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Impact of Collagen Fragments on the Synthesis and Degradation of the Extracellular Matrix of Cartilage Tissue

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Summary

Clinical studies have demonstrated the positive influence of orally administered Collagen Hydrolysate (CH) in the treatment of degenerative joint disease. The therapeutic mechanism of such specific collagen fragments, however, remains essentially unclear. These current results clearly indicate a significant dose-dependent, stimulatory effect of CH on the type II collagen biosynthesis of chondrocytes. Moreover, a significant increase in the amount of pericellular proteoglycans could be observed. Both results suggest that cartilage cells were stimulated by CH to synthesize enhanced amounts of a complete ECM. In contrast, protease activity of the chondrocytes was not affected by CH treatment.
Due to the administration of CH the increased synthesis of cartilage tissue might thus counteract degenerative processes within the ECM. These data might be helpful to explain the positive clinical results observed as a result of CH therapy.

Key words: Collagen Hydrolysate – collagen fragments – Type II collagen synthesis – ECM – osteoarthritis

Introduction

The extracellular matrix (ECM) of cartilage tissue, due to its specific properties and complex structure, is essentially responsible for the unique biomechanical properties of joint cartilage. Collagen fibrils form a three-dimensional, fiberlike, elastic network that represents the basic architecture of the tissue and gives it its stability. Proteoglycans are embedded in the fiber network and render the tissue compressible and elastic (1).
During the lifetime of an individual, the matrix components are continuously formed and degraded, whereby, under normal physiological conditions, there is equilibrium between degradation and synthesis (2). If this equilibrium is disturbed, the cartilage matrix may suffer pathological changes, including severe damage to the cartilage and osteoarthritis (OA) (3). The regulation of these processes and the pathomechanisms involved in OA are still inadequately understood. However, it is indisputable that osteoarthritis therapy can only take place by exerting targeted influence on chondrocyte metabolism in order to compensate for the progressive pathological loss of cartilage substance. Over the past few years, numerous substances have been identified that are involved in the regulation of chondrocyte metabolism (4). Apart from certain cytokines and growth hormones, the effect of structural components of

the ECM and their degradation products have been discussed as having an effect on chondrocyte cell metabolism. In this connection, and within the scope of the present experiments, the question as to whether collagen fragments are principally capable of influencing synthesis and degradation of the ECM should be addressed.
In the experiments carried out, a special type of collagen hydrolysate with relatively short-chained collagen fragments was used.
In clinical studies, the positive influence of orally applied CH on degenerative joint disease has been demonstrated, even if the therapeutic mechanism involved has remained unclear.
With the help of the experimental results obtained, an attempt should also be made to find an explanatory model for the therapeutic mechanism of CH.

Materials and methods

The investigations that took place were carried out using primary porcine chondrocytes and articular cartilage explants.
In preparing the chondrocytes, cartilage tissue was initially taken from the shoulder joints of freshly slaughtered pigs under aseptic conditions. Subsequently, the chondrocytes were isolated from the tissue in several enzymatic digestion steps (hyaluronidase, pronase, collagenase) and placed in Ham's F-12 medium (10 % FCS) under oxygen reduction (5 % O_2) in 12-well culture plates.
At the beginning of the experiment (culture day 3), the cells of the various test substances were added to the culture medium and the cells incubated for a further 8 days.
The chondrocytes of the control group were cultivated in normal culture medium over the entire period of the investigation.
After cultivating for a total of 11 days, type II collagen biosynthesis of the chondrocytes was determined in each case using Arthogen-CIA® Capture Elisa Systems (Chondrex LLC, Redmond, USA).
For quantification of the pericellular proteoglycans in the monolayer cultures, the cells were initially fixed (10 % formalin solution) and then dyed using an Alcian Blue solution (24 h at 4 °C). Finally, the Alcian Blue-labeled proteoglycan complexes were dissolved by adding 8M guanidine hydrochloride. The optical densities of the lysates were then determined at a wavelength of 620 nm.

Fig. 1: *Dose-dependent stimulation of type II collagen synthesis in 11-day old chondrocyte cultures after application of collagen hydrolysate. The mean values and SD for 5 cell preparations, carried out as double determinations, are illustrated; * $p < 0.01$.*

Fig. 2: *Type II collagen biosynthesis of 11-day old porcine chondrocyte cultures after application of various test substances (0.05 mg/ml):*
Coll I — Native type II collagen
PLA — Collagen-free hydrolysate extracted from wheat protein
Coll II — Type II collagen hydrolysate
*The cultivation of the control cells was carried out in normal cell culture medium (BM). The mean values and SD for 5 cell preparations, carried out as double determinations, are illustrated; * $p < 0.01$.*

In additional experiments, porcine articular cartilage explants were cultivated analog to the above cell culture experiments in the presence of extracellular CH. For determining the protease activity in the culture medium, an aliquot of the serum-free supernatants (culture days 9-11) was analyzed using SDS-PAGE zymography (7.5 % polyacrylamide gel containing 0.1 % gelatine).

For the experimental investigations, a special collagen hydrolysate (GELITA AG, Eberbach, Germany) was used. Detailed characterization of the molecular weight distribution of the collagen fragments in the hydrolysate was carried out using gel permeation chromatography.

