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Differential scanning calorimetric examination of the osteoarthritic hyaline cartilage in rabbits

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Abstract

Osteoarthritis of the knee is one of the most common musculoskeletal disorders with major pathological changes occurring in the structure of hyaline cartilage. Differential scanning calorimetric (DSC) examination is a well-established method for the demonstration of thermal consequences of local and global conformational changes in biological systems. With foregoing studies authors have demonstrated the feasibility of DSC in the investigation of the hyaline cartilage. The aim of this study was to establish the thermograms of hyaline cartilage degeneration in the knee joint, experimentally induced in rabbits. The calorimetric experiment of osteoarthritic samples in rabbits resulted in scans similar to those observed earlier in human samples. Measurements were reproducible both in terms of changes in total enthalpy and heat capacity and in the shape of DSC scans themselves.

Keywords: DSC; Hyaline cartilage; Osteoarthritis; Femoral condyle; Rabbit

1. Introduction

Arthritis of major joints especially osteoarthritis of the knee is a very frequent disease of human beings mainly in the developed countries. In osteoarthritis there is a clear pathological abnormality in the tissue elements building up the hyaline cartilage, which is responsible for the disease. The derangement occurring in the cartilage leads to the total destruction of the joints over several steps. The basic histological and biochemical alterations in osteorthritis have been clearly described before [1–4].

The structural changes occurring in the joint cartilage can be described as follows: the amount of chondroitine sulfate is decreasing, the surface shows cracks. There are also significant structural changes in the deeper layers, the number of cartilage cells gradually decreases in certain parts, in other parts, irregular cell proliferation can occur. The cell proliferation results in the increased release of proteolytic enzymes that cause a rapidly increased denaturation of proteins. In certain layers, calcium deposits are formed. As a result of all these changes, the integrity of the cartilage tissue gradually weakens. Due to the mechanical forces affecting the articular surface, parts of the cartilage are torn, ulcerations are developing. Because the cartilage is destroyed, the bony ends of the joints are left partially or completely cartilage-free [5–7].

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With our foregoing study we could demonstrate that differential scanning calorimetric (DSC) examination is a useful and well applicable method for the investigation of hyaline cartilage. Besides describing the characteristic DSC scans of normal human hyaline cartilage, we clearly demonstrated significant differences between the different conditions of cartilage samples. Osteoarthritic human preparates showed typical and well reproducible changes at the melting temperature of 60 °C [8].

2. Aim of study

The aim of our study was to experimentally create osteoarthritis in the knee joint by setting up an appropriate animal experiment model. The calorimetric examination of arthritis created this way was our target, which we hoped could give an answer to the following questions.

1. Is it possible to use calorimetry in the experimental knee arthritis animal model and is it possible to reproduce results even if sample size is expected to be below those of the human experiment?
2. How are the calorimetric results of experimental arthritis correlating with the graphs described earlier in human samples?
3. Is it possible to show differences between samples regarding the duration of osteoarthritis?

3. Materials and methods

3.1. Animal experiment

The experiment was performed under supervision and with permission of the local committee of the national animal experiment board. For the study we used 10 white, New Zealand type rabbits, each being 3–3.5 kg. Animals were approximately 5 months old at the time of first surgery. Anaesthesia was performed using intramuscular injections containing 5 ml of ketamin hydrochlorate (Calypsol®) and 1 ml of diazepam (Seduxen®). After proper preparation of the surgical field (shaving, disinfection), further local anaesthesia was performed by using 2 ml of lidocain hydrochlorate (Lidocain®) in the region of the skin incision.

After a parapatellar arthrotomy of the joint, resection arthroplasty of the patella of one knee (in five cases in the right, in five cases in the left) was carried out with an oscillating saw. Main purpose of this intervention was to remove the cartilage of the patella and to leave a rough surface articulating with the femoral cartilage. After repositioning the resected patellar surface into the femoral trochlea reconstruction of the capsule and the surrounding tissues was carried out. The animals were granted free movement in a cage approximately 1 m × 1 m in size. Complications did not occur, all animals survived the intervention and were able for later examination.

The first animal was sacrificed 12 months after the surgery, by an overdose of ketamin hydrochlorate (Calypsol®), later on animals were sacrificed with 1 month intervals using the same protocol. As could be expected, on the femoral cartilage surface articulating with the resected patella macroscopically justifiable osteoarthritis developed. Formation of osteophytes, loss of cartilage thickness and remarkable fissuration could be observed. Macroscopic signs of osteoarthritis could be verified in all cases. We removed the whole distal femoral end in all cases for further preparation of the cartilage. In three animals, in order to have further verification of osteoarthritis, a part of the condyle underwent histological examination too. These took place after formaline fixation and decalcification with EDTA. Histological methods (hematoxylin–eosin and Giemsa) verified severe arthritis in all examined samples.

