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Lactate Dehydrogenase Isoenzymes in Dupuytren's Contracture

Izoenzymy dehydrogenazy mleczanowej w patogenezie przykurczu Dupuytrena

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Abstract

Background. Dupuytren's contracture is a fibroproliferative disorder of the hand characterized by an abnormal myo/fibroblast proliferation and extracellular matrix deposition, leading to retraction and deformation of the palm. Lactate dehydrogenase (LDH, EC 1.1.1.27) is a well-known glycolytic enzyme that reversibly catalyses the conversion of pyruvate to lactate. It has been recently suggested that lactate promotes cell growth and induces an increase in collagen production.
Objectives. The aim of the present study was to examine the participation of LDH in the pathogenesis of Dupuytren's contracture.
Material and Methods. The relative values of LDH isoenzyme activity, expressed by the LDH-A to LDH-B subunit ratio, were investigated in 39 pathological tissues representing four clinical stages of disease progression and 20 fragments of normal palmar fascia obtained from patients surgically treated for carpal tunnel syndrome, using an original method of native electrophoresis separation. Total LDH activity was evaluated using the COBAS INTEGRA lactate dehydrogenase (P–L) (Roche) cassette.
Results. The study demonstrated a significantly elevated ($p < 0.05$) A/B ratio in the palmar fasciae with Dupuytren's contracture (median: 2.38, range: 1.0–3.25) compared with the normal tissues (median: 1.5, range: 0.68–2.08), which is strictly associated with a shifted LDH isoenzyme pattern towards more the anaerobic isoenzymes LDH-4 and LDH-5. Increased A/B ratio characterized pathological tissues with degree I–III of disease progression. In the group with degree IV, the A/B ratio resembled that of the control values and was significantly decreased compared with the other groups of patients with contracture ($p < 0.05$). Also observed was a statistically significant increase ($p < 0.05$) in total LDH activity in aponeurosis affected by Dupuytren's contracture (median: 0.96, range: 0.11–2.4) compared with normal fasciae (median: 0.62, range: 0.13–1.0).
Conclusions. Since significant differences were found in the A/B ratio and total LDH activity in fibrotic palmar aponeurosis, adaptation of the isoenzyme profile to the altered conditions (hypoxia) and participation of LDH in the pathogenesis of Dupuytren's contracture can be suggested (**Adv Clin Exp Med 2007, 16, 2, 205–211**).

Key words: lactate dehydrogenase, LDH, Dupuytren's contracture, hypoxia.

Streszczenie

Wprowadzenie. Choroba Dupuytrena (*contractura/morbus Dupuytren*) jest przykładem włókniakowatości guzkowatej, pojawiającej się w obrębie rozcięgna dłoni. Charakteryzuje się wzmożoną aktywnością proliferacyjną mio-/fibroblastów oraz nadmierną ekspresją i akumulacją białek macierzy zewnątrzkomórkowej, co prowadzi do trwałego przykurczu palców, a nawet do deformacji stawów śródręczno-paliczkowych i międzypaliczkowych. Dehydrogenaza mleczanowa (LDH, EC 1.1.1.27) jest enzymem szlaku glikolitycznego, katalizującym odwracalnie redukcję pirogronianu do mleczanu.
Cel pracy. Ocena udziału dehydrogenazy mleczanowej w patogenezie przykurczu Dupuytrena w zależności od klinicznego stopnia zaawansowania choroby.
Materiał i metody. Badania przeprowadzono na materiale tkankowym, uzyskanym śródoperacyjnie od 39 pacjentów (z różnym stopniem zaawansowania choroby), leczonych chirurgicznie z powodu przykurczu Dupuytrena. Do celów porównawczych wykorzystano fragmenty powięzi dłoni uzyskane od 20 pacjentów w czasie operacyjnego

leczenia zespołu kanału nadgarstka. Do rozdziału izoform LDH zastosowano natywną elektroforezę w 6% żelu poliakrylamidowym. Udział izoenzymów w aktywności całkowitej LDH wyrażono stosunkiem podjednostki A do podjednostki B. Aktywność całkowitą LDH oznaczono za pomocą biochemicznego analizatora z wykorzystaniem kasetki odczynnikowej COBAS INTEGRA dehydrogenaza mleczanowa (P–L) (Roche).
Wyniki. W tkankach objętych przykurczem Dupuytrena (mediana = 2,38; zakres 1,0–3,25) wykazano istotnie większą wartość A/B ($p < 0,05$) w porównaniu do grupy kontrolnej (mediana = 1,5; zakres 0,68–2,08), co wskazuje na duży udział w aktywności całkowitej LDH podjednostki A, a więc izoenzymów wolno wędrujących w polu elektrycznym (LD5 i LD4). Istotnie wyższy poziom izoenzymów LD5 i LD4 obserwowano w grupie patologicznych tkanek z I, II oraz III stopniem klinicznym przykurczu. Wykazano również istotny ($p < 0,05$) wzrost aktywności całkowitej LDH w patologicznych tkankach (mediana = 0,96; zakres 0,11–2,4) w porównaniu z tkankami prawidłowymi (mediana = 0,62; zakres 0,13–1,0).
Wnioski. Reorganizacja aktywności izoenzymatycznej w kierunku dominacji izoform wolno wędrujących w polu elektrycznym, a więc LDH5 i LDH4 oraz podwyższona aktywność całkowita LDH może sugerować dostosowanie profilu izoenzymatycznego LDH do zmienionych warunków (niedotlenienie) oraz udział dehydrogenazy mleczanowej w patogenezie przykurczu Dupuytrena (**Adv Clin Exp Med 2007, 16, 2, 205–211**).

Słowa kluczowe: dehydrogenaza mleczanowa LDH, choroba Dupuytrena, niedotlenienie.

Lactate dehydrogenase (LDH, EC 1.1.1.27) is a well-known glycolytic enzyme that reversibly catalyses the conversion of pyruvate to lactate. LDH is a tetramer that exists in five isoenzyme forms, LD1 to LD5, as a result of random association of the two subunits A and B encoded by two distinct genes, ldhA and ldhB. The differences in the activities of the isoenzymes are dependent on their subunit composition. The LD1 (B4) isomer functions primarily in aerobic conditions and favors converting lactate to pyruvate. The LD5 (A4) isoform has the highest efficiency among all other isoenzymes to catalyze pyruvate to lactate and thereby plays an important role in compromised oxygen delivery [1]. The expression of LDH genes is generally regulated developmentally in a tissue-specific manner and undergoes changes in pathological conditions [2]. Upregulation of ldhA gen expression is tightly coupled with a strong glycolytic metabolism and reduces the dependence of cells on the presence of oxygen [3, 4]. It was reported that microcirculation disruption, inflammation, or rapid cell growth and subsequent increased oxygen consumption lead to changes in LDH-A activity, resulting in over-production and accumulation of lactate [5]. Recent studies have provided evidence that the functional role of LDH-A might be more complicated [6]. Several observations indicate that the A subunit also displays non-glycolytic functions, such as participation in transcription or DNA replication [6].

Dupuytren's contracture (palmar fibromatosis) is a connective tissue disorder viewed as a progressive pathological process involving significant changes in cell phenotype and function and the deposition of excess matrix proteins, mainly collagens, in the extracellular space of the palmar aponeurosis, resulting in irreversible flexion deformity and contracture of fingers and loss of hand function. The critical feature of the early stage of the Dupuytren's contracture is enhanced proliferation of fibroblasts [7–9]. It is well known that lactate promotes cell growth in cultured fibroblasts in a dose-dependent manner [10]. Moreover, it has been recently suggested that lactate, by down-regulating ADP-ribosylation, stimulates collagen transcription and procollagen synthesis [5, 11, 12]. Therefore, the present authors postulated that LDH may be an important participant in the progression of Dupuytren's contracture. Because the etiopathogenesis of Dupuytren's contracture remains unknown, although there have been numerous theories suggesting that it may be the consequence of abnormal growth factor/cytokine expression, diabetes, epilepsy, traumatic phenomena, or genetic alterations [13, 14], it was decided to investigate LDH activity and isoenzyme composition in palmar fascia with Dupuytren's contracture in relation to different clinical stages of its progression.

Material and Methods

Fragments of pathological palmar aponeurosis taken intraoperatively from 39 patients aged 33–87 years, surgically treated for Dupuytren's contracture at the Department of Traumatic Surgery and Hand Surgery, Silesian Piasts University of Medicine in Wrocław, comprised the study material. Iselin's classification was used to identify four clinical stages of the disease progression (I–IV) [15]. According to this scale, 9 specimens of palmar fascia were classified as first degree contracture, 9 as second degree, 12 as third degree, and 9 as fourth degree. For comparison, 20 fragments of normal palmar fascia obtained from patients surgically treated for carpal tunnel syndrome were analyzed. Highly vascularized tissues were not analyzed because any erythrocyte contamination elevates results, especially the isoenzymes LDH-1 and LDH-2.

The tissue fragments (50 mg) were rinsed with 0.9% NaCl, dried on blotting paper, and homogenized in five volumes of 50 mM Tris-HCl lysis buffer, pH 7.5, containing 250 mM sacharose, in a glass Potter's homogenizer. After 30 minutes of incubation at 4 C, the homogenates were centrifuged for 15 minutes at 13,500 × *g*. These extracts were collected and stored at –20°C and used for isoezymatic studies by non-denaturing gel electrophoresis and to determine total activity of LDH. Native electrophoresis was carried out in 6% polyacrylamide gel in which the wells were covered with an upper gel of the same composition as the separating gel [16]. The isoenzymes were stained by a method modified by Langvad [17] and densitometrically analyzed using Image J software. The ratio of polypeptide A to B was obtained in each specimen from the integral percentage value of each isoenzyme according to the method described by Stagg et al. [18] using the formula:

$$A = [(LDH5) + 0.75\ (LDH4) + 0.50\ (LDH3) + 0.25\ (LDH2)/\ \Sigma(LDH1–5)] \times 100$$

$$B = 100 – A$$

Total LDH activity was determined using the cassette COBAS INTEGRA. Lactate dehydrogenase (P–L) contains an *in vitro* diagnostic reagent system intended for use on COBAS INTEGRA 400 (Roche).

The results were analyzed statistically using the non-parametric Mann-Whitney *U*-test (http://eatworms.swmed.edu/~leon/stats/utest.html). *p* values of less than 0.05 were considered statistically significant.

Results

Figure 1 shows the pattern of the isoenzymes of lactate dehydrogenase in normal and pathological palmar fasciae, randomly selected from the collection of all the investigated samples. In the group of 39 tested palmar aponeurosis with Dupuytren's contracture, the median A/B ratio was 2.38 (range: 1.0–3.25) and 1.5 (range: 0.68–2.08) in the group of 20 normal tissues. The difference between the two groups was statistically significant ($p < 0.05$) (Fig. 2). Total LDH activity in normal and pathological specimens are presented in Fig. 3. It was found that the activity of LDH in the Dupuytren's tissues (median: 0.96, range: 0.11–2.4) was significantly elevated ($p < 0.05$) in comparison with the normal palmar fasciae (median: 0.62, range: 0.13–1.0).

Table 1 shows changes in the A/B ratio and total activity of LDH in the tissues with Dupuytren's contracture depending on the clinical stage of disease progression. It was found that the A/B ratios of tissues in the groups I–III were significantly higher than in the control group. The lowest A/B ratios were noted in the most advanced stage of disease, i.e. in the group of patients with clinical degree IV of contracture (median: 1.7). This group appeared statistically significantly different ($p < 0.05$) in comparison with all the other groups of patients with contracture except for the control group.

