Experimental effects of cardiomyoplasty on stressed normal left ventricle in sheep

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Abstract

Objective: Several basic mechanisms of cardiomyoplasty were discussed in the last years, but the definite underlying mechanism is still unknown. The aim of the present study was to determine the effects of cardiomyoplasty on pressure volume loops of the non-failed left ventricle under different myocardial working conditions.

Methods: We performed cardiomyoplasty in eight female sheep after conditioning of the left latissimus dorsi muscles. To simulate different stress conditions we used myocardial stimulation up to 150/min and an increased afterload up to 140 mm Hg. The changes of left ventricular pressure and volume, arterial pressure and arterial flow were registered and analyzed.

Results: We found a significant decrease direct cardiomyoplasty effects during simulated stress conditions with increased heart rate up to 150/min and an increased afterload up to 140 mm Hg. We have seen direct effects in the non-failing hearts at rest only. Conclusions: These findings do not favor the concept of direct cardiomyoplasty-induced improvement of cardiac function under stress conditions. It seems that the conditioned and transformed skeletal muscle already under normal perfusion conditions in normal hearts is not able to generate enough force for an effective contraction under stress conditions. We conclude that the mechanism of cardiomyoplasty can not be explained by a direct effect of muscular support alone but results also from recovery of failed myocardium.

Keywords: Cardiomyoplasty; Pressure-volume-loop; Left ventricular function

1. Introduction

Since the principle studies of Salmons and Pette concerning the physiological and biochemical basics of skeletal muscle transformation and the clinical introduction of cardiomyoplasty (CMP) by Carpentier and Chachques large efforts have been made to explain the effects of cardiomyoplasty on the failing ventricle. However, the underlying mechanism of action is still poorly understood [1–4].

Numerous clinical studies have demonstrated a beneficial effect of CMP in more than 400 patients with end-stage heart insufficiency worldwide [5–16]. Despite obvious clinical improvement only little differences in left ventricular function were observed using standardized techniques in most of these studies. There is increasing evidence, that the relatively small increase of left ventricular ejection fraction (EF) usually observed with CMP is not sufficient to explain the improvement in clinical status [8,12]. In experimental models the influence of CMP on different parameters (i.e. extent of induced collateralization, reduction of myocardial wall stress and the improvement of myocardial oxygen uptake) has been studied in order to explain this phenomenon [17–19]. But there is still a lack of clarity if the potency of contraction of a conditioned skeletal muscle is high enough to realize an active support even under stress conditions.

Recent studies from Cho et al. and Nakajima et al. showed that mechanical support of the myocardium by the skeletal muscle is important for the effects of CMP [20,21]. Accordingly CMP does not result from a direct mechanical effect on global function parameters, but leads to an indirect improvement by optimization of altered pressure-volume-loops of the insufficient myocardium.

To test this hypothesis and to verify the potential effects of skeletal muscle contraction in physiological stress situations we studied left-ventricular pressure and volume changes before and after acute cardiomyoplasty with differ-
ent myocardial working conditions in an experimental protocol in sheep.

2. Material and methods

All experiments were performed in accordance with the guidelines for the prevention of cruelty to animals of the Federal Republic of Germany’s legislation in the version of 18.08.86 (BGBl. I p. 1319), changed through article 5 of the law of 20.06.1990 (BGBl. I p 1080) and article 3 of the law of 30.08.1990 (BGBl. I p 1762).

2.1. Surgical procedure

In eight female Merino sheep (weight, 40 ± 3.5 kg) the left musculus latissimus dorsi (MLD) was exposed through a lateral skin incision in general anaesthesia using Ketamin (Ketanest®). Two stimulation leads (SP 5577-90/-30, Medtronic Inc., Minneapolis, MN) were placed in the muscle near the neurovascular pedicle and in a distance of about 6 cm, respectively. A myostimulator (ITREL, model 7420, Medtronic Inc. Minneapolis, MN) was implanted subcutaneously between the scapulae and connected to the stimulation leads. Wounds were allowed to heal for a 1 week period. Stimulation was begun (pulse width 210 μs; amplitude 5 V; burst frequency 5 Hz; 'on' time 0.5 s; 'off' time 1.5 s) and values were increased every other week. After 14 weeks of stimulation the following levels were reached: burst frequency 30 Hz, pulse amplitude 10.5 V, 0.5 s 'on', 1.0 s 'off'.

Now the animals were anaesthetized again. Induction of anaesthesia was performed with 0.22 mg/kg of Xylacine (Rampun®) and 11.0 g/kg Ketamin (Ketanest®) and was maintained with appropriate continuous administration. All animals were intubated and put on volume controlled respiration.

