

Myoblast Implantation in Demand Dynamic Cardiomyoplasty: a Bonus or a Must for the Heart Wrap?

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Abstract

Tissue or Cell Cardiac Bioassistance (Cardiac-Bio-Assists) is a biological approach to remedy to cardiac progressive insufficiency based on autologous tissue or cell transplantation. Some of the work-hypotheses are in pre-clinical evaluation (implants of embryonic myocardiocytes and of myoblasts derived from skeletal muscle satellite cell or Skeletal Muscle Ventricle, SMV), others are under preliminary or advanced clinical testing (Dynamic Aortomyoplasty and Dynamic Cardiomyoplasty, DyA and DyC).

Dynamic Cardiomyoplasty is a surgical procedure, which could support myocardial function when cardiac insufficiency would become pharmacologically intractable in the middle period. In this procedure a nonessential muscle, the Latissimus Dorsi (LD) is diverted from its normal role, transferred into the chest, and wrapped around the heart (LD Wrap), conditioned to fatigue and activated during systole to provide cardiac assistance. The clinical results of the international and Italian studies are encouraging, but it is still missing an objective evidence of an advantage of the procedure on the optimised pharmacological treatment. The mechanisms of its action are discussed and it remains the risk of myodystrophic lesions of the LD wrap, which could reduce the work capability of the pericardial muscle prosthesis.

The Italian MURST-funded “Italian Trial of Demand Dynamic Cardiomyoplasty” is addressing some of these issues by clinical research on the group of Italian patients of Demand Dynamic Cardiomyoplasty, and by animal experiments to develop and test new biotechnological, surgical, clinical and engineering approaches. In particular we will here discuss if increase of muscle mass of the distal part of the LD is desirable and feasible or if it is needed.

Key words: activity-rest stimulation, autologous tissue or cell transplantats, Cardiac-Bio-Assists, Demand Dynamic Cardiomyoplasty, echocardiography, LD wrap mechanogram, myoblasts, skeletal muscle satellite cell.

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Morbidity and morbidity of congestive cardiac insufficiency, differently than others cardiac diseases, do not decrease, in spite of significant progress of pharmacological treatments, due to increased longevity of the population. Cardiac failure is the most frequent cause of death in Europe, with 500.000 new cases every year and 50% mortality after five years. Cardiac insufficiency heavily limits autonomy and social relations of the patients, which experience an “early functional ageing”: in spite of their anagraphic age the cardiopathic subjects show performances of an eighty-year-old people. Therefore, two are the goals of any therapy: improve quality of life and increase survival [1]. Alternatives to current pharmacological treatments

for advanced heart failure are mandatory due to clinical needs. Cardiac transplant is the therapy of election when cardiac failure become pharmacologically intractable, but all over the developed world (nothing to say, the situation in the underdeveloped countries) the number of heart transplants has reached a limit set by the availability of donor organs. Though admission to transplant list is more and more selective, 25% of patients still die while awaiting a heart. In the near or far future xenotransplants could solve this problem, but even being optimists on their development and reliability, they would carry the risk of anthroponozoonotic viral infections [2].

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Ventriculoplasty is attracting interest of many cardiac surgeons, but because it involves discarding up to 300 g of not necessarily diseased myocardium, it is difficult to view it as other than an extreme remedy to extreme dilated cardiomyopathies. It currently carries high preoperative mortality and an adequate assessment of its benefits awaits the outcome of properly conducted trials [3, 4]. Mechanical artificial hearts will continue to be used mainly as a bridge to transplant. Even if the problems of hemocompatibility and infection were solved, the need of an external power supply might pose an unacceptable psychological challenge to the patient over the longer time.

Against this background, a biological, surgical alternative to cardiac assists (Cardiac-Bio-Assists), based on use of autologous transplants of skeletal muscle tissue or myogenic cells onto or in myocardium remains an attractive goal, worth being pursued strenuously [5].

