Intra-articular injection of hydrolyzed collagen to treat symptoms of knee osteoarthritis. A functional *in vitro* investigation and a pilot retrospective clinical study.

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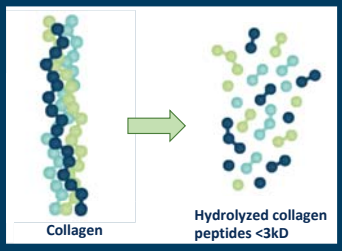
BACKGROUND

- Knee osteoarthritis (OA) is a musculoskeletal disorder that may have a heavy impact on the patients' quality of life. Available pharmacological treatments are effective only on a short-term basis, and their prolonged use has several adverse effects.
- OA-affected joints exhibit a complex range of structural, tissue, cellular and biochemical changes. Inflammation mediators are expressed and in turn activate enzymes degrading the extracellular matrix (ECM) including collagen in the cartilage.
- Collagen is a fibrillar protein that may hierarchically auto-assemble in more complex fibers that are at the same time resistant and elastic, a feature observed both in cartilage, where collagen is the main constituent of the amorphous matrix, and in all specialized connective or supporting tissues, such as tendons, ligaments and bone.
- ChondroGrid (CG; Bioteck S.p.A., Arcugnano, Italy) is a medical device made of lyophilized low molecular weight hydrolyzed bovine collagen for intra- and peri-articular injection.
- Intra-articular collagen injection may be a safe adjuvant, yet evidence concerning its use is limited.

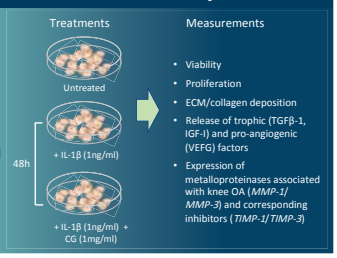


ChondroGrid (CG)

Aims of the study: To assess ① the effect of a hydrolyzed (< 3kDa) bovine collagen formulation, ChondroGrid (CG) on isolated human chondrocytes, as well as ② CG's safety/efficacy profile in intra-articular injections administered to patients suffering from knee osteoarthritis (OA)

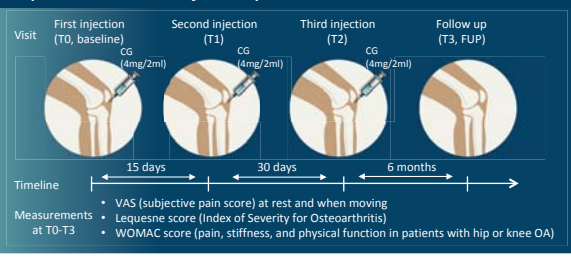
① *In vitro* study on human articular chondrocytes

Human articular chondrocytes were isolated by enzymatic digestion from portions of articular cartilage obtained during total hip arthroplasty from 5 donors affected by grade IV osteoarthritis.



② Retrospective clinical study in OA patients

- Included patients: Kellgren Lawrence (KL) grade 1 - 4 knee OA, treated with CG according to indications for use, 18-75 years old, lack of any potentially interfering disease
- Excluded patients: BMI ≥ 30, knee infection or skin disorder affecting the knee, use of corticosteroids (CS) or intra-articular injections over the three months preceding treatment with CG, surgery over the previous 6 months
- Clinical records of 20 patients that received three 4mg/2ml CG injections as described below were retrospectively assessed



RESULTS

CG concentrations up to 1mg/ml showed no impact on human chondrocyte viability and proliferation

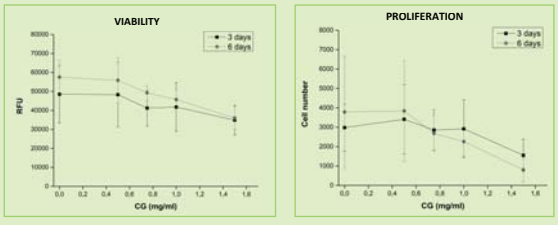


Figure 1. Human chondrocytes viability and proliferation at different CG concentrations. CG 1.5 mg/ml induces a significant decrease in viability ($p<0.001$) and proliferation ($p<0.05$) with respect to control (no CG), only at 6 days.

GENE EXPRESSION data of specific markers indicated that CG had no metabolic or catabolic effect on relevant OA ECM degradation pathways and did not induce any anti-angiogenic or trophic effect

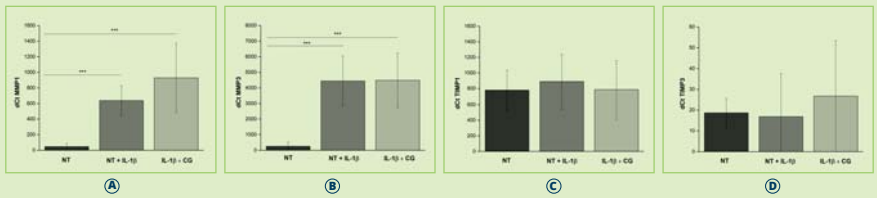


Figure 2a-d. Expression of metalloproteases associated with knee OA (MMP1, MMP3) and corresponding inhibitors (TIMP1, TIMP3). An inflammatory response in chondrocytes was induced by exposure to IL-1β. Consequent exposure to CG 1.0 mg/ml (IL-1β + CG) did not induce a significant change in the expression of MMP1 (a), MMP3 (b), TIMP1 (c), TIMP3 (d). NT: control (Not Treated).

