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145 ANTI-INFLAMMATORY AND CHONDRO-PROTECTIVE EFFECTS of ROSE HIP POWDER and ITS CONSTITUENT GALACTOLIPIDS GOPO

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Purpose: Clinical studies have shown that rose hip powder (RHP) exerts beneficial effects in OA patients. Thus, substances contained in proprietary RHP should have an effect on parameters involved in disease initiation and progression. Until now only one of its constituents – GOPO – was shown to inhibit the migration of polymorphonuclear leukocytes. In this study various in vitro effects of RHP and GOPO related to inflammatory processes, erosion of cartilage tissue and chondro-protection were evaluated.

Methods: Anti-inflammatory effects were measured in LPS-activated macrophage RAW264.7 cells. Cells were cultured for 4–24 hrs with different doses of RHP or GOPO. Gene expression was quantified by RT-PCR; production of inflammatory mediators like PGE2 was measured by ELISA and nitric oxide determined by the Griess reaction. Substances were tested on primary human chondrocytes (without and with concomitant activation of chondrocytes by IL-1beta) and the expression of anabolic and catabolic genes was monitored by real-time PCR.

Results: Rose hip and GOPO inhibit the production of NO (IC50 816 ± 35 mg/mL and 37.0 ± 8.0 µm/L, respectively). We observed weaker effects on PGE2 production (IC50 594 ± 43 mg/mL and >50.0 µm/L). Expression of inflammatory genes like iNOS, II-1alpha, MMP-9 was significantly reduced by GOPO, but to a lower degree by RHP. In activated chondrocytes, GOPO decreased the expression of catabolic genes (e.g. ADAMTS-4, MMP-13), interleukins or chemokines (II-L-alpha, II-C, CS), whereas the expression of collagen or aggrecan genes was slightly increased. We observed a GOPO-dependent increased expression level of SOX-5 or SOX-9. RHP had similar although weaker effects. On a stoichiometric basis, however, the GOPO contents of RHP does not fully account for the observed biological effects and thus for the presence of additional anti-inflammatory and chondro-protective substances in RHP.

Conclusions: The demonstrated anti-inflammatory effects in vitro are consistent with OA-reducing properties of RHP. In addition, its chondro-protective and cartilage-rebuilding activities are at least partially attributed to GOPO, a constituent galactolipid of RHP.

146 DIFFERENTIAL EFFECTS OF HYALURONAN ON FIBRONECTIN FRAGMENT ACTIONS BETWEEN NORMAL AND RHEUMATOID ARTHRITIS CARTILAGES

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Purpose: This study was aimed to compare the inhibitory effects of hyaluronan (HA) on nitric oxide (NO) production by COOH-terminal heparin-binding fibronectin fragment (HBFN-f) between normal and rheumatoid arthritis (RA) cartilages.

Methods: Articular cartilage slices from RA knee joints or normal hip joints were cultured with HBFN-f with or without pretreatment with 2700 kDa HA. Alternatively, isolated RA chondrocytes in monolayer were incubated with HBFN-f. Secreted NO levels in conditioned media were determined. Induction of inducible nitric oxide synthase (iNOS) was assessed with immunoblotting. Immunofluorescence histochemistry was performed using fluorescein isothiocyanate-conjugated anti-CDDA antibody.

Results: HBFN-f stimulated NO production in a dose-dependent manner in association with iNOS induction in normal and RA cartilages. While CD44 expression was up-regulated in RA cartilage, HA significantly blocked HBFN-f-stimulated NO production in RA cartilage. Compared with normal cartilage, cartilage response to HBFN-f and the blocking effects of HA on HBFN-f action were stronger in RA cartilage.

Conclusions: The present study clearly demonstrated that RA cartilage exhibited stronger response to HBFN-f leading to NO induction. HA binding to the increased CD44 in RA cartilage may effectively protect RA joint destruction by fibronectin fragments like HBFN-f.

147 EXPRESSION OF NOVEL EXTRACELLULAR SULFATASES SULF-1 AND SULF-2 IN NORMAL AND OSTEOARTHRITIC ARTICULAR CARTILAGE

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Purpose: The recently identified novel extracellular sulfatases (Sulf) can modulate the activity and signaling of important growth factors in cartilage, such as BMP, bFGF, Wnt and VEGF. The aim of this study was to analyze expression patterns of Sulf-1 and Sulf-2 in articular cartilage and chondrocytes.

Methods: Sulf-1 and Sulf-2 expression in human articular cartilage from normal donors and patients with osteoarthritis (OA), and in normal and aged mouse joints were analyzed by real-time PCR, immunohistochemistry and western blotting.

Results: Sulf-1 and Sulf-2 mRNA expression in articular cartilage were significantly higher in the OA group (Sulf-1: P = 0.001, Sulf-2: P = 0.019).

Figure 1. Sulf-1 and Sulf-2 mRNA expression in articular cartilage were significantly higher in the OA group (Sulf-1: P = 0.001, Sulf-2: P = 0.019).

Figure 2. The OA middle zone had significantly more Sulf-1 positive cells than the other zones (*: P < 0.01). Sulf-2 expression in normal cartilage was significantly higher than Sulf-1 (P = 0.02).

Results: Sulf-1 and Sulf-2 mRNA expression in older OA donors (n = 8; 49–68 years old; Mankin score: 7–10) was significantly higher than in young donors (n = 8; 19–37 years old; Mankin score: 0–2 points) as determined by real-time PCR (Figure 1). Immunohistochemistry showed that young and old normal samples had only a few Sulf positive cells in the superficial zone and no positive cells in the middle and deep zone. In general, the expression of Sulf-2 appeared more intense than Sulf-1 in normal cartilage. In OA cartilage, many positive cells were detected, especially in chondrocyte clusters. The normal areas had only 18.5% Sulf-1 positive and 31.9% Sulf-2 positive cells in the superficial zone, which was greater than in normal cartilage. On the other hand, OA areas had 75.3% Sulf-1 positive and 73.2% Sulf-2 positive cells. Figure 2 shows quantitative analysis of the zonal distribution of Sulf-1 and Sulf-2 expressing cells in eight normal (17–37 years old) and eight OA donors (43–82 years old). The middle zone in OA cartilage had significantly more positive cells than normal (*P < 0.01). Moreover, the number of Sulf-2 positive cells in superficial and middle zone was greater than Sulf-1 expressing cells (P = 0.02). Western blotting revealed higher levels of Sulf-1 and Sulf-2 protein in OA cartilage as compared to normal tissue. Temporomandibular joints (TM) and knee joints from normal C57Bl/6 J mice (n = 14) showed only few cells were positive for Sulf-1 but Sulf-2 positive cells at 6 months of age. There was a marked increase in Sulf-2 expression at 9 months and in Sulf-1 expression at 12 months, associated with the presence of early OA-like lesions.