



Effectiveness of a novel hydrolyzed collagen formulation in treating patients with symptomatic knee osteoarthritis: a multicentric retrospective clinical study

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Abstract

Purpose Knee osteoarthritis (OA) is a musculoskeletal disorder that may have a heavy impact on the patients' quality of life. Intra-articular collagen injection may be a safe adjuvant. Recently, CHondroGrid (CG), a hydrolyzed (< 3 kDa) bovine collagen injectable formulation, has been placed on the market. The aim of this study was to investigate the safety and performance profile of CG.

Methods Patients affected by Kellgren Lawrence grade 1 to 4 knee OA and BMI < 30 were treated by administering three CG injections of 2 ml (4 mg) each (at 15 days and 45 days from the first one, respectively) and were followed up for six months after the last administration. Clinical records were retrospectively assessed to compare VAS, Lequesne and WOMAC total, pain, stiffness, and physical function scores collected at baseline and 15, 45, and 225 days after the first injection.

Results At the last follow-up, 70 patients (37 men and 33 women, aged 57.1 ± 14.5 years) treated with CG showed a 50% reduction in their median Lequesne score, a 50% reduction in their VAS score at rest and moving, and a $\geq 50\%$ reduction for all other scores under consideration.

Conclusions CG may be a safe and effective adjuvant in the treatment of symptomatic knee OA.

Keywords CHondroGrid · Intra-articular injection · Non-pharmacological therapy · Knee osteoarthritis

Introduction

Osteoarthritis (OA) is the most common musculoskeletal disorder affecting both small and large diarthrodial joints [1]. The hand, hip, and knee are the joints being most affected [2]. Knee OA has a prevalence of about 10% in men and 13% in women aged > 60 years [3]. Economic and social costs of knee OA are significantly high, and its impact on the patient quality of life may be devastating [4, 5]. Treatment aiming at regenerating

cartilage is only beginning to be investigated in the clinical setting [6, 7], and current approaches for less severe case not calling for surgery still aim to treat symptoms and improve the patient quality of life. Pharmacological treatment of symptomatic knee OA includes oral or topical administration of non-steroidal anti-inflammatory drugs (NSAIDs) or intra-articular injection of corticosteroids [8]. Yet, effective topical application of NSAIDs may call for multiple daily applications, and NSAIDs and corticosteroid injections may display several side effects while being short-lasting [9]. Consequently, extensive pre-clinical and clinical research is being carried out on non-pharmacological interventions [2, 10–12] including manual and physical therapies [13] and viscosupplementation [14]. OA-affected joints exhibit progressive degradation of the extracellular matrix (ECM), due to the combined action of matrix metalloproteinases (MMPs), a disintegrin, and a metalloproteinase with thrombospondin motifs (ADAMTS) [1] that are activated by inflammation mediators, such as IL-1 α , TNF- β , and IL-6 [15]. As degradation affects even collagen [16], exogenous administration of collagen has been investigated as a possible symptomatic adjuvant or stand-alone treatment. In vitro exposure of animal or human synovial and cartilage cells to collagen

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preparations, with different degree of hydrolyzation or polymerization, increased their production of hyaluronic acid and reduced the release of some inflammatory mediators [17–19]. The injection of a Gly-X-Y collagenic tripeptide in joints of animals suffering from experimentally induced OA led to a significant reduction in cartilage degradation and to a concomitant significant increase in the number of chondrocytes producing type II collagen [20]. Clinical investigations concerning collagen intra-articular injections are still limited to two randomized clinical trials on few patients. Furuzawa-Carballeda et al. ([21], $N = 27$; 2012, $N = 19$) compared 12 bi-weekly intra-articular injections of 2-ml (13.8 mg) pepsin-treated porcine polymerized, type I collagen vs. as many placebo injections and observed a statistically significant improvement on VAS, WOMAC, and Lequesne indexes. Martin Martin and colleagues ([22], $N = 29$) compared five 4-ml (concentration unknown) 300 kDa type I hydrolyzed porcine collagen intra-articular injections at a one week interval versus as many injections of 2.5-ml (25 mg) sodium hyaluronate and observed no significant differences in VAS and Lequesne scores at three or six months after treatment. Recently, a novel injectable collagen formulation (CHondroGrid (CG), Biotech S.p.A., Arcugnano, Italy) consisting in bovine hydrolyzed < 3 kDa type I collagen has been placed on the market for the treatment of OA symptoms. It must be administered through three consecutive injections of 2 ml (4 mg) each, over 45 days. A preclinical in vitro study [23] showed that when human chondrocytes were exposed to CG for 28 days, they deposited more type II and less type I collagen and had a higher Bern score than unexposed cells. These results led the authors to speculate that CG may prompt chondrocytes to produce hyaline cartilage and to counterbalance the normal reparative response that would lead, instead, to fibrous tissue formation [23]. The same authors also presented the results of a preliminary study, involving the retrospective assessment of the records of 20 patients affected by 1 to 4 Kellgren-Lawrence knee OA who received three 4-mg/2-ml CG injections with the same protocol described in the present study. Six months after the third injection, these patients experienced a > 70% reduction of WOMAC total score and all WOMAC sub-scores, together with a 55% and 44% decrease of VAS at moving and Lequesne scores, respectively [23]. The present study extends the results of the above-mentioned pilot investigation, involving a larger number of subjects collected at four different clinical centers and allowing to have a better understanding of CG safety and performance for symptomatic treatment of knee OA.