For control we explored the contralateral, unoperated knee as well, where macroscopically no cartilage degeneration was visible. The three animals, in which histological examination of the operated knee has been performed underwent the same in the contralateral knee as well. The histological slides showed slight signs of arthritis, the hyaline cartilage being intact, but thinner than normal.

3.2. Sample preparation

The samples serving as a basis for research were derived from the femoral trochlear surface of the patellofemoral joint. Since the dimensions in the rabbit knee are much smaller than in human knees,

removed pieces were either. We avoided removing subchondral bone, our aim was to harvest only pure hyaline cartilage.

The samples were obtained by a sharp scalpel, from the same anatomic region in all animals. The shape of samples was flat with 1 mm of thickness and 5 mm of length. Most of the samples were identical by size, and their average wet weight was 20 mg. Samples were put into RPMI-1640 solution (Sigma) containing 10% fetal bovine serum (Hyclone laboratories), antibiotic, antimycotic solution (1 U/ml penicilline, streptomycine, gentamycine and fungisone, Gibco laboratory), non-essential amino acids (Gibco) and sodium carbonate. All the individual samples were stored separately at 4 °C, no longer than 24 h. Then samples were subjected to calorimetric measurement. The total mass (together with buffer) was about 850 mg.

3.3. DSC measurements

DSC was performed in cartilage samples of all operated knees and in five cases in the contralateral, not operated knee joint as well. The calorimetric experiments were done as they were described earlier [9,10]. The thermal denaturation was monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100 °C. The heating rate was 0.3 K/min. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μl sample volume, on average. The pure RPMI-1640 solution served as reference. The sample and reference vessels were equilibrated with a precision of ±0.1 mg and there was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. The data treatment after ASCII conversion was done by Origin 6.0.

4. Results and discussion

Hyaline cartilage is a tissue having a complex and active metabolism from the biochemical point of view. It is composed of chondrocytae, collagens,

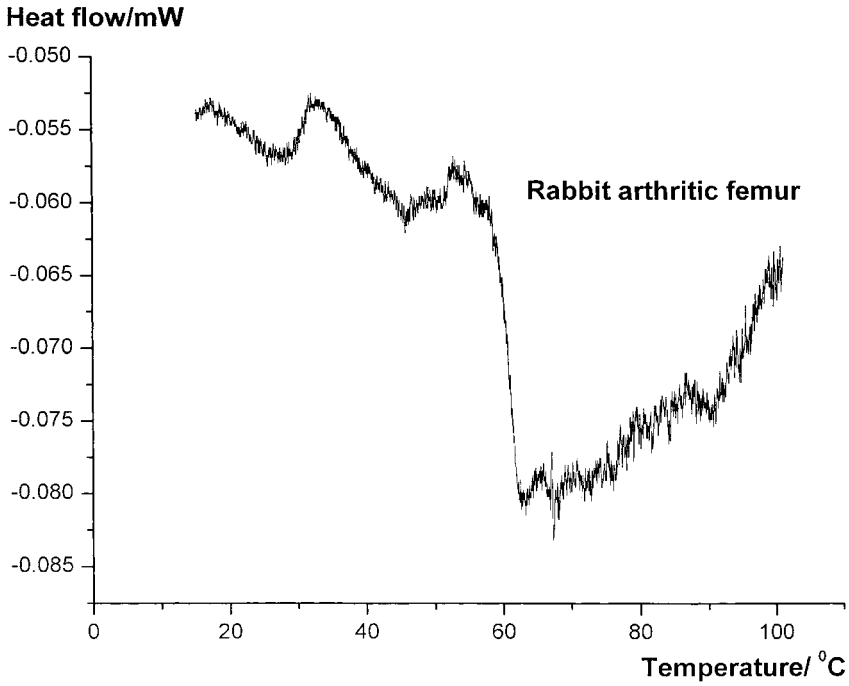

Fig. 1. DSC curve of experimentally induced arthritic femoral cartilage samples in rabbits (the downwards deflection means endothermic effect in SETARAM Micro DSC-II, which can operate in the temperature range of −20 up to +100 °C). The total volume of the investigated samples were 850 μl; the wet weight of cartilages were 20 ± 3 mg.

proteins of non-collagen type (e.g. proteoglycans), inorganic materials and 70–80% of water. The collagen–proteoglycan matrix serves as the mechanical frame of the cartilage and simultaneously determines its mechanical properties [11]. The collagen fibers having a triple helix form are responsible for tensile strength while proteoglycans are responsible for compressibility. There is an increased cellular activity in the arthritic joint at the early stage combined with changes of the matrix and increased water uptake. The homeostasis shifts towards catabolic activity, cartilage is degraded, proteoglycan fragments are liberated which is followed by the fragmentation of collagen fibers and their structural change [1,4]. These structural manifestations result in a remarkable change of thermal stability of hyaline cartilage samples as it could be clearly demonstrated with our earlier investigations.

