In Vitro and *In Vivo* Modulation of Cartilage Degradation by a Standardized *Centella asiatica* Fraction

ANITA HARTOG,*,†,[1] H. FRISO SMIT,* PETER M. VAN DER KRAAN,‡ MAARTEN A. HOIJER,* AND JOHAN GARSSEN*,†

*Danone Research, Centre for Specialised Nutrition, 6704 PH Wageningen, The Netherlands; †Department of Pharmacology & Pathophysiology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, 3584 CA Utrecht, The Netherlands; and ‡Department of Experimental Rheumatology & Advanced Therapeutics, Radboud University Medical Centre, 6525 GA Nijmegen, The Netherlands

Osteoarthritis (OA) is a degenerative joint disease in which focal cartilage destruction is one of the primary features. The present study aims to evaluate the effect of a *Centella asiatica* fraction on *in vitro* and *in vivo* cartilage degradation. Bovine cartilage explants and bovine chondrocytes cultured in alginate were stimulated with IL-1β in the presence or absence of different concentrations (2, 5 and 10 μg/ml) of a standardized *Centella asiatica* triterpenes (CAT) fraction. The CAT fraction inhibited the IL-1β-induced proteoglycan (PG) release and nitric oxide (NO) production by cartilage explants in a dose-dependent manner. The IL-1β-induced reduction in PG synthesis and proliferation of chondrocytes cultured in alginate were counteracted by the CAT fraction at a concentration of 10 μg/ml. In a zymosan-induced acute arthritis model, the CAT fraction inhibited PG depletion without modulating joint swelling and inflammatory cell infiltration. In conclusion, the present study demonstrated for the first time that the tested *Centella asiatica* fraction was able to inhibit the zymosan-induced cartilage degradation *in vivo* without affecting the zymosan-induced inflammatory cell infiltration and joint swelling. The *in vitro* data indicate that the cartilage protective activity might at least partially be induced by the inhibition of NO production. The overall results indicate a possible disease modifying osteoarthritic activity of the *Centella asiatica* fraction. Exp Biol Med 234:617–623, 2009

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[1] To whom correspondence should be addressed at Danone Research, P.O. Box 7005, 6700 CA Wageningen, The Netherlands. E-mail: anita.hartog@danone.com

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Introduction

Osteoarthritis (OA) is one of the most common joint diseases and an important cause of physical disability in elderly (1). It is characterized by degeneration of articular cartilage, limited intra-articular inflammation with synovitis, and changes in periarticular and subchondral bone (2). OA is considered to be caused by an imbalance between anabolic and catabolic processes in the joint leading to a progressive destruction of the tissue, resulting in extensive cartilage damage (3). The biological changes that occur in OA cartilage affect both major matrix components: proteoglycans (PG) and type II collagen.

Pro-inflammatory cytokines, such as IL-1 and TNF-α, play a pivotal role in the initiation and development of OA. Cytokines originating from the inflamed synovial membranes, as well as from the chondrocytes, inhibit cartilage PG synthesis and promote catabolism (4, 5). Especially important in this regard is IL-1β, a cytokine initiating a number of events leading to cartilage destruction. IL-1β promotes cartilage destruction by inducing the production of matrix metalloproteinases (6, 7) and oxygen-derived free radicals including nitric oxide (NO) (8, 9), which initiates matrix degradation and causes chondrocyte apoptosis. Sabotaging the IL-1β-induced processes is thought to be protective for the cartilage and could serve as a target for the development of new disease modifying osteoarthritic drugs (DMOADs) (10).

Pharmacological management of OA is primarily focused on the relief of clinical signs and symptoms. Analgesics and non-steroidal anti-inflammatory drugs

(NSAIDs) represent the mainstay of this treatment (11). NSAIDs bring about a decrease of pain and stiffness. However, alterations of the underlying cartilage changes by the use of these traditional drugs are under debate (12). Identification of DMOADs, which reverse slowdown or stabilize the pathologic changes in OA, is an important research object. A number of such new agents have been developed (13) and are shown to have a cartilage protective potential. These drugs are expected to provide a more long-term symptomatic relief compared to the traditional treatment.

