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Hydrogel of Guar Gum in Experimental Osteoarthritis in Rats

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Summary: Commercial Guar Gum, a galactomannan polysaccharide, was purified by the use of two subsequent methods, including precipitation with Fehling solution in order to eliminate protein impurities. The protein content (3.6%) was totally removed. Glutaraldehyde cross-linked gels were prepared with purified (GGP) and unpurified gum (GGU). The viscosity of the gels is similar to that of Hylan G-F 20, a commercial substitute of hyaluronic acid, used in viscosupplementation in human osteoarthritis. Guar Gums (gel and solution) were injected intra-articularly into the knee joints of rats subjected to experimental OA and the effect in hypernociception and cells influx measured. GGU promoted hypernociception and cell influx in naïve rats. GGP was innocuous to naïve rats and inhibited hypernociception, both as a gel or solution, to the same extent as Hylan G-F20. GGP promotes analgesia in OA due to its carbohydrate component.

Keywords: analgesia; guar galactomannan; hydrogel; osteoarthritis; viscosupplementation

Introduction

Osteoarthritis (OA) is a leading cause of disability in the elderly. Viscosupplementation is a term proposed to indicate the recovery of the viscoelastic properties of the synovial fluid after the administration of high molecular mass hyaluronic acid (HA) solutions or its analogues.[1] HA is a large linear glycosaminoglycan composed of repeating disaccharide units of glucuronic acid and N-acetyl-glucosamine linked via the 1–4 positions of the sugar rings (Figure 1a). It is a component of both articular cartilage and synovial fluid. Hylan G-F 20 is one of the mostly used commercial preparations in viscosupplementation,[2] due to its high viscosity. Though there are claims that the analgesia promoted by viscosupplementation is due to the rheologic characteristics of the compounds, this mechanism is yet to be clarified.

Guar galactomannan (GG) is a water-soluble polysaccharide derived from the endosperms of *Cyamopsis tetragonolobus*.[3] It has a linear backbone of β-1,4-linked mannose units with α-1,6-linked galactose units randomly attached as side chains[3] (Figure 1b). In addition to being a potentially innocuous component, GG is a low cost, easily available biodegradable substance.[4] A hydrogel of unpurified GG, obtained using glutaraldehyde as a cross linking agent, was characterized.[5] It was tested in rats subjected to anterior cruciate ligament transection (ACLT) in the right knee, as an experimental OA model.[6] This paper reports the chemical and rheological properties of GG in solution and as a gel. The effect of the intra-articular (i.art.) injection of these materials and Hylan G-F 20 into the rat knee joint was studied.

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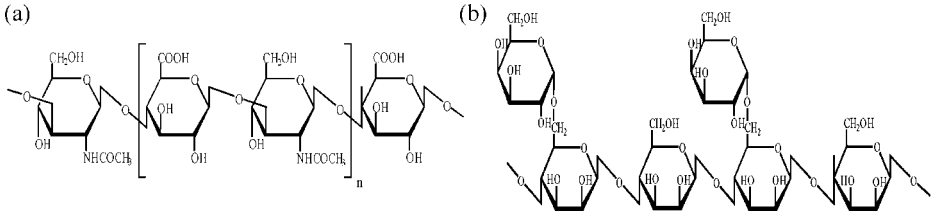

Figure 1.
Structures of (a) hyaluronic acid and (b) guar gum.

Materials and Methods

GG purification: Guar gum from Sigma (denoted as GGU) was purified using the method proposed by Cunha et al.[7] denoted in previous publication as Method 4, and briefly described here. Gum was treated with boiling, aqueous 80% ethanol and the obtained slurry collected on a glass filter and washed with ethanol and acetone. This material was allowed to hydrate, and then stirred and centrifuged. The supernatant was precipitated in acetone. After redissolution in water the solution was centrifuged. The supernatant was precipitated with ethanol and the solid collected on a glass filter and washed with ethanol and acetone. The guar sample was subjected to additional purification procedure by precipitation with Fehling solution. The obtained sample was denoted as GGP.

