activity and an anti-thrombic effect (17,24). With this background, we hypothesized that CC extract will provide symptomatic relief from OA, including the reduction of cartilage damage and inhibition of inflammation. With reference to preliminary evidence, we presently investigated the chondroprotective effects of CC extract on articular cartilage using an ACLT + MMx model in rats.

Materials and Methods

Materials

CC was obtained from Young-Hwa Pharmaceutical (Daegu, Korea) after microscopic confirmation of morphology. For extraction, 1 kg of dried CC was added to 4 L of water and boiled for 2 hours at 100ºC, filtered, and concentrated to 800 mL. The water extract was lyophilized and stored at room temperature until use. Dry yield was 9.52% (w/w). The principle component analysis of CC extract was performed with

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a recently reported HPLC analytical method (6). Cinnamaldehyde was the most abundant marker component (content was 81.25 mg/g), followed by eugenol (12.29 mg/g), coumarin (4.34 mg/g), cinnamyl alcohol (1.13 mg/g), and cinnamic acid (0.87 mg/g).

Diclofenac sodium (DS) was purchased from Sigma-Aldrich (St. Louis, USA). DS was used as a comparator for non-steroidal anti-inflammatory drugs (NSAIDs). Rabbit anti-cleaved caspase-3 polyclonal antibody and anti-cleaved PARP (Asp214) antibody were from Cell Signaling Technology (Beverly, USA). Substrate kit for peroxidase (vector® VIP, SK-4600) and avidin-biotin-peroxidase complex (ABC) reagent (universal Vectastain Elite ABC kit; PK-6200) were from Vector Laboratories (Burlingame, USA), and 5-bromo-2'-deoxyuridine (BrdU) was from MP Biomedicals (Solon, USA). Sheep anti-BrdU polyclonal antibody was from Abcam (Cambridge, UK).

Animals and administration

Male Sprague-Dawley rats (170 ± 10 g, 6-weeks-old; SLC, Japan) were used after acclimatization for 7 days. Feed and water were supplied *ad libitum*. One week after ACLT + MMx, 48 rats were divided into six groups (n = 8 in each group): sham as the negative control (NC) (mock operation with saline), positive control (PC, ACLT + MMx with saline), DS 2 mg/kg (ACLT + MMx with DS 2 mg/kg in saline), CC 25 mg/kg (ACLT + MMx with CC 25 mg/kg in saline), CC 50 mg/kg (ACLT + MMx with CC 50 mg/kg in saline), and CC 100 mg/kg (ACLT + MMx with CC 100 mg/kg in saline). CC extract was dissolved in saline and administered at a dose volume of 5 ml/kg by oral gavage. All treatments were administered once daily for 84 days beginning 7 days after operation. Animals were allocated into social groups of four or five per polycarbonate cage in a temperature (20-25°C) and humidity (40-45%) controlled room with a 12 h/12 h light/dark cycle. This study was approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Korea.

Induction of OA

Each rat was anesthetized with isoflurane (Hana Pharm Co., Korea) and, after being shaved and disinfected, the right knee joint was exposed through a medial parapatellar approach. The patella was dislocated laterally and the knee placed in full flexion, followed by anterior cruciate and medial collateral ligaments transection and MMx resection with micro-scissors (11). After washing the joint surface, both capsule and skin were closed with 4-0 vicryl suture by using a subcuticular pattern. In sham-operated animals, the right knee joint was exposed and incisions were closed after subluxation of the patella and washing the joint surface with saline.

Measurement of maximum knee extensor angle

At sacrifice, knees of all animals were dissected from the coxofemoral region to the ankle region, leaving the articular capsule intact. After dissection, the maximum extension angle of each knee was measured using previously reported methods (29).

Histopathological examination

After disarticulation of the right joint, both the femur and tibia were dissected and fixed in 10% neutral buffered formalin followed by decalcification in a solution of 24.4% formic acid and 0.5N sodium hydroxide for 5 days. Once decalcified, the knee joints were cut into two approximately equal halves in the frontal plane, using the collateral ligament as a landmark. The tissues were embedded in paraffin, cut into 3-4 μm thick sections, and stained with hematoxylin-eosin or Safranin O. The articular cartilage was evaluated and recorded by two independent blinded observers using a modified Mankin score (2,22), examining the surface, cellularity, extent of cloning clones, and Safranin O stain intensity. Each characteristic was scored from 0 to 3, with higher scores reflecting worse degenerative change. Digital images were taken from five areas chosen from the four corners and the center of the articular cartilage of each longitudinally trimmed slice. The thickness of the tibial and femoral articular cartilage was measured in micrometer using an automated digital image analyzer (DMI-300, Seoul, Korea) for each histological sample.

