Fifteen-Month Human Dynamic Cardiomyoplasty: Morphometry, Myosin/Actin Ratio, Collagen, Total Protein, and SDS PAGE of MHC Show Trophic Transformation of Grafted Latissimus Dorsi

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Abstract

Evidence of myofibre abnormalities and replacement with fatty and connective tissue has been described experimentally and clinically in long-term electrostimulated Latissimus Dorsi (LD) muscle transposed for Dynamic Cardiomyoplasty. Such changes were related to the long-term diminished effectiveness of the procedure in patients. Clinically, a few reports have been addressing direct inspection of muscle histology at autopsy after Cardiomyoplasty. We here present a case report of a LD used for cardiomyoplasty fifteen months after operation. Morphologic study shows moderate and non specific changes in muscle fibers associated with moderate numerical increase of capillaries in comparison with contralateral normal LD muscle. Only in the region near to the electrodes there is variation in fibre size with atrophy and focal fiber degeneration indicating suffering by electric damage. The eutrophic state of the LD is confirmed by its total protein concentration, myosin/actin ratio, and collagen content. Isomyosin analysis by SDS PAGE shows that gene expression is homogeneously changed in all the sampled specimens taken from the proximal portion till the peripheral part of the pedicled graft. In conclusion, this case demonstrates that muscle damage is not a compulsory consequence of the unusual pattern of muscle activity imposed to LD by electrostimulation protocol for Dynamic Cardiomyoplasty.

Key words: actin, cardiomyoplasty, circulatory assist, eutrophic transformation, heart failure, human, isomyosin, latissimus dorsi muscle, morphometry, SDS PAGE of MHC, total proteins.

BAM 6 (5), 337-340, 1996

Evidences of myofibre abnormalities and replacement with fatty and connective tissue has been described experimentally and clinically in long-term electrostimulated Latissimus Dorsi (LD) muscle transposed for Dynamic Cardiomyoplasty [10, 11]. Changes were related to the long-term diminished effectiveness of the procedure in patients. Clinically, just a few reports have been addressing indirect evaluation by magnetic resonance imaging [2] or direct inspection of muscle histology [1, 3]. We here present a case report of a LD used for cardiomyoplasty fifteen months after operation.

The patient aged 63 years, presenting a progressive deterioration of chronic heart failure secondary to coronary artery disease, underwent cardiomyoplasty using a clockwise cardio-costal wrapping with the left LD muscle. The stimulation of the LD muscle started two weeks after surgery using a pulse amplitude of 4 volts and a muscle-to-heart contraction ratio of 1 : 4. The LV ejection fraction before the surgery was 31%, six months after cardiomyoplasty was 50%. Despite clinical improvement, the patient died from sudden death 15 months after surgery.

The left LD appeared well wrapped and viable. Fragments were collected from the left LD, the right LD and the myocardium. Left LD specimens were taken from the proximal portion till the peripheral part of the pedicled graft. Muscle morphology and fiber diameter were studied on 8 μm thick paraffin embedded sections, stained with Hematoxylin-Eosin (H-E), using an Automatic Interactive Image Analyzer IBAS I and II (Kontron, Bildanalyse, Munich, FRG). Fibre type composition was studied on cryostat

