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DIFFERENTIAL SCANNING CALORIMETRIC EXAMINATION OF THE DEGENERATED HUMAN PALMAR APONEUROSIS IN DUPUYTREN DISEASE

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The Dupuytren contracture – degenerative shortening of the palmar aponeurosis – is a common disease of the hand in Europe. The aetiology of the degenerative changes in the collagen structures is still not clear. To describe the clinical manifestation of the disease we use an international classification according to Iselin. Our hypothesis was that in Dupuytren disease there is a clear pathological abnormality in the tissue elements building up the palmar aponeurosis, which is responsible for the disease, and could be monitored besides the classical histological methods by differential scanning calorimetry.

The thermal denaturation of different parts of human samples 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^{-1}. DSC scans clearly demonstrated significant differences between the different types and conditions of samples (control: T_m=63°C and ΔH_{cal}=4.1 J g^{-1}, stage I.: T_m= 63°C and ΔH_{cal}=5.1 J g^{-1}, stage II.: T_m=64°C and ΔH_{cal}=5.2 J g^{-1}, stage III.: T_m=60°C and ΔH_{cal}=5.2 J g^{-1}, stage IV.: T_m=60.2°C and ΔH_{cal}=5.3 J g^{-1}). The heat capacity change between native and denatured states of aponeurosis samples increased with the degree of structural alterations indicating significant water loosing. These observations could be explained with the structural alterations caused by the biochemical processes.

With our investigations we could demonstrate that DSC is a useful and well applicable method for the investigation of collagen tissue of the human aponeurosis. Our results may be of clinical relevance in the future i.e. in the choice of the optimal time of surgical therapy of different clinical level Dupuytren contractures.

Keywords: *DSC, Dupuytren contractures*

Introduction

The Dupuytren contracture – degenerative shortening of the palmar aponeurosis – is a common disease of the hand in Europe. The aetiology of the degenerative changes in the collagen structures is still not clear. The genetic factors are the most important in the manifestation of the disease – 72% of the patients are man, 98% are white –, and there are a lot of risk factors – diabetes, epilepsy, alcohol and cigarette abuse – which increases the development of the disease [1–3]. To describe the clinical manifestation of the disease we use an international classification according to Iselin. DSC is a well established method for the demonstration of thermal consequences of local and global conformational changes in biological systems. It has never been applied for the investigation of human palmar aponeurosis. According to the present study the thermograms may prove and follow the changes in the structure of aponeurosis collagen in different stages of Dupuytren disease.

Major biochemical changes in the palmar fascia affected by Dupuytren's disease included increased collagen and hexosamine contents and the presence of galactosamine in the most severely involved tissue. Type-III collagen, which is virtually absent from normal adult palmar fascia, was abundant in the tissue of patients with Dupuytren's disease [4, 5]. All of these biochemical changes are similar to those that occur during the active stages of connective-tissue wound repair. This includes the rapid synthesis and turnover of collagen which leads to newly synthesized, immature collagen being more abundant in the involved tissue than in normal tissue. The shortening of the palmar fascia in Dupuytren's disease is due to an active cellular process that progressively draws the distal extremities of the affected tissue closer together at the same time that the original tissue is being replaced.

Dupuytren's contracture is a fibroproliferative disorder of the hand characterized by an abnormal myofibroblast and fibroblast proliferation and

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extracellular matrix deposition leading to retraction and deformation of the palm. The molecules of extracellular matrix may coordinate morphogenesis, cell differentiation, and most importantly, fibrogenesis in tissue [6].

The therapy of Dupuytren disease depends on the clinical manifestation of structural and functional changes (shortening) of the aponeurosis and the progress in the articular contracture of the metacarpophalangeal and interfalangeal joints of the fingers (Figs 1 and 2). The therapy of the first clinical stage according to Iselin – no shortening and no contracture – is conservative. All the other three stages need surgical intervention like partial or total aponeurectomy, excision of the affected part of the palmar fascia [7, 8].

Our hypothesis was that in Dupuytren disease the clear pathological abnormalities in the tissue elements building up the palmar aponeurosis, can be detected by DSC. Earlier examinations have demonstrated that DSC is a useful and well-applicable method for demonstration of thermal consequences of local and global conformational changes in the organs of the musculoskeletal system. Different authors have demonstrated thermal effects of degenerative processes in various human tissue samples [9–24]. A calorimetric examination of this type has not yet been carried out on international level.

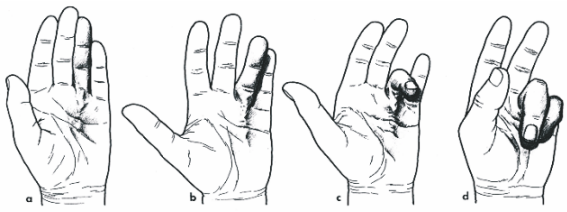

Fig. 1 Clinical stages (Grade I–IV) of the Dupuytren disease according to Iselin

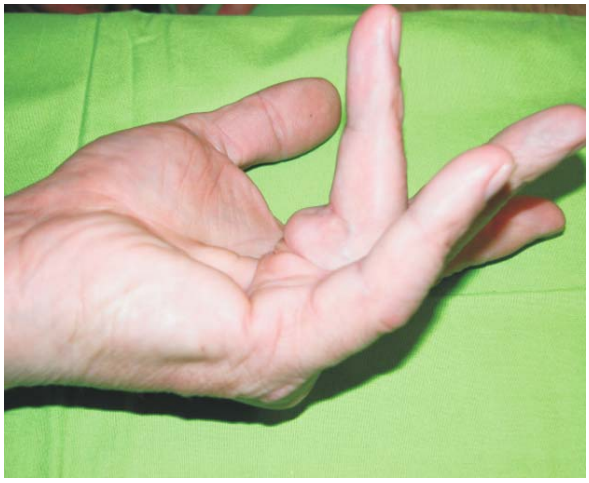

Fig. 2 Clinical appearance of Gr. III Dupuytren disease

Our aim was to prove with the examinations that there is a definitive difference in the structure of the healthy (4 samples from healthy cadaver) and pathological (2-2 samples from all of four stadium according to Iselin) aponeurosis, which can be reproduced.

Experimental

Materials and methods

Sample preparation

The healthy palmar fascias of the human hand were of cadaver origin. We removed both side aponeurosis from two cadaver hands. The donors taken into our study were all under age of 45 at their death, we considered these persons to be free any degenerative changes in their joints. We took samples only from hands where any other kind of degeneration of the aponeurosis or post traumatic changes of the hand could not be verified macroscopically. All the medical interventions were made according to the ethic regulations of the University of Pécs.

The pathologic ligaments were derived during operations of different seriousness of Dupuytren disease (Fig. 3). During the operations from crisscross approach of the palm we prepared the degenerated part of the aponeurosis and cut it out in full thickness and length. With this method we could solve the contractures of the fingers joints. We measured 10 pathologic aponeurosis from two females and eight males being in average 54 years (34–67) of age.

Histological examination

We removed the whole length of degenerated aponeurosis and cut them into two parts. One part has been sent to histological examinations the other

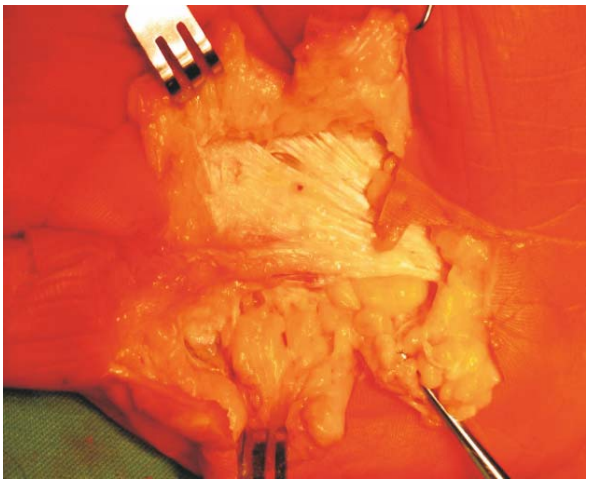

Fig. 3 Intraoperative picture of the degenerated palmar fascia

underwent DSC investigation. The later samples were put into physiological saline solution and were stored at 4°C, no longer than 24 h. The samples subject for histological examination were fixed in 4% formaldehyde, longitudinal and cross section slides have been made and stained with picrosyrius. Light microscopic control has been performed.

DSC investigation

The pieces of different samples have been prepared and measured within 6 hours of removal. The thermal denaturation of different parts of human samples 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^{-1}. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume (samples plus buffer) in average. Typical sample wet masses for calorimetric experiments were between 100–200 mg. RPMI-1640 solution was used as a reference sample. 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. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration. The data treatment after ASCII conversion was done by Origin 6.0.

Result and discussion

With our histological examination we could demonstrate that cadaveric palmar aponeurosis tissues showed no sign of degeneration, regular collagenous structure could be seen (Fig. 4) The pathologic

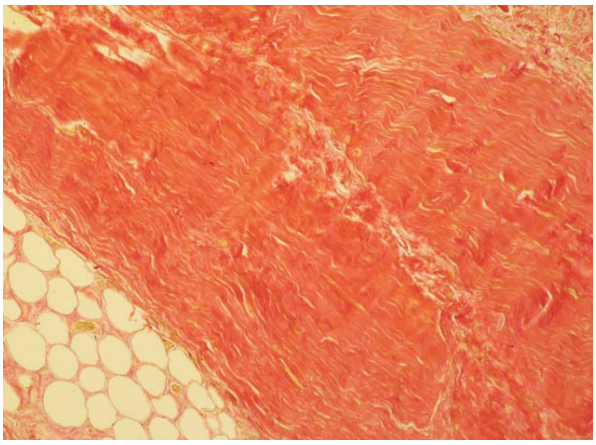

Fig. 4 Histological examination of the healthy human palmar fascia, normal collagen fibers with picrosyrius stain (200×)

