The Effects of Hydrolyzed Collagen and Collagen Peptide in the Treatment of Superficial Chondral Lesions: An Experimental Study

Os efeitos do colágeno hidrolisado e do peptídeo de colágeno no tratamento de lesões condrais superficiais: Um estudo experimental

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Abstract

Objective To evaluate the effects of hydrolyzed collagen and collagen peptide in the treatment of superficial chondral lesions in rats.

Method This research employed 18 *Rattus norvegicus*. A single intraarticular infiltration of sodium iodoacetate (2 mg solution) through the patellar ligament induced joint damage in previously anesthetized animals. We divided the animals into three groups: a control group, a collagen peptide group, and a hydrolyzed collagen group. Treatment consisted of oral administration of collagen peptide or hydrolyzed collagen for 30 days. Afterwards, we euthanized the animals and studied the joint chondral changes. We evaluated the results according to the chondrocyte clusters count and a histological evaluation, as per Pritzker et al.

Results There was no statistical significance in injury stages between the control, hydrolyzed collagen, and collagen peptide groups ($p = 0.11$). Regarding scores, there was a statistical significance between the groups treated with hydrolyzed collagen and collagen peptide ($p < 0.05$), but not in comparison with the control group.

Keywords
- chondral lesion
- collagen
- knee
- rats
- treatment

Multicentric study developed at the Experimental Surgery Laboratory from the Universidade do Estado do Pará (UEPA) and the Porto Dias Hospital, Belém, Pará, Brazil.

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Conclusion The proposed treatments of the induced chondral lesion with the oral administration of hydrolyzed collagen or collagen peptides were effective, resulting in lesion stabilization or regression, and warranting further experimental research to understand and improve the primary outcome of this study.

Resumo

Objetivo Avaliar os efeitos do colágeno hidrolisado e do peptídeo de colágeno no tratamento de lesões condrais superficiais de ratos.

Método Foram utilizados 18 *Rattus norvegicus* nesta pesquisa. O dano articular foi induzido por uma única infiltração intra-articular de iodoacetato de sódio (solução 2 mg), injetada através do ligamento patelar da articulação dos animais previamente anestesiados. Os animais foram distribuídos em três grupos: grupo controle, grupo peptídeo de colágeno e grupo colágeno hidrolisado. O tratamento foi realizado por 30 dias com a administração via oral do peptídeo de colágeno ou do colágeno hidrolisado. Posteriormente, foi realizada a eutanásia dos experimentos e seguiu-se para o estudo das alterações condrais articulares. Os resultados foram avaliados conforme contagem de condrócitos por *cluster* e através da avaliação histológica segundo Pritzker et al.

Resultados Ao observar os estágios de lesão, não foi observada significância estatística entre os grupos controle, colágeno hidrolisado e peptídeo de colágeno ($p = 0{,}11$). Ao observar os escores, houve significância estatística na comparação do grupo tratado com colágeno hidrolisado e o grupo peptídeo colágeno ($p < 0{,}05$), porém sem diferença estatística em relação ao grupo controle.

Conclusão Os tratamentos propostos da lesão condral induzida com uso de colágeno hidrolisado ou peptídeos de colágeno via oral mostraram-se eficazes, com estabilização ou regressão da lesão apresentada em ratos, merecendo novas pesquisas experimentais com o intuito de compreender e melhorar o desfecho primário deste trabalho.

Palavras-chave
- colágeno
- joelho
- lesão condral
- ratos
- tratamento

Introduction

The treatment of chondral lesions remains a challenge for orthopedists because of the low regenerative ability of the cartilaginous tissue.[1,2]

These injuries result from several metabolic, genetic, vascular, and/or traumatic factors. Their classification considers the size and thickness of the affected cartilage.[3,4] Its actual incidence remains unknown, since most of these injuries are asymptomatic. Therefore, many patients do not seek medical help, even when the disease is in its more advanced stages. Chondral lesions are common joint conditions, especially in elderly and obese subjects, causing pain, stiffness, and functional limitation. Joints under mechanical overload are the most compromised, particularly the knee.[3]

It is estimated that more than 10% of the population over 60-years-old have severe joint issues due to some degree of articular impairment.[4] Senna et al.[5] and Henrotin et al.[6] reported a prevalence of 4.14% in the Brazilian population. Moreover, radiological studies show changes in 30% of men and women over 65-years-old; among them, one-third is symptomatic.

