Brief Communication

Direct Effect of Increased Hemodynamic Load on Cardiac Mass

Borivoj Korecky and Mary Masika

The hearts of rats were transplanted into the abdomens of recipients of the same inbred strain by attaching the stumps of aorta and pulmonary artery end to side to the abdominal aorta and inferior vena cava, respectively, of the recipient. The transplant functions as a denervated “nonworking” Langendorff heart; the recipient in situ heart serves as a normal control. One week after surgery, an onset of atrophy is observed in the transplanted heart, which stabilizes after 2 weeks; the in situ heart grows normally. Using this model, we increased the load of the left ventricle (LV) in the transplant by inserting a permanent polyethylene cannula into its aortic orifice during surgery to induce valvular incompetence and/or stenosis (TPE group). This resulted in significantly increased LV systolic pressure (115±5 versus 95±3 mm Hg) and a significantly increased rate–pressure product (34.7±1.7 versus 24.4±1.4 mm Hg ∙ min⁻¹ ∙ 1₀⁻²) as compared with rats with control transplants (TC group). The LV mass in the TPE group decreased to only 85±4.8% of the mass of the corresponding in situ recipient heart as compared with 59±2.6% in the TC group (p<0.001). In three cases in the TPE group with highest overload, we observed about 20% larger LV mass in transplanted hearts as compared with the corresponding recipient in situ hearts. These results indicate that the increased load significantly attenuated the atrophy observed in LV of the isotransplants. This attenuation could be correlated with the increment of load as indicated by higher peak LV pressures and higher rate–pressure products. (Circulation Research 1991;68:1174–1178)

A hemodynamic overload leading to cardiac hypertrophy may have either a direct effect on the myocardium or an indirect one through cardiovascular reflexes and/or circulating soluble growth factors. The isotransplanted rat heart heterotopically located in the recipient’s body represents a unique model for examination of the direct humoral effects on the myocardium, because the transplanted heart is exposed to the same hormonal milieu as the in situ heart but does not support the hemodynamic load. In our model, the heart of an inbred rat is transplanted into the abdomen of a recipient of the same inbred strain by attaching the stumps of aorta and pulmonary artery end to side to the abdominal aorta and to the inferior vena cava, respectively, of the recipient. The transplanted heart is perfused by the recipient’s blood through the coronary vessels; its left ventricle beats mostly isovolumetrically and at a slower rate than does the heart in situ. The transplant functions as a denervated “nonworking” Langendorff heart and does not appreciably contribute to the hemodynamics; the recipient in situ heart supports the hemodynamics and serves as a control heart in situ. Several days after surgery, an onset of atrophy (loss of existing mass) is observed in the left ventricle of the transplanted heart, which stabilizes after 2 weeks; the in situ heart grows normally. Since both hearts remain in the same hormonal environment, they represent a unique model to test interventions acting directly on the transplanted heart, which is unloaded and relatively independent of changes in hemodynamics and neural stimulation.

In the present experiments, we created experimental aortic insufficiency in the donor heart at the time of transplantation surgery. We hypothesized that the increased filling of the left ventricle during diastole might lead to increased left ventricular stress during systole and that the hemodynamic load on the transplant would increase. We also hypothesized that this might ultimately attenuate the progression of atrophy as compared with the in situ heart of the recipient or might even lead to the development of hypertrophy

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of the left ventricle in the transplant as compared with the heart in situ.

The results of the experiments indicate that the development of an otherwise predictable degree of atrophy of the left ventricle in the heterotopic isotransplant was attenuated or minimized by application of the additional load. In addition, hypertrophy of the left ventricle was observed in several transplanted hearts in which aortic insufficiency led to a major overload as compared with the recipient hearts in situ.

