Effect of collagen hydrolysates on human osteoarthritic cartilage explants

**ABSTRACT INTRODUCTION:**

Collagen hydrolysates are used as nutriceuticals for long-term treatment of osteoarthritis (OA). Pharmacokinetisch studies using mice showed that orally administered radioactive collagen hydrolysate is resorbed and some radioactivity is recovered within joints. Collagen hydrolysates have been reported to stimulate the biosynthesis of collagen and proteoglycans using cultured bovine articular chondrocytes. However, using collagen type II fragments isolated from human cartilage, increased matrix degradation and gelatinase activity was found which may either contribute to the osteoarthritic destruction of cartilage or be just a normal endogenous metabolic feedback. Also, a ligand-receptor interaction of small collagen fragments with the α2α domain of integrin was recently reported which might serve as a molecular mode of action of collagen hydrolysates.

The aim of our study was to determine for the first time whether and to what extent collagen hydrolysates obtained from different sources 1) modulate the synthesis of collagen from human articular osteoarthritic cartilage, 2) regulate the degradation of collagen and proteoglycans from human osteoarthritic cartilage, 3) have similar effects on cartilage metabolism, and 4) modulate the collagen biosynthesis of cartilage explants independently of the OA changes of cartilage obtained from human knee joints.

**METHODS:**

1. **Treatment of human cartilage explants with collagen hydrolysates:** Collagen hydrolysates from bovine (RDH, RDH-N from Rousselot), fish (FGH, FGH-N from Rousselot) or porcine origin (Mobiforte®, Astrid Twardy) were used for our experiments. Using MALDI-TOF mass spectrometry the molecular weight distribution of collagen hydrolysate peptides was determined. Before starting the experiments, approval by the ethical board of our university and the written informed consent of the patients were obtained. Full-thickness cartilage explants of the lateral femoral condyles were taken from OA patients undergoing knee replacement surgery. 4-mm-diameter articular cartilage discs were obtained using a biopsy punch. The degree of OA changes of femoral condyles was determined according to Collins. Explants with mild or moderate OA alterations were cultured in serum-free Ham’s F12 media together with the serum substitute CR-ITS™ for 4 days in a CO2-incubator under sterile conditions in order to first stabilize the cartilage metabolism at 37°C, 5% CO2 and 95% relative humidity. In order to determine the collagen synthesis, media were changed, explants were radiolabeled for 24 h with 20 µCi/ml [3H]-proline, washed several times, and radiolabeled again for 4 h with 10 µCi/ml [14C]-proline in the presence of 0, 0.1, 0.5, 1.0, 2.0 or 10 mg/ml collagen hydrolysates. In order to measure cartilage degradation, the metabolism of additionally cultured cartilage explants was first stabilized for 4 days followed by a culture period of 6 days in the presence of 0-10 mg/ml collagen hydrolysates. Explants were radiolabeled for 24 h with 20 µCi/ml [3H]-proline, washed several times, and radiolabeled again for 4 h with 10 µCi/ml [14C]-proline in the presence of 0, 0.1, 0.5, 1.0, 2.0 or 10 mg/ml collagen hydrolysates. In order to measure cartilage degradation, the metabolism of additionally cultured cartilage explants was first stabilized for 4 days followed by a culture period of 6 days in the presence of 0-10mg/ml collagen hydrolysates with a media change after 3 days. Media and explants were frozen at -20°C in the presence of protease inhibitors until analysis.

2. **Analytical procedures and chondrocyte viability:** Collagen synthesis was determined by isolating radioactive hydroxyproline and analyzing the data according to a novel dual labelling procedure being previously described [1]. Endogenous proteoglycans within explants and media were determined by the DMMB-method, whereas MMP-1,-3,-13 and collagen type II within media was measured using commercially available ELISA kits. NO in nutrient media was quantified using the Griess reaction. Data were normalized by the cartilage wet weight. The viability of chondrocytes within each of the three different anatomical zones of cartilage explants (superficial, intermediate and deep layer) was determined in separately cultured explants treated with or without 10 mg/ml collagen hydrolysates. Using fluorescein diacetate and propidium iodide Cell viability was evaluated microscopically. Briefly, at two sites of each of the 3 slices per explant, viable and/or dead cells, as indicated by a green or red fluorescence, were counted and documented.

3. **Statistical analysis:** Results were compared to untreated explants from the same joint. Each experimental condition was repeated five times using explants always obtained from different patients (n=6). Data presented are mean ± SD. Groups of data were evaluated using one-way analysis of variance (ANOVA) and the Friedman test. Significance was set to p < 0.05.

**RESULTS:**

MALDI-TOF analysis revealed differences between collagen hydrolysate peptides obtained from different sources with respect to both the width of molecular weight distribution and the average molecular weight. The molecular weight distribution of peptides obtained from RDH and FGH was up to 2 fold larger than those obtained from RDH-N, FGH-N and Mobiforte. The average molecular weight were similar between RDH (3700 kDa) and FGH (3600 kDa) but larger than those found for RDH-N (3100 kDa), FGH-N (2900 kDa) and Mobiforte® (3300 kDa).

The collagen synthesis of human articular cartilage explants was significantly inhibited by RDH (Fig. 1), whereas the other collagen hydrolysates displayed no effect. The degree of OA alterations had no impact on the collagen synthesis as modulated by RDH. Only Mobiforte® induced an increased loss of proteoglycans (Fig. 2), whereas an elevated level of NO was found for Mobiforte®, RDH and FGH-N. None of the investigated collagen hydrolysates induced an increased loss of collagen even though significantly elevated levels of MMP-1, -3, -13 and/or PGE2 were found for RDH, FGH and Mobiforte®. Analysis of chondrocyte viability revealed that none of the collagen hydrolysates tested at the high concentration of 10 mg/ml are cytotoxic.

**DISCUSSION:**

Our investigation show for the first time that none of the collagen hydrolysates tested has any stimulatory effect on the biosynthesis of collagen by human articular knee cartilage. This observation was even independent from the degree of OA alterations as found for cartilage explants. Our findings are in contrast to one previous study in which a collagen hydrolysate was reported to stimulate collagen biosynthesis by bovine chondrocytes [2]. Even though RDH and FGH can induce elevated levels of MMP-1, -3, -13 and/or PGE2, no elevated loss of proteoglycans or collagen was observed. These findings indicate either induction of TIMPs or absence of proteolytic processing of proMMPs to active MMPs. However, only Mobiforte®, in contrast to other collagen hydrolysates with similar molecular weight distribution (RDH-N, FGH-N), significantly induced cartilage degradation in that elevated levels of proteoglycans, NO, MMP-1 and -3 was found within nutrient media.

In conclusion, our study show for the first time that collagen hydrolysates differ both with respect to the molecular weight distribution of peptides and by their biological activities on human chondrocytes.

**SIGNIFICANCE:**

Collagen hydrolysates from various sources differ significantly with respect to both their chemical composition of peptides as well as their effects on human articular cartilage. Since collagen hydrolysates used as nutriceuticals might be either therapeutically useful, ineffective or even detrimental to OA cartilage their biomedical properties have to be studied thoroughly before being used in patients.

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**REFERENCES:**