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NOVEL CALORIMETRIC PROPERTIES OF HUMAN CARTILAGE SAMPLES IN RHEUMATOID ARTHRITIS

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During recent years, knowledge of rheumatoid arthritis has increased, and management of the disease has improved. A limited number of papers have been published before on the subject of thermal analysis of degenerative cartilage but rheumatoid arthritis (RA) has not been studied previously. A new protocol had to be established before the investigation. The purpose of this study was to further characterize the altered metabolism in human RA cartilage that promotes disease progression.

Previously, these methods have not been used for this purpose. The use of thermal analysis could be an effective method for controlling the relationship between biomarkers and disease progression.

Keywords: *DSC, human hyaline cartilage, rheumatoid arthritis*

Introduction

During recent years, knowledge of the nature and pathogenesis of rheumatoid arthritis (RA) has increased, and management of the disease has improved. RA is a chronic (long-term) disease that causes inflammation of the joints and surrounding tissues, affecting approximately 0.8% of the adult population worldwide. Persistent joint synovial tissue inflammation causes pain, swelling and stiffness. Over time, progressive bone erosion, destruction of cartilage, and complete loss of joint integrity can occur, resulting in chronic pain, loss of function and disability. Eventually, multiple organ systems may be affected. It is the most common form of inflammatory arthritis, and has a substantial social effect in terms of cost, disability and lost productivity [1–3]. Pathogenic mechanisms have showed that irreversible loss of articular cartilage begins relatively early. The formation of locally invasive synovial tissue (pannus) is a characteristic feature of RA. This tissue is involved in the joint erosions [4].

The purpose of this study was to further characterize the altered metabolism in human RA that promotes disease progression in order to characterize the changes in human RA cartilage. Normal and degenerative human hyaline cartilage has already been investigated by different thermoanalytical studies [5, 6]. The first paper from this field was the study of Than *et al*. [7]. The limited number of published papers concluded that structural manifestation of osteoarthritis appears as a remarkable change of thermal stability of hyaline cartilage samples. The healthy cartilage samples used in these studies were of cadaver origin as waste material, pathological cartilage was derived as intraoperative tissue fragments [8–10]. RA has not been studied previously by thermal analysis of degenerative human hyaline cartilage. Current knowledge of the pathophysiology of tissue destruction and the macromolecular changes that occur in cartilage and bone in RA has been gained mainly from studies in vitro and of animal models. The conclusions reached in such experiments do not necessarily apply to the human situation. Studies of tissue metabolism in vivo in humans are therefore needed to substantiate experimental data [11].

Thermoanalytical techniques measure the change in physical or chemical properties of the sample as a function of temperature. Conventional differential thermal analysis instruments subject a sample and an inert reference material to a controlled heating program and measure the differential temperature between sample and reference material. In differential scanning calorimetry (DSC), the sample material is subjected to a linear temperature program, and the heat flow rate into the sample is continuously measured; this heat flow rate is proportional to the instantaneous specific heat of the sample [12].

DSC directly measures the transition energy of the sample analyzed calorimetric data can be used to gain fundamental insight into a process or property of a material. Current uses of calorimetry are primarily on complex, often poorly defined, systems, and the most common use is probably kinetics. Calorimetry can be used for qualitative and quantitative analyses. ΔH is the enthalpy change of the process initiated by the tem-

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Table 1 Thermal parameters of denaturation (mean±SD) of normal and RA samples

Sample group	Sample number	ΔH/J g^{-1}	DSC peak/°C	Beginning/°C	Ending/°C
Normal	11	–1493.31 SD=193.04	49.79 SD=5.09	21.82 SD=3.64	55.20 SD=5.43
RA	24	–1359.03 SD=156.13	47.01 SD=6.86	19.99 SD=6.08	52.48 SD=7.00

perature change. ΔH can often be determined for an unknown reaction in a complex system, and the value of ΔH can then be used to assist in identifying the reaction of the system. The change of energy in thermal processes can be measured. Analysis of data from calorimetry always involves a model for the property as a function of temperature, pressure, or composition. Calorimetric data will be fitted to the model to obtain model parameters, and thus provide a description of the system as a function of the experimental variables [13, 14]. The advantage of DSC is the speed of the measurements and the small amount of sample that is required. However, DSC may require modification to assess the stability of compounds to various environmental conditions such as oxygen or humidity.

Experimental

Materials

In order to conduct the thermoanalytical study, 35 samples were collected. During arthroplasty procedures performed at the Orthopedic Department, University of Szeged, 24 RA human hyaline cartilage sample was obtained and normal cartilage from 11 knees. The mean age of the RA patients was 61 years (SD=5.2), while the average age of the normal group was 64 years (SD=4.2). Normal samples were derived when total knee arthroplasty was performed because of osteoarthritis and the unaffected femoral condyle had to be sacrificed for the procedure. Usually, in arthritis of both medial and lateral knee compartments total knee replacement is performed, when only one compartment is affected and ligamental stability is intact unicondylar prosthesis is implanted. We were able to obtain normal cartilage from those cases where one compartment was degenerated and the other was normal but ligamental instability was the indication for total knee arthroplasty. All tissues were yielded in accordance to legal regulation, international ethical concerns, and patients' consent. After the operation, a disc (5 mm in diameter) was removed from the unhealthy and healthy cartilage surface. The sample was taken under sterile conditions, and excess bone was removed. The disc was first washed in sterile saline, then stored in 20 mL saline for transportation at room temperature. Mean storage time was 6 h (min: 1 h, max: 26 h). Preoperatively the diagnosis of the patient was established on basis of the patient history, clinical signs, laboratory tests and radiological findings. The state of the hyaline cartilage was determined intraoperatively.