Despite the role of mechanical injuries in articular cartilage degradation, the inflammatory process plays a critical role in the pathophysiology of the disease, triggering it or accelerating its evolution.[4] The exact sequence of pathological events remains poorly understood, making consensus on the best therapeutic approach difficult. There is still no cure, and treatments only alleviate symptoms.[7]

Recent oral treatments with nutraceutical agents have a weak scientific recommendation. This supplementary treatment consists of using laboratory-produced nutrients to strengthen the articular cartilage and delay or prevent the evolution of the disease.[6]

In this context, the hydrolyzed collagen resulting from the hydrolysis of crude collagen fibers or powder stands out. It has a proven role in maintaining and reconstituting the skin, bones, cartilage, and extracellular matrix. Hydrolyzed collagen is safe and has minimal adverse effects. Its amino acid composition is rich in proline, which preferentially accumulates in cartilage and presents a chondroprotective effect.[6] Factors such as aging and a poor diet can affect the demand for collagen in the body, contributing to the risk of bone and joint dysfunctions, and the eventual need for dietary supplementation.[5,7,8]

Additionally, it is possible to isolate collagen peptide fragments through enzymatic hydrolysis.[6] Collagen peptides have bioactive properties and may direct the production of specific proteins, scavenge free radicals, prevent lipid oxidation, and act as chelators for transition metals. As food supplements, they have a role in skin aging and osteoporosis prevention.[9]

Some studies demonstrated the role of nutraceuticals by comparing the time of chondral injury evolution, prevention, or both, between two groups with knee chondral injury, the first one being treated with oral hyaluronic acid and an exercise program, and the second one submitted only to physical exercises. At a 1-year follow-up, the first group showed a different outcome, with fewer symptoms and lower use of analgesics.[10–12]

Thus, this study evaluates the effects of hydrolyzed collagen and collagen peptide in treating chondral lesions 30 days after their induction in the knee articular cartilage of rats.

Method

Ethical Considerations

This research followed the precepts of the Brazilian legislation regarding animal husbandry and use (Federal Law number 11.794, of 2008) and the standards from the Brazilian College of Animal Experimentation. The Ethics Committee on the Use of Animals of the Center for Biological and Health Sciences from Universidade do Estado do Pará (UEPA) approved this research project under protocol number 09/2019.

Study Type

This experimental study used 18 male, 120-day-old *Rattus norvegicus* from the Wistar strain, weighing approximately 240 g. The rats came from the animal facility of Instituto Evandro Chagas. They were placed in plastic cages and kept in a controlled environment for temperature, humidity, light, and noise.

We divided the animals into three groups, each with 6 subjects: the control group (CG), collagen peptide group (CPG), and hydrolyzed collagen group (HCG). Drug doses for each group were based on previous studies published in the literature.[10–13]

Surgical Procedures

The articular damage was induced across the patellar ligament through a single intraarticular infiltration of a solution containing 2 mg of sodium iodoacetate, in a total volume of 25 µl in the right knee joint of previously anesthetized rats, with a flexed at a 90-degree angle, using a 26G X 3/8-gauge needle.

Treatment

Anesthesia for subsequent knee infiltration consisted of intraperitoneal injection of ketamine and xylazine at doses of 70 mg/kg and 7 mg/kg, respectively.[14] A booster, if required, consisted of an intramuscular application of ¼ of the initial dose.

After joint injury induction, all animals received analgesia with intraperitoneal tramadol at a 2 mg/kg dose. They were kept in cages identified by groups, with food and water ad libitum, for 30 days.

Animals from the CG (n = 6) received no treatment after the chondral lesion.

Those from the CPG (n = 6) received collagen peptide (0.16 g/kg/day) by gavage after chondral lesion induction.

Animals from the HCG (n = 6) received hydrolyzed collagen (0.14 g/kg/day) by gavage after chondral lesion induction.

The vehicle for component dilution was purified water. The rats were weighed daily for potential dose adjustments.

Histological Processing

The animals were euthanized by anesthetic overdose. The right knee was dissected from the hip to the ankle region, leaving the joint capsule intact. The samples were fixed in 10% formalin and submitted to histopathological analysis. The knees were kept in this solution for 1 day and demineralized in 5% nitric acid for 2 to 3 days. Tissues were embedded into paraffin blocks to prepare tibial sections for staining with hematoxylin-eosin and the Masson trichrome stain.

