Chondroprotective Effects of Resveratrol on Matrix Metalloproteinase-13 Conditioned Articular Cartilage

*Chondroprotective agents slow the progression of osteoarthritis by promoting cartilage metabolism and decreasing degradative enzymes. Resveratrol, a flavonoid present in red wine and grape skins, has been identified as a potential chondroprotective agent due to its inhibition of NF-kappaB activation. The objective of this study was to determine if resveratrol would protect cartilage against the degradative effects of matrix metalloproteinase-13 (MMP-13) using a cartilage explant/synoviocyte co-culture model. The hypothesis was that resveratrol would increase collagen type IIB (col IIB) expression and decrease proteoglycan loss, prostaglandin E2 (PGE2) production, matrix metalloproteinase (-1, -3, and -13) expression and MMP-13 activity in MMP-13 conditioned cartilage explants co-cultured with synoviocytes.

METHODS:

Explants and synovial membrane were harvested aseptically from the femoropatellar joints of 3 juvenile (1-4 years of age) horses euthanatized for reasons other than sepsis or musculoskeletal problems associated with the femoropatellar joint. Institutional Animal Care and Use committee approval was obtained prior to start of this project. Synoviocyte monolayer cultures were established in the bottom of split-well plates specifically designed for co-culture as previously described. Co-cultures were established by suspending 2 cartilage explants in the medium of each well using low protein-binding polyester membrane inserts (pore size=5µm).

The treatment groups were performed in triplicate and included 1) no treatment 100µM DMSO control, 2) 25 ng/ml equine MMP-13 (Fortier LA, Cornell University) plus 100µM DMSO no treatment control, 3) 25 ng/ml equine MMP-13 plus 0.1 µM, 1.0 µM, 10 µM, or 100 µM resveratrol dissolved in 100µM DMSO. Co-cultures of cartilage explants and synoviocytes were treated for 96 hours with medium exchange at 48 hours. Media was harvested at 48 hours and 96 hours for proteoglycan content, MMP-13 activity, and PGE2 analysis. The cartilage explants and synoviocytes were harvested at 96 hours for RNA isolation and biochemical analysis. Total RNA was isolated from synoviocytes and pulverized cartilage using phenol and guanidine extraction followed by purification on RNeasy spin columns.

Explants and media were analyzed for proteoglycan content using the 1,9-dimethyl-methylene blue dye binding (DMMB) microwell spectrophotometric assay, with results normalized to cartilage DNA (Hoechst method). Media samples were evaluated for latent and endogenous MMP-13 activity using a fluorimetric assay (R&D Systems, Inc., Minneapolis, MN). Media samples were analyzed for PGE2 using an enzyme immunoassay (Cayman Chemical, Ann Arbor, MI). Real-time quantitative RT-PCR analysis (two-step) was performed for equine specific collagen type IIB, aggrecan, MMP-1, MMP-3, MMP-13, and 18S RNA. Real time RT-PCR was performed in duplicate and the results were quantified using the comparative Ct method.

Statistical analysis was performed using a one-way ANOVA to compare values between treatment groups (p<0.05). For ANOVAs with a significant F test value, a Tukey post hoc test was performed.

RESULTS:

Resveratrol at the 10µM concentration caused a significant increase in cartilage proteoglycan content in explants conditioned with MMP-13 (p=0.019) (Fig. 1). Proteoglycan content in the media was not significantly affected by treatment with resveratrol (p=0.46). The 10µM concentration of resveratrol significantly decreased MMP-13 levels in MMP-13 conditioned media after 96 hours of treatment (Fig. 2).

Similarly, treatment with 10µM or 100µM resveratrol significantly reduced PGE2 concentration in the media after 48 hours (p=0.0002) and 96 hours (p=0.006) of treatment (Fig. 3).

DISCUSSION:

Using this co-culture model for osteoarthritis, resveratrol shows promising chondroprotective effects by increasing cartilage proteoglycan content and decreasing media levels of MMP-13 and PGE2. The effects of resveratrol appear to be concentration-dependent, with the 10µM concentration giving the most significant effects. These findings support previous studies that have shown anti-inflammatory properties of resveratrol in chondrocytes and other cell types. Proposed mechanisms for these anti-inflammatory effects include inhibition of NF-kappaB activation by IL-1β and TNF-α, suppression of PGE2 via COX-2 inhibition, blocking reactive oxygen species, and decreasing iNOS expression. Further studies with more animals may be necessary to determine differences in gene expression of col IIB, aggrecan and MMPs.

REFERENCES: