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Sympathetic re-innervation after heart transplantation: dual-isotope neurotransmitter scintigraphy, norepinephrine content and histological examination

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Abstract. Cardiac transplantation entails surgical disruption of the sympathetic nerve fibres from their somata, resulting in sympathetic denervation. In order to investigate the occurrence of sympathetic re-innervation, neurotransmitter scintigraphy using the norepinephrine analogue iodine-123 metaiodobenzylguanidine (MIBG) was performed in 15 patients 2–69 months after transplantation. In addition, norepinephrine content and immunohistochemical reactions of antibodies to Schwann cell-associated S100 protein, to neuron-specific enolase (NSE) and to norepinephrine were examined in 34 endomyocardial biopsies of 29 patients 1–88 months after transplantation. Anterobasal ^{123}I-MIBG uptake indicating partial sympathetic re-innervation could be shown in 40% of the scintigraphically investigated patients 37–69 months after transplantation. In immunohistochemical studies 83% of the patients investigated 1–72 months after transplantation showed nerve fibres in their biopsies but not positive reaction to norepinephrine. Significant norepinephrine content indicating re-innervation could not be detected in any biopsy. It was concluded that in spite of the lack of norepinephrine content there seemed to be immunohistological and scintigraphic evidence of sympathetic re-innervation. An explanation for this contradictory finding may be the reduced or missing norepinephrine storage ability compared to the restored uptake ability of regenerated sympathetic nerve fibres.

Key words: Cardiac transplantation – Sympathetic re-innervation – Iodine-123 metaiodobenzylguanidine – Thallium-201 – Dual-isotope technique

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Introduction

Cardiac transplantation has become an accepted treatment for end-stage cardiac disease. In spite of increased survival rates of up to 92% 1 year after transplantation and 78% 5 years after transplantation [1] due to improved immunosuppressive therapy, physical performance remains impaired [2]. The major reason for the persistent impairment of physical function seems to be the denervation of the donor heart. In transplanted hearts the sympathetic neurons are separated from their nerve cell bodies in the thoracic and cervical ganglia. This causes rapid depletion of norepinephrine stores in the cardiac nerve terminals [3] because the vesicles and enzymes needed for synthesis of norepinephrine must be transported from the cell body in the ganglia to the nerve terminal via an intact axon [4]. Due to denervation, transplanted hearts lack the ability to increase heart rate upon exercise [5]. Therefore a stress-associated increase in ejection fraction results from an increase in stroke volume [6]. However, in long-term cardiac transplants a tendency toward an improved heart rate response to exercise has been shown [7, 27] and has been interpreted as a sign of sympathetic re-innervation [8].

In animal models sympathetic re-innervation was proved by electrophysiological examination and by iodine-123 metaiodobenzylguanidine (MIBG) scintigraphy 4 months [9] and 9–12 months after transplantation [10, 11]. Using quantitative positron emission tomography (PET) studies employing the norepinephrine analogue carbon-11 hydroxyepinephrine (HED), sympathetic re-innervation could be shown in the proximal anterior wall in humans 2 years after transplantation. In the re-innervated areas the norepinephrine content was 60% of that in normals [12].

The goal of this study was to investigate the occur-

rence of sympathetic re-innervation after human heart transplantation by reference to scintigraphic intraneuronal ^{123}I-MIBG uptake, to morphological structures as assessed by immunohistochemical examination and to myocardial norepinephrine content as assessed by biochemical analysis and by immunohistochemical reaction to anti-norepinephrine. Biochemical analysis and immunohistochemical examination were based on endomyocardial biopsies of the right ventricle.

The scintigraphic investigation was based on the intraneural ^{123}I-MIBG uptake. ^{123}I-MIBG serves as a norepinephrine analogue, enters the sympathetic postganglionic presynaptic neurons by an energy-dependent uptake mechanism and is trapped in the vesicles. Since the intraneuronal I-^{123}I-MIBG uptake corresponds to the integrity of the sympathetic nervous system, it can be used as an indicator of sympathetic re-innervation [13–16]. In order to localize the myocardial region, thallium-201 was administered simultaneously and acquisition was performed by a dual-isotope method.

Materials and methods

Patients. Fifteen patients (13 males, two females) between 19 and 63 years old (average age 48±4 years) were examined by scintigraphy 2–69 months after orthotopic heart transplantation. The mean organ age since transplantation was 31.9±5.9 months. At the time of examination no rejection episode was known to have occurred in any patient. In none of the patients did medication consist of ^{123}I-MIBG uptake-reducing substances. Calcium channel blockers that were taken by three patients were withdrawn 24 h prior to the ^{123}I-MIBG scintigraphy. The immunosuppressive therapy, which is known not to interfere with ^{123}I-MIBG [9], consisted of cyclosporine 2–4 mg/kg, prednisone 0.15 mg/kg and azathioprine 0.5–1.5 mg/kg.

