Journal of Thermal Analysis and Calorimetry, Vol. 95 (2009) 3, 809–811

CHARACTERIZATION OF HUMAN CARTILAGE IN DEGENERATED SPINE DISEASE WITH DIFFERENTIAL SCANNING CALORIMETRY

L. Mécs[1], *Z. Aigner*[2], *G. Sohár*[1], *Piroska Szabó-Révész*[2] *and K. Tóth*[1]*

[1]Department of Orthopaedics, University of Szeged, Faculty of Medicine, Semmelweis u. 6, 6725 Szeged, Hungary
[2]Department of Pharmaceutical Technology, University of Szeged, Faculty of Pharmacy, Eötvös u. 6, 6720 Szeged, Hungary

The purpose of this study was to further characterize the altered metabolism spondylolisthesis that promotes disease progression. Degenerative human cartilage (intervertebral disc, facet joint and vertebral end-plate) was obtained during 15 posterior lumbar spine interbody fusion procedures performed at the University of Szeged. The thermal properties of samples were determined by differential scanning calorimetry (Mettler-Toledo DSC 821^e). Greatest change in the enthalpy was observed in the intervertebral disc samples: –1600.78 J g^{-1}. Denaturation caused by heating in the normal human hyaline cartilage needed –1493.31 J g^{-1} energy. Characterization of the altered metabolism that promotes disease progression should lead to future treatment options.

Keywords: *DSC, enthalpy, human hyaline cartilage, spondylolisthesis*

Introduction

Every level of the spine is composed of a disc in the front and paired facet joints in the back. The disc acts as a shock absorber in between the vertebrae, whereas the paired facet joints restrain motion. They allow the spine to bend forwards (flexion) and backwards (extension) but do not allow for a lot of rotation. As the facet joints age, they can become incompetent and allow too much flexion, allowing one vertebral body to slip forward on the other. This slippage is known as a degenerative spondylolisthesis. Spondylolisthesis is also associated with deterioration of the facet joints connecting the two vertebra. As the facet joints become arthritic due to this deterioration, cartilage surfaces damaged. Degenerative spondylolisthesis is far more common in individuals older than 65 and is more common in females than males by a 3:1 margin. It is most common at the L4–L5 level of the lower spine. Back pain and/or leg pain are typical symptoms [1–3].

There is a range of non-surgical treatment options (such as pain medications, ice or heat application) that may help with some of the pain. Patients with severe pain and difficulty functioning, surgery can be done that includes a decompression with spine fusion. Posterior lumbar spine interbody fusion is a procedure used to treat problems of spine instability. The goal of the procedure is to stimulate the vertebrae to fuse together in order to create a rigid and immovable column of bone in the problem section of the spine [1–3].

The new paradigm of osteoarthritis considers it as a heterogeneous disease with numerous factors leading to its pathologic hallmark of cartilage loss [4]. The first alteration seen within days after joint destabilization is an increase in cartilage water content. The increase in water content in OA cartilage is due to loss of the collagen network's elastic restraint, enabling the hydrophilic polyanionic proteoglycans to swell more than normal. Very shortly after the increase in cartilage water, newly synthesized proteoglycans are characterized by a higher proportion of chondroitin sulfate. Once proteoglycan loss reaches a critical threshold, water content, which initially increased, falls below normal [5–7].

Over time, for reasons not well understood, the water content of the gelatinous nucleus matrix of the disc decreases, with a decreased and altered proteoglycan composition. The normal adult disc has a large amount of extracellular matrix and a few cells that account for about 1 vol%. The biochemical environment of the intervertebral disc changes remarkably, as people age. The disc loses its water and alters its chemical content, with the overall content of proteoglycan, collagen, and water decreasing [8–10].

There are many possible applications of thermoanalytical techniques: characterizations of active and inactive ingredients, routine analysis, and qualitative control. Differential scanning calorimetry (DSC) involves the heating or cooling of a sample and reference and the measurement of the differential heat flow. Calorimetry can be used for qualitative and quantitative analyses [11, 12]. The change of energy in thermal processes can be measured. ΔH is the enthalpy change of the process initiated by the temperature change [13].