In OA cartilage chondrocytes display phenotypic changes. These cells have been indicated to display a dedifferentiated phenotype by the production of type I and III collagen (14, 15). *In vitro* culturing of chondrocytes can result in comparable changes including a transition to a fibroblastic cell shape (16). *Centella asiatica* (L.) Urb. (Apiaceae) (CA) has been traditionally used for the treatment of different types of diseases in various parts of the world including Eastern Asia, China and India (17). More recent experimental investigations showed stimulatory effects of extracts, triterpenes fractions or isolated active components of CA, on collagen, glycosaminoglycan and fibronectin production by fibroblasts (*in vitro*). Whereas positive effects on wound healing were shown *in vivo* (18–22). In view of the biological properties of the CA preparations in combination with the fibroblast-like phenotype of at least a part of the OA chondrocytes, the effect of a CA fraction on articular chondrocytes and arthritis was studied.

In the present study the *in vitro* effects of a standardized *Centella asiatica* triterpenes (CAT) fraction were studied on PG synthesis and degradation, NO production and chondrocyte proliferation, using bovine explants or bovine chondrocytes cultured in alginate. The *in vivo* effect of the standardized CAT fraction on cartilage degradation and joint inflammation was histologically evaluated in a mouse zymosan-induced acute arthritis model.

Materials and Methods

All experimental procedures using laboratory animals were approved by an independent animal experiments committee (DEC Consult, Bilthoven, The Netherlands). The standardized *Centella asiatica* triterpenes (CAT) fraction, containing 42% asiaticoside (w/w) and 55% genins (w/w), was obtained from Cognis (Barcelona, Spain). The microbiological analysis of the CAT fraction revealed that the total count of aerobes (bacteria, yeast, and mould) was very low (<1000 cfu/gram). No *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* or *Escherichia coli* were detectable in 1 gram of the CAT fraction.

Preparation of Test Agents. The CAT fraction was dissolved in 70% ethanol at a concentration of 150 mg/ml. The stock solution was sonicated for 10 minutes and subsequently centrifuged at 13,000 rpm in an Eppendorf centrifuge. The supernatant was further diluted in culture medium to the appropriate test concentrations. Solvent concentrations used in the various assays were included in the experiments as a negative control.

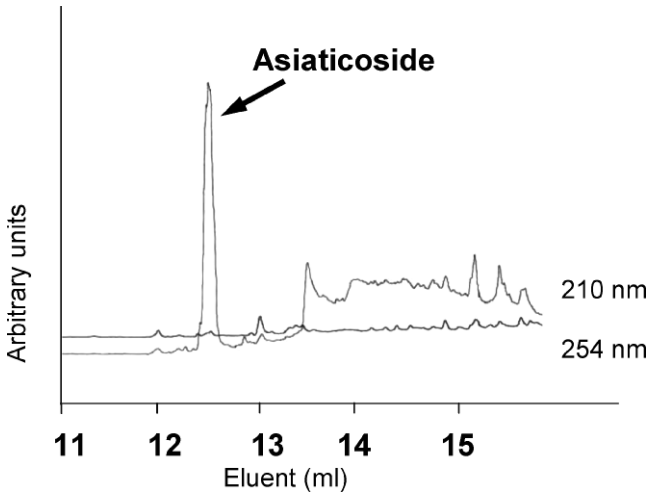

Figure 1. HPLC fingerprints of the ethanolic extract of CAT at the wavelength of 210 and 254 nm.

