Incomplete sympathetic reinnervation of the orthotopically transplanted human heart: Observation up to 13 years after heart transplantation

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Abstract

Objective: Heart transplantation (HTx) is associated with autonomic denervation of the donor heart. Sympathetic reinnervation (RI) as defined by the presence of functional nerve terminals occurs only if sympathetic ganglia outside the heart are connected with nerve terminals in the transplanted heart. The purpose of this study was to define the incidence and functional consequences of RI over time after HTx. The activity and distribution of norepinephrine (NE) uptake sites were assessed by positron emission tomography (PET) imaging. Symptom limited exercise testing was performed by bicycle-ergometer. Methods: Forty-seven patients (m/f = 42:5, 47.6 ± 8.2 years, age range 27–65 years) were investigated between 2 months and 13.6 years after HTx using PET and the NE analogue C-11-hydroxyephedrine (HED). Tracer uptake was quantified using dynamic imaging protocols yielding regional HED retention fraction. A regional value above 7%/min (±2.5 SD above the mean value of denervated hearts) was considered evidence for RI. The functional significance of RI was investigated in 34 patients (m/f = 30:4, 49.3 ± 8.4 age range 27–62 years) by symptom limited exercise testing. Cardiac catheterization was performed at the time of PET imaging. Results: RI could not be assessed in the first year after HTx, in 11% in the second year and in 80% of the patients from the third year on. Retention values plateaued then. In all time intervals, beyond the third year, not reinnervated patients were found. RI remained incomplete and was always restricted to the anterior wall of the left ventricle. Extent of retention of the left ventricle revealed a large individual range up to 66%, averaging of 20%. Recipient age at the time of HTx, reinnervated patients were 5.5 years younger than not reinnervated ones, proved as the only significant influencing factor for RI (P < 0.05). Dividing patients into scintigraphically reinnervated (n = 20) and not reinnervated (n = 14), reinnervated patients displayed during exercise a higher maximal heart rate (137 ± 14 vs. 123 ± 20/min, P < 0.05), heart rate increase (40 ± 15 vs. 28 ± 13/min, P < 0.05), max. oxygen consumption (1674 ± 424 vs. 1279 ± 308 ml/min, P < 0.01) and anaerobic threshold (887 ± 170 vs. 717 ± 183 mlO2/min, P < 0.01) than not reinnervated ones. A correlation between transplant vasculopathy and RI could not be demonstrated. Conclusion: RI assessed by PET and the NE analogue HED is time dependent, incomplete, displays a typical pattern and demonstrates a broad individual spread. Furthermore, RI enhances functional parameters of exercise testing. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Heart transplantation; Sympathetic reinnervation; Exercise testing; Positron emission tomography; Time course; Transplant vasculopathy

1. Introduction

Explantation of a donor heart for heart transplantation (HTx) causes autonomic denervation, i.e. axonal degeneration and depletion of norepinephrine (NE) stores in the presynaptic nerve terminal [1]. Functional reinnervation and its clinical relevance has been discussed in the past based on animal experiments and clinical observations after transplantation [2–10]. Recently, scintigraphic studies using the NE analogues C-11-meta-hydroxyephedrine (HED) and I-123 meta-iodobenzylguanidine (MIBG) indicated regional reoccurrence of NE uptake sites in the human heart [11–13]. Furthermore, tyramine induced transcardiac NE spillover was demonstrated in patients after HTX and interpreted as an indicator of sympathetic reinnervation (RI) [14–17]. These results, derived from two different methods, indicate that regional RI by sympathetic nerve fibers occurs in the human transplanted heart with functional integrity of the nerve terminals. Little is known about the time course and the clinical significance of such partial reinnervation of the transplanted heart [18].

The purpose of this study was to define the incidence of cardiac RI over time after transplantation. Thus, the intensity and extent of NE uptake sites was measured by PET imaging.
imaging and compared with functional parameters of exercise testing.

2. Methods

2.1. Patients

Forty-seven patients were selected out of a population of 421 patients who received a HTx between August 1981 and December 1994. All patients were operated on according to Lower and Shumway’s technique [19]. In order to investigate RI over time in a cross sectional study we selected patients 2 months to more than 13 years after HTx, aiming at an even distribution in time. Patients were asked for participation in the study during their yearly follow-up at the clinic for cardiac surgery and were scanned immediately if PET was available, or if the patient’s residency was close enough to return to the clinic later. Patients had to be older than 18 years.