CG increased ECM deposition and type-II collagen production (typical of hyaline cartilage) in human chondrocytes

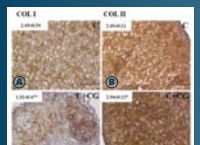


Figure 3a-d. Immunohistochemical analysis of human chondrocytes exposed to a chondrogenic medium (C) or to a chondrogenic medium and CG 1.0 mg/ml (C + CG). Avidin-biotin detection method. Bar = 100 μm. When chondrocytes were exposed to the chondrogenic medium only, type-I and type-II collagen expression was not significantly different; when they were exposed also to CG 1.0 mg/ml, expression of type-I collagen was inhibited, and that of type-II collagen enhanced.

Treatment	A Cell density/matrix amount (score 0-3)	B Cell morphology (score 0-3)	A+B (score 0-6)
NT	0.85±0.76	0.40±0.38	1.25±1.00
NT+CG	0.60±0.57	0.75±0.83	1.35±1.28
C	2.50±0.31***§§§	2.10±0.42	4.60±0.63***§§§
C+CG	2.05±0.41*§§	2.20±0.45*	4.25±0.77***§§§

Table 1. Bern Scores of human chondrocytes. NT, not treated; NT+CG, CG 1.0 mg/ml added; C, chondrogenic medium; C+CG, chondrogenic medium and CG 1.0 mg/ml. *, vs NT; §, vs NT+CG. * or §, $p<0.05$; *** or §§§, $p<0.001$.

Intra-articular injections of CG in knee OA patients significantly reduced OA signs and symptoms as measured by VAS, Lequesne and WOMAC scores

Parameter	Mean ± SD or Y/N (%/%)
Age (years)	58.1±11.1
Weight (Kg)	76.5±14.1
Height (cm)	173.7±9.0
BMI (Kg/m²)	25.2±3.3
KL score 1,2,3,4 (%)	4 (20); 11 (55); 3 (15); 3 (10)
M/F (%)	14/6 (70/30)
Diabetes Y/N (%)	2/18 (10/90)
Cardiovascular disorders Y/N (%)	7/13 (35/65)
Metabolic disorders Y/N (%)	4/16 (20/80)
Concomitant treatment Y/N (%)	11/9 (55/45)

Table 2. Baseline characteristics of patients.

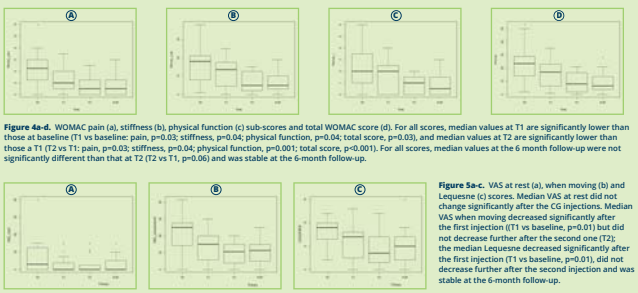


Figure 4a-d. WOMAC pain (a), stiffness (b), physical function (c) sub-scores and total WOMAC score (d). For all scores, median values at T1 are significantly lower than those at baseline (T1 vs baseline: pain, $p=0.03$; stiffness, $p=0.04$; physical function, $p=0.04$; total score, $p=0.03$), and median values at T2 are significantly lower than those a T1 (T2 vs T1: pain, $p=0.03$; stiffness, $p=0.04$; physical function, $p=0.001$; total score, $p<0.001$). For all scores, median values at the 6 month follow-up were not significantly different than that at T2 (T2 vs T1, $p=0.06$) and was stable at the 6-month follow-up.

Figure 5a-c. VAS at rest (a), when moving (b) and Lequesne (c) scores. Median VAS at rest did not change significantly after the CG injections. Median VAS when moving decreased significantly after the first injection (T1 vs baseline, $p<0.01$) but did not decrease further after the second one (T2); the median Lequesne decreased significantly after the first injection (T1 vs baseline, $p=0.01$), did not decrease further after the second injection and was stable at the 6-month follow-up.

CONCLUSIONS

CG may prompt chondrocytes to produce hyaline cartilage, and counterbalance the normal reparative response that would lead, instead, to fibrous tissue formation.

CG may be a safe and effective adjuvant in the treatment of symptomatic knee OA by intra-articular injection.