Detection of activated caspase-3 and cleaved PARP

Other sections were immunostained using the ABC method (32). The tissue sections were deparaffinized with xylene and rehydrated with a series of ethanol and boiled in 10 mM of citrate buffer (pH 6.0) for 10 minutes for antigen retrieval. Then, sections were immersed in 0.3% hydrogen peroxide in phosphate buffered saline to block endogenous peroxidase activity and 10% normal horse serum to block non-specific bonding, followed by incubation overnight in rabbits polyclonal anti-active caspase-3 and anti-cleaved PARP antibody diluted 1:100 in blocking solution. After washes, the sections were incubated for 1 hour in biotin-labeled goat anti-rabbit secondary antiserum (1:50) and then reacted for 1 hour in the dark with ABC reagents. The vector® VIP reagent was added and samples incubated for 0.5 minute and counter-stained with Mayer's hematoxylin. In the histomorphometric analysis, the frequency of immunoreactive cells was determined as the mean and standard deviation of eight fields of view per articular cartilage region by automated digital image as percentages, with one field of view recorded for each animal.

Cell proliferation index

One hour prior to administration, rats were given intraperitoneal injections of BrdU 50 mg/kg and the animals were sacrificed 72 hour later. BrdU uptake was detected with an anti-BrdU antibody as reported previously (25). Briefly, the tissues were deparaffinized through a series of washes with xylene and graded alcohols. After epitope retrieval by pre-

treatment with 2N HCl, sections were immunostained using sheep anti-BrdU polyclonal antibody (1:100). The percentage of chondrocytes showing BrdU immunoreactivity was histomorphometrically calculated using the same process as adopted for assessing the active caspase-3 and cleaved PARP immunoreactivity (15).

Statistical analysis

All data were expressed as mean ± SD. The Mann-Whitney *U*-Wilcoxon Rank Sum W test was used to analyze the significance of data with SPSS for Windows (Release 14k; SPSS, Chicago, USA). A $p < 0.05$ was considered statistically significant.

Results

Changes in knee maximum extension angle and articular cartilage thickness

The functional analysis of each knee was performed by analyzing the maximum extension angle, which reflects the degree of articular stiffness. The maximum extension angle of the operated knee joint was significantly increased in the PC group compared to that of the NC group ($p < 0.01$, Table 1). DS- and CC extract-treated groups showed a significant decrease in knee angle compared with the PC group ($p < 0.01$). Significant decreases in the thickness of the tibial and femoral articular cartilage were detected in the PC group compared to the NC group ($p < 0.01$). These decreases were significantly minimized in response to treatment with CC extract or DS ($p < 0.05$).

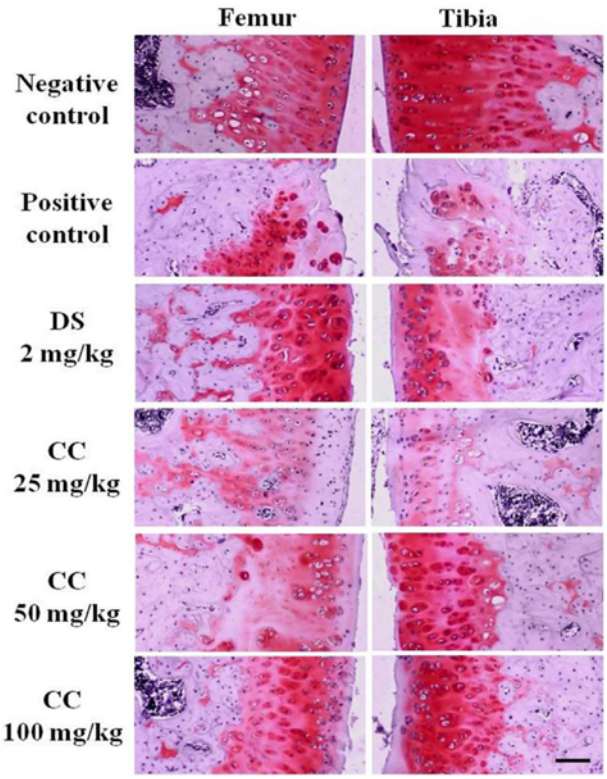

Fig 1. Microphotos of articular cartilage in osteoarthritic rats treated with diclofenac sodium (DS) and *Cinnamomum cassia* Blume (CC). DS or CC treatment was a less reduction in the severity of lesions to the femoral-tibial knee joint compartment in response to controls. Scale bar = 80 μm. 40 ×, Safranin O stain.

