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Structural characteristics are crucial to the benefits of guar gum in experimental osteoarthritis

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ABSTRACT

Protein-free guar gum (DGG) was oxidized (DGGOX) or sulfated (DGGSU) by insertion of new groups in C-6 (manose) and C-6 (galactose), for DGGOX and DGGSU, respectively. Rats were subjected to anterior cruciate ligament transection (ACLT) of the knee, joint pain recorded using the articular incapacitation test, and the analgesic effect of intraarticular 100 μg DGG, DGGOX or DGGSU solutions at days 4–7 was evaluated. Other groups received DGG or saline weekly, from days 7 to 70 and joint damage assessed using histology and biochemistry as the chondroitin sulfate (CS) content of cartilage. The molar mass of CS samples was obtained by comparing their relative electrophoretic mobility to standard CS. DGG but not DGGOX or DGGSU significantly inhibited joint pain. DGG significantly reversed the increase in CS, its reduced electrophoretic mobility, and histological changes following ACLT, as compared to vehicle. Structural integrity accounts for DGG benefits in experimental osteoarthritis.

1. Introduction

Osteoarthritis (OA) is a leading cause of disability and chronic pain with increasing prevalence and costs to health care systems. The recognition that inflammatory cells play a major role in OA pathogenesis has led to the concept of an important immunomediated inflammatory component in OA pathogenesis (Berenbaum, 2013). OA treatment still relies on attempts to provide symptom improvement whereas chondroprotective compounds, meaning treatments to alter OA progression and/or outcome, are an unmet need (McAlindon et al., 2014).

Viscosupplementation has been proposed as a term to indicate the recovery of the viscoelastic properties of the synovial fluid after the administration of high molar mass hyaluronic acid solutions or its analogues (Hunter, 2015). Despite a persistent debate on the clinical efficacy of viscosupplementation, current OA treatment guidelines recommend this strategy, particularly to treat patients with knee OA (Bruyère et al., 2014; Hochberg et al., 2012; McAlindon et al., 2014). Systematic reviews and meta-analysis of data on viscosupplementation efficacy are controversial probably because of different selection of articles and a lack of standardization of patient selection and evaluation (Hunter, 2015).

The mechanisms responsible for the viscosupplementation efficacy are yet to be demonstrated. Though some claim that the higher the molar mass of the agent, coupled to the gel state, the better the results, there are no definitive data to prove this assumption (Bannuru et al., 2015). Indeed, high molar mass hylans (around 10^6 g/mol) had similar efficacy, as compared to lower mass compounds ($5–7.5 \times 10^5$ g/mol) in providing pain relief in OA (Pasquali Ronchetti et al., 2001). The sustained clinical relief

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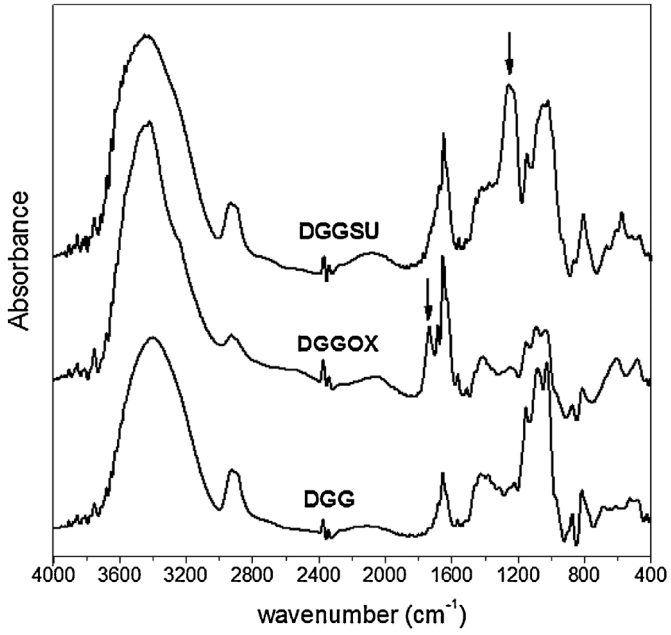

Fig 1. FTIR spectrum of deproteinized guar gum (DGG), oxidized (DGGOX), and sulfated (DGGSU) derivatives.

that may be achieved after a set of 3–5 injections or even a single injection of these compounds may last for up to 8–12 months (Kirwan, 2001) argues against a local rheological effect to explain the therapeutic mechanism. Considering that endogenous hyaluronic acid clearance from the joint fluid is estimated at 20 h and in OA joint this clearance time is even less (Hunter, 2015), the possibility that exogenously administered hyaluronates last less than 1 day in the joint is very likely. Furthermore, the issue of whether viscosupplementation agents display a chondroprotective effect in OA is also yet to be defined.