Materials and methods

Clinical records were selected among those of patients suffering from knee OA and referred to the Knee Surgery and Sports Traumatology Unit, Humanitas Clinical and Research Center, Milan, Italy; to the Policlinico San Marco Hospital, Mestre,

Italy; to the Maria Cecilia Hospital, Cotignola, Ravenna, Italy; and to Bressanone/Brixen Hospital, Bressanone, Bolzano, Italy. Patients included in this retrospective study were (a) suffering from Kellgren Lawrence (KL) grade 1 to 4 knee OA and (b) underwent treatment with CG according to its indications for use. Other inclusion criteria were an age > 18 years; the lack of any disease that may interfere with the assessment of knee symptom and function indexes, as fibromyalgia, Reiter's syndrome, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, lymphoma, sarcoidosis, amyloidosis, chondrocalcinosis. Patients were excluded if having a BMI ≥ 30 or presenting any clinical sign of knee infection as well as any skin disorder/issue (such as topical infections, cuts, bruises, pimples) affecting the knee to be treated. Patients were also excluded if having taken corticosteroids or being subjected to intra-articular injections of corticosteroids, hyaluronate, or other formulations, including NSAIDs, over the 3 months preceding treatment with CG, as well as if they had been subjected to surgery over the previous six months. All patients were not presenting any of the following: cancer, HIV, HCV, drug, or alcohol abuse. Clinical records were analyzed only if reporting the following: complete anamnestic and demographic patient's data; knee anteroposterior (AP) weight-bearing X-rays collected before treatment; OA severity grade measured by the KL score; the Lequesne indexes; the WOMAC scores; and the subjective VAS scores, measured at rest and moving, recorded just before the first (baseline/T0), the second (T1), and the third (T2) CG injection as well as six months after the third CG injection (FUP). All patients provided their informed consent to treatment with CG. No ethical committee approval was sought for this study given its retrospective nature and the use of CG according to its manufacturer's indications for use. The study meets the ethical standard of the journal [24].

Treatment

The patients received three 2-ml (4 mg) CG injections, the first two 15 days apart and the third one 30 days after the second. After confirming the knee skin was healthy, the injection area was disinfected using a povidone-iodine spray (Betadine Spray, Meda Pharma, Milan, Italy). The CG injection was then carried out according to a superolateral approach to the patella. After injecting CG, the needle was removed, and a sterile gauze applied over the injection site.

Statistical analysis

Patients' demographic and characteristics at baseline were described by means of descriptive statistics. To investigate if treatment with CG caused any change among scores collected at the observation time points, distribution of VAS, Lequesne and WOMAC total, pain, stiffness, and physical function scores was first checked for normality using the Shapiro-

Wilk test and, as the distribution was found to be not normal for any of them, scores for each parameter at all different time points were compared using a non-parametric ANOVA Friedman test, followed by pairwise comparisons using the Wilcoxon signed rank test.

Results of parametric tests are provided as mean \pm standard deviation; results of non-parametric tests are provided as medians and the corresponding interquartile ranges (IQRs). All statistical tests were regarded as significant if $p < 0.05$. All statistical calculations were performed using standard statistical software programs (R System, Ver. 3.3.2 with RMS libraries, R Core Team, 2017 [25] or GraphPad Prism v5.00; GraphPad software, La Jolla, CA, USA).

Results

Records were analyzed for 70 patients (37 men and 33 women) with a mean age of 57.1 ± 14.5 (range 22 to 83) years. Descriptive statistics concerning patient characteristics at baseline are provided in Table 1. No patients experienced any complication or side effect following CG injections.