Patients were excluded, if their medication interfered with catecholamine uptake, i.e. tricyclic antidepressants, or if they presented diabetes mellitus. None of the patients showed signs of rejection in the endomyocardial biopsy 24–48 h before the PET study. Hypertensive drugs were discontinued 1 day before the study.

All patients received immunosuppressive therapy consisting of cyclosporine A, azathioprine and prednisone. Thirteen patients had a combination of antithymocyteglobulin (ATG) in combination with cyclosporine A. Graft rejections were assessed by biopsy and rated according to Billingham or ISHLT [21]. Clinical findings triggering rejection therapy were defined as rejection episodes.

Forty-seven patients (male n = 42, female n = 5) with a mean age of 47.6 ± 8.2 years (27–65 years) were studied by PET. At the time of investigation left ventricular ejection fraction was 73 ± 11% (n = 39), as determined by left ventricular angiography. While six of the patients had no acute rejection episodes after HTx, 24 had one or two and 17 patients had more than two rejection episodes. PET, as well as exercise testing was performed from 2.0 months to 13.6 years after transplantation. A subgroup of 34 patients (m:f = 30:4, 49.3 ± 8.4, age range 27–62 years) underwent bicycle-ergometer testing (Table 1).

The protocol was approved by the ethics committee of the Technische Universität München, and informed consent was obtained by all patients before entering the study.

2.2. Positron emission tomography (PET)

The radiolabeled catecholamine analogue C-11-hydroxyephedrine (HED) was synthesized according to Rosenspire et al. [22,23].

Imaging was performed with a PET scanner (951 R CTI/ Siemens Knoxville, TN). Myocardial perfusion was assessed by studying N-13-ammonia (370 MBq) [24,25]. After physical decay of N-13, HED (740 MBq) was injected as a slow bolus over 30 s and dynamic data acquisition was initiated over 40 min [11].

Attenuation corrected, transaxial images were reconstructed (spatial resolution of 9 mm) and circumferential activity profiles were used to quantify regional tracer distributions [25]. From the dynamic PET images, retention was defined as HED activity at 40 min divided by the integral of the blood activity curve [26]. Previous studies showed that hearts transplanted within 6 months were completely denervated. Therefore, these hearts served as controls and all values below the upper 1% confidence limit of the mean (7%/min) were considered to represent denervated areas of the heart.

The fraction of left ventricular myocardium that showed HED retention above this threshold was used to define the extent of reinnervation.

Retention values derived from healthy age-matched volunteers were available in the Clinic of Nuclear Medicine, Technische Universität München.

2.3. Spiroergometric exercise test

Symptom limited exercise testing was performed using a bicycle ergometer (Ergotest Jaeger, Germany), increasing the work load by 20–30 W every 3 min. Exhaled air was continuously monitored, oxygen uptake was measured paramagnetically, and carbon dioxide release was determined using infrared analysis. Maximal work load, heart rate, maximal oxygen consumption, and anaerobic threshold [27,28] were determined in each patient as parameters for response to exercise.

2.4. Statistical analysis

All values are given as mean ± SD. Statistical comparisons between HED-positive and negative patients were performed using Student’s t-test. One way ANOVA was used to determine significant differences between HED retention and functional parameters of exercise testing in different time intervals. Posthoc analysis was done by the
Scheffe test. Multiple t-tests were performed including Bonferroni–Holm correction. A P-value of less than 0.05 was considered statistically significant. Correlation between status of RI and the occurrence of transplant vasculopathy (TVP) was tested in a contingency table.

3. Results

3.1. Positron emission tomography

All 47 patients underwent N-13-ammonia and HED scanning. Relative regional N-13-ammonia values were within 2.5 standard deviations of normal flow in all patients. In contrast, HED uptake was absent or heterogeneous. Fig. 1 depicts examples of PET images of two patients: 1.0 and 8.0 years after transplantation. Both showed normal perfusion in the left ventricle. Early after transplantation there was no scintigraphic evidence of HED uptake, while late after transplantation regionally increased retention was noticeable. Normal volunteers displayed an equal distribution of the entire heart, the average retention value in all segments amounted to 17%/min [11].