High levels of total LDH activity characterized tissues with first and second degree palmar contracture (groups I and II). Significant differ-

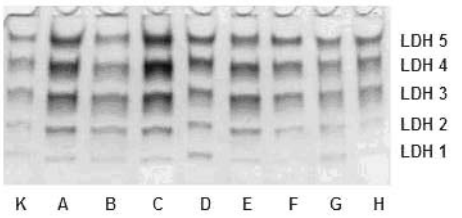

Fig. 1. Lactate isoenzyme pattern in tissue extracts of palmar fascia from a healthy donor (lane K) and from four patients with Dupuytren's contracture (lanes A–H). Lanes A–B, C–D, E–F, and G–H show, successively, clinical degrees I–IV of disease

Ryc. 1. Profil izoenzymatyczanej aktywności LDH w tkankach prawidłowych (K) i tkankach objętych przykurczem Dupuytrena (A–H). Linie A–B; C–D; E–F i G–H odpowiednio obrazują I–IV stopień kliniczny choroby

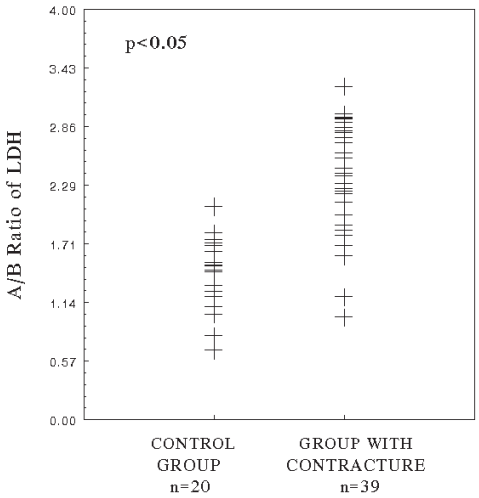

Fig. 2. A/B ratio of the LDH subunits in normal fasciae (control group) and in tissues of palmar aponeurosis with Dupuytren's contracture; *p* – probability value in relation to the control group ($p = 2.364 \times 10^{-5}$)

Ryc. 2. Stosunek podjednostki A/B LDH w grupie kontrolnej oraz w grupie patologicznych tkanek objętych przykurczem Dupuytrena; *p* – wartość prawdopodobieństwa w stosunku do grupy kontrolnej ($p = 2.364 \times 10^{-5}$)

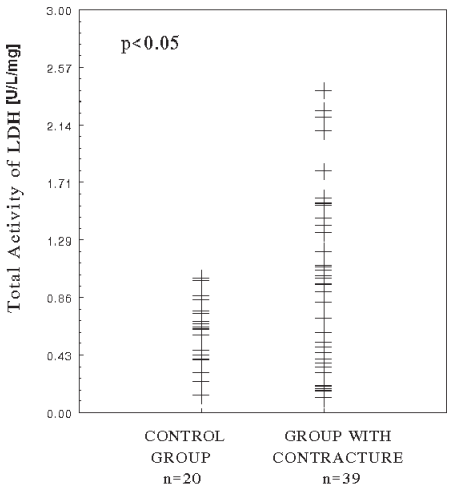

Fig. 3. Total activity of LDH in normal fasciae (control group) and in tissues of palmar aponeurosis with Dupuytren's contracture; *p* – probability value in relation to the control group ($p = 0.02602$)

Ryc. 3. Aktywność całkowita LDH w grupie kontrolnej oraz w grupie patologicznych tkanek objętych przykurczem Dupuytrena; *p* – wartość prawdopodobieństwa w stosunku do grupy kontrolnej ($p = 2.364 \times 10^{-5}$)

Table 1. A/B ratio of LDH subunits and total activity of LDH in the group of normal tissues of palmar fascia (control group) and in the four patient groups with degrees I, II, and III, IV of disease progression

Tabela 1. Stosunek podjednostki A do B i aktywność całkowita LDH w tkankach prawidłowych oraz w grupie patologicznych tkanek z I, II, III i IV stopniem klinicznym przykurczu Dupuytrena