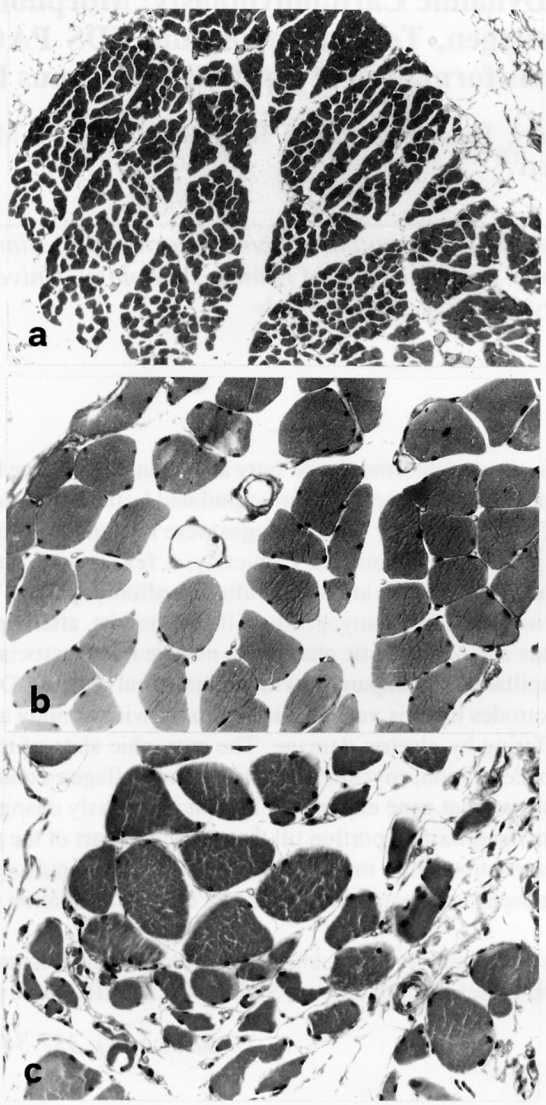

Figure 1. Histology of the left LD 15 months after cardiomyoplasty. Hematoxylin and eosin. a, normal general architecture of the grafted left LD, x60; b, moderate variation in the fibre diameter and internal nuclei in scattered fibres, x250; c, muscle fibre alterations induced by electric stimulation near the electrodes, atrophy and degeneration/regeneration of grouped fibres is evident, x250.

sections of muscles cooled in liquid nitrogen and stained for myosin ATPase at pH 9.6. The intramuscular capillarity was studied on transverse sections stained with Gomori-silver impregnation for reticulin and reported as capillary density for mm^2 (CD) and as number of capillaries/muscle fibre (C/F) [8, 9]. All molecular analyses were performed on cryostatic sections 20 μm thick. Their total protein and collagen content, myosin/actin ratio and myosin heavy chain composition were as described in [4, 5]. For electrophoretic analyses three cryostatic sections were solubilized and boiled. Separation of MHC was performed on 7.5% polyacrylamide gel slabs according to Rossini et al. [6]. Densitometric scanning of MHC and actin was performed using a GS 300 Transmittance Reflectance Scanning Densitometer (Hoefer Scientific Instruments) connected to a Mcintosh SE Apple Computer. The data were processed using the GS370 Densitometry Software [7].

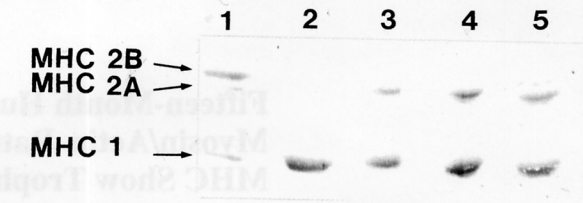

Figure 2. SDS PAGE of MHC of normal right LD, grafted left LD, and myocardium 15 months after cardiomyoplasty. 1, normal right LD; 2, myocardium; 3, transposed LD, at about 4 cm from distal aponeurosis; 4, transposed LD, at about 6 cm from humeral tendon; 5, transposed LD, at about 3 cm from humeral tendon. MHC2B: myosin heavy chain characteristic of the fast fatigable fibres; MHC2A: myosin heavy chain characteristic of the fast fatigue-resistant fibres; MHC1: myosin heavy chain characteristic of the slow fatigue-resistant fibres.

At autopsy the heart was moderately dilated and moderate myocardial thickness of the left ventricle was observed. The left LD appeared well wrapped to epicardium and stable. A total of 6 specimens were collected from the proximal till the peripheral part of the pedicled graft, taking care to include even some specimens of the heart-muscle interface. Five specimens from the contralateral normal LD were also collected as control for morphological analysis and morphometry.

Contralateral normal LD was composed by fascicles of polygonal muscle fibers (mean diameter 40 ± 3.0 μm with interposed lobules of fat tissue (18% of the total cross sectional area). A moderate variation in muscle fibre size was seen, but no structural alterations were observed. In the superficial area of muscle, a more pronounced variation in fibre size and occasional fibres with internal nuclei were seen. Reactions for myosin ATPase showed type 2B fibre predominance. Distribution of intramuscular capillaries was almost normal and they frequently appeared with restricted lumen.

