Serial Assessment of Sympathetic Reinnervation After Orthotopic Heart Transplantation
A Longitudinal Study Using PET and C-11 Hydroxyephedrine

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Background—Little is known about the progressiveness of sympathetic reinnervation late after cardiac transplantation (HTX). The aim of the present study was to describe individual growth of sympathetic terminals after HTX by a longitudinal quantitative assessment.

Methods and Results—In 20 patients after HTX, dynamic PET with C-11 hydroxyephedrine (HED) was performed twice within 3.0±0.5 years. According to the time interval between HTX and first PET, subgroups of patients early (group A, <1.5 years; n=7), intermediate (group B, 1.5 to 7 years; n=7) and late (group C, >7 years; n=6) after HTX were defined. At the time of first HED PET, 10 patients were completely denervated (7 in group A, 2 in group B, and 1 in group C). Only 3 remained denervated at second PET. A significant increase of reinnervated myocardium between first and second PET was found in all 3 groups (0% to 9±9% of left ventricle for group A, P<0.05; 13±12% to 23±17% for group B, P<0.05; 21±21% to 37±23% for group C, P<0.05). The magnitude of increase was similar between groups. Reinnervation was first surveyed in the basal anterior region, then toward apex, septal, and lateral wall. Inferior wall remained denervated. The largest reinnervated area surveyed in an individual was 66% of the left ventricle.

Conclusions—The present data confirm the low likelihood of sympathetic reinnervation within 18 months after HTX. Once the reinnervation process is initiated, a continuous growth is observed even late after HTX, suggesting a progressive nature of reinnervation. Reinnervation, however, remained regionally heterogeneous, and a complete restoration was not found until 15 years after HTX. (Circulation. 1999;99:1866-1871.)

Key Words: transplantation ■ nervous system, autonomic ■ positron emission tomography

At cardiac transplantation, postganglionic sympathetic nerve fibers of the donor heart are interrupted, causing rapid depletion of norepinephrine within the nerve terminal and resulting in complete denervation.1 Various approaches have been applied to demonstrate subsequent restoration of cardiac catecholamine uptake and storage sites after heart transplantation (HTX) in humans. Evidence for sympathetic reinnervation has been derived from invasive measurements of transcardiac norepinephrine spillover,2 from power spectral analysis of heart rate variability,3 and from radionuclide studies using catecholamine-analogs such as radioiodinated metaiodobenzylguanidine,4 or the positron emitter C-11 hydroxyephedrine (HED).5 These studies confirmed complete denervation within the first year after HTX. The prevalence of signs of reinnervation increased later after HTX, but complete restoration of innervation to normal levels was not found, suggesting that sympathetic reinnervation remains incomplete.

Despite this incomplete pattern, the potential clinical importance of reinnervation has been supported by demonstration of its relevance for regulation of myocardial blood flow6,7 and by an association of reinnervation with improved exercise performance compared with denervated patients.8 However, questions regarding the progressive nature of reinnervation late after HTX remain, as most of the previous studies were cross-sectionally designed. Currently, little is known about the individual development of localization, extent and density of sympathetic nerve terminals. Quantitative measurements of regional sympathetic reinnervation have not yet been applied longitudinally. It remains unclear whether a constant growth is found once the reinnervation process is initiated or whether a standstill or even decrease of the ingrowth of sympathetic fibers occurs late after HTX.

It was thus the aim of the present study to longitudinally assess sympathetic reinnervation in a series of patients at different time points after HTX. PET and HED were applied for noninvasive global and regional quantification of the individual reappearance and growth of sympathetic innervation.
Methods

Patients and Study Design
From a group of 47 patients enrolled in a previous cross-sectional study with PET and HED,²⁰ 20 patients (2 women, 18 men) at various time points after orthotopic heart transplantation were asked to undergo a second PET study approximately 3 years later. Six patients were transplanted because of ischemic cardiomyopathy and 14 because of idiopathic dilated cardiomyopathy. At the time of both PET measurements, all patients were symptom-free, and none showed signs of acute rejection as documented by clinical follow-up and endomyocardial biopsy. None received medication known to interfere with catecholamine uptake in presynaptic nerve terminals (eg, antidepressants, clonidine, or reserpine). All patients underwent immunosuppressive therapy consisting of cyclosporine A. Mild to moderate graft vessel disease, defined by angiographic presence of circumscript narrowing of vessels or dilated angiopathy was present in 5 of the 20 patients.