Tested groups (Badane grupy)		A/B ratio of LDH subunits (Stosunek podjednostki A/B LDH)	Total activity of LDH [U/l/mg] (Aktywność całkowita LDH)
Control group (Grupa kontrolna) n = 20	median range	1.5 0.68–2.08	0.625 0.13–1.0
Group I (Grupa I) First-degree contracture (Pierwszy stopień przykurczu) n = 9	median range p k	2.3 1.9–3.25 5.033×10^{-5}*	1.1 0.82–2.4 9.128×10^{-5}*
Group II (Grupa II) Second-degree contracture (Drugi stopień przykurczu) n = 9	median range p k p I	2.55 2.12–2.85 0.0004* 0.452	1.34 0.2–2.25 0.03* 0.691
Group III (Grupa III) Third-degree contracture (Trzeci stopień przykurczu) n = 12	median range p k p I p II	2.57 2.2–2.95 0.0006* 0.669 0.886	0.65 0.3–2.2 0.242 0.022* 0.319
Group IV (Grupa IV) Fourth-degree contracture (Czwarty stopień przykurczu) n = 9	median range p k p I p II p III	1.7 1.0–2.3 0.2203 0.003* 0.0009* 0.0004*	0.34 0.11–1.6 0.238 0.011* 0.057 0.117

p k – probability value in relation to the control group.
p I – probability value in relation to degree I of disease.
p II – probability value in relation to degree II of disease.
p III – probability value in relation to degree III of disease.
* statistically significant difference.

p k – wartość prawdopodobieństwa w stosunku do grupy kontrolnej.
p I° – wartość prawdopodobieństwa w stosunku do grupy z I stopniem klinicznym przykurczu.
p II° – wartość prawdopodobieństwa w stosunku do grupy z II stopniem klinicznym przykurczu.
p III° – wartość prawdopodobieństwa w stosunku do grupy z III stopniem klinicznym przykurczu.
* różnica istotna statystycznie.

ences were found between these groups and all others. Groups with advanced stages of disease (III and IV) did not differ statistically significant from the control group.

Discussion

Dupuytren's contracture is a palmar fibromatosis that leads to a heavy flexion deformity of the fingers. The deformity occurs as a consequence of progressive scarring and shortening of the palmar and digital fascia [19]. From the clinical point of view there are four degrees in the course of Dupuytren's contracture according to Iselin's classification [15]. Each of them characterizes a different stage of palm contracture and tissue architecture [7]. The first degree is related with the appearance of nodules composed of proliferative cells, mostly of myofibroblastic phenotype, and small cords without signs of contracture in the interphalangeal joints. Degree II shows a little contracture in the metacarpophalangeal (MCP) and the proximal interphalangeal joints (PIP). Degree III has contracture in the MCP, PIP, and the distal interphalangeal (DIP) joints, and degree IV severe contracture in the MCP and PIP joints with hyperextension of the DIP joints together with advanced lesions in the osseous system [15].

The appearance of highly specialized myofibroblasts rich in smooth muscle α-actin in the initial stage of disease suggests that these cells are probably responsible for generating contractile forces and deposition of abnormally large amounts of extracellular matrix components [8]. Substantial experimental evidence supports the idea that myofibroblasts are the key players in the process of response to tissue injury, especially under hypoxic conditions as a result of microvascular compromise. Hypoxia can directly upregulate α-smooth muscle actin expression in fibroblast subpopulations, suggesting that lack of oxygen may result in mediating the "transdifferentiation" of fibroblasts into myofibroblasts. In addition, chronic hypoxia causes stable phenotypic changes in the cells that appear to be associated with the signaling pathways used to elicit a proliferation [20]. Some authors have suggested a significant role of local hypoxia in the progression of aponeurosis contracture. The increased cellularity in Dupuytren's tissue causes strangulation of the vascular architecture, producing further localized ischemia and hypoxic conditions. Such conditions trigger an increased level of xanthine oxidase, which allows the synthesis of free radicals. The free radicals subsequently cause a proliferation of fibroblasts and deposition of collagen, leading to tissue fibrosis and even lower local oxygen concentrations [21, 22]. Additionally, hypoxia may significantly decrease the rate of beta-oxidation and enhance triglyceride accumulation. Increased levels of free fatty acids and short-chain fatty acids in fibrotic fascia in patients with Dupuytren's contracture are suggestive of the local hypoxia that exists [23, 24].