Histopathological findings

Sham-operated joints had articular cartilage with a smooth surface with flat superficial chondrocytes orientated parallel to the cartilage surface. No proteoglycan depletion was observed. Cartilage in PC exhibited severe degradation changes including surface fibrillation, chondrocyte loss, subchondral bone resorption and loss of Safranin-O staining compared to the CC- and DS-treated cartilages (Fig 1). Only dose-dependent superficial irregularities, decreased erosions and mild local fibrillation extending to the superficial layer of cartilage were observed following these treatments.

Changes in the Mankin score

Various degrees of articular cartilage surface damage were apparent in all OA-induced groups as evidenced by the extent of hypocellularity, cloning, and the Safranin O stain intensity (Table 2). This was reflected in the higher total Mankin scores in both the tibia and femur of the PC group compared to the NC group ($p < 0.01$). The total Mankin scores revealed a significant dose-dependent decrease by

Table 1. Evaluation of maximum extension level and cartilages thickness in the knee joints

Group	Maximum extensor angles (degree)	Thickness of articular cartilages (μm)	
		Femur	Tibia
Negative control	28.63 ± 3.20	569.43 ± 95.77	780.16 ± 145.83
Positive control	74.25 ± 4.13[a]	303.07 ± 91.40[a]	296.51 ± 73.69[a]
DS 2 mg/kg	60.75 ± 6.78[ac]	409.48 ± 69.80[b]	500.53 ± 196.69[ad]
CC 25 mg/kg	61.13 ± 7.99[ac]	438.28 ± 195.00[d]	437.93 ± 163.81[a]
CC 50 mg/kg	56.13 ± 4.22[ac]	455.06 ± 129.93[d]	497.98 ± 192.80[ad]
CC 100 mg/kg	52.63 ± 10.01[ac]	508.30 ± 159.25[c]	494.44 ± 116.69[ad]

Mean ± SD (n = 8). [a]$p < 0.01$, [b]$p < 0.05$ *vs* negative control; [c]$p < 0.01$, [d]$p < 0.05$ *vs* positive control.
DS: Diclofenac sodium, CC: *Cinnamomum cassia* Blume

Table 2. Grades of articular cartilage damage in osteoarthritic lesions

Groups	Surface	Hypocellularity	Clones	Safranin O stain	Totals
Femur					
Negative control	0.25 ± 0.46	0.25 ± 0.46	0.00 ± 0.00	0.25 ± 0.46	0.75 ± 0.89
Positive control	2.75 ± 0.46[a]	2.38 ± 0.52[a]	2.25 ± 1.16[a]	2.50 ± 0.53[a]	9.88 ± 1.55[a]
DS 2 mg/kg	2.13 ± 0.35[ad]	1.38 ± 0.52[ac]	2.38 ± 0.92[a]	1.25 ± 0.46[ac]	7.13 ± 1.36[ac]
CC 25 mg/kg	2.13 ± 0.64[ad]	1.75 ± 0.71[ad]	0.63 ± 0.74[d]	1.63 ± 0.74[ad]	6.13 ± 1.55[ac]
CC 50 mg/kg	2.13 ± 0.83[ad]	1.63 ± 0.52[ad]	1.63 ± 1.19[b]	1.00 ± 0.76[bc]	6.38 ± 2.77[ac]
CC 100 mg/kg	1.63 ± 0.52[ac]	1.25 ± 0.71[ac]	1.25 ± 1.04[b]	1.25 ± 1.04[ac]	5.38 ± 2.77[ac]
Tibia					
Negative control	0.25 ± 0.46	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.35	0.38 ± 0.74
Positive control	2.63 ± 0.52[a]	2.00 ± 0.53[a]	2.38 ± 0.52[a]	1.50 ± 0.93[a]	8.50 ± 1.93[a]
DS 2 mg/kg	2.00 ± 0.53[ad]	1.63 ± 0.52[a]	1.75 ± 0.71[a]	1.38 ± 0.74[a]	6.75 ± 1.16[ad]
CC 25 mg/kg	2.13 ± 0.64[a]	1.75 ± 0.71[a]	0.63 ± 0.74[c]	1.63 ± 0.74[a]	6.13 ± 1.55[ac]
CC 50 mg/kg	2.13 ± 0.35[a]	1.25 ± 0.71[a]	1.63 ± 1.06[a]	1.00 ± 0.76[b]	6.00 ± 1.85[ac]
CC 100 mg/kg	1.75 ± 0.46[ac]	1.38 ± 0.52[a]	1.25 ± 0.89[ad]	1.00 ± 0.53[b]	5.38 ± 1.69[ac]

