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CURCUMIN AND RESVERATROL REDUCE LIPOPOLYSACCHARIDE MEDIATED GLYCOSAMINOGLYCAN RELEASE IN AN EXPLANT MODEL OF CANINE ARTICULAR CARTILAGE

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Purpose: The aim of this study was to determine the effects of co-treatment with the dietary phytochemical curcumin (derived from *Curcuma longa*) and the polyphenolic phytoalexin stilbene resveratrol (found in red grapes, red wine, peanuts and some berries) on glycosaminoglycan (GAG) release from explants of canine articular cartilage challenged with lipopolysaccharide (LPS).
Methods: Samples of canine articular cartilage were harvested from the stifle joints and used to establish an *ex vivo* explant model. The explants were incubated in serum-free DMEM medium containing antibiotics and challenged with LPS at concentrations of 0.25, 0.5, 1.25, 2.5 and 5 μg/ml thus creating a model of joint inflammation. LPS stimulated samples and controls were co-treated with either curcumin or resveratrol, both at 2.5 μM for a period of 5 days. The tissue culture medium and the papain digested cartilage explants were analysed for GAG content by the dimethylmethylene blue (DMMB) assay. LPS mediated GAG release into the culture medium was then expressed as a percentage of the total GAG present in the explants prior to experimentation.
Results: The DMMB assay confirmed the degradative effect of LPS on canine cartilage explants. Challenge with LPS significantly increased GAG release into the culture medium; increasing the concentration of LPS increased GAG release in a dose dependent manner. Co-treatment with 2.5 μM curcumin and 2.5 μM resveratrol antagonized the degradative actions of LPS and decreased GAG release from the explants. GraphPad InStat software was used for statistical analysis of data obtained with LPS at 0.25 μg/ml (One-way ANOVA, Student-Newman-Keuls test). There was a significant difference in GAG release between control and LPS stimulated samples ($P<0.001$). There was a marked difference between the LPS stimulated samples with and without 2.5 μM resveratrol ($P<0.001$). Statistically significant differences were also noted between the LPS stimulated samples with and without 2.5 μM curcumin ($P<0.001$). The results are summarized in Figure 1.

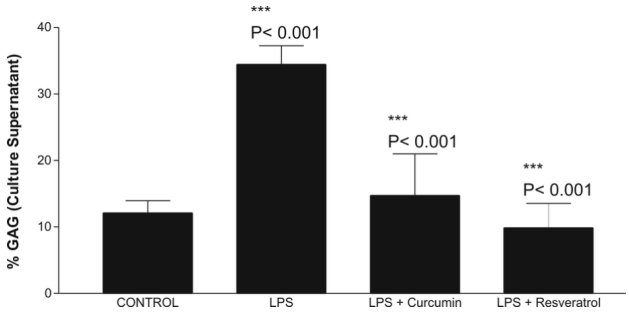

Fig. 1

Conclusions: We have shown for the first time that micromolar concentrations of curcumin and resveratrol down-regulate the catabolic and degradative effects of LPS, particularly its effects on GAG release in explant cultures of canine articular cartilage. Therefore dietary phytochemicals and phytoalexin stilbenes such as curcumin and resveratrol may have nutritional potential as naturally occurring anti-inflammatory agents for treating osteoarthritis (OA). Future experiments will investigate the effects of these compounds on the activity of inflammatory enzymes including matrix metalloproteinases (MMPs) and cyclooxygenase 2 (COX-2) that modulate the catabolic status of articular cartilage.

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THE EFFECTS OF INTRA-ARTICULAR INJECTION OF P38 MAPK INHIBITOR ON MATRIX METALLOPROTEINASE IN CARTILAGE OF EXPERIMENTAL OSTEOARTHRITIS MODEL

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Purpose: The primary aim of this study was to investigate, using an experimental rat model of osteoarthritis(OA), the effect of a selective p38 mitogen activatedprotein kinase inhibitor,SB203580, on the development of structural changes. Additional aims were to assess the effects of the inhibitor on levels of matrixmetalloproteinase 3 (MMP-3) and MMP-13(collagenase 3) in OA cartilage and to explore the relation between the MMP-3,13 expression and the severity of OA.
Methods: OA was induced in 40 SD rats by anterior cruciate ligament transection (ACLT). After surgical, rats with OA were randomly divided into A_D groups: Rats of group A received 0.1 ml intra-articular injection of SB203580 at high oncentration of 100um/L. Each treatment started immediately after surgery, once a week; those in group B were treated under the same condition using SB203580 with low oncentration of 10um/L and those in group C received 0.1ml of intra-articular 0.9% Sodium Chloride injection, animals of group D were not injected as controls after ACLT. The animals were killed 8 weeks after surgery. Macroscopic and histological studies were performed on the cartilage. The levels of MMP-3, 13 in OA cartilage chondrocytes were evaluated by immunohistochemistry and western blotting.
Results: All ACLT knees demonstrated osteoarthritic changes. Cartilage degradation in the control group was significantly severer than that in the experimental group both on the macroscopic grading scale and on Mankin's grading scale ($P<0.05$). Immunohistochemical study showed that in the experimental group MMP-3, 13 was predominantly expressed in the superficial and upper intermediate layers of cartilage, and the amount of MMP-3, 13 in the experimental group was also lower than that in control group($P<0.05$). In western blotting, the amount of MMP-3, 13 was reduced by the treatment of the inhibitor. The protein levels of MMP-3 and MMP-13 in cartilage of inhibitor injection groups were significantly lower than those of Sodium Chloride group and untreated group. There was no significant difference in MMP-3 and MMP-13 expression between the different concentration inhibitor injection groups. No significant difference in MMP-3 and MMP-13 expression in cartilage was found between Sodium Chloride group and control group.
Conclusions: This study demonstrates that, in vivo, SB203580, a selective inhibitor of p38MAPK, can partially decrease the development of some of the structural changes in the early phases of experimental OA and significantly reduces the severity of cartilage degradation. This effect was associated with a reduction in the level of MMP-3,13 in OA cartilage, which probably explains the action of the drug and thus may be a potential drug for the treatment of OA.