A HPLC fingerprint analysis was recorded from the supernatant of the CAT solution in 70% ethanol (150 mg/ml), processed as described above. A 50 µl aliquot of the extract was injected on a Superspher 100 RP18 column, 125 × 3 mm (Bischoff YC17841230, Leonberg, Germany), with a precolumn 10 × 3 mm (Bischoff YC17840130). Mobile phases were 0.01% TFA (v/v) in demiwater (A) and 0.01% TFA (v/v) in Acetonitril (B). The gradient elution program started with 2.3 column volumes (CV) A, followed by a linear gradient to 25% B in 9 CVs and after that a linear gradient to 100% B in 3.5 CVs. The column effluent was monitored at UV 210 and 254 nm (Fig. 1).

Bovine Cartilage Explant Isolation. Full thickness bovine articular cartilage slices were aseptically dissected from the metacarpophalangeal joint of young bulls (1–2 years) and placed in PBS (Life Technologies, Merelbeke, Belgium). Cartilage explants were prepared using a 3 mm biopsy punch (Stiefel Laboratories, Coral Gables, Florida, USA) and transferred to 24-wells plates, each well contained 3 randomly picked explants. Explants were incubated in 1 ml culture medium (DMEM/F12 and penicillin 100 U/ml / streptomycin 100 µg/ml; Life Technologies, Merelbeke, Belgium) containing 1% heat-inactivated fetal calf serum (FCS^{hi}) at 37°C in a humidified atmosphere containing 5% CO_2 in air. After a stabilization period of 24 hours, explants were cultured for 7 days in 1 ml culture medium in the presence or absence of the CAT fraction at 2, 5 and 10 µg/ml. After one hour pre-incubation with the different concentrations of the CAT fraction, recombinant human (rh) IL-1β (Tebu-Bio, Heerhugowaard, The Netherlands) was added to a final concentration of 10 ng/ml. At day 4, the culture medium and components were refreshed. Culture supernatant obtained on day 7, was

collected for PG and NO detection and stored at –20°C until analysis.

Bovine Chondrocyte Isolation. Bovine articular chondrocytes were isolated by collagenase digestion; cartilage slices were prepared as described above. The slices were minced and digested overnight at 37°C in 1.5% (w/v) collagenase B (Roche Applied Science, Almere, The Netherlands), in DMEM/F12 (DMEM/F12, containing penicillin 100 U/ml / streptomycin 100 µg/ml; Life Technologies). After removing undigested cartilage pieces by filtration through a 40 µM gauze (Becton Dickinson BV, Breda, The Netherlands), cells were washed twice with a physiological salt (PS) solution after which cell entrapment was performed. Briefly, cells were suspended in 1.2% (w/v) alginate (Keltone® LV, ISP alginates, Sint-Niklaas, Belgium) in PS at a density of 8×10^6 cells/ml and passed by drip through a 22-gauge needle into a 102 mM $CaCl_2$ solution. After 10 minutes of polymerization, beads were rinsed twice in PS. The beads were cultured, four beads per well in a 24-well plate, for 2 weeks in culture medium with 10% FCS^{hi}, at 37°C in a humidified atmosphere containing 5% CO_2 in air; the medium was refreshed twice weekly. After the stabilization period of 2 weeks the beads were cultured in the presence or absence of 2, 5 and 10 µg/ml of the CAT fraction. After one hour pre-incubation with the different concentrations of the CAT fraction, rhIL-1β was added to the beads. The culture medium with the different components was replaced after 3 days. Four and 5 days after start of the incubation with the CAT fraction, the cell proliferation and PG synthesis assays were executed, respectively.

Proteoglycan Measurement. The amount of PG released by the explants into the medium was detected by a modified 1,9-dimethylmethylene blue (DMB) assay (Sigma, Zwijndrecht, The Netherlands) according to Farndale (23). Briefly, 50 µl of 5 times in culture medium diluted culture supernatant was mixed with 100 µl DMB reagents (48 mg/l DMB, 40 mM glycine, 40 mM NaCl, 10 mM HCl, pH 3.0). Absorbance was measured at 595 nm within 5 minutes after addition of the dye. A standard curve of chondroitin 6-sulfate from shark cartilage (Sigma) in the range of 0–35 µg/ml was used for quantification.