In these 15 scintigraphically investigated patients and in another 14 patients – giving a total of 29 patients (24 males five females) between 19 and 65 years old and 1–88 months (median 27 months) after transplantation – 34 right heart catherizations were performed between July 1992 and March 1994. At each catheterization endomycocardial biopsies were obtained from the right ventricular septum for histological and biochemical examination.

Patients gave their informed consent to all investigations.

Scintigraphy. Thyroid uptake was blocked by giving 1.2 g sodium perchlorate 30 min prior to the administration of 220 MBq ^{123}I-MIBG and 37 MBq ^{201}Tl.

Fifteen minutes and 4 h after the application of ^{201}Tl and ^{123}I-MIBG static dual-isotope anterior acquisitions of the chest were performed. We used a Sophy DS7 gamma camera equipped with a low-energy very high resolution collimator (8 mm FWHM at 10 cm) and a 128×128 acquisition matrix. Acquisition time was 10 min with an average of 200 counts per pixel. For ^{201}Tl we chose a symmetrical 25% energy window (70 keV) and for ^{123}I a symmetrical 17% energy window (159 keV).

Scintigraphic images were processed semiquantitatively. A cardiac shape-adapted region of interest (ROI) (12×12 pixel) was drawn over the myocardium on the ^{201}Tl images. The ROI was then automatically translated to the ^{123}I image. Patient motion did not affect the positioning of the myocardial ROIs, since according to our protocol the dual-isotope studies were performed simultaneously. On the ^{123}I image a reference ROI was drawn within the mediastinum (8×8 pixel). The myocardial ^{123}I-MIBG uptake ratio was calculated by the division of ^{123}I counts in the heart ROI by the counts in the mediastinal ROI.

Cross-over assessment. In the framework of this study cross-over was quantified in phantom and patient studies. Cross-over was calculated by the ratio: ^{201}Tl counts in the ^{123}I energy window/^{201}Tl counts in the ^{201}Tl window. Phantom studies were performed using a rectangular phantom (RP) and a cardiac phantom (CP). In order to confirm that experimental cross-over results were equal to those obtained in patient studies, 15 patients underwent myocardial scintigraphy. After injection of ^{201}Tl a simultaneous dual-window acquisition was performed to assess cross-over from ^{201}Tl into the ^{123}I window. In order to reduce cross-over from ^{201}Tl into the ^{123}I energy window, ^{123}I activities were 6 times as high as ^{201}Tl activities. Relative cross-over from ^{201}Tl into the ^{123}I window was expressed as the ratio: ^{201}Tl counts in the ^{123}I window/total counts in the ^{123}I window. Three patients underwent a modified protocol. After injection of ^{201}Tl and a subsequent dual-window acquisition, ^{123}I MIBG was injected (^{123}I/^{201}Tl activity ratio 6:1) and a second dual-window acquisition was performed.

Immunohistochemistry. Immunohistochemical studies were performed in 29 biopsies with a modified avidin-biotin technique as described elsewhere [42]. As primary antibodies we used anti-S100 (rabbit; Dakopatts, Copenhagen), anti-NSI (rabbit; Dakopatts, Copenhagen) and a monoclonal anti-norepinephrine (rabbit; Biodesign, Kennebuuk, Me.). Additionally routine haematoxylin and eosin staining was performed in two biopsies.

Biochemical method. Tissue samples were extracted overnight in 1 ml of 0.4 *M* perchloric acid. Tissue extracts were spiked with 3,4-dihydroxybenzylamine (DHBA) as internal standard and processed by extraction on alumina. After addition of 100 µl of a solution containing 10% Na_2-EDTA, of 40 mg Al_2O_3, of 1 ml TRIS buffer (2 *M*, pH 8.68) and of 100 µl of a solution containing 12.5% Na_2SO_3, the samples were mixed for 10 min. Afterwards the supernatant was discarded. The alumina was transferred into filtration units and washed 4 times with 1 ml of water. The catechols were desorbed from Al_2O_3 with twice 100 µl of 0.1 *M* perchloric acid. Portions of the perchloric acic eluates from the alumina were analysed by high-performance liquid chromatography (HPLC) and their catecholamine contents quantified by electrochemical detection. The HPLC system consisted of a Waters M 460 electrochemical detector (Millipore GmbH Eschborn, Germany) equipped with a glassy carbon working electrode, a P 1000 pump, an AS 3000 automated sample processor and a Chrom Jet integrator, all from Thermo Separation Products, GmbH (Darmstadt, Germany). Separation was accomplished by a 25×4.6 mm, 3 µm particle size, Spherisorb ODS 2 reversed phase column from GROM (Herrenberg-Kayh, Germany) which was maintained at a temperature of 32° C. The mobile phase (NaH_2PO_4 95.2 m*M*, sodium octanesulfonate 2.3 m*M*, EDTA 0.3 m*M*, methanol 7.4% (v/v), pH adjusted to 3.0 using perchloric acid) was filtered (0.2 µm), degassed with helium and pumped through the system at a flow rate of 0.6 ml/min. The oxidation potential was 0.7 V versus Ag/AgCl. Quantitation of peaks was carried out by comparing the height with that of standard solutions, taking into account recovery of an internal standard (DHBA) in the individual analysis.