Intrinsic viscosity determination: Aqueous solutions of GGU and GGP at 0.07% (w/v) concentration were filtrated and the flow time determined at 25 °C in an Ubberlohde CANNON Model I-71 viscosimeter. Intrinsic viscosity $[\eta]$ was obtained with five gum solution dilutions. Average viscosity molar masses (Mv) were calculated by the use of Mark-Houwink equation, $[\eta] = 3.8 \times 10^{-4}\ Mv^{0.72}$, in dL/g.[8]

Elemental microanalysis: Nitrogen content was determined in CARLO ERBA EA1108 microanalyzer and related to protein content by the conversion factor 5.87.[9]

Cross linking of gum: GGU and GGP were cross linked as described by Cunha et al.,[5] briefly reported here. Acidified aqueous dispersions of gums (0.8% w/v, pH 2) were mixed with 25% (w/v) aqueous solution of glutaraldehyde. The system was put to react until the desired viscosity was reached. The obtained hydrogels were dialyzed initially against water and then against phosphate/NaCl (pH = 7.3, 0.2M/0.15M) buffer. The residual amount of glutaraldehyde (GA) was determined by UV as described by Cunha et al.[5]

Rheology of Guar Gels and Hylan G-F 20: Viscosity measurements were carried out at 36 °C, on a BROOKFIELD D V-III rheometer (shear rate of 0.04 s^{-1}). Guar gel and solution (0.8% in a buffer/saline) were analyzed. Hylan G-F 20 was used as commercially available (Synvisc®). Viscoelasticity measurements were carried out at 36 °C, on an AR 555 TA Instruments rheometer at frequency range 0.1–10 rad/s.

Scanning Electron Microscopy: Guar solution (0.8% in water) and GelGG were frozen in liquid N_2, freeze-dried and the morphology investigated using Phillips X-L 30 Scanning Electron Microscope (SEM).

Gait disturbance: The rat knee incapacitation test, as described previously[10] was used. After guar gum injection (100 μg in physiological solution) into knee joint, Wistar rats (groups of six) were put to walk on a steel rotary drum (30 cm wide × 50 cm diameter) covered with a fine-mesh wire screen, which rotates at 3 rpm. Metal gaiters were wrapped around both hind paws and the right paw connected to a microcomputer. The paw elevation time (PET) is the time that the animal walks failing to touch the cylinder with the injected hindpaw, during a 10 min period, which is proportional to the gait disturbance that reflects joint hypernociception.[6] PET

for untreated animals was measured for control.

Analysis of cell influx: After sacrifice, the synovial cavity of the knee joints was washed with 0.4 mL saline containing EDTA (10 mM). Total and differential cell counting was assessed, using a Neubauer chamber and stained smears, respectively.

Osteoarthritis model: Anterior cruciate ligament transection (ACLT) either secondary to joint trauma in humans or experimentally induced is a well-known secondary cause of knee Osteoarthritis. Rats subjected to ACLT, under anesthesia, received one single intra-articular injection of Guar Gum (gel and solution) and Hylan G-F 20, used as a gold-standard comparator, (100 μg polysaccharide/50 μL) 4 days after ACLT. Sham-operated and naïve animals were controls. The effect of GG or Hylan preparations in joint hypernociception was measured at day 7 after ACLT using the increase in PET (see above).

Results and Discussion

Guar Gum from Sigma (GGU) contains 3.6% of protein (Table 1). The purification procedure that gives GGP sample eliminated all protein present. The viscosity average molar mass (Mv) of the galactomannan decreases after purification. It could be due to the cleavage in polysaccharide-protein complex bonding and subsequent release of protein. Depolymerization of polysaccharide during Fehling purification step could also occurs, caused by the high medium alkalinity.

Purified and unpurified Guar Gum samples were cross-linked with glutaraldehyde. The kinetics of GGP and GGU cross linking was followed by the variation of the mixture viscosity with reaction time

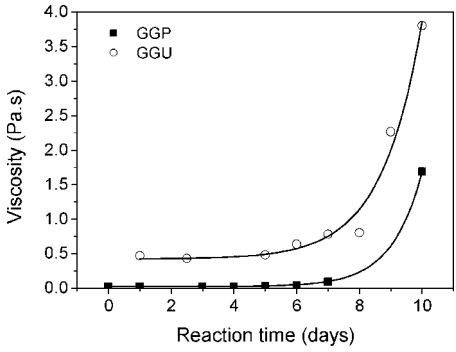

Figure 2.
Cross linking kinetics of Guar Gum samples (GGU and GGP) with glutaraldehyde.

(Figure 2). The GGP has initial viscosity lower than that of GGU, due to the molar mass difference. A significant viscosity increase was observed after the sixth day of reaction. The reactions were stopped after 16 days, when the apparent viscosity was similar to that of Hylan G-F 20.

The viscosity of the GG gels was similar to that of Hylan G-F 20 and approximately 40 times the solution viscosity at the same polymer concentration and shear rate (Table 2). The amount of residual glutaraldehyde in gel is very low (0.04 mg/kg of rat) and considered not toxic.[11]

The Figure 3 shows that the viscoelastic parameters (G′ and G″) of Hylan G-F 20 were higher than that of GelGGP values. In the frequency range of 0.1-10 rad/s, healthy synovial fluid behaves as a viscoelastic fluid and, in the frequency range corresponding to the motion of the knee joint in walking and in running (approximately 2-8 rad/s), G′ is higher than G″.[12] In this frequency range, the GelGGP and the Hylan G-F 20 presented a behavior similar to the synovial fluid, with G′ higher than G″ though Hylan G-F 20 is more elastic than guar hydrogel.