Median values at the different time points for all scores under investigation are reported in Table 2 and shown in Fig. 1 (VAS and Lequesne scores) and Fig. 2 (WOMAC scores). Median VAS values at rest decreased significantly with respect to baseline after the CG injections (T1 vs. T0, $p < 0.001$). After the second injection, a further decrease of VAS at rest was observed (T2 vs. T1, $p < 0.001$); VAS at rest did not change significantly at follow-up (FUP vs. T2, $p = 0.15$). Median VAS values when moving decreased significantly after the first injection (T1 vs. T0, $p < 0.001$), decreased further after the second injection (T2 vs. T1, $p < 0.01$), and remained stable at the six month follow-up (FUP vs. T2, $p = 0.12$). Median Lequesne index decreased significantly after

the first injection (T1 vs. T0, $p < 0.001$), decreased further after the second injection (T2 vs. T1, $p < 0.001$), and was slightly increased at the 6-month follow-up (FUP vs. T2, $p = 0.02$). All four WOMAC score median values were significantly lower at T1 than those at baseline (T1 vs. baseline: pain, $p = 0.006$; stiffness, $p < 0.001$; physical function, $p < 0.001$; total score, $p < 0.001$), and median values at T2 were significantly lower than those at T1 (T2 vs. T1: pain, $p < 0.001$; stiffness, $p < 0.001$; physical function, $p < 0.001$; total score, $p < 0.001$). For all WOMAC scores, median values at the six month follow-up were not significantly different than those at T2 (FUP vs. T2: pain, $p = 0.19$; stiffness, $p = 0.20$; physical function, $p = 0.07$; total score, $p = 0.08$).

Discussion

Knee OA is one of the most common presentations of osteoarthritis and it has high social costs, heavily impacting on the patients' quality of life. Pharmacological treatments alone are effective only for a limited amount of time and show long-term adverse effects. Accordingly, adjuvant non-pharmacological treatments may be of interest, allowing to devise different and possibly more effective combined therapeutic strategies. Studies on intra-articular collagen injections are lacking, and the few available ones provide limited information [21–23, 26], with only a single study on CG by De Luca et al. [23] showing that this < 3 kDa hydrolyzed type I collagen formulation possibly modulates chondrocytes metabolism toward hyaline cartilage regeneration. Results of the present clinical study provide stronger evidence, on a larger number of subjects than previously published [23], that CG is effective in managing symptoms of knee OA in adult patients affected by grade 1 to 4 KL knee OA and having a BMI < 30 . Such findings are consistent with an inflammatory-modulating effect, or even with a pro-regenerative effect on cartilage by CG that should be the subject of further, dedicated investigations.

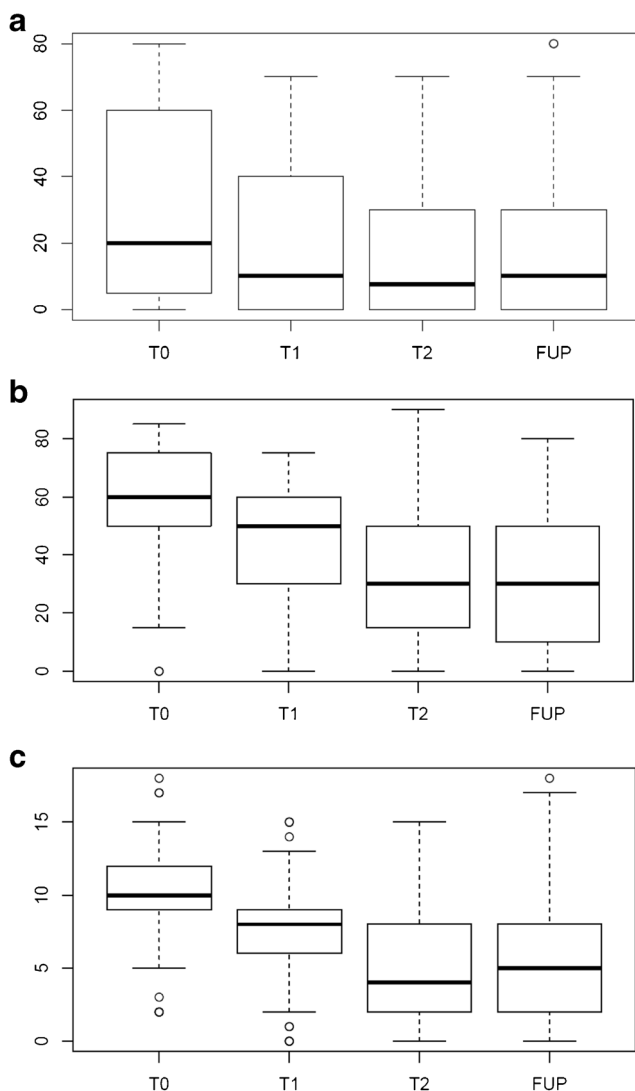
Results of the present study also confirm the findings by De Luca et al. [23] concerning CG safety, showing that CG is well-tolerated as its use is not associated with any significant side effect. When compared to the preliminary results reported by De Luca et al. [23], the findings here reported show that, when considering a larger number of records, CG injections are not as effective in reducing VAS at rest, with a 50% reduction versus the 100% reduction previously reported; further, on a larger number of subjects, it was less effective in modulating the WOMAC stiffness (50% vs. 75% reduction). Such reductions, yet, are still clinically important. Differences concerning all the other scores considered (total WOMAC, WOMAC pain, WOMAC physical function, the Lequesne Index) were minor. Thus, the results of this final retrospective analysis may be regarded as overlapping to those of the