Dynamic Cardiomyoplasty (which sounds like, but it is different from cardiomyoplasty or ventriculoplasty, that is the ablation of some ventricular wall) is a surgical treatment for heart failure, which uses a pedicled graft of the latissimus dorsi to wrap the heart. After one-two healing weeks the LD is conditioned to fatigue resistance and the permanently activated every second systole [6-9]. Transposition of LD is without functional consequences, and indeed it is a standard procedure used by plastic surgeons to transplant pedicled skin graft or for breast reconstruction after mastectomy. When pharmacological therapy fails to prevent recurrent episodes of cardiac congestive failure, Dynamic Cardiomyoplasty offers several potential advantages over alternative options. Unlike mechanical supports, skeletal muscle requires no external power source. Each patient serves as "donor", so rejection is not at all a problem, and therefore immunosuppression is not required. The implanted device is a pacemaker coupled to a neurostimulator.

Though mechanisms of cardiac support are not fully understood, dynamic cardiomyoplasty probably assists the damaged myocardium, thus preventing systolic bulging, and it girdles the ventricle, thereby inhibiting progressive ventricular enlargement. Both mechanisms probably contribute to the subjective decrease in symptoms experienced by patients. More importantly, these mechanisms explain the results of the phase II and III clinical trials, in which, end-diastolic volume is unaltered, i.e., progressive left ventricular enlargement is inhibited, in spite of poor or absent evidence of increased systolic support [9-11]. The lacking evidence of systolic augmentation in dynamic cardiomyoplasty may partly be due to myodystrophic lesion of the LD wrap, which may reduce mass and power of the LD wrap [13-17]. On the other hand, the clinical

improvement is clear in a large majority of subjects, and appreciated by patients and their parents [6-9, 18-20].

Historically, palpating the left axillary region for the presence of the muscle twitch has done verification of muscle contraction. LD contraction can also be verified by fluoroscopy to note heart displacement during assisted beats or shortening of the distance between the intramuscular electrodes or metal clips fixed to the LD wrap. More invasive techniques involving catheterization, such as pressure-volume loop analysis, can also document LD contractile activity, though in an indirect way, and provide information about the optimal delay setting. Such invasive techniques are not practical on a routine basis. Proper synchronisation is typically performed by using M-mode echocardiography. The timing of the LD contraction is tuned based on observing the time of mitral valve closure and the onset of the electrical impulses delivered by the myostimulator. However, this method is limited to tuning the electrical events rather than the actual mechanical events of LD contraction and relaxation. We developed a new method for non-invasive, bedside monitoring of LD function using a standard polygraph, previously used for monitoring cardiac apical motion and heart sounds. ECG and heart tones are registered simultaneously with the pressure changes due to LD wrap contraction and relaxation that are measured near the rib window using the probe normally used for recording an apicocardiogram. From the LD "mechanogram," we can determine: i) LD activation threshold; ii) the duration of the full LD contraction-relaxation cycle; iii) optimal synchronisation delay between cardiac events and "contraction-relaxation" of the LD wrap; iv) dynamic contractile characteristics of the LD wrap based on the determination of the tetanic fusion frequency, and their changes over time; and v) efficacy of Demand Stimulation to induce daily activity-rest stimulation periods (interval stimulation) able to retro-differentiate LD wrap dynamic characteristics after full slow transformation.

The clinical observations that activity-rest stimulation tunes the dynamic characteristics of the LD wrap are in full agreement with results of long-term training-detraining experiments in rodents, rabbit, goat, sheep and man. The effects of the intermittent stimulation are also corroborated by additional evidence, using the pattern of stimulation mandatory for Dynamic Cardiomyoplasty. In these animal experiments, increased speed of wrap contraction is accompanied by significant increase of muscle power. It is important to note that in the last experiments measurements of peak isometric force do not equate to the maximum force-generating capability of these muscles but rather indicate their capacity to perform work under stimulation conditions generally accepted for clinical