Mean ± SD (n = 8). [a]$p < 0.01$, [b]$p < 0.05$ *vs* negative control; [c]$p < 0.01$, [d]$p < 0.05$ *vs* positive control.
DS: Diclofenac sodium, CC: *Cinnamomum cassia* Blume

treatment with CC ($p < 0.01$) or DS ($p < 0.05$) as compared with that of the PC group. More marked changes were evident in the CC-treated groups compared to that of DS-treated group

Immunohistochemical expression of activated caspase-3 and cleaved PARP

In the osteoarthritic knees, the numbers of caspase-3- and PARP-immunoreactive cells were increased. These cells were diffusely distributed throughout the entire cartilaginous tissue, but these abnormal increases in the expression levels of the two apoptotic markers were dramatically and dose-dependently decreased by CC treatment (Fig 2). At immunohistochemical analysis, an increase of caspase-3- and PARP-immunoreactive cells were detected in the PC group compared to that of the NC group. However, this increase was significantly and dose-dependently reduced by CC treatment ($p < 0.05$). No statistically significant differences were found between PC group and the DS group (Table 3).

Changes in cell proliferation

In the osteoarthritic knees, the numbers of BrdU-immunoreactive cells were reduced and they were distributed in restricted cartilage layer (Fig 2). No CC- and DS-treatment related changes on the BrdU immunoreactivity were detected. Immunohistochemical analysis revealed a significant decrease in the frequency of BrdU-immunoreactive cells in the PC compared to that of the NC ($p < 0.01$). However, the number of these cells was unaffected by treatment with DS or any dose of CC (Table 3).

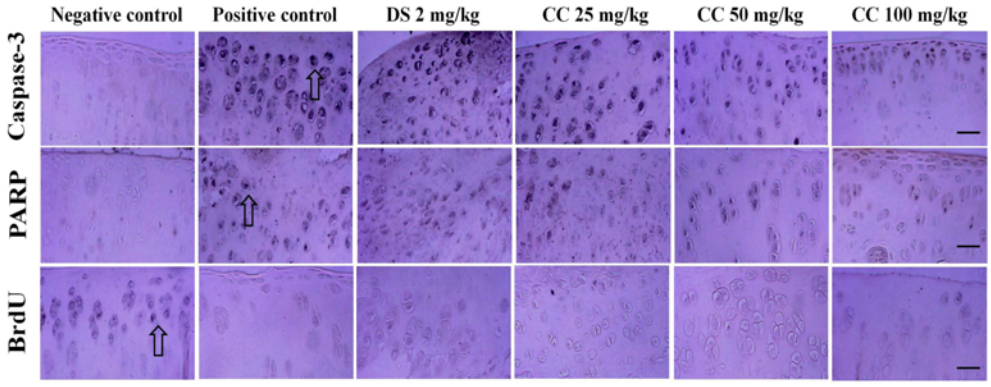

Fig 2. Photomicrographs of active caspase-3, cleaved poly-(ADP-ribose) polymerase (PARP) and 5-bromo-2'-deoxyuridine (BrdU) expression in the femoral articular surface cartilage. The level of caspase-3- and cleaved PARP-positive chondrocytes was significantly reduced in the CC-treated cartilage compared with the positive control. In contrast, compared to positive control, the level of BrdU immnoreactivity was unaffected by treatment with DS or any dose of CC. Open arrows indicate cells positive for the marker. scale bar = 80 μm. 100 ×, avidin-biotin-peroxidase complex stain. DS: Diclofenac sodium, CC: *Cinnamomum cassia* Blume.