After a left lateral skin incision the MLD was dissected from the origin. The insertion at the intertubercular groove of the humerus was preserved to serve as a contrapoint and to maintain the integrity of the thoraco-dorsal neuro-vascular pedicle. The muscle flap and the leads were then transferred to the thoracic cavity by way of a partial rib resection of the third rib. The heart was exposed through a left thoracotomy at the 4.–6. intercostal space and the pericardiotomy was performed. Subsequently the origin of the aorta was dissected and an Ultrasonic Transit-Time-Flowmeter (T 208, Transonic Systems Inc., Ithaka, NY) was placed proximal to the truncus brachiocephalicus. A Tipmanometer (SPC 370, Millar Instruments, TX) was placed in the aorta. A 7.5 F conductance catheter with pressure-sensor (ANP 098, Cordis) was implanted into the left ventricle via the dissected left internal carotid artery and connected to a conductance voltmeter (Leycom 2, Sigma 5 DF, CardioDynamics, Rijnsburg, The Netherlands). A stimulation lead (Temporary Pacing Electrode, Biotronik, Germany) was inserted in the right ventricle over the internal jugular vein.

All volume measurements were performed according to the guidelines of Baan et al. [22]. The time-varying segmental conductances are measured and converted to total left ventricular volume using the formula,

\[ V(t) = \frac{1}{\alpha} \left( \frac{L^2}{\sigma} \right) \left[ G(t) - G^0 \right] \]

where \( \alpha \) is a slope constant and \( L \) is the inter-electrode constant; \( G(t) \) represents the sum of the five segmental conductances.

The pressure-volume area represents the mechanical energy generated during LV contraction, and adds external work and potential energy. We used the area surrounded by the pressure-volume loop itself, which is the external work and corresponds to the stroke work [21].

After a stabilization period registration of pre-cardiomyoplasty values was performed (Table 1). Myocardial pacing in V00-mode was performed with an external pacemaker (EDP 30/A, Biotronik, Germany). The aortic root was partially obstructed distal to the truncus brachiocephalicus

<table>
<thead>
<tr>
<th>Time course (T min)</th>
<th>Operative-experimental situation</th>
<th>Measurement conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Original situation)</td>
<td>Implantation of a tip- and a conductance-catheter, inflating electrode, implantation of a flowprobe</td>
<td>Frequency 90/min, frequency 120/min, frequency 150/min, aortal occlusion</td>
</tr>
<tr>
<td>10 (Baseline value)</td>
<td>After performed CMP</td>
<td>Frequency 90/min; CMP (−); CMP (+); aortal occlusion</td>
</tr>
<tr>
<td>10</td>
<td>Measurement</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Recovery</td>
<td>Frequency 120/min; CMP (−); CMP (+); aortal occlusion</td>
</tr>
<tr>
<td>10</td>
<td>Measurement</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Recovery</td>
<td>Frequency 150/min; CMP (−); CMP (+); aortal occlusion</td>
</tr>
<tr>
<td>0</td>
<td>Measurement</td>
<td></td>
</tr>
</tbody>
</table>

* All measurements were performed at first at a fixed stimulation rate with and without skeletal muscle support and then with increased afterload up to 140 mm Hg. Before CMP a baseline measurement was performed to get the baseline values at all stimulation rates. CMP (−), cardiomyostimulator ‘off’ CMP (+), cardiomyostimulator ‘on’.
(about 60%) as long as needed to increase afterload according to the protocol.

After implantation of a sensing electrode at the right ventricle the ‘reinforcement’-cardiomyoplasty was performed clockwise. The skeletal muscle was fixed by suturing it to the lipid tissue of the atrioventricular sulcus. The cardiomyoplasty was completed by a side-to-side anastomosis of the free sides of the muscle. Both ventricles were completely included. Then the muscle leads and the trigger electrode were connected with a cardiomyostimulator (SP 1005; Medtronic Inc., Minneapolis, MN). Following CMP all parameters were registered again as baseline values (Table 1). Stimulation of the skeletal muscle was performed frequency-dependent triggered in relation to the electrical heart action (90/min, 1:2; 120/min, 1:3; 150/min, 1:4). The synchronization delay was downregulated with increasing stimulation frequency (60, 50, and 40 ms, respectively).

2.2. Statistics

All results are reported as mean ± standard error. Wilcoxon’s signed rank test was used to test for the differences of means. A P-value < 0.05 was considered significant. To verify the effects of cardiomyoplasty the data of CMP (+) and CMP (−) were compared each other for every level of myocardial stimulation (90, 120 and 150/min, respectively). Comparisons between the three levels were performed for the data of CMP (+) only.