The LD used for cardiomyoplasty showed normal histological aspect with moderate and non-specific changes in muscle fibres. Results were homogeneous in the specimens obtained from the different regions, except for the muscle areas near the electrodes. In the remaining specimens obtained from the proximal portion till the peripheral part of the pedicled graft, fascicles composed by polygonal fibres with a moderate variation in size (mean fibre diameter: 36

Table 1. Fibre diameter and capillary in normal right LD and grafted left LD 15 months after cardiomyoplasty. Mean ± SD on 30 fields at 40x objective; C/F, number of capillaries per fibre; CD, number of capillaries per mm^2; n.d., not determined.

	Fibre diameter	Capillarity		Interstitial fat
	(μm)	C/F	CD	(%)
Normal right LD	40 ± 3	1.2 ± 0.1	220 ± 36	18
Grafted left LD	36 ± 2	1.6 ± 0.1	280 ± 40	24
Grafted left LD at the site of electrodes	28 ± 4	n.d.	n.d.	n.d.

Table 2. Total protein and collagen content, Myosin/Actin (M/A) ratio, and MHC composition of normal right LD, grafted left LD, and myocardium 15 months after cardiomyoplasty.

	Protein %	Collagen %	MHC/Actin ratio	MHC %		
				2B	2A	1
Contralateral LD	8.5	1.8	2.0	52	20	28
Conditioned LD 3 cm from the humeral tendon	9.0	1.1	1.7	19	24	57
Conditioned LD 6 cm from the humeral tendon	6.0	1.4	1.6	15	36	49
Conditioned LD 9 cm from the humeral tendon	7.6	0.8	1.8	9	34	57
Conditioned LD 4 cm from the distal aponeurosis	14.5	0.6	1.7	9	35	56
Myocardium	7.7	0.4	1.4	0	0	100

± 2 μm), and occasionally fibres with internal nuclei were shown (Fig 1 a and b). The percentage of intrafascicular fat tissue was 24% of the total cross sectional area. Dilated capillaries were seen in all studied specimens and their number per fiber was moderately increased in respect to those of the contralateral normal LD (Table 1). Atrophy and focal degeneration/regeneration which result in variation in fibre size is present only near the electrodes, in which marked variation in fibre diameter (mean fibre diameter: 28 ± 4 μm), degenerative changes of scattered fibres, and interstitial fibrosis were evident (Fig 1 c); these alterations may be interpreted as the result of the physical electric damage, since they were confined to muscle fibres near the electrodes. Enzyme-histochemical reactions for ATPase reveal a pattern of fibre type transformation with very significant increase of slow type fibres. Fast to slow transformation (up to 60% of type 1 fibres) is present in all the specimens of the grafted LD.

Two previously reported cases showed that nine months and two years after cardiomyoplasty the LD flap stimulated every cardiac systole with 30 Hz bursts lasting 185 msec showed preservation of muscle structure without fibrosis, but histochemical analysis revealed a considerably higher percentage of type 1 fibres (up to 98%) than in the LD here presented [1].

The morphological results are confirmed by molecular characterization of the specimens taken from the grafted left LD. Total protein and collagen contents and myosin/actin ratio confirm that the conditioned LD is an almost normotrophic muscle (Table 2). Of particular interest is the evidence of change in MHC composition. In agreement with enzyme-histochemical analysis, gel electrophoresis shows that in all the specimens of grafted left LD MHC2B is drastically reduced and substituted by MHC2A and MHC1, the gene products expressed in fatigue resistant fast and slow fibres, respectively (Figure 2 and Table 2). Such a myosin composition suggests that though transformed the conditioned latissimus dorsi could have been faster contracting than a fully transformed muscle, i.e., a muscle which contains only type 1 fibres.

In conclusion, this case demonstrates that human latissimus dorsi is homogeneously, but not fully transformed after fifteen months of electrostimulation, and that muscle damage is not a compulsory consequence of the unusual pattern of muscle activity imposed to latissimus dorsi by electrostimulation protocol for Cardiomyoplasty.

Acknowledgements

Supported in part by funds from the Italian C.N.R. to the Unit for Muscle Biology and Physiopathology, and by funds of the Italian M.U.R.S.T. to U. C. and R.S..

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