The first PET study for initial determination of reappearance of myocardial norepinephrine uptake sites had been performed at 0.2 to 11.8 years after HTX. The second PET study was performed 3.0±0.5 years (range 2.4 to 4.2 years) later to measure additional individual growth of sympathetic reinnervation. Cardiac events (episodes of acute rejection, heart failure, or exacerbation of severe graft vessel disease) did not occur in any of the patients between first and second PET study.

According to the time interval between HTX and first PET, groups of patients early (group A, <18 months; n=7), intermediate (group B, 18 months to 7 years; n=7), and late (group C, >7 years; n=6) after HTX were generated. Characteristics are shown in Table. The groups were matched for patient age, age of the donor heart, and time between first and second PET study. No significant difference for the number of treated rejection episodes before inclusion in the study was present; the incidence of graft vessel disease was expectedly higher in group C late after HTX compared with the other groups. No significant difference for the number of treated rejection episodes before inclusion in the study was present; the incidence of graft vessel disease was expectedly higher in group C late after HTX compared with the other groups.

PET
The radiolabeled catecholamine analogue C-11 HED was synthesized according to Rosenspire et al.¹⁰ Imaging was performed using an ECAT 951 or ECAT EXACT PET scanner (CTI/Siemens). Performance characteristics of these scanners have been described previously.¹¹,¹² In brief, these scanners have an axial field of view of 10.8 cm and 16.2 cm, respectively, yielding 31 and 47 transaxial slices to cover the whole cardiac region. After adequate positioning, a transmission scan of 15 minutes was acquired for correction of photon attenuation using external germanium-68 rod sources. Myocardial perfusion was assessed qualitatively using 370 MBq of N-13 ammonia¹³ or C-11 acetate.¹⁴ After 5 half-lives to allow for decay of the perfusion tracer, 600 to 740 MBq of C-11 HED at high specific activity of >300 Ci/mmol were injected as a slow bolus over 30 s, and a dynamic imaging sequence of 14 frames over 40 minutes (6×30, 2×60, 2×150, 2×300, 2×600 s) was initiated. Heart rate and blood pressure were monitored continuously throughout the imaging procedure.

Data Analysis
Attenuation-corrected transaxial images were reconstructed by filtered backprojection using a Hannig Filter with 0.3 cycles/bin cutoff frequency, resulting in a spatial resolution of 8 to 9 mm full width at half maximum (allowing accurate quantification of tracer retention in normal left ventricular myocardium). Using current PET methodology, quantification for structures below this resolution, such as atria and right ventricle, or calculation of a transmural gradient for the left ventricle is not feasible.

A volumetric sampling tool was applied to create polar maps of activity distribution throughout the entire left ventricle.¹⁵ Additionally, the arterial input function was derived from a small circular region of interest in the left ventricular cavity. From the dynamic PET images, myocardial HED retention was defined as activity at 40 minutes divided by the integral of the arterial blood curve.¹⁶

On the basis of results in denervated hearts,⁵ myocardium showing HED retention below 7% min was defined as denervated. The global extent of reinnervation was quantified by the percentage of polar map showing retention above this threshold. Maximal left ventricular HED retention was chosen to define the global intensity of reinnervation.

In addition to global analysis, myocardial HED retention was regionally assessed to describe the localization of reinnervation. A 9-segment model (Figure 1) was applied to the polar maps, and the percentage of reinnervated myocardium for each segment was calculated.

Statistical Analysis
Values are expressed as mean±SD. A paired Student’s t test was applied to compare results from first and second PET. Differences between subgroups of patients were assessed by 1-way factorial
ANOVA and the post hoc t test according to Bonferroni/Dunn. Correlation between continuous variables was described by Pearson's correlation coefficient and tested for significance by Fisher's r to z transformation. \( P < 0.05 \) was generally defined as significant. For the post hoc t test according to Bonferroni/Dunn, \( P < 0.0167 \) was defined as significant.

Results

First PET and Initial Reappearance of Catecholamine Uptake Sites

At the initial PET evaluation, relative regional myocardial perfusion was within 2.5 SD of normal flow in all patients. Individual maximal myocardial HED retention ranged from 3.8% to 13.7%/min, and the reinnervated area was between 0% and 55% of the left ventricle. Maximal myocardial HED retention and reinnervated area correlated highly significantly \((r = 0.88; P < 0.001)\). Complete myocardial denervation, defined as HED retention \(< 7\% / \text{min} \), was found in 10 of the 20 patients: all patients of group A early after HTX \((n = 7)\), 2 patients in group B, and 1 patient in group C. Average values for reinnervated area and maximal HED retention in the 3 groups early, intermediate, and late after HTX are shown in Figure 2. The initial extent of reinnervation was significantly correlated with time between first PET study and HTX \((r = 0.47; P = 0.03)\).