Nitric Oxide Assay. NO production was determined by measurement of nitrite released in the culture supernatants of the explants using the Griess reaction (24). Briefly, 100 µl conditioned culture medium or sodium nitrite ($NaNO_2$) standard dilution was mixed with 100 µl Griess reagent (1% sulphanilamide (Sigma), 0.1% naphtylethylenediamide dihydrochloride (Sigma), 10.2% H_3PO_4). Absorbance was measured within 15 minutes at 550 nm.

Proliferation. Proliferation of chondrocytes cultured in alginate was detected by thymidine incorporation. After the cells were stimulated with rhIL-1β and incubated with various concentrations of the CAT fraction for 4 days, ^{3}H-Thymidine (PerkinElmer Life Sciences, Zaventem, Belgium) was added to the culture medium to a final concentration of 5 µCi/ml. After 16 hours the beads were rinsed 5 times in PBS and each bead was dissolved in 500 µl Lumasolve® (PerkinElmer Life Sciences) at 60°C, during 16 hours. Ten ml Lipoluma plus® (PerkinElmer Life Sciences) was added and the incorporated radioactivity was counted (LKB Wallac liquid scintillation counter, PerkinElmer Life Sciences).

Proteoglycan Synthesis. PG synthesis by chondrocytes cultured in alginate was detected by sulphate incorporation. After 5 days of incubation with the different concentrations of the CAT fraction in the presence of rhIL-1β, a two-hour pulse with $^{35}SO_4^{2-}$ ($Na_2{}^{35}SO_4$, 5 µCi/ml; PerkinElmer Life Sciences) was executed. Afterwards the beads were rinsed 5 times in PBS, and each bead was dissolved in 500 µl Lumasolve® during 16 hours at 60°C. Ten ml Lipoluma plus® was added to each dissolved sample and incorporated radioactivity was counted (LKB Wallac liquid scintillation counter, PerkinElmer Life Sciences).

Prompting Zymosan-Induced Arthritis. Female C57Bl/6 mice (Charles River, Maastricht, The Netherlands), aged 14 weeks at the start of the experiment, were acclimatized to the animal housing one week prior to the start of the experiment. All animals had free access to a standard rodent diet and tap water. CAT (0.3 mg/mouse/day) was administered daily for 11 days through gavage. Tap water (vehicle) was applied by gavage in the same volume, 200 µl, to control mice. At day 7, 6 µl zymosan (3% suspension in PBS) was injected into the joint cavity of the right knee to induce arthritis. The left knee joint served as an internal control. The injections were performed by highly trained personnel. At the training stage the intra-articular location of the injection was confirmed by the injection of dye. A "butterfly" distribution of dye in the knee joint confirms even intra-articular distribution. Only personnel 100% successful with this procedure performed experimental intra-articular injections.

Joint inflammation was detected 24 hours after zymosan injection. At day 11, 4 days after zymosan injection, mice were bled under general anesthesia and sacrificed. The knees were processed for histological analysis.

Assessment of Joint Inflammation. Joint inflammation (swelling) was measured 1 day after zymosan injection by 99mTc-pertechnetate (99mTc) uptake in the knee joints (25, 26). Briefly, 10 µCi 99mTc in 0.2 ml saline was injected subcutaneously in the scruff of the neck. After 15 minutes the accumulation of the isotope in the knee due to increased blood flow and edema was determined by external gamma counting. The severity of inflammation was expressed as the ratio of the 99mTc uptake in the inflamed knee over its non-inflamed counterpart.