Results

The collagen hydrolysate (CH) used in the experiments was produced from type I collagen using a special method of enzymatic hydrolysis. GPC analysis of the hydrolysate resulted in a mean molecular weight of 3.3 kDa, whereby fragment sizes from 0.5 – 15 kDa were determined.
The cell culture experiments clearly showed that enriching the culture medium with CH resulted in a significant ($p = < 0.01$), dose-dependent increase in type II collagen synthesis of the chondrocytes (fig. 1). Under the selected experimental conditions, a significant increase in type II collagen synthesis was determined (1.64 ± 0.15 µg/µg DNA) as from a concentration of 0.25 mg CH/ml. Within the concentration range 0.5 - 5 mg CH/ml cell medium, a significant increase of the collagen synthesis of the chondrocytes by a factor of 1.9 was determined in comparison with the untreated control cells. In increasing the concentration further to 10 mg/ml CH, a decrease in the type II collagen biosynthesis to the extent of approx. 25 % was established in comparison with the maximum values determined.
In a further experiment, after application of 0.5 mg of type II collagen hydrolysate (CH II)/ml medium, a significant increase ($p = 0.01$) of collagen type II synthesis of the chondrocytes in comparison with the control cells was established (fig. 2). This was consistent with previous results obtained. Here, the values (2.45 ± 0.21 µg/µg DNA) were only insignificantly above the results determined subsequent to CH treatment. In contrast, neither the cells treated with collagen-free hydrolysate (PLA) nor those incubated with native type I collagen (Coll I) showed an increase in type II collagen biosynthesis (fig. 2). The amounts of collagen determined here were insignificantly different from those of the untreated control cells (BM).
Analog to the determined stimulation of type II collagen synthesis after application of CH, a significant increase of pericellular proteoglycan brought about by collagen fragments was determined (fig. 3). Here, however, as from a concentration of 0.5 mg CH/ml medium, significantly higher ($p = 0.05$) optical density values were established for the test reactions in comparison with the control cells.
Further experiments showed that the application of CH had no influence on the protease activity of the chondrocytes (fig. 4). Densitometric evaluation of the zymographs of the culture supernatants showed no significant differences between the explants treated with various concentrations of CH and the untreated controls.

***Fig. 3**: Significant increase of pericellular proteoglycan in 11-day old chondrocyte cultures after application of collagen hydrolysate. The optical density of an Alcian Blue-dyed monolayer was determined as parameter. The mean values and SD for 5 cell preparations, carried out as triple determinations, are illustrated; * $p < 0.05$*

***Fig. 4**:SDS-PAGE gelatine zymography in the culture supernatants of porcine cartilage explants after application of collagen hydrolysate. The mean values and SD of the densitometric evaluation of the zymographs of 4 preparations are illustrated.*

Discussion

In the experimental investigation carried out, the effect of collagen fragments on cartilage metabolism was clearly confirmed. The results obtained from porcine chondrocytes confirmed the dose-dependent stimulation of type II collagen biosynthesis of the cells by CH and thus essentially confirm the results obtained from other investigations (5, 6). Under the selected experimental conditions, a maximum increase in collagen type II synthesis was obtained within the concentration range of 0.5 – 5 mg CH/ml medium. In addition, it was shown that collagen fragments were exclusively responsible for the stimulating effect on cartilage cell metabolism as both native collagen and collagen-free protein hydrolysate had no effect on the synthesis of the chondrocytes. The fact that, in addition to type I collagen fragments (CH), degraded type II collagen (CH II) also induced a significant increase in the collagen synthesis can be explained by the small differences that exist between fibrous collagens in terms of structure and composition (7). Due to the extensive degree of degradation of type I and type II collagens, it is conceivable that, during hydrolysis, very similar or even identical collagen fragments are produced.

A more detailed characterization of the effect specificity of collagen hydrolysate could not be established due to the heterogeneity of the collagen fragments. However, it may be assumed that both the peptide length as well as the sequence of the terminal amino acids play a major role in the stimulating effect. Thus, not all of the collagen fragments of a hydrolysate have a corresponding effect on cartilage cell metabolism.

Within the scope of this investigation, in addition to increased type II collagen synthesis brought about by CH, a significant dose-dependent increase in pericellular proteoglycan could be demonstrated in the monolayer cultures. Both findings indicate increased synthesis of a complete extracellular matrix.

In recent years, in various clinical studies, the positive effect of orally applied CH in osteoarthritis in terms of pain reduction and improved mobility of patients has been demonstrated (8, 9). Resorption studies have shown that CH, even in highly molecular form, is resorbed to a certain extent and that the resorbed peptides are preferentially enriched in cartilage tissue (10). These results show that orally applied collagen fragments reach the targeted organ cartilage tissue and that they can bring about increased synthesis of extracellular matrix.

Analysis of the protease activity of chondrocytes after application of CH gave no indication of significantly changed enzyme activity in the cartilage tissue. Thus, the application of CH fragments brings about no undesired increase in the degradation processes within the ECM.

In summary, it can be said that, due to collagen fragment-induced stimulation of synthesis of extracellular matrix, progressive degradation of joint cartilage can in principle be counteracted. This could well serve as an explanatory model for the positive clinical findings of CH therapy.

Literature references