DSC scans of arthritic samples clearly demonstrated that in every case a characteristic high temperature endothermic reaction could be traced at $62.8 \pm 0.9\,^{\circ}C$. This could be observed in every sample, the small size of sample did not affect the result of the examination (Fig. 1). The graphs were identical in several aspects with those observed by us earlier with examinations of markedly degenerated human knee joints [12]. Interestingly, the curve detected in case of non-operated, thus seen as to be "intact" knees also showed an endothermic reaction at $66.5\,^{\circ}C$ (Fig. 2). These curves were not similar to those which we observed earlier with intact human samples, but rather identical to those which we have seen in slight human arthritis [8,12]. One possible explanation for this finding of ours might be that the 1-year-old animals might already have had degeneration in the hyaline cartilage of the unoperated knee. The fact of spontaneous progression of arthritis in the knee of elder rabbits is well known and could also be verified with the histological investigation of our samples.

According to the duration of the pathologic state no differences could be proven. Graphs showed similar characteristics in all animals, independently from the time period between the operation and the DSC investigation.

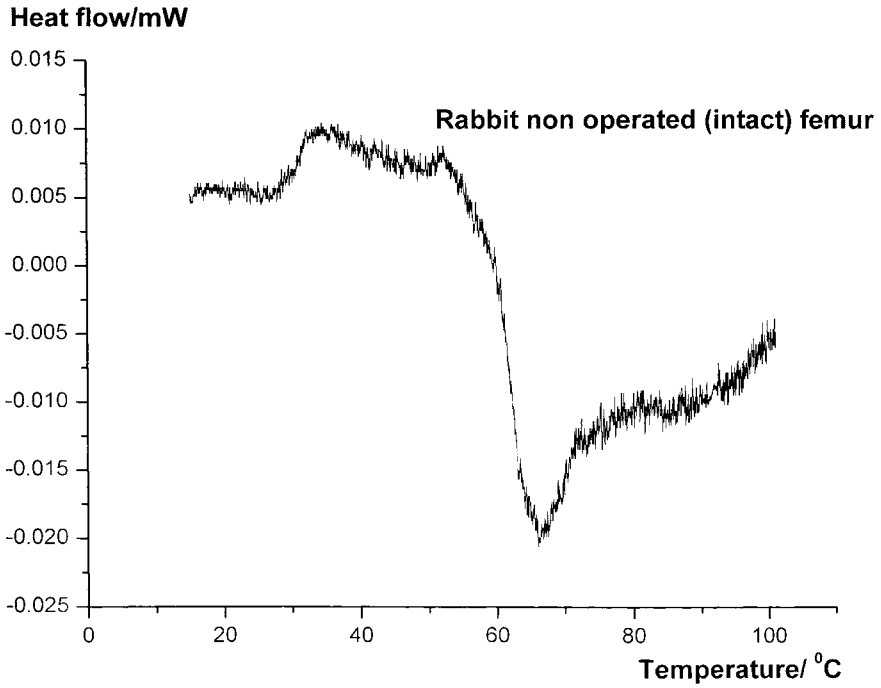

Fig. 2. DSC curve of contralateral, not operated (intact) femoral cartilage samples.

Table 1
The main melting temperatures and transition enthalpies (mean ± S.D.) of rabbit cartilages

	T_{m1} (°C)	ΔH_1 (mJ/g)	T_{m2} (°C)	ΔH_{m2} (mJ/g)	T_{m3} (°C)	ΔH_{m3} (mJ/g)
Non-operated (intact)	27.1 ± 0.3	9.1 ± 0.8	35.9 ± 0.7	4.7 ± 0.8	66.5 ± 0.9	114.4 ± 9.8
Arthritic	28.1 ± 0.4	12.5 ± 1.0	45.9 ± 0.9	22.5 ± 1.4	62.8 ± 0.9	183.5 ± 12.8

The pronounced heat capacity change (between the native and denaturated state) in arthritic samples can be explained with the structural alterations. It is supported by the increased transition enthalpies at each melting temperature (Table 1), which is the sign that during the degenerative processes the structure of cartilage became more densely packed. The values of main melting temperature fell into the same range like in human samples, namely of stable biological macromolecules. This is a further verification of the possible cause being the denaturation of collagen and protein compounds of the cartilage.

With our investigations we could repeatedly demonstrate that DSC is a well applicable method for the investigation of hyaline cartilage. The scans demonstrated that osteoarthritis has its characteristic calorimetric appearance being very similar in human and experimental animal samples.

As stated earlier we believe that the most important question to be cleared in the future is: which components are mainly responsible for the demonstrated DSC findings. This needs the additional separations of different components with biochemical methods and the establishment of DSC features of each component. Our future studies will head towards this direction.

Acknowledgements

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