The histopathological analysis consisted of chondrocyte clusters counts in the superficial and intermediate layers of the cartilage. The Osteoarthritis Cartilage Histopathology Assessment System (OARSI) classified the depth of the lesion in six degrees, and the extension of the joint surface lesion in four stages (►**Fig. 1**).

The extent of the articular cartilage lesion determined the stage of chondral injury (►**Table 1**).

Next, we crossed the data on groups and injury stages in a table to determine the grade of a standardized score (►**Table 2**).

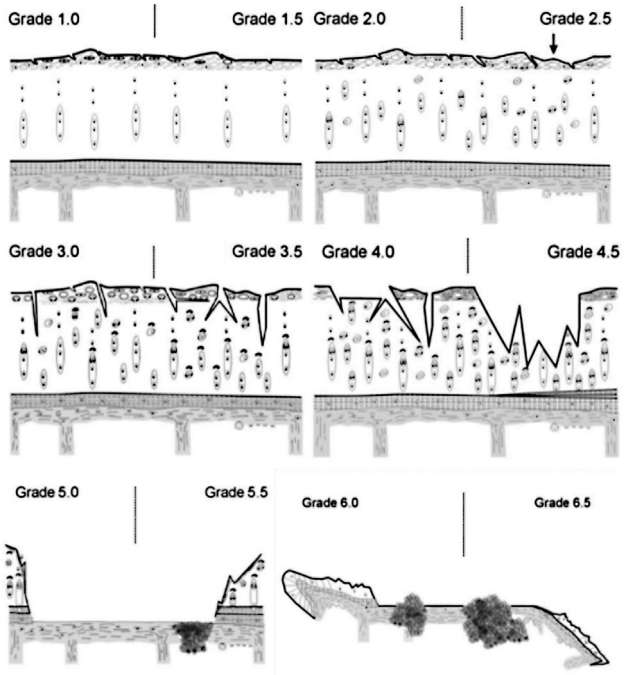

Fig. 1 Histopathological analysis. Group 1: normal articular cartilage. Group 2: joint surface discontinuity. Group 3: formation of fissures or gaps on the joint surface. Group 4: erosion of the articular cartilage surface. Group 5: denudation of articular cartilage. Group 6: deformation of the subchondral bone (reproduced from Pritzker et al., 2006).

Table 1 Lesion stages (cartilage involvement extension) per Pritzker et al., 2006.

Stage	% of involvement (surface, area, volume)
Stage 0	No osteoarticular activity
Stage 1	< 10%
Stage 2	10–25%
Stage 3	25–50%
Stage 4	> 50%

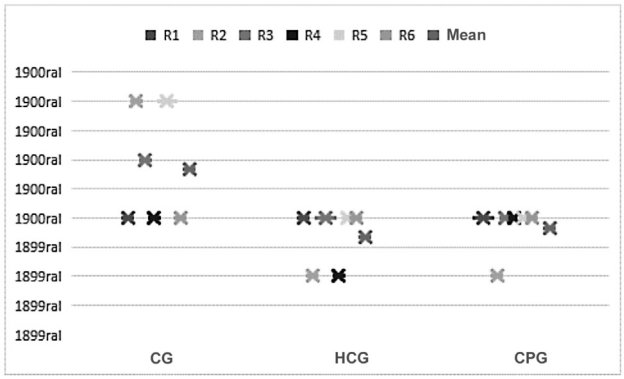

Fig. 2 Graph of the correspondence of the joint injury groups and each rat from the experimental groups ($p < 0.05$ for all groups; $p = 0.3632$ when comparing HCG and CPG).

The Kolmogorov-Smirnov test assessed normality. Statistical analysis of the study data used the Kruskal-Wallis test. If there was a statistically significant difference, the Student-Newman-Keuls test was used, adopting a significance level of $\alpha = 0.05$. Analysis was performed with the BioEstat 5.4 software.

We used Word 2016 (Microsoft Corp., Redmond, WA, USA) to prepare this manuscript, Excel 2016 (Microsoft Corp., Redmond, WA, USA) to organize and construct tables and graphs, and PowerPoint 2016 (Microsoft Corp., Redmond, WA, USA) to create the slide show.

Results

►**Table 3** shows the OARSI classification. Initially, the Lilliefors test verified data normality and revealed a nonparametric distribution. Then, we applied a Kruskal-Wallis test that detected a difference among groups ($p = 0.0008$). The Student-Newman-Keuls test confirmed this difference.