Guar gum is a highly viscous polysaccharide derived from the seed endosperm of the plant *Cyamopsis tetragonolobus*. It is predominantly composed of a galactomannan with a long central chain of manose residues, joined by type β-(1 → 4) glycosidic links and residues of galactose joined by type α-(1 → 6) links attached to the sides of the mannan central core. This gum was purified by four different methods in order to reduce the impurities, especially protein residues (Cunha, Maciel, Sierakowski, de Paula, & Feitosa, 2007).

Using an experimental OA model in rats, we demonstrated that the intraarticular (i.a.) injection of the purified DGG, meaning a protein-free guar gum derivative, provided analgesia similar to that of Hylan G-F20, a commercially available viscosupplementation agent (Castro et al., 2006). Additionally, the galactomannan provided analgesia when it was given as a viscous or saline solution. That viscous preparation presents a viscosity similar to that of Hylan G-F 20, 110 and 120 Pa s, respectively, but the viscosity of the galactomannan solution was only 3.6 Pa s, at the same shear rate and temperature (Cunha, Castro, Rocha, de Paula, & Feitosa, 2005). Thus, we proposed that viscosupplementation does not depend only on the viscoelastic properties of the compound. Rather, it could derive from a local pharmacological effect yet to be described.

In the present study, through derivatization of the DGG (oxidation or sulfation) we provide further evidence that the biochemical structure, rather than the viscosity, accounts to explain the clinical effects of this polysaccharide in experimental OA. Additionally, the data show that i.a. administration of a DGG solution prevents joint damage in an OA model.

2. Experimental

2.1. Materials

2.1.1. Chemical materials

Guar gum was supplied by Sigma-Aldrich Brasil Ltda. and subjected to purification, as follows: The gum was purified free of protein (DGG), according to the physical and Fehling procedures, described elsewhere, with a mannose/galactose ratio of 1.61 (Cunha et al., 2005). Chlorosulfonic acid and TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) were supplied by Sigma Corporation. Chondroitin 4-sulfate (C4S), chondroitin 6-sulfate (C6S), and heparan sulfate were purchased from Sigma-Aldrich Brasil Ltda.

2.1.2. Animals

Male Wistar rats (180–200 g) from our own animal facilities were used throughout the experiments. Animals were housed in cages (6/cage) in temperature-controlled rooms with a 12 h light/dark cycle with free access to water and food. At the start of any experiments, rats were 2.5 months of age. All efforts were made to minimize animal suffering and the number of animals used. The experimental protocol was approved by our local ethics committee (protocol number 113/07) that followed the guidelines of the Brazilian College of Animal Experimentation (COBEA).

2.2. Methods

2.2.1. Sulfation of guar gum

The protein-free DGG was sulfated using methodology earlier reported by Moura Neto, Maciel, Cunha, de Paula, and Feitosa (2011) and recently used for sulfation of a galactomannan from *Dimorphandra gardneriana* (Moura Neto et al., 2014). Some modifications were performed, as follows: a mass of 3 g of DGG was dissolved in 225 mL of formamide overnight, followed by the addition of 60 mL pyridine. The solution was cooled to 4 °C and 18 mL of chlorosulfonic acid (CSA) was dropped over a period of 3 h. The proportion SR/SU (molar ratio of sulfation reagent to sugar unit) was maintained in 14.6. After 12 h at 13 °C, the solution was neutralized with $NaHCO_3$, dialyzed against water, the solid precipitated, washed many times with ethanol, and filtered. The dry product was denoted as DGGSU.

2.2.2. Oxidation of guar gum

The protein-free DGG was oxidized following the method reported by Sierakowski, Milas, Desbrières, and Rinaudo (2000) and Cunha et al. (2007), as follows: a mass of DGG (2 g) was dissolved in 1 L distilled water under stirring overnight. The solution was cooled in an ice bath, sodium hypochlorite (9 mL) was added, and pH adjusted to 9.2. TEMPO reagent (18.4 mg) and NaBr (160 mg) were added. The oxidation proceeded at constant pH, adjusted with NaOH. Borohydride (50 mL) was included to stop the reaction. The pH was decreased to 7, the gum precipitated with EtOH, and after 12 h in refrigerator, filtered, and washed with EtOH. DGGOX is the designation of the product.

2.2.3. Characterization of guar gum derivatives

The derivatives were characterized by FT-IR, NMR, rheology, and static light scattering. The percentage of nitrogen and sulfur was determined by elemental microanalysis in a Carlo Erba EA 1108 micro analyzer and the N% related to protein content through the conversion factor of 5.87 (Azero & Andrade, 2002). The degree of sulfation was calculated from the equation proposed by Melo, Feitosa, Freitas, and de Paula (2002). The degree of oxidation was determined by potentiometer titration with NaOH 0.1 mol/L. The FT-IR spectra were recorded in solid state using KBr pellet with a