Materials and Methods

Hearts of Lewis inbred male rats (Charles River Laboratories, Inc., Wilmington, Mass.) weighing 330–350 g body wt were transplanted into the abdominal cavity of recipients of the same strain by using a modified technique of Ono and Lindsey, as described in detail by us in 1983. In the present experiments, the donor heart was exposed to cold (3–5°C) cardioplegia using a modified solution (Plegisol, Abbott Laboratories, North Chicago) with a potassium concentration to 16 mM. The donor heart received 15–25 ml of the above solution by retrograde perfusion through the aortic stump with pressures not exceeding 50 mm Hg.

Two groups of rats with transplanted hearts were prepared: 1) In the control group (TC group, n=17), the donor heart was transplanted as above. 2) In the experimental group (TPE group, n=16), the aortic orifice of the arrested donor heart was cannulated with polyethylene tubing (PE-20; 1.09 mm o.d.). The cannula, which was about 15 mm long, was advanced into the apex of the left ventricle and protruded into the aortic stump of the donor heart. It was then left inside as a permanent stent, and the transplantation surgery was performed. The middle part of the cannula was previously flattened to prevent its ejection into the abdominal aorta of the recipient and also to minimize formation of clots.

After recovery, the rats were put on a standard diet and received antibiotics for 7 days (Gentocin 1.5 mg/day). The beating of the transplants in both groups was monitored by palpation. The control TC group was compared with the aortic-stented TPE group. To cover the time span of anticipated development of atrophy and/or hypertrophy, rats were killed between 4 and 20 days (see Figure 1). In all TPE rats and in eight TC rats, the following protocol to assess the hemodynamic status was attempted: After general anesthesia (40 mg/kg i.p. Nembutal, Abbott Laboratories), the carotid artery of the recipient was cannulated with an 18-gauge needle cannula, and the arterial pressure was monitored using a Gould-Statham pressure transducer (model P23, Gould, Cleveland, Ohio) and displayed on a recorder (model RPS 7C8, Grass Instrument Co., Quincy, Mass.). After stabilization, the abdominal cavity was opened, the transplant was inspected, a short blunt 18-gauge needle was advanced through the apex into the left ventricle, and the left ventricular pressure was recorded as above. A simultaneous high-speed tracing of both pressures was obtained. This was used to measure the peak systolic pressure, the lowest diastolic arterial pressure, and the heart rate of the in situ heart and the initial diastolic pressure, the end-diastolic pressure, the peak systolic pressure, and the heart rate of the transplanted heart. The rate–pressure product (heart rate times peak systolic pressure) in the left ventricle of the transplant was later calculated and used as an index of hemodynamic load (mm Hg×min⁻¹×10⁻³). The rat was then bled, and both hearts were removed, dissected, and weighed. Samples of the left ventricles were taken for determination of dry weight. The reported mass of the left ventricles included the free wall plus septum.

Statistical Analysis

Results were statistically evaluated by Student’s t test, linear regression, and standard correlation.

Results

As can be seen from Figure 2, the mass of the left ventricle of the control isotransplanted hearts started to lag behind the corresponding in situ normal hearts in the TC group (100%, dashed line) on the fourth day after surgery; this lag gradually increased with time, reaching its maximum by about 2 weeks (p<0.01). On the other hand, no such relation could be observed in the TPE group. In three transplants in the TPE group, hypertrophy of the left ventricle could be observed in spite of the fact that by this time after surgery the transplants of the TC group had regressed to their lowest weights. When we compared the mean values of both groups of isotransplants, a significantly lower left ventricular mass in the TC group was observed than that in the TPE group (p<0.01) (Table 1). The attenuation of the process of atrophy and development of the moderate
hypertrophy in the transplants observed in the TPE group could be correlated with the value of the left ventricular peak systolic pressure (y=29+0.529x; r=0.573; p<0.05) as displayed in Figure 2. A similar positive correlation was found between the left ventricular end-diastolic pressure (x) and cardiac mass (y) in the TPE group (y=72+1.024x; r=0.722; p<0.01). Therefore, it seems that these two important indexes of load could be closely related to the difference existing between the left ventricular mass of the transplant in the two groups.