Histological Processing and Analysis of Knee Joint. Knee joints were dissected, fixed, decalcified, dehydrated and embedded in paraffin (27). Standard frontal sections of 7 µM were prepared and semi-serial sections were stained with Safranin O and counterstained with Fast Green for cartilage PG measurements. Detection of cartilage

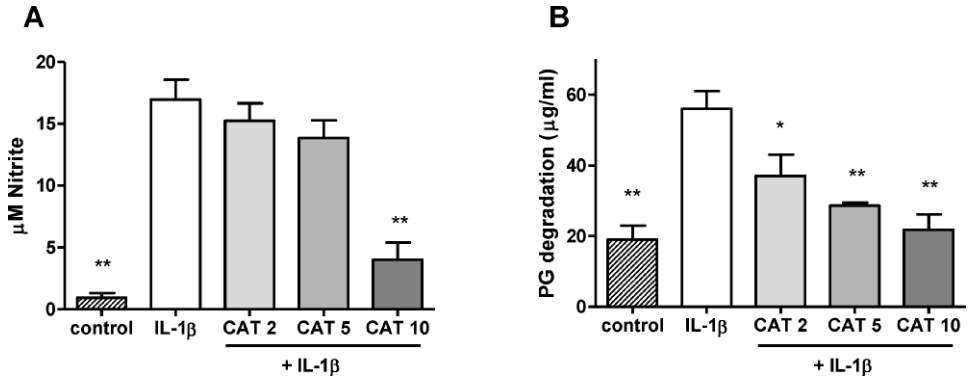

Figure 2. Concentration dependent effects of CAT on rhIL-1β (10 ng/ml) induced NO_2 production as a measure for NO production (A) and PG release (B) by bovine cartilage explants. Values are expressed as the mean ± SD of at least three independent experiments. Significant differences to the IL-1β group were indicated by * $P < 0.05$ and ** $P < 0.01$.

PG depletion was carried out on the patella. The mean score of the sections of each animal was calculated. Cartilage PG depletion was visualized by a diminished staining of the matrix and quantified by Zeiss image analysis software (KS300 version 3.0, Carl Zeiss, Sliedrecht, The Netherlands), as described previously (28). The average value of the non-injected knees was calculated and subtracted from the average of each zymosan-injected knee, resulting in a change of PG content. A corresponding group of sections was stained with heamatoxylin and eosin (HE) for the detection and evaluation of inflammation. Inflammation was scored by influx of inflammatory cells in the joint cavity and synovium. A score of 0 indicated no cell influx; 1 to 4 was scored according to the degree of cell infiltration.

Statistical Analysis. NO production, proliferation and PG synthesis and degradation, of explants or chondrocytes cultured in alginate were compared to the rhIL-1β-stimulated condition using one-way ANOVA. The overall significance of differences for all calculations was tested using the post hoc Dunnett's test. The joint swelling, PG content and cell infiltration of the zymosan-injected, CAT supplemented animals were compared to the zymosan-injected sham supplemented condition by Student's *t* test. *P* values < 0.05 were considered statistically significant.

Results

The *in vitro* effect of CAT on rhIL-1β-induced NO production and PG degradation was detected on bovine cartilage explants. The *in vitro* effect of CAT on rhIL-1β-reduced proliferation and PG synthesis was detected in bovine chondrocytes cultured in alginate beads. The zymosan-induced arthritis model was used to study the *in vivo* effect of CAT on the zymosan-induced joint inflammation (99mTc-pertechnetate uptake and zymosan-induced inflammatory cell infiltration) and cartilage PG depletion.

Nitric Oxide Production by Cartilage Explants. To determine whether CAT alters the rhIL-1β-induced NO release by bovine cartilage explants, the nitrite concentration in the culture supernatant at day 7 was analyzed. RhIL-1β in the absence of test agents increased the NO production from 0.92 μM to 16.92 μM ($P < 0.01$). CAT dose-dependently inhibited the NO production (Fig. 2A). At a concentration of 10 μg/ml, CAT inhibited the rhIL-1β-induced NO production significantly to 4.0 μM ($P < 0.001$).