3. Results

3.1. Pacing

After cardiomyoplasty a significant increase of endsystolic left ventricular pressure (LVESP), stroke volume (SV), mean aortic pressure (MAP) and aortic flow (Qaorta) was found for supported versus non-supported cardiac cycles at a pacing rate of 90/min and a ratio of 1:2. A significant increase for dp/dt max during contraction and a decrease of dp/dt min during relaxation (P < 0.05) could be demonstrated. There was no difference in end diastolic pressure (LVEDP) between supported and non-supported cycles. However, there was a significant increase of LVEDP in comparison with data registered prior to CMP (Table 2; Fig. 1a,b). The observed increase of SV and LVESP shifts the calculated pressure volume loops to the left.

After increasing the stimulation frequency up to 120/min the effect of muscle support became non-significant. The left ventricular end-systolic and aortal pressure changes of the supported cycles were smaller as compared to the values obtained at a stimulation rate of 90/min and did not differ significantly for supported and non-supported cycles (Table 2). However, a marked increase of stroke volume (P > 0.05), increase of maximum pressure rise velocity (dp/dt max, P < 0.05) and faster relaxation (dp/dt min) was found. The changes in the area surrounded by pressure-volume-loops with active skeletal support was less pronounced at a stimulation rate of 120/min as compared to the data obtained with a rate of 90/min (Fig. 1c).

Increasing the pacing rate up to 150/min resulted in a further decrease of skeletal muscular support. Both pressure changes and changes of stroke volume as well as stroke work and aortic flow differed little for supported versus non-supported cycles (Table 2; Fig. 1d). The observed increase in LVESP, mean aortal pressure and stroke volume for supported cycles at a rate of 150/min was significantly smaller as compared to the values for supported cycles at a frequency of 90/min (P < 0.05).

3.2. Aortal occlusion

With increased afterload produced by partial aortic occlusion the effect of CMP on changes in pressure and volume was significantly altered (Fig. 2, Table 3). Following partial

Table 2

Effect of CMP on hemodynamic parameters*:

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>F = 90/min</th>
<th>F = 120/min</th>
<th>F = 150/min</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>F 80/min</td>
<td>CMP (−)</td>
<td>CMP (+)</td>
<td>CMP (−)</td>
</tr>
<tr>
<td>LVEDP</td>
<td>12 ± 3</td>
<td>14 ± 3</td>
<td>18 ± 4</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>LVESP</td>
<td>86 ± 5</td>
<td>88 ± 4</td>
<td>105 ± 6*</td>
<td>81 ± 7</td>
</tr>
<tr>
<td>SV</td>
<td>33 ± 3</td>
<td>32 ± 4</td>
<td>42 ± 5*</td>
<td>28 ± 6</td>
</tr>
<tr>
<td>dp/dt max</td>
<td>2144 ± 433</td>
<td>2256 ± 712</td>
<td>2587 ± 431*</td>
<td>2189 ± 564</td>
</tr>
<tr>
<td>dp/dt min</td>
<td>1845 ± 512</td>
<td>1968 ± 474</td>
<td>2047 ± 323*</td>
<td>1693 ± 459</td>
</tr>
<tr>
<td>P aorta mean</td>
<td>83 ± 9</td>
<td>82 ± 10</td>
<td>88 ± 8*</td>
<td>81 ± 6</td>
</tr>
<tr>
<td>Q aorta</td>
<td>2.5 ± 0.3</td>
<td>2.7 ± 0.4</td>
<td>3.8 ± 0.5*</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>PVA</td>
<td>1337 ± 988</td>
<td>967 ± 345</td>
<td>2156 ± 688*</td>
<td>1035 ± 876</td>
</tr>
</tbody>
</table>

* Stimulation frequencies 90/min, 120/min and 150/min; data are shown as the mean ± standard error; *P < 0.05 to CMP (−); #P < 0.05 to CMP (+) for F = 90/min; dp/dt max and dp/dt min, maximal positive or negative pressure changes (mm Hg/s); LVEDP, left ventricular end diastolic pressure (mm Hg); LVESP, left ventricular end systolic pressure (mm Hg); P aorta mean, mean aortal pressure (mm Hg); PVA, area surrounded by pressure-volume loops (mm Hg × ml); Q aorta, aortic flow (l/min); SV, stroke volume (ml).
Fig. 1. Effects of CMP at different stimulation rates in generated pressure-volume-loops (—, supported cycles; ..., unsupported cycles). (a) Representative original registration of hemodynamic parameters of supported (arrows) and unsupported cycles (ECG, electrocardiogram; LVP, left ventricular pressure; $dp/dt$, pressure rise velocity; Vol., left ventricular volume changes). (b) Pressure-volume-loop generated from the data shown in Fig. 1a; (—, supported cycles; ..., unsupported cycles). (c) Effect of CMP at a stimulation rate of 120/min (ratio of muscle support rate to myocardial contraction rate 1:3). (d) Pressure-volume-loops of left ventricular function with Kardiomyoplastie at a stimulation rate of 150/min, (ratio of supported to unsupported cycles was 1:4).
aortic occlusion with increasing left ventricular end systolic and mean aortal pressure a decrease of stroke volume for supported cycles was found \((P < 0.05)\). As a result aortic flow did not differ for unsupported cycles and was significantly lower for supported cycles after aortic occlusion. The maximum pressure rise velocity was unchanged, while relaxation \((dp/dt_{min})\) was significant increased (Table 3). Concerning pressure-volume-loops we observed an increase of area surrounded by pressure-volume-loops following occlusion, but during maximal occlusion no difference was seen for supported and non-supported cycles (Fig. 2).