use. Under activity-rest pattern of stimulation LD wrap provides higher power than continually stimulated LD, and of the left ventricle, too. Furthermore, the muscle is fast enough to contract and relax during cardiac systole [21-28]. We shown that LD wrap slowness reverses by the activity-rest regime even after years of standard stimulation (Tetanic fusion frequency of 11 ± 2 Hz after standard stimulation vs. 30 ± 3 Hz after demand regime, $p < 0.0001$. After Demand Dynamic Cardiomyoplasty there are no deaths (eight subjects, 42 ± 8 months post-operation, of which 14 ± 3 months of demand stimulation). Quality of life is substantially improved with significant reduction of heart failure symptoms (NYAH class: pre-op 3.0 ± 0.0 , post-Demand Dynamic Cardiomyoplasty 1.5 ± 0.2 , $p < 0.0001$). In the subgroup of patients light stimulated from LD conditioning, exercise capacity tends to increase over pre-op values more than two years after operation (VO₂ max: pre-op 12.3 ± 0.7 vs. 16.6 ± 1.7 post-Demand Dynamic Cardiomyoplasty, $p = 0.05$). In this group of patients the excitation threshold of the LD wrap is not increased throughout the two years of follow-up. The conclusions of the Italian Trial of Demand Dynamic Cardiomyoplasty, a phase two study, are that demand stimulation and mechanography of the LD wrap are safe procedures, which could offer long-term the benefits of Dynamic Cardiomyoplasty to patients with pharmacologically intractable heart failure [20].

On the other hand, this study leaves open several questions, which we hope to answer with an incoming research plan: the Cardiac-Bio-Assists/rT-DDyC project. We hope during this study to: 1. Confirm statistically by a randomised trial the clinical results already reached from the previous Italian study; 2. Optimise surgical procedures and electrical stimulation protocols of the LD wrap to reduce the risk of myodystrophic lesions of the muscle tissue wrapped to heart; 3. Sustain “dedicated” Italian industrial research by investing needed resources to the study and develop a new demand myo&cardio stimulator (Demand LD/Cardio Pacer), a new device to measure by non invasive analyses the dynamic characteristics of the muscle tissue transposed around the heart (Meccanography of the LD Wrap), and an implantable converter of mechanical to electrical power; 4. Explore the potentials of myoblast implantation to augment the distal part of LD, which is the one wrapped around the heart.

These objectives will be pursued with clinical research on the group of Italian patients of Demand Dynamic Cardiomyoplasty, and by animal experiments to develop and test new biotechnological, surgical, clinical and engineering approaches. The program will address several issues related to: i) the mechanisms of action of dynamic cardiomyoplasty, ii) those which control

muscle dynamic characteristics in the peculiar conditions dictated by clinical constrains, iii) and prevention of myodystrophic changes related to the peculiar use of the LD wrap. To the last purpose, we will test pre-stimulation of LD [29, 30], with or without combined pre-transposition vascular delay surgery [31, 32]. The program will include studies to take advantage of the recent developments of cell therapy approach [33-37]. In particular, we will explore in rodent models feasibility of increasing muscle mass of the LD wrap by injecting autologous myoblasts derived from muscle satellite cells [33-37]. Their growth to new reinnervated myofibers will be analysed. Muscle growth through hyperplasia will be also induced with electrostimulation protocols derived from rehabilitation management of muscle lesions in elite sportsmen. To this goal, local continuous delivery of growth factors by means of osmotic pumps will be also tested.

A major issue of dynamic cardiomyoplasty continue to be whether muscle damage is induced by the chronic abnormal stimulation, in particular when a muscle-to-heart contraction ratio of 1:1 or 1:2 are applied. Sport scientists and physiatrists are well aware that spontaneous exercise per se could be a trauma to muscle fibers [38, 41].

The unusual work performed in cardiomyoplasty by the wrap may damage LD muscle tissue. Direct histological evidence of muscle damage had been collected in sheep and goat experiments [39]. There are reports explaining long-term ceased effect of the procedure with indirect [42] or direct [43, 44] evidence of major muscle atrophy, fibrosis and fat infiltration.

On the contrary, autoptic cases directly show that this is not an obligatory event. 15 months or even 8 years after cardiomyoplasty morphological and molecular analyses of the LD wrap showed preserved muscle mass and patent vessels with normal endothelial and smooth muscle walls [16, 40]. Interestingly, in these two cases LD wrap was activated every second or fourth sensed QRS, and clinical results were excellent [16].

Pre-operative Vascular Delay and/or of low-frequency electrical pre-stimulation of LD to prevent/reduce myodystrophy of LD wrap will be tested in a simplified sheep model of dynamic cardiomyoplasty. Sheep will be randomised in groups of six animals. Operative and post-operative care will be along “The principles of laboratory animal care” NIH (# 86-36, 1985).