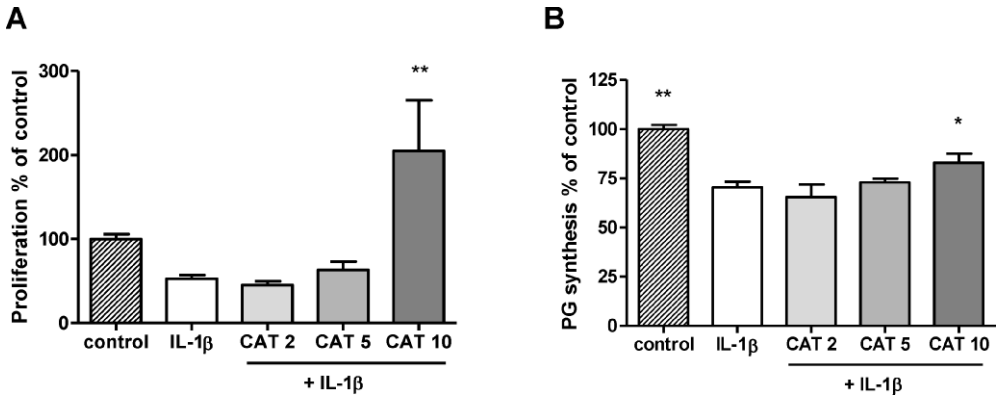

Figure 3. Effects of CAT on the proliferation (A) and PG synthesis (B) by bovine chondrocytes cultured in alginate, in the presence of rhIL-1β (10 ng/ml). Values are expressed as the mean of the % of the control ± SD of three independent experiments. Significant differences to the IL-1β group were indicated by * $P < 0.05$ and ** $P < 0.01$.

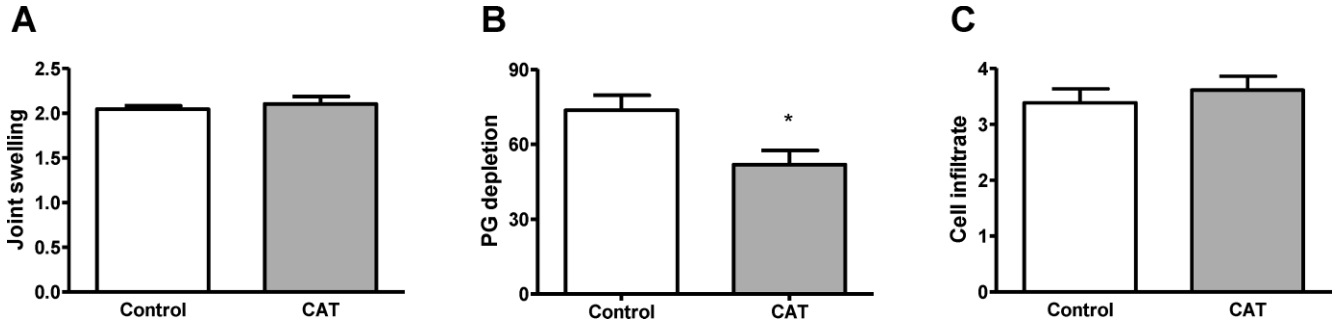

Figure 4. Vehicle (Control) and CAT (0.3 mg/mouse/day) were orally administered for 11 days, and at day 7 the right joint was injected with zymosan. Joint swelling was detected by 99mTc-pertechnetate (99mTc) uptake measurements at 24 hours after the injection and was depicted as the ratio of the inflamed knee above the non-injected knee (A). At day 11 the knee joints were isolated and processed for histology. PG depletion was quantified by computer analysis. The results were expressed in arbitrary units expressing the change in inversed color intensity (B). Cell infiltration was scored at a scale from 0 (no cells) to 4 (severe cell influx in joint cavity and synovium) (C). Results are shown as means ± SEM, $n = 6$. Significant differences to the corresponding zymosan-injected control group were indicated by * $P < 0.05$.

Proteoglycan Degradation by Cartilage Explants. The effects of increasing doses of CAT on rhIL-1β-induced PG release by bovine cartilage explants were established by analyzing the PG concentration in the supernatant at day 7. RhIL-1β in the absence of test agents increased the PG release from 19 μg/ml to 56 μg/ml ($P < 0.05$). The different doses of CAT, 2, 5 and 10 μg/ml, inhibited the PG release dose-dependently to 37, 29 and 22 μg/ml, respectively (Fig. 2B).