Fig. 2 depicts the maximal HED retention of each patient vs. the postoperative time. The scatterplot demonstrates that there is an increase in retention in the first years after HTx in the majority of patients, but a further increase does not occur after the fourth year. Some patients do not show any signs of reinnervation, not only early but even late, e.g. our very first patient.

3.2. Time course of RI

In order to define the time point, at which the majority of patients show reinnervation, the mean retention value ± SD in eight patient groups was calculated.

The threshold of 7%/min was exceeded on average in the third year after HTx as depicted in Fig. 3. Up to the third year there is still an increase of retention.

Due to the small number of included patients in the fifth year...
year after HTx this group missed the significance level. So did the cohort transplanted longer than 11 years. Only one of the four patients could be classified as reinnervated. This explains the decline of the average retention value below the threshold of 7%/min in this time period.

During the first year after HTx none of the patients exceeded the threshold, between the third and the fifth year approximately 80% of all patients showed reinnervation. The number of patients reaching the threshold of 7%/min or not at different time intervals is noted in Fig. 3.

3.3. Retention pattern

Tracer retention shows a typical pattern. It starts at the basis of the anterior wall of the left ventricle to expand then time dependent to the septal and lateral parts and to the apex. Homogeneous retention of HED in the entire heart, as in normals, was never observed. It was always confined to the anterior wall and never detected at the posterior wall and the apex. Polar maps confirm these observations. Fig. 4 depicts that retention occurs primarily in the left anterior descending artery (LAD)-area – excluding the apex – and partially in the circumflex artery (RCX)-area and not in other coronary areas.

3.4.Extent of reinnervation

The extent of HED above 7%/min ranged from 0 to 66% and increased with time. No reinnervated area could be found in the first year after HTx, but up to the fifth year a steady increase was measured, reaching an average of 20 ± 18%.

The reduction of extent in patients transplanted longer than 11 years was due to the fact that 3 out of 4 patients in this cohort did not show any signs of reinnervation.

The whole group of all 47 patients showed a large individual spread, e.g. in the subset 6–8 years post HTx only one patient did not exceed 7%/min, never the less the extent of reinnervation of three further patients was between 1 and 5% and only one reached 21%.

3.5. Factors influencing RI

Our very first patient (13.6 years after HTx) did not show any signs of reinnervation. The retention value was 4.3%/min. A second investigation within 3 months yielded the same result. On the contrary, one patient 1.4 years after HTx achieved 10.9%/min HED retention. This suggests that other factors may influence RI. Analyzing factors influencing RI (Table 2), only recipient age was found to be significant. Reinnervated patients were at the time of HTx 5.5 years younger than not reinnervated ones. Difference in rejection episodes, on average nearly one time more frequent in not reinnervated than in reinnervated patients, did not reach significance.

3.6. Exercise test

Maximum work load, maximum oxygen consumption, peak heart rate increase, and anaerobic threshold ranged from 40 to 180 W, 780 to 2570 ml/min, 7 to 74/min and 420 to 1360 mlO2/min, respectively. In order to evaluate a time course of exercise data the patients were grouped according to their postoperative period in five groups. In Table 3 the results are summarized for these groups after HTx. Without selection of innervated patients according to positive HED accumulation, a steady increase up to the 60th month of each parameter was found. Beyond the fifth year post HTx however, results amount to a level achieved in the first year. No significant correlation between these parameters and time after transplantation was found. The only exception was heart rate increase between the patient groups transplanted less than 1 year and in the third year after HTx.

Using the HED signal, the patients were grouped to ‘reinnervated’ or ‘not reinnervated’, and the functional parameters in these two groups were compared. As displayed in Table 4 significant differences between these two groups became evident. The differences in maximal oxygen consumption (1674 ± 424 vs. 1279 ± 308 ml/min) and anaerobic threshold (887 ± 170 vs. 717 ± 183 ml O2/min) were even highly significant \( P < 0.01 \). Time after HTx (4.4 ± 3.0 vs. 2.5 ± 3.8 years) proved to be a significant factor too.