In this study, total LDH activity in tissues of palmar aponeurosis with Dupuytren's contracture was measured. It was found that the level of LDH activity in pathologic tissues was significantly elevated, especially in tissues at early stages of the contracture. It was reported that the biosynthesis of both non-collagen and collagen proteins, which are elevated in the initial phases of Dupuytren's contracture and decrease during the final stages of fibrosis, tightly correlates with the presence of myofibroblasts. During disease progression, myofibroblasts gradually disappear and are not observed in the most advanced phase [7].

The present study showed that elevated levels of total LDH activity were coupled with alterations in the A/B subunits ratio. In the group of palmar aponeurosis with symptoms of Dupuytren's contracture, an increased A/B ratio in comparison with normal palmar fasciae was found. This was strictly associated with teh shifted LDH isoenzyme pattern towards the more anaerobic isoenzymes, i.e. LDH-4 and LDH-5, with a simultaneous reduction in the LDH-1 and LDH-2 proportion. These findings are not surprising. It is known that hypoxia, a reduction in the normal level of tissue oxygen tension, may disturb cellular activity by changes in metabolism [25]. Under hypoxic conditions, cells switch glucose metabolism from the oxygen-dependent tricarboxylic acid (TCA) cycle to glycolysis, the oxygen-independent metabolic pathway [25].

Changes in the ratio of A/B in relation to different clinical phases of the progression of Dupuytren's contracture were also observed in the present study. The significantly higher ratio of A/B than that seen in the control group characterized tissues with first, second, and third degree palmar contracture progression. In the specimens taken from the patients with fourth degree contracture, regression to the control level of the A/B ratio was observed. This might prove that the metabolism of glucose ultimately returns to the state observed in normal palmar aponeurosis, where mature fibroblasts constitute a small proportion of cells. Additionally it seems that only myofibroblasts appear to be uniquely equipped to proliferate and migrate under hypoxic conditions [20].

Harris et al. [25] and Koukorakis et al. [4] showed that the overexpression of *LDH-A* gene

enhances lactate production. *LDH-A* gene transcription is directly regulated by hypoxia-inducible factor I (HIF I), whose expression is induced under conditions of reduced oxygen tension [26, 27]. It is well known that increased lactate level is connected with enhanced collagen synthesis, whose excessive expression is a major contributor to Dupuytren′s contracture [11, 12]. Gladden et al. [12] and Trabold et al. [11] proposed two separate mechanisms to explain the stimulation of collagen synthesis by lactate. First, lactate induces an increase in collagen promoter activity that leads to elevated pro-collagen mRNA production and collagen synthesis. Lactate may also increase the activity of prolyl hydroxylase, a crucial enzyme of collagen biosynthesis. Both mechanisms are based on down-regulation of ADP-ribosylation, a widespread form of the post-translational modification of proteins. These authors presumed that collagen gene transcription in fibroblasts is normally down-regulated by polyadenosinediphosphoribose (pADPR) and that lactate accumulation decreases pADPR levels by depleting intracellular NAD+, the substrate for pADPR [5, 11, 12].

It is known that reorientation of the LDH isoenzyme pattern, with characteristically elevated levels of the cathodic isoenzyme LDH-5, has been observed in cancer tissues [3,4]. The analogy between Dupuytren's contracture (palmar fibromatosis) and benign fibroproliferative tumors may be presumably associated with marked cell (fibro/myofibroblast) proliferative activity and high metabolic demands involving significant alterations in microenvironmental conditions of hypoxia and acidity. Hence the results of this investigation are consistent with the concept that the course of Dupuytren's contracture resembles benign tumor formation.

The authors conclude that the data of this study showed that the fasciae of Dupuytern's contracture are characterized by an elevated level of the A/B ratio of the LDH subunits and increased total activity of LDH which directly correlate with an imbalance in energy metabolism and enhanced anaerobic glycolysis. The participation of LDH in the progress of Dupuytren's contracture can be suggested.

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