Table 1.
Protein Content, [η] and Mv of the guar samples.

Gum	Protein Content (%)	[η] (dL/g)	Mv (g/mol)
GGU	3.6	12.8	1.9×10^6
GGP	0.0	5.2	5.5×10^5

Table 2.
Apparent viscosity of materials at 36°C and shear rate 0.04 s^{-1}.

	GGU	GelGGP	GelGGU	Hylan G-F 20
Viscosity (Pa.s)	3.6	116	116	116

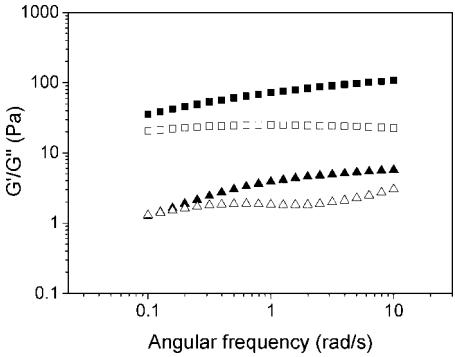

Figure 3.
Viscoelasticity curve of Hylan G-F 20 (■ G′, □ G″) and GelGGP (▲ G′, △ G″) at 36 °C.

The purified Guar gum gel (GelGGP) and Hylan G-F 20 showed different morphologies (Figure 4). The GelGGP has a more homogeneous and interconnected structure. The heterogeneous morphology of Hylan is probably due to the product formulation, a mixture of hyaluronate solution and gel. Compact and porous regions are observed.

Effect of Guar Gum Purification in the Naïve Knee-Joint

The intra-articular injection into the rat knee joint of unpurified Guar Gum (GGU) solution promoted an increase in PET, as compared to saline (Figure 5a). A slight though significant increase in cell influx, as compared to saline, was also observed ($p < 0.001$, using one-way ANOVA), (Figure 5b). The purified Guar gum, i.e. the protein free preparation, neither altered PET nor cell counts in the joints, as compared to saline. Thus, the protein content present in the original unpurified GG account for the inflammation observed. The contribution of viscosity or molar mass difference on gait disturbance could not be evaluated because of the lower values for the purified solution in comparison with those for GGU (Table 1). The gelation of unpurified gum induced still more pain and cell influx. Injection of the purified GG, both as a gel or solution, did not promote cell influx or hypernociception. These results indicate that the hypernociception observed in naïve joints depends on the protein contamination rather than on the viscosity of the liquid injected into the naïve joints.

Effect of Guar Gum in Joint Hypernocicetion in Experimental Knee Osteoarthritis

The hypernociception secondary to ACLT in rats was evaluated in 4 groups, as follows: NAIVE = animals with no manipulation except for saline i.art. injection; SHAM = animals subjected to the surgical procedure without ACLT; NT = animals subjected to ACLT that received i.art. saline. Animals subjected to ACLT received GGP (gel and solution) or Hylan G-F 20. The i.art. injection of GGP in both forms (gel and solution) and of Hylan G-F 20 significantly reduced the pain, as compared to vehicle (NT) ($^{***}p = 0{,}001$), leading PET values to return to baseline (Figure 6). Considering the great viscosity difference between gel materials (Hylan G-H 20 and Guar Gel) and Guar solution

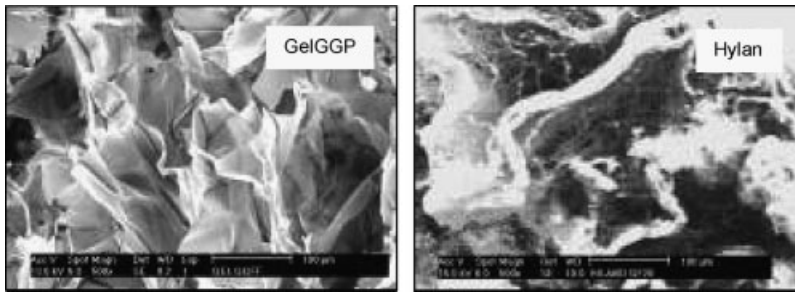

Figure 4.
SEM of GelGGP and Hylan G-F 20. Amplification 500×.