3.7. Transplant vasculopathy (TVP)

Thirteen of the 47 PET investigated patients demonstrated coronary changes in cardiac catheterization. These changes developed after baseline catheterization after HTx and were diagnosed as TVP. Five patients have been treated by PTCA, some even several times. The first appearance was 17 months post HTx in a female transplant, all other patients experienced TVP between 3.5 up to 13.6 years. According to PET results, four were not reinnervated, nine
Table 2  
Symphatic reinnervation proven on the basis of HED retention in relation to potentially influencing factors

<table>
<thead>
<tr>
<th></th>
<th>R (reinnervated)</th>
<th>NR (not reinnervated)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11C-HED &gt;7%/min</td>
<td>11C-HED &lt;7%/min</td>
<td>P</td>
</tr>
<tr>
<td>No. of patients</td>
<td>26</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Gender (m:f)</td>
<td>24:2</td>
<td>18:3</td>
<td>ns</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>27.2 ± 8.3</td>
<td>27.5 ± 9.3</td>
<td>ns</td>
</tr>
<tr>
<td>Recipient age (years)</td>
<td>45.2 ± 8.4</td>
<td>50.7 ± 7.2</td>
<td>0.024</td>
</tr>
<tr>
<td>No. of rejection episodes</td>
<td>2.3 ± 1.4</td>
<td>3.2 ± 2.3</td>
<td>ns</td>
</tr>
</tbody>
</table>

*a t-Test: P < 0.05, group R vs. group NR.

reinnervated. The average time after HTx was for the first group 8.7 years, for the latter 7.9 years. The two patients living the longest time with a transplanted heart (11.8 and 13.6 years) did not show RI, but revealed TVP.

There was no correlation between the extent of TVP and HED-retention (P = 0.24), e.g. a patient 6.3 years post HTx with massive TVP showed a retention of 11.1%/min, another 11.8 years post HTx with several PTCA’s and myocardial infarction a value of 5.6%/min.

Two of the 13 patients were successfully retransplanted (11.8 years post HTx not reinnervated, 7 years post HTx reinnervated).

4. Discussion

This study addresses the incidence and the time course of RI after HTx. PET and HED offer the advantage for quantitative measurement of RI. The extent of reinnervation increased in general over time, paralleling maximal retention, but showed a large individual spread. Furthermore, sympathetic RI proved to be functional significant.

There is limited scintigraphic evidence for reinnervation in the first year after the operation and the most dramatic increase of tracer retention occurs between 1 and 3 years after surgery and increases only little thereafter. About 80% of the population demonstrated reinnervation late after transplantation.

The retention values increased up to the fourth year after HTx and stayed constant thereafter. Some individuals did not show any evidence of RI even in the long term, others demonstrated an early and strong but always partial reinnervation.

Cardiac transplantation leads to denervation of the donor heart because surgical interruption of the postganglionic sympathetic nerve axon causes rapid depletion of the cardiac neurotransmitter NE within nerve terminals [29]. RI as defined by the return of functioning nerve terminals can occur only if sympathetic ganglia outside the heart are connected with nerve terminals in the transplanted heart.

This PET-study with the NE analogue HED confirms earlier work of Schwaiger et al. identifying catecholamine uptake sites in the transplanted heart, which become more prevalent and involve larger parts of the heart as time after transplantation progresses [11].

However, in our observed time period of up to more than 13 years after transplantation there was no patient with complete restoration of neuronal ventricular HED uptake. Thus, only partial RI takes place in a majority of patients with HTX.

Due to limitations in spatial resolution, only the left...
Ventricular tracer distribution can be adequately imaged by PET, not atria or right ventricle. Therefore, conclusions regarding sinus node innervation cannot directly be drawn from PET images.

There is no obvious explanation for the regional pattern of HED uptake, other than that reinnervation may initiate along the great arteries (pulmonary artery and aorta) and slowly progress from the base to the apex of the left ventricle, as demonstrated in animals [2].

However, this process remains incomplete and surgical techniques – all patients were transplanted according to Lower and Shumway’s technique – may limit the growth of fibers across anastomoses causing heterogeneous structural reinnervation.

Analyzing factors for RI recipient age was found to be significant. The innervated patients were on average 5.5 years younger than the not reinnervated ones. Rejection episodes only showed a trend and were approximately one time more frequent in not reinnervated patients.