The purpose of this study was to further characterize the altered metabolism in human degenerated

* Author for correspondence: tothk@orto.u-szeged.hu

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

cartilage that promotes disease progression. Previously, calorimetric studies were only used for the investigation of normal and degenerative human hyaline cartilage and intervertebral disc [14, 15]. The first paper from this field was the study of Than *et al.* [16], further studies measured the calorimetric properties of human intervertebral disc [17, 18].

Experimental

Materials

Most patients present with both back and leg complaints, therefore nerve decompression and fusion with or without spinal instrumentation is usually required to adequately treat degenerative spondylolisthesis. Because the fusion operation will reestablish stability after decompression, there is no limitation to how much bone can be removed and therefore a more complete decompression can be accomplished. A posterior spinal fusion is then performed by transplanting bone from the iliac crest to the bones of the back of the spine.

Degenerative human cartilage (intervertebral disc, facet joint and vertebral end-plate) was obtained during 15 posterior lumbar spine interbody fusion procedures performed at the Orthopedic Department, University of Szeged. Normal samples were derived when total knee arthroplasty was performed and the unaffected femoral condyle had to be sacrificed for the procedure. Usually, in osteoarthritis 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 5 h (min: 1 h, max: 19 h). The state of the hyaline cartilage was determined intraoperatively. In order to conduct the thermoanalytical study 14 intervertebral disc, eight degenerative facet joint cartilage, eight vertebral end-plate samples were collected. Based on the patient diagnosis, eleven samples were analyzed as normal hyaline cartilage.

Methods

The thermal 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. The enthalpy change of the process initiated by the temperature change showed marked difference between the normal and pathological groups.

Greatest change in the enthalpy was observed in the intervertebral disc samples: –1600.78 J g^{-1} (SD:141.25). Consequently these samples required the largest amount of energy for decomposition. Denaturation caused by heating in the normal human hyaline cartilage needed –1493.31 J g^{-1} (SD: 193.04) energy. The average enthalpy change during the calorimetric measurements in the vertebral end-plate samples was –1196.91 J g^{-1} (SD: 361.10) and

Table 1 Thermal parameters of denaturation (mean ±SD) of normal and degenerated 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
Intervertebral disc	8	–1600.78 SD: 141.25	50.36 SD: 4.40	19.66 SD: 3.07	56.09 SD: 4.28
End-plate	8	–1196.91 SD: 361.10	46.43 SD: 9.34	18.45 SD: 9.03	52.73 SD: 7.94
Facet joint	14	–1063.62 SD: 321.01	44.54 SD: 7.53	16.87 SD: 4.81	51.71 SD: 7.36

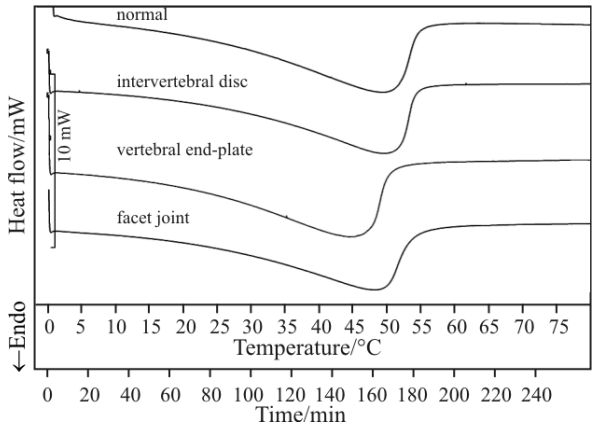

Fig. 1 DSC curve of normal and degenerated human hyaline cartilage samples (the downwards deflection means endothermic effect)

–1063.62 J g^{-1} (SD: 321.01) in the facet joint. Statistical tests proved these calculations to be significantly different (p<0.05). All samples showed a clear denaturation peak on the calorimetric curve.