Table 1 Patients' characteristics at baseline

Parameter	Mean \pm SD or yes or no (%/%)
Age (years)	57.1 \pm 14.5
Weight (kg)	73.2 \pm 11.9
Height (cm)	172.2 \pm 7.9
BMI (kg/m ²)	24.6 \pm 2.9
KL score 1,2,3,4 (%)	25 (35.7); 31 (44.3); 11 (15.7); 3 (4.3)
M/F (%)	37/33 (52.8/47.2)
Diabetes Y/N (%)	3/67 (4.5/95.5)
Cardiovascular disorders Y/N (%)	11/59 (15.7/84.3)
Metabolic disorders Y/N (%)	7/63 (10/90)
Concomitant treatment Y/N (%)	14/56 (20/80)

Table 2 Median values at all time points for the parameters under consideration: VAS at rest and when moving, Lequesne index, WOMAC subscores concerning pain, stiffness and physical function, and total WOMAC score. Time is provided as mean \pm SD; all other values are provided as median (IQR)

	Baseline (before first injection)	T1 (15 days after first injection)	T2 (30 days after second injection)	FUP (about 6 months after third injection)	FUP vs baseline (%)
Time (days)	N/A	14.7 \pm 2.4	27.0 \pm 10.3	186.4 \pm 35.5	N/A
VAS at rest	20 (53.8)	10 (40.0)	7.5 (30.0)	10 (30.0)	- 50.0%
VAS when moving	60 (25.0)	50 (30.0)	30 (31.3)	30 (40.0)	- 50.0%
Lequesne Index	10 (3.0)	8 (3.0)	4 (5.8)	5 (5.8)	- 50.0%
WOMAC (pain)	5 (4.0)	3 (4.0)	1 (2.0)	1 (2.0)	- 80.0%
WOMAC (stiffness)	2 (2.0)	2 (1.0)	1 (2.0)	1 (2.0)	- 50.0%
WOMAC (physical function)	16 (12.8)	11 (11.0)	5 (6.8)	6 (7)	- 62.5%
WOMAC (total)	23.5 (17.0)	16 (17.0)	7 (11.0)	8.5 (11.8)	- 63.8%

**Fig. 1** a–c VAS at rest (a), when moving (b) and Lequesne (c) scores. Median VAS at rest and moving decreased significantly after both CG injections and it remained stable at follow-up. Median Lequesne decreased significantly after the first injection, decreased further after the second injection, and slightly increased at the 6-month follow-up

preliminary one [23], confirming CG is effective in managing knee OA symptoms.

Results concerning the VAS, Lequesne, and WOMAC scores show a significant reduction of both parameters following CG injection, consistently with results published by Furuzawa et al. [21, 26] concerning all three indexes and by Martin Martin et al. [22] concerning the Lequesne and VAS scores. As a whole, the results of the present study as well as those of the preliminary analysis by De Luca et al. [23], together with those by Furuzawa-Carballeda et al. [21, 26], and by Martin Martin et al. [22], consistently show that intra-articular collagen injections may be beneficial in alleviating symptoms of knee OA and consequently improve physical function. Overall, results of these studies indirectly confirm that intra-articular collagen injections may be regarded as a viable approach to manage knee OA symptoms, again calling for further investigations on the underlying mechanism through which collagen might exert such beneficial effects.