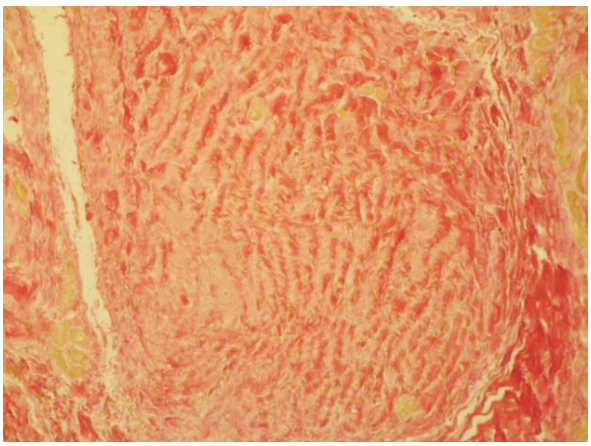

Fig. 5 Histological examination of a degenerated (Gr. IV) human palmar fascia: disorganised collagen in the field of myofibroblastic proliferation with picrosyrius stain (200×)

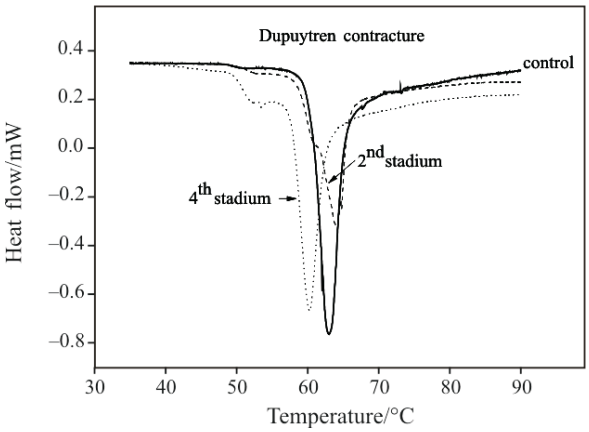

Fig. 6 Thermal denaturation scans of normal and clinical Grade II and Grade IV palmar fascia in Dupuytren disease

samples showed marked signs of degeneration and myofibroblastic proliferation microscopically (Fig. 5). In three cases stage II type degeneration, in the remaining five cases type III degeneration could be seen in the collagenous structure. All samples from the clinical stadium III and IV showed a type III degeneration and fibrillation of the collagenous tissue.

According to our knowledge this study is the first in the line of Dupuytren contracture research that used thermal analytical method. In Fig. 6 one can see the thermal denaturation of control, Gr. II and IV samples. In case of control we obtained a well cooperative single endotherm with slight heat capacity change. These thermal parameters indicate the presence of a single thermal domain with a weak water release after the denaturation. In Gr. II we have observed the presence of a second thermal structural unit that became more pronounced in Gr. IV. The DSC scans clearly demonstrate the significant

Table 1 Thermal parameters of various clinical stages (Grade I–IV) of the Dupuytren disease, according to Iselin

	Control	Gr. I.	Gr. II.	Gr. III.	Gr. IV.
T_m/°C	63	63	64	60	60
ΔH_{cal}/ J g^{-1}	4.1	5.1	5.2	5.2	5.3

differences between the different stages of contracture (Table 1): control: T_m=63°C and ΔH_{cal}= 4.1 J g^{-1}, stage I.: T_m=63°C and ΔH_{cal}=5.1 J g^{-1}, stage II.: T_m=64°C and ΔH_{cal}=5.2 J g^{-1}, stage III.: T_m=60°C and ΔH_{cal}=5.2 J g^{-1}, stage IV.: T_m=60.2°C and ΔH_{cal}=5.3 J g^{-1} respectively. The heat capacity change between native and denatured states of aponeurosis samples increased with the degree of structural alterations indicating significant water loosing. These observations could be explained with the structural alterations caused by the biochemical processes.

The thermal parameters of the healthy and pathologic aponeurosis were absolutely different. There was no significant difference between the clinical grade I and II and the clinical grade III and IV samples. In grade III and IV samples the appearance of a second thermal structural unit is an evidence of the presence of the Type-III collagen, which is virtually absent from normal adult palmar fascia.

With our investigations we could demonstrate that DSC is a useful and well applicable method for the investigation of collagen tissue of the human aponeurosis. Our results may be of clinical relevance in the future i.e. in the choice of the optimal time of surgical therapy of different clinical level Dupuytren contractures.

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