The values of the left ventricular systolic pressure of the transplant as seen in Table 2 depend, among other factors, on the perfusion pressure through its coronary vasculature, which is due to the arterial pressure of the recipient. To relate the two oscillatory pressures, which do not cycle in phase because of the difference in heart rates, we related the peak systolic pressure of the transplanted heart to the peak systolic arterial pressure of the in situ heart, which was taken as 100%. The average value of this pressure ratio expressed in percent was somewhat lower in the TC group (94%), but its value was significantly higher (see Table 2) in the TPE group (116%). These data indicate that in the latter group, conditions allowing for ejecting beats (i.e., positive pressure gradients from the left ventricular cavities of the transplants into their aortic stumps) must have occurred quite frequently.

**Discussion**

The left ventricle of a heterotopic isotransplanted heart loses a predictable degree of existing cardiac mass within the first weeks after transplantation, and subsequently the transplant does not follow the usual allometric organ growth depending on the growth of body mass as is usually observed in the heart in situ. In our previous experiments, we could follow this model for a period of up to 1 year. Similar cardiac growth patterns in an identical model were recently reported in younger animals (150–200 g body wt) in the same strain by others.

The following are perhaps the main reasons for the initial net loss of existing cardiac mass and subsequent cessation of growth after transplantation: 1) the reduction of hemodynamic load, which may be due to lower filling volumes, lower peak systolic left ventricular pressures, and lower heart rate, 2) a complete denervation of the heart, which may be responsible for the decreased heart rate and other reduced values, and 3) the heterotopic location of the transplant, which may expose the heart to different ambient pressures and different frictional resistance to movement as compared with the original location in the thorax.

Several indirect attempts have been made in our laboratory to increase the load on the transplant to stimulate its growth. We were unable to change the pattern of developing atrophy either by administration of norepinephrine or isoproterenol in euthyroid rats (authors’ unpublished observation). When we transplanted the atrophied hearts of hypothyroid rats into the abdomen of hypothyroid recipients and subjected them to large doses of thyroxin, we could observe a predictable growth stimulation of the heart in situ; however, in the transplant, the only change was the predictable shift of the isomyosin spectrum, but no stimulation of actual organ growth could be observed. Similar findings using thyroxin in isotransplants of younger euthyroid male rats of the same strain were reported by others.3 Inbred female Fischer 344 rats with isotransplants located in the abdominal cavity were subjected to swimming; no attenuation of atrophy was observed in transplanted hearts, whereas a mild hypertrophy was observed in the in situ hearts.7 In all the above attempts to indirectly increase the load of the transplant, a significant shift to a predominantly α-isomyosin was observed, and no direct effect on stimulation of the transplant growth was observed in spite of the
Table 2. Characteristics of Transplanted Rat Hearts

<table>
<thead>
<tr>
<th></th>
<th>TC (n)</th>
<th>TPE (n)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVP (mm Hg)</td>
<td>95±3.4</td>
<td>115±5.2</td>
<td>2,471</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PSP (%)</td>
<td>94±1.6</td>
<td>116±6.2</td>
<td>2,532</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>HR (%)</td>
<td>73±3.0</td>
<td>83±3.8</td>
<td>1,781</td>
<td>NS</td>
</tr>
<tr>
<td>RPP (mm Hg/sec×10³)</td>
<td>24.4±1.4</td>
<td>34.7±1.7</td>
<td>3,857</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVS (%)</td>
<td>59±2.6</td>
<td>85±4.8</td>
<td>4,905</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean±SEM and were obtained from rats 4–20 days after surgery. TC, control transplanted hearts; TPE, transplanted hearts in which a permanent polyethylene aortic stent was inserted; LVP, peak systolic left ventricular pressure of the transplanted heart; PSP, LVP expressed as percentage of peak systolic pressure in the arteries of the corresponding recipient hearts; HR, heart rate of the transplanted heart expressed as percentage of the heart rate of the in situ recipient heart; RPP, rate–pressure product of the transplanted heart; LVS, mass of the left ventricle and septum of the transplanted heart expressed as percentage of mass of the left ventricle and septum of the respective in situ recipient heart; NS, not significant.