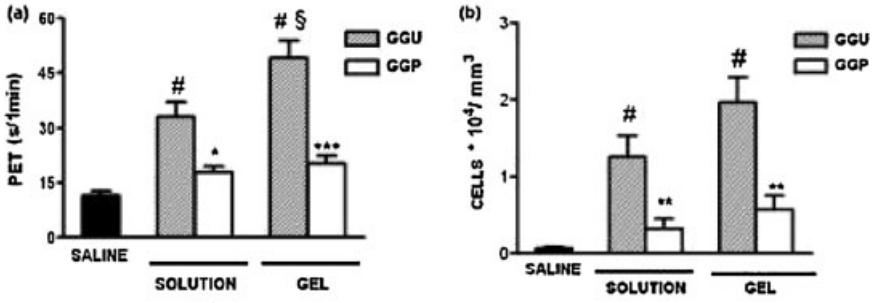

Figure 5.
Effect of GGU and GGP in: (a) Gait disturbance; (b) Cell influx. $^{\#}p < 0{,}001$ *vs* Saline; $^{*}p < 0{,}01$; $^{**}p < 0{,}001$ *vs* GGU at the same dose; $^{\S}p < 0{,}001$ *vs* 400 μg GGU solution.

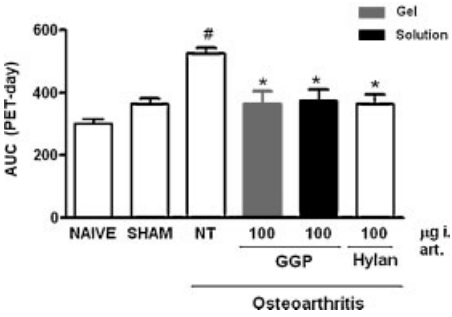

Figure 6.
Effect of Guar Gum and Hylan G-F 20 in joint hypernociception in experimental OA. $^{*}p < 0{,}001$ *vs* NT; $\#p < 0{,}001$ compared to the SHAM group.

(Table 2) with no reflection on PET difference, the role of high viscosity as responsible for the analgesia in viscosupplementation is clearly questioned.[13] In addition, the differences in the chain charge (guar is neutral and Hylan G-F 20 is negative) and in the chemical structure between the two polysaccharides rules out these parameters as responsible for the analgesia.

Conclusion

The analgesia promoted by the injection of the purified GG solution was similar to that of GelGG, which has a 40 times higher viscosity. Both GGP preparations displayed a similar analgesia, as compared to Hylan G-F 20. The great viscosity difference between gel materials (Hylan G-F 20 and guar) and guar solution with no reflection on PET values indicates that the analgesia provided by the guar gum can not be attributed to the rheological properties of these materials. Rather, pharmacological activity of the carbohydrate components, possibly coupling to specific receptors present in the joint would account for the analgesia provided. These issues are being the subject of further investigation.

[1] J. Kirwan, *The Knee* **2001**, *8*, 93.
[2] M. Wobig, G. Bach, P. Beks, A. Dickhut, A. Runzheimer, G. Schwieger, G. Vettere, E. Balazs, *Clin. Ther.* **1999**, *21*, 1546.
[3] Y. Cheng, R. Prud'homme, J. Chik, D. Rau, *Macromolecules* **2002**, *35*, 10155.
[4] K. Soppirnath, T. Aminabhavi, *Eur. J. of Pharm. and Biopharm.* **2002**, *53*, 87.
[5] P. L. R. Cunha, R. C. M. de Paula, J. P. A. Feitosa, *Int J Biol Macrom* **2005**, *37*, 99.
[6] R. R. Castro, F. Cunha, F. Silva, Jr, F. A. C. Rocha, *Osteoarthritis Cart* **2006**, *14*, 769.
[7] P. L. R. Cunha, R. R. Castro, R. C. M. de Paula, F. A. C. Rocha, J. P. A. Feitosa, *Int J Biol Macrom* **2007**, *41*, 324.
[8] G. Robinson, S. Ross-Murphy, E. Morris, *Carbohydr. Res* **1982**, *107*, 17.
[9] E. G. Azero, C. T. Andrade, *Polym. Test.* **2002**, *21*, 551.
[10] C. A. Tonussi, S. H. Ferreira, *Pain* **1992**, *48*, 421.
[11] physchem.ox.ac.uk/MSDS/GL/glutaric_dialdehyde.html, accessed at November 11, 2007 and updated on January 22, 2005.
[12] S. Pelletier, P. Hubert, E. Payan, P. Marchal, L. Choplin, E. Dellacherie, *J. Biomed. Mater. Res.* **2001**, *54*, 102.
[13] R. Barbucci, S. Lamponi, A. Borzacchiello, L. Ambrosio, M. Fini, P. Torricelli, R. Giardino, *Biomaterials* **2002**, *23*, 4503.