The OARSI classification proposed by Pritzker et al.[15] revealed the correspondence among groups (CG, HCG, and CPG; $p < 0.05$) and the statistical difference between HCG and CPG ($p = 0.3632$) (►**Fig. 2**).

There was no statistically significant difference between the groups regarding stages of injury ($p = 0.11$) (►**Fig. 3**).

Regarding the scores, there was a statistical difference when comparing HCG and CPG to CG ($p < 0.05$) (►**Fig. 4**). The scores were 1.66, 1.83, and 3.5 for HCG, CPG, and CG, respectively.

Table 2 Histopathological lesion grading score based on the cross-referencing of injury grades and stages (reproduced from Pritzker et al., 2006)

Grades	Stages			
	E1	E2	E3	E4
G1	1	2	3	4
G2	2	4	6	8
G3	3	6	9	12
G4	4	8	12	16
G5	5	10	15	20
G6	6	12	18	24

Table 3 Knee osteoarthritis grade in rats and their respective groups, stages, and scores

Rats	Control Group			Hydrolyzed Collagen Group			Collagen Peptide Group		
	Group	Stage	Score	Group	Stage	Score	Group	Stage	Score
R1	2	1	2	2	1	2	2	1	2
R2	4	1	4	1	1	1	1	1	1
R3	3	1	3	2	1	2	2	1	2
R4	2	2	4	1	1	1	2	1	2
R5	4	1	4	2	1	2	2	1	2
R6	2	2	4	2	1	2	2	1	2
Mean	2.83	1.33	3.5	1.66	1	1.66	1.83	1	1.83

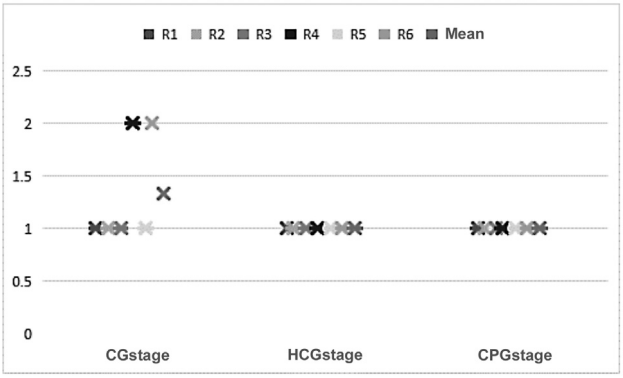

Fig. 3 Graph of the correspondence of joint injury stages and each rat from the experimental groups (CG, HCG, and CPG) ($p = 0.11$).

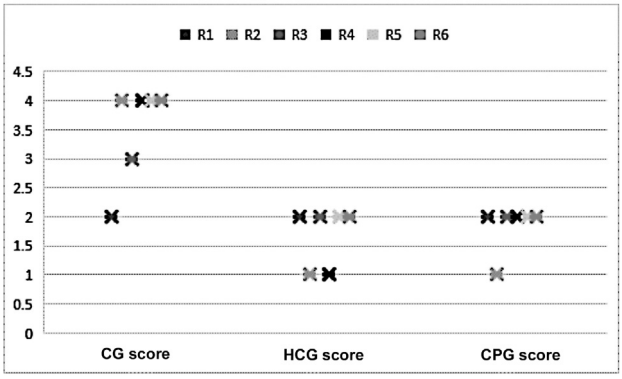

Fig. 4 Graph of the correspondence of joint injury scores and each rat from the experimental groups (CG], HCG, and CPG) ($p < 0.05$).

►**Table 4** shows the number of chondrocyte clusters in the superficial and intermediary layers of the cartilage.

Discussion

The literature supports the beneficial effect of the oral administration of hydrolyzed collagen in joint diseases. A double-blinded, randomized, clinical study demonstrated that treatment with 5 g of collagen peptides twice a day in patients with chondral injury of the knee promoted a statistically significant reduction in Western Ontario McMaster Universities Osteoarthritis Index (WOMAC) scores, a quality-of-life questionnaire specific to this population.[15]

Our study revealed an improvement in the induced knee injury, indicating that the compounds reached the chondrocytes and with a proper recognition by their surface proteins. The literature reports the accumulation of hydrolyzed collagen in murine joints. The beneficial action of hydrolyzed collagen may be associated with the stimulation of chondrocyte metabolism, activating collagen biosynthesis.[7]

Table 4 Chondrocyte clusters numbers at the superficial and intermediary layers of the cartilage

Cartilage layer	Control group	HCG	CPG
Superficial 12h	2 to 3	3 to 4	3 to 4
Intermediary 12h	4 to 5	4 to 5	4 to 5

Abbreviations: HCG, hydrolyzed collagen group; CPG, collagen peptide group.