Proliferation of Chondrocytes in Alginate Beads. The effect of different doses of CAT on proliferation was detected by ^{3}H-Thymidine incorporation in chondrocytes cultured in alginate in the presence of rhIL-1β. The ^{3}H-Thymidine incorporation in the absence of rhIL-1β (control) was set at 100%. All values were expressed as % of the control. RhIL-1β decreased the proliferation from 100.0% to 45.1%. Only the highest concentration of the CAT fraction was able to influence the rhIL-1β-reduced proliferation significantly from 45.1% to 204.8% ($P < 0.01$) (Fig. 3A).

Proteoglycan Synthesis by Chondrocytes in Alginate Beads. The effect of different doses of the CAT fraction on the PG synthesis by chondrocytes cultured in alginate was detected by sulphate incorporation. The sulfate incorporation in the absence of rhIL-1β (control) was set at 100%. All values were expressed as % of the control. RhIL-1β decreased the PG synthesis from 100.0% to 70.5%. Only the highest tested concentration of the CAT fraction (10 μg/ml) was able to stimulate the rhIL-1β-reduced PG synthesis significantly from 70.5% to 83.0% ($P < 0.05$) (Fig. 3B).

Joint Inflammation. To investigate the effect of CAT on zymosan-induced joint swelling in mice, the CAT fraction was administered orally for 11 days. At day 7, zymosan was injected into the right knee joint. Knee swelling was measured after 24 hours by 99mTc-pertechnetate uptake. CAT 0.3 mg/mouse/day did not influence the zymosan-induced joint swelling (Fig. 4A).

Proteoglycan Depletion, *In Vivo*. To investigate the effect of the CAT fraction on zymosan-induced proteoglycan depletion in mice, CAT was administered orally for 11 days. At day 7, zymosan was injected into the right knee joint. At day 11 the joints were processed for histology and semi-serial sections were stained with Safranin O and counterstained with Fast Green. The average of the PG content in the patella of the non-injected knee, 49.9 ± 6.1, was subtracted from the zymosan-injected sham and the CAT fraction (0.3 mg/mouse/day) treated values. No differences were indicated between the proteoglycan content in the non-injected knees of the sham and the CAT treated animals (data not shown). The CAT fraction was able to inhibit the zymosan-induced decrease in PG content in a significant manner from 73.6 ± 6.03 to 51.9 ± 5.6 (Fig. 4B).

Inflammatory Cell Infiltration, *In Vivo*. To investigate the effect of the CAT fraction on zymosan-induced inflammatory cell infiltration in mice, The CAT fraction was administered orally for 11 days. At day 7, zymosan was injected into the right knee joint. At day 11 the joints were processed for histology and semi-serial sections were stained with HE. CAT supplementation had no effect on the zymosan-induced infiltration of inflammatory cells into the joint (Fig. 4C).

Discussion

OA is, in the context of the aging Western population, a serious and highly significant medical problem. It has a major impact on the quality of life for a rapidly increasing number of patients. Therefore identification of new DMOADs, which reverse, slow down or stabilize the pathologic changes, is an important target in OA research.

The present study demonstrated an inhibitory effect of the *Centella asiatica* triterpenes fraction on the zymosan-induced PG degradation in mouse knee joints (*in vivo*). *In vitro*, the CAT fraction was able to inhibit the rhIL-1β-induced decrease in cartilage synthesis and cartilage degradation, indicating a cartilage protective effect. Furthermore, a strong inhibition of the rhIL-1β-induced NO production by bovine chondrocytes was demonstrated. NO