Before HED became available, Jodine-123-MIBG and SPECT (single photon emission computed tomography) enabled assessment of RI. Applying this qualitative scintigraphic analysis Dae et al. found evidence in four of ten patients for RI within the first year after HTx and in further two in the second year [30]. In another study by de Marco et al. 11 of 23 patients revealed RI between the first and the second year [13]. The findings of our own PET results are more consistent with an MIBG study by Gürtner et al. [31].

The two scintigraphic methods demonstrate the same pattern of RI (anterobasal, -septal and -lateral) and the lack of RI in some patients up to 13 years p.o. De Marco et al. demonstrated that ischemic cardiomyopathy is more favorable for RI than dilated disease, which could not confirmed by our results.

There was also close agreement between the scintigraphic results and NE release after tyramine. NE release has been investigated in heart transplant recipients and control subjects previously [14]. Seventy-eight percent of recipients beyond the first year yielded evidence for significant, although partial, reinnervation with average NE release of 25% of control values. The results of these investigations also suggested regional differences in NE release. In 14 long-term transplants, which were classified reinnervated in a former investigation, Wilson et al. intubated selectively LAD and RCX. After application of intracoronary tyramine they found by NE release a regional pattern, which was similar to the scintigraphic results, confirming them [15].

De Marco et al. compared the assessment of RI by MIBG with transmyocardial NE release in 16 patients. All ten patients with a clear NE release demonstrated an anterobasal MIBG retention. Six patients displaying no retention did not show a transcardial release [13]. Own PET results vs. NE release were as good as in the aforementioned study [32].

These findings not only confirm the specificity of the tracer approach for neuronal tissue but also the reoccurrence of amine uptake sites as well as tyramine sensitive NE release from storage vesicles as a sign of normally responding sympathetic nerve terminals.

Angiographically assessed TVP could not be correlated with RI. Of the 13 patients with TVP, 9 (69%) were classifi-
fied as reinnervated and 11 (85%) of the 13 patients were transplanted more than 6 years ago. This suggests that TVP is emerging in the long term follow-up independent from RI.

The pattern and time course of transplant reinnervation was first described in animal studies. Adrenergic nerves were found in biopsies taken from dogs 1 year after transplantation [1]. Dog studies using direct electrical stimulation had shown RI as early as 74 days after transplantation starting at the atria spreading to the apex [2,33]. Functional effects were noticed 9–12 months after transplantation, showing normal response to autonomic nerve stimulation but less than normal catecholamine content in tissue [2,3,33].

In humans histologic evidence for RI failed [11,34]. RI was concluded from clinical signs, like an altered heart rate response of the transplanted patients. Several authors found a lower heart rate in remote compared to early transplanted patients and an increased heart rate during exercise in long term transplants [7–10].

Although cardiac transplantation has been shown to improve survival of patients with endstage heart failure considerably, recovery of exercise tolerance remains unsatisfactory. In accordance with data by Stevenson [28], the achievable work load in the studied patients was low. Exercise capacity remained unchanged in the different groups after transplantation, in agreement with a recent longitudinal study [35]. This persistent limitation of exercise tolerance probably reflects multiple factors. Since heart rate response is blunted, the transplanted heart relies more heavily on preload/volume changes to increase cardiac output during exercise. However, work load significantly increased by 24%, paralleled by about 30% increase in maximal oxygen consumption in patients with scintigraphic evidence of reinnervation. The exercise induced heart rate increased to a significantly greater extent in these patients, which may explain the enhanced performance. However, the positive inotropic response of partial innervation may also contribute to an improved contractile response to exercise. In contrast, De Marco et al. [13] did not find significant differences in peak exercise heart rate or peak oxygen consumption in the reinnervated patients. Since the average time after transplantation in our patients was longer and functional effects may occur delayed, this may explain the discrepancy.

This study indicates time dependent RI of the human heart after orthotopic transplantation. Despite only partial RI, there is evidence of a significant correlation of scintigraphic results with functional parameters of autonomic innervation.

The data suggest further, that using current surgical techniques and standard immunotherapy, structural RI takes place in the first 3 years after transplantation and remains constantly incomplete thereafter. The occurrence of TVP seems to be independent from RI. The apparent inhomogeneity of the reinnervation process sparing the posterior wall of the left ventricle and lack of RI in some individuals need further investigations.

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