There was no difference among groups regarding the number of chondrocyte clusters in the superficial and intermediary layers of the articular cartilage, except for CG, in which the superficial layer presented fewer cells. This finding contradicts the literature, which describes the classic histological pattern of cartilage affected by joint injury with an increase of cell clusters in both size and number.[7,16,17] The experimental model of injury induction may justify this finding. The fact that joint damage results from an external agent, instead of an intrinsic issue with the balance between anabolism and cartilage catabolism, may account for this discrepancy. Furthermore, although apoptosis is a component of the local response to injury, the decrease in cluster count may be related to it, either as an inducing factor or a product of osteoarticular degeneration.

There was a statistically significant difference in the classification of histological groups and lesion scores ($p < 0.05$). This finding suggests a delay in the inherent inflammatory process and the stabilization of the extracellular matrix degradation due to an increased synthesis of its components by chondrocytes. An experimental study in bovine and human chondrocyte models reports that treatment with hydrolyzed collagen inhibited the production of inflammatory mediators (e.g., nitric oxide and prostaglandin E-2). This inhibition occurred even in the presence of proinflammatory cytokines (interleukin β-2). Additionally, the treatment decreased the production of metalloproteinases (which degrade the extracellular matrix) and the expression of the enzyme cyclooxygenase 2. All these findings were more prominent in human chondrocytes.[18,19]

On the other hand, a metanalysis by Bakilan et al.[20] concluded that there was no evidence the oral administration of hydrolyzed collagen reduced cartilage destruction. It is noteworthy, however, that this study considered clinical trials alone. The authors stated that the oral administration of type II collagen is a new treatment with the potential to prevent joint destruction, pain, and loss of function, warranting further studies.[18,21,22]

There was no difference between the results from HCG and CPG, which is consistent with the results from a randomized clinical trial by Kumar et al.[23] Therefore, despite the origin of collagen peptides, their efficacy remains the same in our study.

For humans, pain reduction indirectly indicates an improvement in the joint conditions of patients with knee chondral injury. Moreover, it may be associated with the initiation of the repair process by collagen peptide accumulation in the cartilaginous tissue. Accumulated collagen helps maintain cartilage structure and function.[24]

Regarding lesion stage (extension), there was no statistically significant difference among groups. In fact, stage 1 injury prevailed even among CG specimens. We can interpret this finding according to a study analyzing chondral lesion defects in human knees with spectroscopy and histopathology. In this report, Spahn et al.[24] showed that the cartilage surrounding the defect and the remaining joint presented changes even if its appearance was intact. Thus, the entire joint is compromised by degeneration even though it remains histologically unchanged to a considerable extent.[20]

In preclinical studies, 24% of patients with joint damage who received 5 to 7 g of hydrolyzed collagen orally experienced substantial improvement with complete absence of pain, and 44% reported remarkable improvement.[11] Despite the noteworthy findings and the lack of side effects, this study had no statistical analysis.[11] There were few side effects descriptions in humans taking more than 10 g/day. The most common side effects included headaches and mild gastrointestinal disorders, and their presence did not contraindicate the treatment.

We cannot conclude whether oral collagen administration is effective in cases with higher joint surface wear. However, preclinical research indicates that the effects of collagen supplementation in improving joint damage are dose- and time-dependent. As such, future investigations may contribute to elucidating these data, with longer treatment times for the objective evaluation of the long-term evolution of the disease with the use of the studied therapeutic interventions.

Conclusion

The proposed treatment of the induced chondral lesion with the oral administration of hydrolyzed collagen and collagen peptides was effective. It resulted in stabilization or regression of the lesion, deserving further experimental studies to understand and improve the primary outcome of our study.

Author's contributions
DRS and RSMB: study conception and design. DRS, DBG, and LAPA: performance of experimental procedures. LGBB and RSL: laboratorial analysis. DPX, DBG, and DRS: data analysis and histology. RSMB, DRS, and DPX: manuscript preparation, final review, and submission.

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Conflict of Interests
The authors have no conflict of interests to declare.

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