Under anaesthesia, LD will be isolated to ligare lumbar perforating arteries. After seven/fourteen days, left and right LD will be isolated, distal aponeuroses will be cut, the LD left to spontaneously shorten and resutured to costal wall. ITREL (Medtronic) neurostimulators or prototypes of Demand LD/Cardio Pacer (MEDICO spa) are implanted, and electrodes

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secured to the proximal part of the LD. Six days after, LD are stimulated according to the new "activity/rest" protocol (Arpesella et al. 1998. Ann Thorac Surg). The same day and one and two months after, dynamic characteristics and sustainable power of the LD are determined under anaesthesia. Three months after stimulation, the sheep are sacrificed after measuring the mechanical power testing. LD are isolated, weighed, photographed and biopsed at the proximal middle and distal thirds to perform morphologic and molecular analyses. Extent of fibber-type transformation (isomyosins) and of myodystrophic lesions (by molecular markers of muscle damage/regeneration/repair) are quantitated. In the other two sheep groups vascular delay is preceded or substituted by a period of low-frequency continual pre-stimulation according to Tang et al. Ann Thorac Surg 1999 (Salmons approach).

MEDICO spa of Rubano (Padova) is collaborating with human and financial resources to develop a new cardiomyostimulator for Demand Dynamic Cardiomyoplasty, the Demand LD-Cardio Pacer. An accessory for echocardiography, a phono/pressure transducer optimised for the LD wrap recordings, will be also developed to non-invasively analyse dynamic characteristics of the LD wrap according to Carraro et al. J Cardiovasc Diagn P (1998).

With the commercial cardiostimulator, the activity-rest protocol of LD wrap stimulation are obtained using a frequency cut-off, which could only be programmed in steps of ten beat per minute (bpm). To avoid the worst case of almost no daily stimulation, the cardiostimulator ought to be set to allow sparse periods of rest. Solutions to this is either to decrease to 1 bpm the setting steps or add clock and counter to the Demand LD-Cardio Pacer, so that to limit to 30,000 per day the electrical impulses delivered to the LD wrap. Indeed the characteristics are: 1. Interpulse intervals from 0 to 700 msec; 2. Number of impulses per train from one to six; 3. During activity periods, LD wrap is stimulated by cycles of 3 min activity followed by 6 or 9 min rest; 4. Activity-rest periods programmable in steps of one hour; 5. Impulses per day: 30,000 maximum.

The options of the Demand LD/ Cardio Pacer would also include sequential settings, which will allow to perform non invasive mechanography of LD wrap. It would be possible to automatically analysed stimulation threshold, optimise synchronisation of LD wrap contraction-relaxation during systole, and determine tetanic fusion frequency to follow over time changing dynamic contractile characteristics of the LD wrap.

To increase muscle mass of the distal part of the LD, in a rodent model of dynamic cardiomyoplasty, we will test implants of satellite cell-derived autologous myoblasts. Skeletal muscle will be explanted from legs,

and myoblasts expanded in vitro in the presence of myogenic factors released from macrophages. After transfection of a reporter gene, they will be injected in the LD (Cantini et al. In Vitro 1994; Cantini&Carraro J Neuropathol Exp Neurol 1995). Extent of myofiber hyperplasia and reinnervation will be quantitated in the presence or absence of growth factors locally released by means of osmotic pumps. We also plan stimulation of the new (if any) muscle by mini implantable electro stimulators for animal use provided by Prof. Salmons, Liverpool University. In case of positive results the experiment will be repeated in the rabbit and finally performed in sheep with the goal of increasing to clinically significant levels the distal third of the LD.

The final goal of these studies is to test clinical efficacy of Demand Dynamic Cardiomyoplasty, after such improved procedures will be secured by animal tests. The study will be performed according standards of randomised trial (as this is possible in case of surgical

Table 1. Selection criteria

Typology:

Age: minimum 18, optimum 40-60, maximum 70 years
Personal motivation: high
Family support: appropriate
Non-alcohol nor toxic intake
Contraindication for heart transplantation, personal opposition or organ unavailability, in patients with whom the procedure might be predictable in the middle term

Inclusion clinical data:

Dilated cardiomyopathy documented for at least one year NYHA class III (or IV intermittent)
Functional integrity of LD (neither neuropathies nor familiar acquired myodystrophies)
Adequate pharmacological treatment after AHA protocol
Left ventricular function seriously reduced with episodes of VT and/or VF, which require ICD (independent group)