Follow-Up PET and Individual Growth of Sympathetic Reinnervation

Approximately 3 years later, intensity and extent of reinnervation increased in 17 of the 20 patients. Again, no abnormalities of regional relative perfusion were surveyed. Overall, only 3 patients \((1 \text{ in every group}) \) remained denervated. A decrease of the reinnervated area was not found in any of the individuals. The largest area of reinnervation in an individual at follow-up was 66% of the left ventricle.

Results of the second PET study and changes between first and second study for the 3 groups are illustrated in Figure 2. In all 3 patient groups \((\text{early}, \text{intermediate, and late after HTX})\), both maximal HED retention and reinnervated area increased significantly. The magnitude of increase for all 3 groups was similar even late after HTX \((\text{increase of maximal HED retention: 3.8} \pm 2.5\% / \text{min for group A versus} 2.1 \pm 1.9\% / \text{min for group B versus} 4.3 \pm 3.7\% / \text{min for group C;} P = \text{NS between groups; increase of reinnervated area:} 9 \pm 9\% \text{ of LV for group A versus} 10 \pm 10\% \text{ for group B versus} 16 \pm 11\% \text{ for group C; P = NS})\).

Regional Extent and Growth of HED Retention

Regional extent of reinnervation in 9 myocardial segments at first and second PET, and increase from first to second study for the 3 groups are shown in Figure 3. After initial complete denervation, the basal anterior wall showed the highest percentage of reinnervation at follow-up in group A early after HTX. A less substantial increase was surveyed in the distal anterior and basal septal wall. The anterior basal wall remained the segment with the highest degree of reinnervation in groups B and C, both initially and at follow-up. In contrast to group A, the highest increase of reinnervation in group B intermediate after HTX was not seen in the basal but in the distal anterior wall, whereas both septal segments also showed substantial increase. Later, in group C late after HTX, growth shifted from the septal segments to the lateral wall.

Some evidence of reinnervation in the apical region was found at follow-up in group B and at both time points in group C. The inferior wall remained completely denervated, except at the latest time of observation in group C late after HTX, where marginal parts of the inferior segments showed minor evidence of reinnervation.

Two individual examples of the development of regional HED retention are shown in Figure 4. A substantial increase of sympathetic reinnervation is demonstrated not only in a patient early after HTX \((\text{group A})\) but also in a patient late after HTX \((\text{group C})\).

Discussion

In summary, this longitudinal study confirms that the likelihood of reinnervation is low within the first 12 to 18 months after HTX but increases with time after HTX. For the first time, however, it has been shown that, once initiated, sympathetic reinnervation progressively increases even late after HTX. This late increase was surveyed despite the presence of mild to moderate graft vessel disease. First evidence of myocardial reinnervation is found in basal parts of the
anterior wall. Further growth then occurs from basal to distal parts of the myocardium, from anterior to septal, and then lateral wall. Late after HTX, apex and inferior wall may demonstrate some signs of reappearing sympathetic nerve terminals. Up to 15 years after HTX, however, a complete reinnervation was not surveyed in this study.

**Time Course of Sympathetic Reinnervation**

HTX results in cardiac denervation by surgical interruption of postganglionic sympathetic fibers and subsequent rapid depletion of catecholamine storage in nerve terminals.\(^1\) It has been demonstrated, in various animal models, that sympathetic reinnervation does occur.\(^{17-19}\) In humans, the presence of structural sympathetic reinnervation has been derived from invasive measurements of tyramine- or handgrip-induced cardiac spillover of norepinephrine\(^2\) and myocardial uptake of radiolabeled norepinephrine analogues.\(^4,5\) The functional integrity of sympathetic nerves after reinnervation has been supported by a concomitant increase in heart rate variability.\(^3\) Furthermore, it has been shown that results of invasively measured norepinephrine spillover correlate with scintigraphic evidence of reinnervation.\(^4,9\)

In the present study, no evidence of reinnervation was found earlier than 18 months after HTX. The number of patients imaged within this period, however, was low. It should be pointed out that sympathetic reinnervation within 12 to 18 months has been described previously,\(^2,9\) but the overall likelihood of reinnervation early after HTX in these studies was also low, confirming the present results.