The direct cannulation of the left ventricle through the apex induces some bleeding. Because the transplant is located in proximity to the inferior vena cava of the recipient, a temporary interference with the venous blood return to the in situ heart is unavoidable during the measurement of left ventricular pressure. This was why some of the hemodynamic measurements obtained from the transplanted heart were expressed in relation to concurrent values obtained from the in situ heart; also, they are, to a point, interdependent (see Table 2).

From our present experiments, we conclude that the growth of cardiac mass can be stimulated in the whole denervated isointransplanted heart by imposing an additional load on the left ventricle by creating experimental aortic insufficiency and/or stenosis. This isointransplanted heart will continue to beat in a normal, stable environment of a recipient animal whose in situ heart follows a normal allometric growth. Therefore, we can discount the role of cardiovascular reflexes and the role of circulatory growth factors as necessary prerequisites for the growth of cardiac myocytes. The stimulated growth of myocardium in our case was induced by a local mechanism that responded by increasing the ratio of protein synthesis to degradation of the total amount of contractile and other myocardial proteins.

Our results do not preclude the possibility that in the in situ heart cardiovascular reflexes or circulating growth factors may play an additional or even an initiating role. It is also possible that in other isolated heart systems⁸⁻¹¹ alternate factors leading to increased protein synthesis, resulting in stimulating growth of the myocytes in vitro, may be operational. However, our results reiterate the importance of local wall stress in inductions of stimulated growth of the heart, as reported in other experimental models as well.¹¹,¹²,¹³

Since the power-producing ventricular myocytes represent more than 70% of the volume of myocardial tissue,¹⁴ it is the ratio of biosynthesis to biodegradation of their proteins¹⁵ that will primarily determine the reported alteration of cardiac mass in the transplanted hearts.

The fact that an increased density of β-adrenergic receptors in this experimental model was reported.⁸

In our present experiments, we opted for a direct increase of the hemodynamic load of the left ventricle by experimentally induced insufficiency and/or stenosis of the aortic valve, but no other attempt was made to interfere with the cardiovascular reflexes or hormonal status of the recipient. Our treatment resulted in a significant increase in end-diastolic pressure, peak systolic pressure, and rate–pressure product in the left ventricle of the transplanted heart of the TPE group as compared with that of the TC group. As a result of the above treatment, the average left ventricular mass of the transplanted heart of the TPE group decreased significantly less than that of the transplanted heart of the TC group.

When evaluating the response in individual rats, we have to take into consideration the time after surgery and the response of the transplanted heart to the permanent stent located in its left ventricle and protruding through the aortic valve into the aortic stump. As can be seen from the individual data, some of the transplants showed only modest increase in indexes of load, whereas the majority exhibited a significant increase in load. Consequently, the cardiac mass of the transplanted hearts exhibited a spectrum of responses ranging from none or modest attenuation of the process of atrophy to true cardiac hypertrophy as compared with the in situ hearts.

In the transplanted heart that showed the greatest hypertrophy (122%), left ventricular systolic pressure was 153 mm Hg and left ventricular end-diastolic pressure was 48 mm Hg; these values show a considerable overload of the left ventricle. This heart probably would have failed in the not too distant future, since its rate of beating at the time of measurement was only 188 beats/min as compared with 395 beats/min for the in situ heart; consequently, its rate–pressure product was only 30. This example illustrates the need of indexing the load by various measurements at the same time and the limitations of the validity of assessment of the long-term postoperative load by one final measurement only.
References


KEY WORDS • atrophy • cardiac growth • hypertrophy
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