Journal of Thermal Analysis and Calorimetry, Vol. 82 (2005) 221–223

# DSC MEASUREMENT OF CARTILAGE DESTRUCTION CAUSED BY SEPTIC ARTHRITIS

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Treatment of a bacterial arthritis is a challenging task for a clinician as inadequate therapy can cause cartilage destruction and can result in severe osteoarthritis of the affected joint. The development of cartilage destruction in septic arthritis is not known in details. The aim of this study was to follow this process by calorimetric method.

We induced experimental septic arthritis in knee joints of seven New Zealand rabbits by single inocculation of *Staphylococcus aureus* OKI 112001 culture (1.5 mL $8 \cdot 10^8 \pm 5\%$ c.f.u.). The first rabbit died on the 11th day. At that time all the other subjects were made overslept and samples were isolated from the cartilage of the femurs for calorimetric measurement. The DSC scans clearly demonstrated the development of infective structural destruction in cartilage from the first to the tenth day of incubation. In case of healthy control the melting temperatures ($T_m$) were: 49.7, 55 and 63.4°C and the total calorimetric enthalpy change ($\Delta H$) was 0.55 J $g^{-1}$. After the first day the enthalpy decreased (0.375 J $g^{-1}$), the first two transition temperature shifted towards higher temperature: 57 and 63.15°C. Up to the fourth day the effect of infection culminated with $T_m$ of 49.3, 55.9, 59.4, 62.8°C and further decrease of the $\Delta H$. At the fifth day the effect of infection is culminated in two separable thermal denaturation events (with 55 and 63.3°C $T_m$s) with high jump in $\Delta H$ indicating the dramatic change of the structure of rabbit cartilage, so this time elapsed seems to be critical from the point of view of practical clinical relevance too. Between the 7th and 11th days practically we had same melting temperatures (50 and 63°C) with low (~0.24 J $g^{-1}$) enthalpy.

***Keywords:*** *DSC, rabbit cartilage, septic arthritis, Staphylococcus aureus*

## Introduction

Physicians specialised in orthopaedic surgery or rheumatology still find any kind of bacterial arthritis difficult to treat. It is still a clinical problem and needs thorough grounding, as inadequate treatment can cause cartilage destruction and can result in severe osteoarthritis of the affected joint [1]. In adults the knee is the most commonly affected joint. The goals of treatment are elimination of joint infection, decompression and restoration of function. Publications regarding the good effect of arthroscopic debridement show some direction of these procedure, but do not focus on the cartilage destruction and the time elapsed since the infection started [2, 3]. During the treatment of experimentally infected joints by *Staphylococci* some authors have applied antibiotics alone or in combination with non-steroid anti-inflammatory drugs, as well. These experiments showed that antibiotics applied at any phase could not stop the cartilage destruction, but decrease it [4, 5].

During the infection of the joint, the proteolytic enzyme mechanisms of the localising leukocytes resulted in a fibrous cover on the surface, which prevented the nutrients from diffusing into the cartilage. Consequently, destruction of the cartilage developed soon [6]. The degeneration of human knee-joint cartilage in osteoarthritis is well-known, we were able to investigate degenerative changes with the help of calorimetry and could determine the calorimetric standards of human hyaline cartilage [7, 8]. In our earlier experiments – besides of describing calorimetric standards of healthy cartilage of the rabbit knee [9] – we investigated the thermodynamic features of the cartilage in experimentally induced osteoarthritis. Differential scanning calorimetric (DSC) scans clearly demonstrated a characteristic endothermic reaction in the range between 50–60°C. This could be observed in every sample, the small size of samples did not affect the result of the examination. The graphs were identical in several aspects with those observed earlier with human examinations [8].

In the recent study we made experiments to prove the cartilage destruction caused by septic arthritis. *Staphylococcus aureus* that is responsible for human septic arthritis in 80 per cent of the cases has been injected into rabbit knee-joints. We assumed

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1388–6150/$20.00

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that arthritis would arise soon and it would be followed by structural alteration in hyaline cartilage. That was to be proved by calorimetry.