The reduction of WOMAC and Lequesne scores observed at six months in the present study was greater than that observed by Furuzawa-Carballeda et al. [21, 26] and was achieved through less injections (three instead of five). The reduction in the Lequesne index observed in the present study was also greater than that observed by Martin Martin [22]. Noteworthy, Furuzawa included patients having any degree of knee OA, and Martin Martin included only KL two and three patients; accordingly, their results and those of the present study should be compared with caution. Still, differences in the outcome between this study and those by Furuzawa et al. [21, 26] and by Martin Martin et al. [22] may be also partially explained by the different collagen formulations being used in the above-mentioned studies. In fact, while CG consists of hydrolyzed (< 3 kDa) type I collagen, the formulation used by Furuzawa [21, 26] was a γ -irradiated mixture of atelopectidic porcine type I collagen and polyvinylpyrrolidone (PVP), and the one used by Martin Martin [22] consisted of pure type I collagen with a much greater (300 kDa)

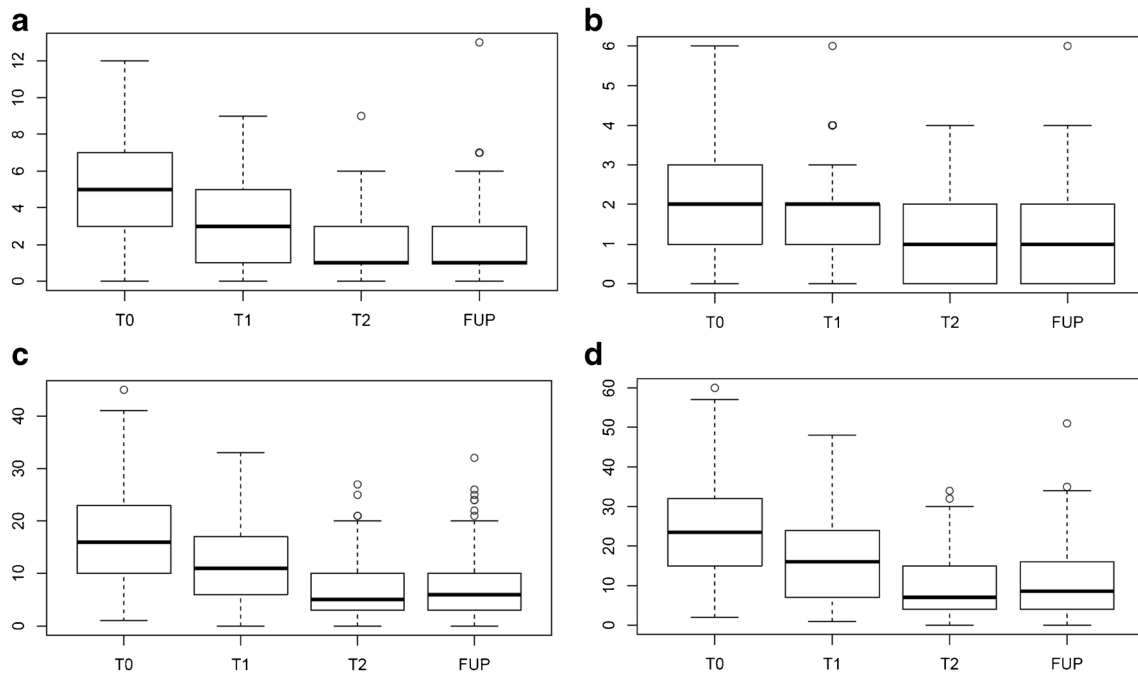


Fig. 2 a–d WOMAC pain (a), stiffness (b), physical function (c), subscores and total WOMAC score (d). For all scores, median values at T1 are significantly lower than those at baseline, and median values at T2

are significantly lower than those at T1. For all scores, median values at the 6-month follow-up were not significantly different than that at T2

molecular weight. How such differences may account for distinct effects should be the subject of targeted *in vitro* studies, and the clinical effects of their intra-articular injection should be compared through prospective, controlled randomized clinical trials.

Main limitations of the present study relate to the fact that it was retrospective in nature and patients were followed up for a relatively short period. While promising, in fact, results of the present study do not provide any indication concerning long-term effects of intra-articular injections of CG as well as do not allow any comparison with other available non-pharmacological treatments, like viscosupplementation using hyaluronate or other agents; furthermore, the limited sample size of this study did not allow to observe any correlation between the clinical outcome and the degree of patients' OA; therefore, long-term prospective, and possibly comparative studies should be carried out to further investigate if intra-articular CG injection may be more beneficial than other non-pharmacological treatments already available in the clinical practice.

Conclusions

Results of the present study indicate that CG intra-articular injection is a safe and effective short-term adjuvant in the treatment of symptomatic knee OA. Further, controlled prospective studies should be carried out to investigate CG effectiveness in specific knee OA patient subgroups as well as to

compare it with other non-pharmacological treatments already available in the clinical practice.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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