EGCG - A CONSTITUENT OF GREEN TEA - INHIBITS CHONDROCYTE APOPTOSIS

Introduction: Rheumatoid arthritis (RA), a disease of unknown etiology, affects approximately 1% of the world population. This chronic inflammatory disease attacks multiple joints, causing severe disability. Local levels of inflammatory cytokines, such as interleukin-1 and tumor necrosis factor-α (TNF-α), correlate strongly with the induction and perpetuation of the disease. Apoptosis of chondrocytes induced by these cytokines could be related to the pathogenesis and severity of arthritis. It has recently been shown that a polyphenolic fraction isolated from green tea (GTP) can reduce the incidence and severity of arthritis in a mouse model for RA (type-II collagen-induced arthritis). Mice given GTP in their drinking water had significantly lower levels of the pro-inflammatory cytokine TNF-α in the affected joints. Other studies have shown that epigallocatechin-3-gallate (EGCG), the biologically active constituent of GTP, can protect articular chondrocytes from TNF-α induced apoptosis (unpublished). In the present study we investigated how EGCG modulates TNF-α signaling in human articular chondrocytes. We examine the effect of EGCG on TNF-α mediated induction of nitric oxide synthase activity in cultured human articular chondrocytes, as NO is suspected of playing a role in chondrocyte apoptosis. We also examine its effect on the activity of caspase-3, and on the activation of the apoptosis associated transcription factor NF-κB (NF-κB).

Materials and Methods: Primary articular chondrocytes were prepared from cartilage samples obtained from fracture patients with no history of RA. Cells were isolated by Pepsin-Collagenase digestion, plated at 1x10^5 cells per 60mm dish, and serum starved overnight. For NO production and caspase-3 activity studies, the cells were treated for 4 hours with either TNF-α (10ng/ml) or EGCG (5 µg/ml), or both, or were left untreated as controls. They were then incubated with complete medium for 24 hours. NO concentration in the supernatant was determined using a commercially available kit (R&D systems). Cell lysates were prepared for caspase-3 activity assays, and activity was determined using a commercially available kit (BioMol). Protein concentrations were determined by Bradford assay. For electrophoretic mobility shift assays (EMSA), chondrocytes were treated with either TNF-α (10ng/ml) or EGCG (5 µg/ml) or both for 5 minutes. Control cells were left untreated. Nuclear extracts were prepared immediately according to previously published protocols. The 22mer, double stranded oligonucleotide (5'-AGTTGAGGGGACTTTCCCAGGC-3') containing the consensus NF-κB binding site was end labeled with 32P, purified and reacted with 6 µg of nuclear protein extract for 15 minutes at room temperature. EMSA were performed in 6% 0.25x TBE native polyacrylamide gels.

Results: NO production was significantly increased in chondrocytes treated with TNF-α. This increase was inhibited by co-treatment with EGCG. (Fig. 1) Similarly, TNF-α increased caspase-3 activity in chondrocytes by about 4-fold, and again co-treatment with EGCG inhibited caspase-3 activity in chondrocytes. (Fig. 2).

Discussion: Our results indicate that in human articular chondrocytes, TNF-α signals cause apoptosis, preceded by the activation of the transcription factor NF-κB and NO production. Both of these events have been implicated in chondrocyte apoptosis. EGCG - the biologically active constituent of green tea polyphenols can significantly block TNF-α induced activation of nitric oxide synthase and NF-κB in human chondrocytes in vitro. Our results also show that EGCG inhibits the TNF-α induced activation of the cell death effector enzyme caspase-3. These studies indicate a potentially important application for a dietary constituent – EGCG - in protecting articular chondrocytes from inflammatory cytokine-induced apoptosis in RA.

References:

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