Table 3. Analyses of immunoreactive cells in osteoarthritic knees

Group	Immunoreactive cells†					
	Caspase-3		*Poly*-(ADP-ribose) polymerase		5-Bromo-2'-Deoxyuridine	
	Femur	Tibia	Femur	Tibia	Femur	Tibia
Negative control	6.75 ± 5.01	6.38 ± 3.85	4.63 ± 3.29	5.75 ± 3.73	45.50 ± 9.49	40.88 ± 8.43
Positive control	76.63 ± 9.43[a]	73.63 ± 11.26[a]	76.25 ± 9.21[a]	67.25 ± 7.03[a]	7.88 ± 3.56[a]	8.00 ± 4.24[a]
DS 2 mg/kg	74.13 ± 9.45[a]	72.63 ± 8.18[a]	71.13 ± 10.38[a]	62.88 ± 13.59[a]	8.63 ± 4.03[a]	8.13 ± 3.52[a]
CC 25 mg/kg	63.68 ± 9.90[ab]	61.00 ± 10.07[ab]	65.63 ± 7.17[ac]	58.38 ± 7.54[ac]	8.00 ± 1.77[a]	8.25 ± 3.54[a]
CC 50 mg/kg	55.75 ± 9.21[ab]	57.63 ± 9.86[ab]	51.75 ± 7.63[ab]	51.25 ± 10.55[ab]	8.38 ± 3.93[a]	8.63 ± 3.25[a]
CC 100 mg/kg	31.00 ± 11.03[ab]	49.63 ± 6.44[ab]	32.38 ± 13.75[ab]	26.75 ± 9.35[ab]	8.25 ± 2.96[a]	8.50 ± 2.96[a]

Mean ± SD (n = 8). †Number (%) of immunoreactive cells in 100 chondrocytes (1 field of view for each animal, n = 8 fields of view). [a]$p < 0.01$ *vs* negative control; [b]$p < 0.01$, [c]$p < 0.05$ *vs* positive control. DS: Diclofenac sodium, CC: *Cinnamomum cassia* Blume

Discussion

OA is the most common form of arthritis and is a primary cause of disability. A number of natural substances have been reported to exert a favourable influence on the course and symptomatic relief of OA (26). Presently, the ACLT + MMx joints showed pathological changes of classical OA, a symptom which includes cartilage degradation, subchondral bone sclerosis, and cleft and irregular bony trabeculum (11). In this study, the first degenerative changes were found in the matrix and chondrocytes of the superficial zone of the articular cartilage, as has also been reported in human (13) and other experimental OA animal models (9). These changes consisted of a loss of superficial chondrocytes resulting in empty lacunae, while those that remained had a more enlarged appearance and resembled chondrocytes from deeper zones (31). These changes often occurred within the first 3-6 weeks after surgery and are the potential basic cause of the previously-observed changes in cartilage mechanical function (30). A subsequent 12-week treatment with CC or DS induced a significant and dose-dependent decrease in the severity of OA such as loss of cartilage matrix proteoglycans and increase of Mankin score for the histological grading of cartilage degeneration. No marked changes were detected in the thicknesses of knee joint after joint capsule exposure, indicative of the absence of hyperplasia of chondrocytes induced by treatment of CC or DS, respectively. A previous study reported that the fibrosis occurring in OA from chronic inflammatory processes limits joint motion and, therefore, joint stiffness is one of the major symptoms of OA, a stage which is typically reached around 3 months after the inducement of OA (29). We observed that the increases in the maximum extension angle resulting from knee instability were markedly reduced in CC groups compared to that in the PC group. The symptomatic relief from OA induced by CC was paralleled by a similar, or more favorable, reduction in overall degeneration score compared with that achieved using 2 mg/kg of DS. The reparative effects of CC are considered to originate from its anti-inflammatory properties, which have previously been described (17,21). More specifically, these beneficial effects may be due to its interleukin-1α regulatory activity and inhibitory actions against kinin formation and arachidonic acid cascade (17,19).

Each chondrocyte is a functional unit of cartilage that is responsible for the synthesis and degradation of the extracellular matrix in its immediate vicinity (18). Understanding the interactions that promote chondrocyte apoptosis and cartilage hypocellularity is essential for the development of appropriately targeted therapies for the inhibition of chondrocyte apoptosis and the treatment of OA. Apoptosis of chondrocytes together with increases of caspase-3 and PARP have been implicated in the pathogenesis of OA (8). The protease caspase-3 is most critical in the apoptotic process as it is required for the degradation of chromosomes into nucleosomal fragments (3). It also promotes the activation of other effector caspases, such as caspase 8 and 9, and leads to cell death by cleaving vital intracellular proteins such as α-fodrin and PARP; the latter inhibits caspase-activated gene expression (5). Previous study has suggested that caspase-dependent chondrocyte apoptosis also occurs in ACLT-induced OA, and that moderate impact exercise in rats is associated with the decreased severity of chondral lesions and apoptotic events (7). Expression of activated caspase-3 and concomitantly cleaved PARP was present in our rat ACLT + MMx model at a rate 10-13 times higher than that in non-arthritic sham control with the significant increase dependent upon the treatment and severity of degenerative lesions. This is in accordance with previous observations using ACLT rat models (32). Although DS did not affect the increase in caspase-3 and PARP, cells reactive to these apoptotic markers were decreased by treatment with all doses of CC. CC therefore protect against the apoptosis of chondrocytes in the surface articular cartilage, which appears to be one of their main mechanisms of action. In previous report, DS was found to be chondroprotective, although this effect was only prominent and statistically significant at high doses and after prolonged use (16). Further studies using other doses and durations are required to elucidate the nature of this drug's effect on carti-

lage metabolism.

The integrity of the cartilage matrix depends on the equilibrium between anabolic synthesis and degenerative processes (12). Immunohistochemical assessment of BrdU has proven to be one of the preferable ways for the quantification of cell proliferation in histological sections (26). In the present study, a similar pattern of immunoreactive chondrocyte distribution on the articular surface was found in the CC- and DS-treatment, as seen in the PC, together with little or no staining for BrdU uptake. This is evidence that none of the dosages of CC increased chondrocyte proliferation or caused any significant increase in BrdU uptake in the marginal zones or in chondrocyte number in the weight-bearing areas. These results suggest that CC does not exert anabolic activities following pathological changes to the structure and function of the cartilage tissue.

Management of OA includes conventional pharmacological treatments consisting primarily of the administration of NSAIDs and physiotherapy. While these medications often relieve symptoms, they are not ideal therapeutic agents and do not prevent or delay the progression of OA. NSAIDs, in particular, can cause serious side effects, including peptic ulcers and hepatic or renal failure (28). CC is commonly used in traditional Chinese medicine for treating dyspepsia, gastritis, blood circulation disturbances, and inflammatory disease (17,24). Among its main active components, cinnamaldehyde, cinnamic acid and eugenol have anti-inflammatory and anti-pyretic activities (33). Although the exact chemical compound responsible for the anti-OA effect of CC remains the subject of speculation, experimental evidence obtained in the present study demonstrates that CC can reduce the severity of progression of OA by inhibiting cartilage matrix degradation in osteoarthritic rat knees.

Based on these results, we conclude that CC has a therapeutic potential to exert a chondroprotective action on OA possibly through the anti-apoptotic and anti-inflammatory properties. In addition, CC 25 mg/kg appears to have similar effects to that of DS 2 mg/kg. More detailed studies to identify the active compounds within CC responsible for these effects and further long-term studies with other relevant models will be necessary.

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골관절염 랫드 모델에서 계피의 연골보호 효과

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요 약 : 본 연구는 내측반월판 절제와 전십자인대 단열로 유발된 랫드 골관절염 모델을 이용하여 계피 (*Cinnamomum cassia* Blume, 肉桂) 추출물의 연골손상 방지에 대한 효과를 평가하였다. 골관절염유발 랫드 48마리를 군당 8마리씩 6군으로 음성 대조군과 골관절염 대조군, 체중당 diclofenac 2 mg 투여군, 계피 추출물 25 mg과 50 mg, 100 mg 투여군으로 각각 분류하여 수술 1주 후부터 12주동안 투여하였다. 연골 소실과 관절의 불안정성은 계피 추출물과 diclofenac 투여군이 골관절염 대조군과 비교할 때 유의하게 감소하였다($p < 0.05$). 병리조직학적 평가에서 연골의 퇴행은 계피 추출물 투여량에 따라 용량 의존적으로 개선됨이 확인되었다($p < 0.01$). 계피 추출물 투여군에서 관절구조 퇴행성 변화의 감소와 Safranin-O 염색 정도의 용량 의존적인 증가를 보여 연골 퇴행이 억제됨을 확인하였다. Diclofenac이 골관절염 진행에 있어 활성화된 caspase-3와 절단된 poly(ADP-ribose) 중합효소에 유도된 세포자멸사 표지율 증가에 별 다른 영향을 주지 않았지만 계피 추출물 투여군에서는 이들 세포자멸 표지자에 대한 반응세포는 유의하게 감소되었다($p < 0.05$). 그러나, diclofenac과 계피 추출물 투여군은 5-bromo-2-deoxyuridine의 섭취에는 영향을 주지 않았다. 이러한 결과에서 계피추출물이 항염활성과 항세포 자멸 활성을 통해 관절연골에 보호 효과가 있음을 보였다.

주요어 : 계피, 골관절염, 세포자멸사, Caspase-3