## Experimental

*Materials and methods*

Seven New Zeland rabbits (mean mass 3 kg) were kept under indifferent conditions for 2 weeks and were inoculated intraarticularly by *Staphylococcus aureus* OKI 112001 strain. This standard strain was originally isolated from septic arthritic wound [10] and cultivated on blood agar plate. Its identity was controlled by biochemical testings [11]. For the inoculation experiment the test strain was cultivated in Mueller Hinton broth (Oxoid Ltd. UK) [12] overnight at 37°C and washed in physiological saline solution three times. At last the colony forming unit (c.f.u.) of the test strain was checked by tube dilution method [13]: $8 \cdot 10^8 \pm 5\%$ c.f.u. 1.5 mL of this bacterial suspension was injected into the knee joint of back legs of rabbits. The injection was carried out day by day. We inoculated only the left back legs of the animals. One rabbit was left for healthy control. The duration of this experiment was 12 days from the first to the last injection. The animals were kept in normal circumstances, they could eat and drink as often they wanted. No parenteral intake of food, liquid or medicine was carried out. After the injection, the subjects were observed closely, the swelling of the joint as a clear sign of synovitis was registered.

The first rabbit spontaneously died on the 11$^{th}$ day. After killing the first subject all the other subjects were made overslept and samples were isolated from the cartilage of the femurs for calorimetric measurements by surgical intervention. The samples contained no bone or synovial membrane, only the cartilage from the macroscopically well seen border of the bone and cartilage was removed. At least 4 samples were taken from each joint, the size of the samples was about 2×2 mm. The joints showed macroscopic signs of inflammation. We cultivated the fluid of joints on blood agar plates and we could repeatedly isolate the original inoculating *S. aureus* OKI 112001 strain from each knee joint. The samples were fixed in phosphate buffer and the thermal analysis started at once.

DSC measurements

The progress of infection was checked by Setaram Micro DSC-II calorimeter. Uniform pieces of rabbit cartilages underwent a heating–cooling cycle in the 0–100°C temperature range with 0.3 K min$^{-1}$ scanning rate. Conventional Hastelloy batch vessels were used during the denaturation experiments with an average 850 μL sample volume. The sample and reference vessels were equilibrated with a precision of 0.1 mg. It was not necessary to correct for heat capacity between the sample and reference vessels. The calorimetric enthalpy was calculated by the Setaram two points fitting integrating software.

## Results and discussion

In the period of observation after inoculation not only the behaviour of rabbits have changed but also the swelling of the knee-joint developed soon, within 23 (16.5–28) h in average. The first subject died due to sepsis spontaneously 270 h after injection of *Staphylococcus aureus*, which was determined to be the end of the experimental period. Samples were taken without delay in order to avoid post mortem alterations. The tendency of the progression of experimental arthritis in the function of time monitored by the thermal consequences can be seen in Fig. 1. The finer details of infected cases can be read from Table 1 too. In the DSC scan of healthy control we can separate at least three melted domains with 49.7; 55 and 63.4°C melting temperatures ($T_m$) and an 0.55 J g$^{-1}$ total calorimetric enthalpy change ($\Delta H$). The main transition has a wide $T_{1/2}$ (transition temperature interval at the half of melting temperature), which is the sign of a weak cooperativity of those structural elements, which are melt in this range. In infected cases after the injection at the first day the macroscopically observed swelling of the knee joint appeared as a significant decrease of the calorimetric enthalpy (0.375 J g$^{-1}$), the third transition temperature remained unchanged (63.15°C) but the second shifted towards to the higher temperatures (57°C) indicating the structural changes in these subunits caused by the infection (Table 1). At the third day the two low meltings were unified in one wider endotherm with a $T_m$ about 50°C and decreased $\Delta H$. At the fifth day the effect of infection is culminated in two separable thermal denaturation events (55 and 63.3°C $T_m$s) with high jump in $\Delta H$ indicating the dramatic change of the structure of rabbit car-

**Table 1** The melting temperatures and calorimetric enthalpy changes of healthy and infected rabbit cartilages

| Stage of animal | | $T_m$/°C | | $\Delta H$/J g$^{-1}$ |
|---|---|---|---|---|
| 1$^{st}$ day | | 57.0 | 63.15 | 0.3754 |
| 4$^{th}$ day | 49.3 | 55.9 | 59.40 | 0.3420 |
| 5$^{th}$ day | | 55.0 | 63.30 | 0.5510 |
| 6$^{th}$ day | after inoculation | 53.5 | 64.45 | 0.2110 |
| 7$^{th}$ day | | 50.2 | 63.15 | 0.2400 |
| 9$^{th}$ day | | 50.0 | 63.70 | 0.2360 |
| 11$^{th}$ day | | 50.0 | 63.70 | 0.2410 |