Exclusion instrumental data:

Severe obesity
AF with average response > 100 bpm (refractory to pharmacological treatment)
Left ventricular volume > 110 ml/m² (echography)
Primary or secondary valvular heart diseases hemodynamically significant (in particular mitral insufficiency), which presumably would not reduce the volume after surgical treatment
Maximum VO₂ < 11 > 16 ml/kg/min
CV < 60%
Non reversible pulmonary hypertension
PCWP > 27 ml Hg
Serum creatinine > 2,8 mg/dl (traditional units)
Coagulopatopathies
Major hepatopathies

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Table 2. Protocol of instrumental and clinical analyses at admission and during follow-up.

Clinical workup at time Zero and at each 4/6 months of follow up:
Cardiological examination
Evaluation of NYHA class
Registration of the current therapy
Digitalis
Diuretics
ACE-inhibitors
Beta-blocker
Others
Instrumental workup at time Zero:
Hemodynamical evaluation
Left and right cardiac catheterization
Coronary radiography
Instrumental workup at time Zero and at each 6 months of follow up:
Evaluation of the electrical stability:
EKG
HOLTER
Signal averaged ECG (SAECG)
Evaluation of the cardiac function state and pulmonary pressure
Doppler echocardiography
Psycho-social follow up
Evaluation of the quality life by psycho-social questionnaires developed by Dr. Daliento

Table 3. Admission Centres (by 11.02.2000).

Division of Cardiology, Legnago General Hospital, (Verona)
Contact Person(s): M. Barbiero and G. Rigatelli
Division of Cardiology, University of Padova
Contact Person(s): L. Daliento and R. Razzolini
Cardiology and Division of Internal Medicine, Geriatric Hospital, Padova
Contact Person(s): M. Trivellato and F. Tamellini
Division Cardiology, Fondazione Maugeri IRCCS, C.M. di Montescano, (Pavia)
Contact Person(s): R. Riccardi and F. Cobelli
Division of Internal Medicine, Adria General Hospital, (Rovigo)
Contact Person(s): G. Vescovo
Division of Cardiology, Pavia University
Contact Person(s): Laura Scelsi

Admission Centres agree with the Registry contents. Even if a few instrumental analyses ought to be performed at a nearest or available Centre (Hosting Centre), follow-up will be responsibility of the Admission Centre.

procedures) to objectively confirm good results already

obtained in a group of Italian subjects [18-21]. A Demand Dynamic Cardiomyoplasty Registry (rT-DDyC Registry) is implemented, and it will allow, if not a randomised trial, comparison of results between groups

under common optimised pharmacological treatments. Specialists with the most experience in Dynamic Cardiomyoplasty from Italian Medical Universities, i.e., Padova, Brescia, Pavia, and Bologna and from well qualified Hospitals of Veneto and Lombardia will perform the study.

Objective 1.1 The Registry of Demand Dynamic cardiomyoplasty has been planned during late 1999 and approved by participating Scientists early during 2000. Responsible of the Registry is Prof. Sergio Dalla Volta, University of Padova.

Subjects, selected according to Table 1 criteria, are followed-up for one year (Table 2) to set up two groups: the control group under optimised pharmacological treatment and the experimental group under optimised pharmacological treatment and Demand Dynamic cardiomyoplasty.

Selection criteria are essentially those of the Cardiomyoplasty Group of the Division of Cardiology of the Legnago General Hospital (MEDICUS News 1997; 4: 22-25), with a few additions according to the Heidelberg Cardiac Surgery (as presented in R Mariotti, M Mariani: Cardiomioplastica Dinamica. Cardiologia 1999 (December); 24 Suppl 3: 53-57), and the suggestions of Luciano Daliento, Division of Cardiology of the University of Padova. Tables 1 and 2 were approved during the 4° TiCCD Workshop (Padova, 11 Febbraio 2000). Table 3 lists the Centres, which will follow-up the subjects admitted to the Registry.

In conclusion, the program will test potentials of Dynamic Cardiomyoplasty to support a declining heart function under optimised surgical procedures, LD wrap/heart synchronisation, and evaluation of clinical results. The preliminary studies are encouraging and rise new hopes that associated to optimised pharmacological therapy "demand" stimulation could offer long-standing benefits, which could delay long-term pharmacologically intractable heart failure.

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