production is known to be elevated in synovial fluid of OA patients (29). Immunostaining of biopsy samples of OA patients showed a high expression of iNOS in cartilage as compared to a low or absent expression of iNOS in the synovial membrane (30). These data suggest that the main source of iNOS-derived NO in the joint is the cartilage. The role of NO in cartilage degradation in OA is comprehensive as reviewed by Vuolteenaho *et al*. (31). NO seems to be a pro-inflammatory and destructive mediator in cartilage, involved in processes leading to chondrocyte death. In addition, NO has been found to regulate various mediators and processes related to the pathogenesis of cartilage degradation. It has been reported to activate metalloproteinases and cyclo-oxygenase, to inhibit collagen and proteoglycan synthesis, and to increase susceptibility to injury by other oxidants. As a consequence of the aforementioned, it is believed that reducing the NO production will reduce the symptoms and also slow the disease progression of OA. This hypothesis is supported by *in vivo* findings in which van de Loo *et al*. demonstrated that, in iNOS gene knockout mice, the zymosan-induced PG loss was markedly ameliorated compared to the wild-type mice (32). Moreover, Pelletier and collaborators demonstrated that L-NIL, a specific inhibitor of iNOS, is able to prevent cartilage degradation in a dog model of OA (9). Therefore, the inhibition of NO, as observed in the present *in vitro* study, might contribute to the cartilage protective effect of the CAT fraction *in vitro* as well as *in vivo*.

The finding that the CAT fraction did not influence the joint swelling in the zymosan-injected joint is in line with a possible activity of the CAT fraction via inhibition of NO production. Van de Loo *et al*. demonstrated that the joint swelling of the zymosan-injected joint of the iNOS knockout mice was equal to the wild-type mice, indicating that NO plays a minor role in oedema (32).

Inhibition of NO production by triterpenes or genins derived from *Centella asiatica* was indicated *in vitro* in macrophages (RAW 264.7) and *in vivo* during gastric ulcer healing in rats (33, 34). A study of Punturee *et al*. showed a condition-dependent increase or decrease of NO production by a *Centella asiatica* extract on mouse macrophages (J774.2) (35). The present data, however, are the first indicating a CA induced inhibition of the NO production by chondrocytes. These studies all show a decrease in iNOS expression or activity. Yun *et al*. showed in the RAW 264.7 cells an inhibition of the lipopolysaccharide (LPS) induced NF-kappaB via suppression of IKK and MAP kinase phosphorylation by asiatic acid (34).

Literature indicates that one of the main constituents of the CAT extract, asiaticoside, by itself is able to modulate wound healing and fibroblast activity (18, 19, 21). The *in vitro* effect of asiaticoside (Apin Chemicals Ltd, Abingdon, Oxon, England) on rhIL-1β-induced NO production and cartilage degradation by bovine cartilage explants was tested using asiaticoside concentrations comparable to the concentrations present in the CAT extract. The asiaticoside, however, was not able to bring about any effect on chondrocytes (data not shown). These results indicate that another constituent or a specific combination of constituents of the extract give rise to the cartilage protective effects seen in this study.

Articular cartilage is exposed to continuous mechanical wear; there is, however, a surprisingly low turnover in cells and extracellular matrix (36, 37). This low turnover could be a reason for the inability of adult cartilage to respond to injuries and subsequently repair lesions. It has been shown that there is an increased, although still very low, proliferative activity in osteoarthritic chondrocytes. Translating the *in vitro* induction of chondrocyte proliferation, as indicated in the present study, to the *in vivo* situation may lead to an increased cartilage repair in patients suffering from OA. However, more research is needed to reveal the *in vivo* effect of the CAT fraction on chondrocyte proliferation and to picture the consequences of increased cell proliferation on cartilage repair.

In conclusion, the present study demonstrated that the tested *Centella asiatica* fraction was able to inhibit the zymosan-induced cartilage degradation *in vivo* without affecting the zymosan-induced inflammatory cell infiltration and joint swelling. The *in vitro* data indicate that this cartilage protective activity might at least partially be brought about by an inhibition of the NO production. The tested *Centella asiatica* fraction pictures to be cartilage protective, indicating a possible disease modifying osteoarthritic activity which could be beneficial for OA patients.

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