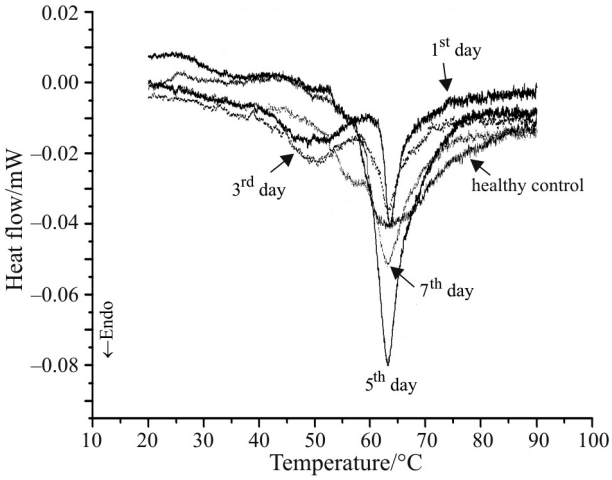


**Fig. 1** Thermal denaturation curves of experimentally evoked arthritic rabbit cartilages

tilage. On the seventh day two meltings with the lowest enthalpy change remained and there was no significant change in $T_m$s and $\Delta H$ at the 9th and 11th days. It could mean that the fifth day seems to be critical in our experiments during the development of fatal septic arthritis (very probably from this point becomes irreversible the structural change) and the remaining six days are the last stage during the development of experimental arthritis.

We could prove in our experiment that during bacterial infection the destruction of hyaline cartilage develops soon, in the first day. This destruction can be measured by calorimetric examinations that can be successfully applied for biological problems [14, 15]. From the experiment we did not get information weather this destruction is reversible or not, it was not our aim. To further explore the tissue degradation caused by infection more experiments including histological examinations are necessary. Furthermore additional examinations are necessary to determine the timespan after which the intraarticular septic process gets irreversible and because of this not treatable by either drugs or surgical interventions e.g. lavage [16].

We would like to call the attention of surgeons dealing with septic joint processes to our experiments. We believe that our findings underline the importance of early treatment of infected joints, which might prevent the irreversible damage of the hyaline cartilage that can later cause osteoarthritis.

## Acknowledgements

This work was supported by grant of Hungarian Research Fund (OTKA CO-272).

## References

1 T. Sillinger and A. Óvári, Jahrestagung der Österreichischen Gesellschaft für Orthopädie und Orthopädische Chirurgie 29. 11.–12. 01. 2001. Salzburg.
2 M. Ivey and R. Clark, Clin. Ortop., 199 (1985) 201.
3 R. W. Jackson and C. J. Parsons, Clin. Ortop., 96 (1973) 160.
4 P. Riegels-Nielsen, N. Frimodt-Moller, M. Sorensen and J. S. Jensen, Acta Orthop. Scand., 60 (1989) 113.
5 R. L. Smith, G. Kajiyama and D. J. Schurman, J. Ortop. Res., 15 (1997) 919.
6 J. Jerosch, I. Hofstetter, M. Schröder and W. H. M. Castro, Acta Orthop. Belgica, 61 (1995) 126.
7 P. Than, Cs. Vermes, B. Schäffer and D. Lőrinczy, Thermochim. Acta, 346 (2000) 147.
8 P. Than, I. Domán and D. Lőrinczy, Thermochim. Acta, 415 (2004) 83.
9 P. Than and D. Lőrinczy, Thermochim. Acta, 404 (2003) 149.
10 B. Lányi and M. Konkoly-Thege, Hungarian National Collection of Medical Bacteria. National Institute of Hygiene Catalogue 1978, p. 154.
11 W. E. Kloos and T. L. Bannerman, *Staphylococcus* and *Micrococcus*, In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, Eds, Manual of Clinical Microbiology, ASM. Washington DC, USA 1999; pp. 264–82.
12 The Oxoid Manual 5th Edition, Oxoid Ltd. Basingstoke, Hampshire, UK 1982, p. 214.
13 É. Czirók, Clinical and Epidemiological Bacteriology Handbook, Melania, Budapest 1999, p. 144.
14 M. Kiss, J. Belágyi and D. Lőrinczy, J. Therm. Anal. Cal., 72 (2003) 565.
15 D. Lőrinczy, M. Kiss and J. Belágyi, J. Therm. Anal. Cal., 72 (2003) 573.
16 M. J. Smith, Arthroscopy, 2 (1986) 30.

DOI: 10.1007/s10973-005-6549-3