4. Discussion

Since the clinical introduction good functional results have been documented in numerous studies \([1,2,5,6,8-12,15,16]\). In patients with end-stage myocardial insufficiency Carpentier et al. \([1]\) observed a 7-year-survival rate of 70.4\% and a significant improvement in NYHA functional class from a mean of 3.3 to a mean of 1.8 after cardiomyoplasty. These excellent results are in a striking contrast to the minor changes of global left ventricular function parameters that have been observed in clinical and experimental studies \([1,12,23]\).

Cheng et al. \([24,25]\) have shown a markedly improvement of global and regional ejection fraction for both the left and (after biventricular CMP) right ventricle after maximal stimulation of the transformed skeletal muscle. The reported increase of EF from a mean of 18\%-31\% with a considerable standard deviation was significant, but cannot answer the question of the principal therapeutic effect of CMP.

The changes of left ventricular pressure-volume loops following active CMP have been described earlier by Cho et al. \([20]\) in animal studies. The beneficial effects of a supporting skeletal muscle on myocardial performance could not be convincingly explained solely by interpreting simple function parameters as the left ventricular ejection fraction.

Our data suggest, that in the sheep model under physiological conditions at rest an acute hemodynamic effect of the contracting skeletal muscle is reflected in the changes of pressure and volume parameters as well as for the resulting loops.

Kass et al. \([26]\) recently reported similar results after chronic CMP. They demonstrated a left-shift of end-diastolic pressure-volume relations in patients at 12 months after cardiomyoplasty and interpreted these results as a return of ‘remodeling’ of the insufficiency ventricle with optimization of myocardial working conditions. The clinical study of Schreuder et al. \([27]\) showed that the delay between QRS-complex and stimulation of the skeletal muscle is important in order to achieve the best support. In comparison of assisted and unassisted beats they demonstrated similar pressure-volume-loops at rest as we have found in this experimental study. However, because of the relative small direct effect of cardiomyoplasty on pressure and volume changes at rest they concluded, that cardiomyoplasty acts as an elastic girdle around the heart. We found in a pre-experiment that in sheep a delay between 40 and 80 ms was the best delay for an effective skeletal muscle support.

Under these conditions we found a markedly active support by the skeletal muscle for heart rates up to 120/ min. It became obvious, that the transformed muscle was indeed capable of fast contractions. Contrary to these findings the muscle already caused impairment of ventricular relaxation even at frequencies between 90 and 120/min.
 Obviously a stepwise frequency-dependent decrease of the delay preserved also not enough time for skeletal muscle relaxation. With increasing stimulated heart rate the effect of cardiomyoplasty on systolic contraction further decreased while at the same time impairment of relaxation was worse. Thus, at a frequency of 150/min there was no more evidence of an active mechanical support by CMP. On the contrary, diastolic function became adversely affected.

With increasing afterload we found a continuous decrease of the pressure and volume changes induced by CMP. With steady-state conditions at an aortic pressure of 140 mm Hg there was no more change in left ventricular pressure and only a small change in stroke volume detectable. As a consequence the area under the pressure-volume loop was nearly the same for supported and non-supported cycles with increasing afterload.

However, clinical studies with long-lasting support by cardiomyoplasty have shown, that especially under stress conditions with a subsequent increase of afterload left ventricular ejection fraction improves [6,10,28]. But it is not clear whether this effect following an active skeletal muscle contraction or the observed effect is a result of a myocardial recovery. Therefore the aim of our study was not to show the chronic effect of cardiomyoplasty as in clinical use. The question was: how potent is a conditioned skeletal muscle at rest could be the basic for a functional recovery with a higher stress tolerance for a long time.

The limitation of our study is of course the non-failing heart model, which does not allow for a transfer of the results on clinical cardiomyoplasty without restrictions. A similar study is currently conducted in an animal model with depressed cardiac function and preliminary results are similar to those described above.

In the present study we have shown, that the effect of cardiomyoplasty can not be interpreted as a direct supporting mechanical system only. To explain the excellent clinical results additional functional recovery of the myocardium with changes at a cellular or subcellular level has to be postulated. This issue will be addressed in ongoing experimental studies.

References