Methods

The calorimetric properties of samples were determined by differential scanning calorimetry (Mettler-Toledo DSC 821^e apparatus, Mettler-Toledo GmbH, Switzerland). Samples were heated from 0 to 80°C. The heating rate was 0.3°C min^{-1}. Conventional Hastelloy batch vessels were used with 40 μL sample volume. All the DSC measurements were preceded in Ar atmosphere and the flow rate was 100 mL min^{-1}. From the DSC curves the decomposition temperature, the transition temperature range and the total calorimetric enthalpy change were calculated. Fisher LSD method by the Statistica for Windows statistical program was used to compare enthalpy change in the different groups.

Results and discussion

With the rise of temperature an endothermic reaction was observed in all of the cases. Although the enthalpy change of the process (ΔH) initiated by the temperature change showed marked difference between the normal and pathological groups (Table 1) statistical analysis did not show significant alteration $p \leq 0.05$.

All samples showed a clear denaturation peak on the calorimetric curve. Greater change in the enthalpy was observed in normal cartilage: –1493.31 J g^{-1} (SD=193.04). In cases of RA –1359.03 J g^{-1} (SD=156.13) was measured. Therefore, denaturation

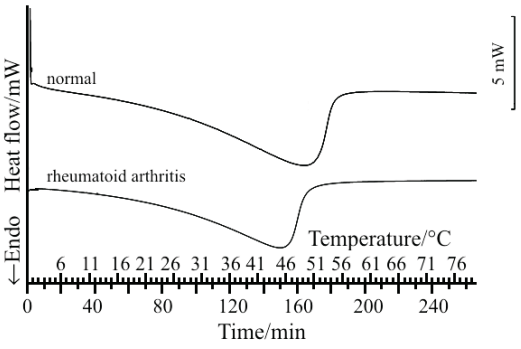

Fig. 1 DSC curve of normal necrotic and osteoarthritic human hyaline cartilage samples

caused by heating was higher in the normal human hyaline cartilage. Consequently these samples required the largest amount of energy for decomposition. Denaturation peak in normal cartilage was at 49.79°C (SD=5.09), however in RA 47.01°C (SD=6.86) was similar to the normal (Fig. 1).

The etiology of RA is not fully understood. Molecular pathology of RA is under intense investigation since research has greatly increased our understanding of the immune system, genetics, and biology. The pathogenesis of RA progression points to a complex interplay between environmental and genetic factors. Over time, inflamed synovial tissue begins to grow irregularly, then the invasive pannus invades and destroys cartilage and bone. Prevention is not possible without the knowledge of the basic pathomorphological mechanism leading to cartilage destruction. Since damaged hyaline cartilage has a very limited potential for healing, prevention is fundamental in treatment.

The use differential scanning calorimetry as part of thermal analysis was a reliable method for differentiating normal hyaline cartilage from rheumatoid samples. All samples showed a clear denaturation peak on the calorimetric curve, therefore a volume of the curve was easily calculated giving the enthalpy change of the sample. These changes correlated with the water content of the samples. The calorimeter that was available for use proved to be adequate for these measurements.

DSC techniques are still developing and many new variants and applications are reported each year. Combined techniques with microscopy or spectroscopic instruments are of obvious value to the pharmaceutical scientist, although commercially available units are not widely available [12].

Similar sample environment was provided by acquiring normal cartilage from live surgery. The investigation was performed in a relatively short period of time to minimize extracorporal degeneration. All samples showed a clear denaturation peak on the calorimetric curve, therefore a volume of the curve was easily calculated giving the enthalpy change of the sample. Since the increased number of samples acquired for this study did not produce statistically large enough deviation from the mean results, further investigations are needed to significantly differentiate normal and RA samples.

Conclusions

Characterization of the altered metabolism in cartilage that promotes disease progression should lead to future treatment options that can prevent structural damage. Since damaged articular cartilage has a very limited potential for healing, prevention is fundamental in treatment. However, prevention is not possible without the knowledge of the basic pathomorphological mechanism leading to cartilage degeneration. Further investigation is needed to examine the effectiveness of currently used for resolving cartilage matrix degeneration.

Although numerous clinical studies have suggested that specific or combinations of biomarkers can have predictive value in terms of disease presence and severity, the wide variability in these values limits its use for individual patients. The use of thermal analysis could be a simple and effective method for controlling the relationship between these markers and disease progression. The revised protocol for sample taking eliminates the presence of disturbing substances during the examination. A detailed thermal examination is needed on the same joint surface with samples taken from different grades of degeneration within the same joint.

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