The pathogenesis of degenerative spine disease likely revolves around a complex interplay of numerous factors. The major contributors include genetic influences, local mechanical factors, and inflammation. The use of differential scanning calorimetry as part of thermal analysis was a reliable method for differentiating normal hyaline cartilage from degenerated samples. The calorimeter that was available for use proved to be adequate for these measurements.

The purpose of this study was not to duplicate what was previously reported in the literature, but to clarify the thermoanalytical results with acquiring normal cartilage from live surgery was important to provide similar sample environment, and to perform the investigation in a relatively short period of time compared to the earlier reports. This way extracorpore degeneration was minimized.

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. When comparing normal and degraded facet joint hyaline cartilage a sharp decrease in the enthalpy change was observed. This is most likely the result of end stage cartilage erosion as part of spondylolisthesis. At this point of the disease the water content probably decrease below a critical point, after, which dejoint is completely destroyed. Physiological water content of the intervertebral discus is high. The biochemical environment of the intervertebral disc changes remarkably, as the disease progresses. Of the four sample types examined, still these samples needed the highest level of energy for denaturation. The cartilage of the end-plate showed similar properties to the osteoarthritic facet joint.

Conclusions

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 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. Further understanding of the initiating events in spondylolisthesis, the relationship between the different pathologic influences, will be necessary to reveal potential targets of therapy.

Acknowledgements

Study was supported by OTKA T-047166.

References

1 S. J. Atlas, R. A. Deyo, D. L. Patrick, K. Conveny, R. B. Keller and D. E. Singer, Spine, 21 (1996) 2885.
2 P. Brinkman, Spine, 21 (1986) 2885.
3 H. N. Herkowitz, Spine, 20 (1995) 1084.
4 G. S. Firestein, R. C. Budd, E. D. Harris, I. B. McInnes, S. Ruddy and J. S. Sergent, Kelley's Textbook of Rheuma- tology, 7th Ed., Elsevier Saunders, Philadelphia 2005, p. 1496.
5 H. Muir, Ann. Rheum. Dis., 36 (1977) 199.
6 H. J. Mankin, V. C. Mow, J. A. Buckwalter, J. P. Iannati and A. Ratcliffe, Orthopaedic Basic Science, AAOS, Chicago 1994, p. 1.
7 P. Than and D. Lőrinczy, Thermochim. Acta, 404 (2003) 149.
8 J. C. Iatridis, J. J. MacLean, M. O'Brien and I. A. Stokes, Spine, 32 (2007) 1493.
9 S. J. Lipson and H. Muir, Arthritis Rheum., 24 (1981) 12.
10 R. H. Pearce, B. J. Grimmer and M. E. Adams, J. Orthopaedic Res., 5 (1987) 198.
11 D. Lőrinczy and J. Belágyi, J. Therm. Anal. Cal., 90 (2007) 611.
12 T. Dergez, D. Lőrinczy, F. Könczöl, N. Farkas and J. Belágyi, BMC Struct. Biol., 7 (2007) 41.
13 L. D. Hansen and D. J. Russell, Thermochim. Acta, 450 (2006) 71.
14 G. Sohár, E. Pallagi, P. Szabó-Révész and K. Tóth, J. Therm. Anal. Cal., 89 (2007) 853.
15 K. Tóth, G. Sohár, E. Pallagi and P. Szabó-Révész, Thermochim. Acta, 464 (2007) 75.
16 P. Than, C. Vermes, B. Schäffer and D. Lőrinczy, Thermochim. Acta, 346 (2000) 147.
17 P. Than, I. Domán and D. Lőrinczy, Thermochim. Acta, 415 (2004) 83.
18 P. Than, I. Domán and D. Lőrinczy, The nature of biological systems as revealed by thermal methods, D. Lőrinczy, Ed., Kluwer Academic Publishers, 2004, p. 285.

DOI: 10.1007/s10973-008-9415-2