Most of the previous studies, however, were designed cross-sectionally and investigated 2 groups early and later after HTX, reflecting denervated and potentially innervated patients.\(^2,3,5,6,20\) Thus, accurate information about the individual time course and progressive nature of reinnervation remained scarce. Although De Marco and colleagues performed serial investigations before and after 1 year after HTX using I-123 metaiodobenzylguanidine and conventional scintigraphy, results were interpreted qualitatively without measurements of extent and growth of reinnervation. Addition-

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**Figure 3.** Regional extent of reinnervation in a 9-segment model in 3 groups early, intermediate, and late after HTX. Numbers represent mean ± SD of the percentage of reinnervation (defined by an HED retention >7%/min) for each segment at first and second PET study and the difference between both studies. For each group, 3 segments demonstrating the largest increase are marked (*).

**Figure 4.** Two examples for global and regional growth of sympathetic reinnervation in patients early (A) and late after HTX (B). The polar maps of left ventricular HED retention (upper rows) show an initial complete denervation in patient A at first PET 0.3 years after HTX, whereas 3 years later, substantial HED retention is found in the basal anterior and septal wall. The area of reinnervation (shown in white in the second row for each patient), increased from 0% to 18% of the left ventricle (LV). In patient B, 23% of LV were reinnervated at first PET 8.9 years after HTX. The reinnervated area increased further within 3 years to 48%. Although initial evidence of reinnervation was found in anterior and septal basal wall, further growth occurred toward the lateral wall and toward the apex and inferoseptal wall.
Serial Assessment of Sympathetic Reinnervation

ally, the latest observation time after HTX was 3 years in the latter study. Thus, no information about the development of reinnervation later after HTX could be derived. In a recent study using PET and C-11 HED, a large series of 47 patients was investigated to build 4 groups at different times after HTX. Although reinnervation increased most prominently from a group less than 1 year after HTX to a group between 1 and 3 years after HTX, no definite conclusions about the individual development could be drawn because of the cross-sectional design.

The present longitudinal study demonstrates, for the first time, that sympathetic reinnervation is progressive, and that the extent of reinnervation continues to increase even late after HTX, up to 15 years after surgery. In confirming the results of previous studies, however, a complete restoration of innervation was not found. Although this observation cannot be readily explained, it may be speculated that the process of sympathetic reinnervation with respect to the limited life expectancy is too slow to survey a complete normalization of innervation after HTX. Additionally, regional heterogeneity may contribute to the lack of complete reinnervation. Growth seems to be limited in some parts of the myocardium, as suggested for the inferior wall in this study.

Regional Heterogeneity

Heterogeneity of sympathetic reinnervation after HTX has been demonstrated in previous studies. Regional HED retention in normals, on the other hand, is known to be relatively homogeneous. The present study confirms previous findings but describes, for the first time, regional growth of reinnervation in detail. Sympathetic nerve terminals reappear in the basal parts of the myocardium first and then extend further into distal parts, whereas the apex may be involved late after HTX. This finding is not surprising because sympathetic neurons are known to travel along arterial structures. If reinnervation occurs, basal parts are reached first by the newly ingrowing fibers.

In addition to the gradient from base to apex, however, anterior and septal wall were reinnervated earlier, whereas the lateral wall was involved later. These results suggest that sympathetic nerves are first restored in the territory of the left anterior descending artery with the left circumflex territory additionally involved later. In the territory of the right coronary artery, however, no substantial reinnervation was observed in the present study. The small percent of reinnervated myocardium in the inferior segments late after HTX seem more likely to represent border zones from other vascular territories.

To rule out the influence of nonspecific uptake, a threshold based on denervated hearts was used to define reinnervation in the present study, thus specific uptake in some small amounts of sympathetic fibers below the threshold may not have been detected. Nevertheless, regional differences are obvious, independent of this threshold.

In a study by Stark et al, chest pain was described in a patient with a collateralized, occluded right coronary. However, ischemia in this case may have occurred in additional vascular territories as the patient later demonstrated vasospasm in multiple vessels, including left and right coronary arteries. Additionally, although transcardiac norepinephrine spillover suggested sympathetic reinnervation in general, tyramin was not injected selectively into coronaries to derive regional information. Direct evidence for reinnervation of the right coronary artery cannot be derived from this case report.

The regional differences between vascular territories in the present study cannot be readily explained and require further investigation. At the present time, it can only be speculated that surgical techniques may limit growth of fibers along the coronaries to a different degree or that regional variations in local regulatory mechanisms, eg, the expression of growth factors, may influence the reinnervation process.

Conclusions

Using serial noninvasive quantitative assessment by PET and C-11 HED, the progressive nature of sympathetic reinnervation until late after HTX has been demonstrated. Sympathetic reinnervation, however, remained regionally heterogeneous, and a complete reinnervation has not been found up to 15 years after HTX. The underlying mechanisms for this regional heterogeneity remain to be elucidated.

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