The conversion of myocardial norepinephrine content from mg wet weight in non-collagen protein (NCP) was based on the calculation that 1 mg wet weight equals 150 µg NCP [18, 19]. Average

weight of the biopsy was 1.78 mg. The norepinephrine detection threshold for our assay was at approximately 2 pg/mg wet weight.

Results

Scintigraphy

Myocardial region was delineated according to ^{201}Tl uptake (Fig. 1). The cardiac to mediastinal ^{123}I-MIBG uptake ratios are listed in Table 1. The extraneuronal (15 min) and intraneuronal (4 h) ^{123}I-MIBG uptake showed slightly higher extraneuronal ^{123}I-MIBG uptake than intraneuronal ^{123}I-MIBG uptake but also a possitive correlation between them (Fig. 2). The intraneuronal ^{123}I-MIBG uptake ranged from 0.83 to 1.37. Since the number of patients was too small to apply proper statistical analysis, we divided the patients into two groups based on half of the maximum of organ age (69 months) since transplantation. The first group consisted of eight patients 2–34 months and the second group of seven patients 37–69 months after transplantation. In the first group the average ^{123}I-MIBG uptake ratio was 1.02 and in the second group, 1.18. The criterion of partial sympathetic re-innervation consisted in an increased ^{123}I-MIBG uptake ratio, which was found in six patients of group 2, who had an average ^{123}I-MIBG uptake ratio of 1.18. These six patients also showed anterior basal ^{123}I-MIBG uptake on the anterior chest wall images (Fig. 3). Therefore six out of 15 patients, which is 40%, showed evidence of partial sympathetic re-innervation of the anterior basal region of the heart. Lower septal, posterolateral and apical regions of the myocardium did not show ^{123}I-MIBG uptake. Uptake could not be verified in one patient of group 2 or in any of the patients of group 1 (Fig. 4).

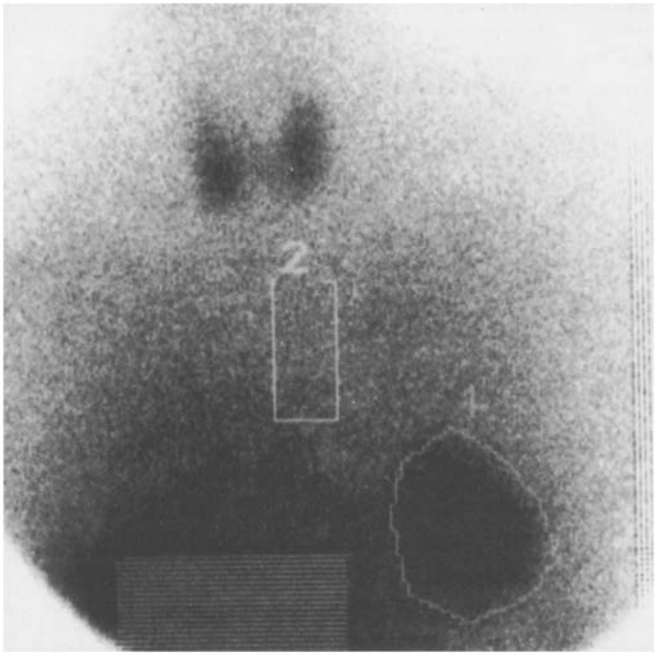

Fig. 1. ^{201}Tl uptake for landmarking

Table 1. ^{123}I-MIBG uptake ratios (ROI left ventricle/ROI mediastinum)

Patient no.	Months after transplantation	15 min p.i.	4 h p.i.
1	2	1.04	1.12
2	2	0.84	1.00
3	2	1.41	1.17
4	5	1.15	0.99
5	14	1.12	1.04
6	21	1.00	1.02
7	34	1.14	0.96
8	34	1.07	0.84
9	37	1.07	1.06
10	44	1.17	1.00
11	49	1.18	1.19
12	53	1.46	1.21
13	54	1.40	1.37
14	59	0.99	0.83
15	69	1.21	1.22

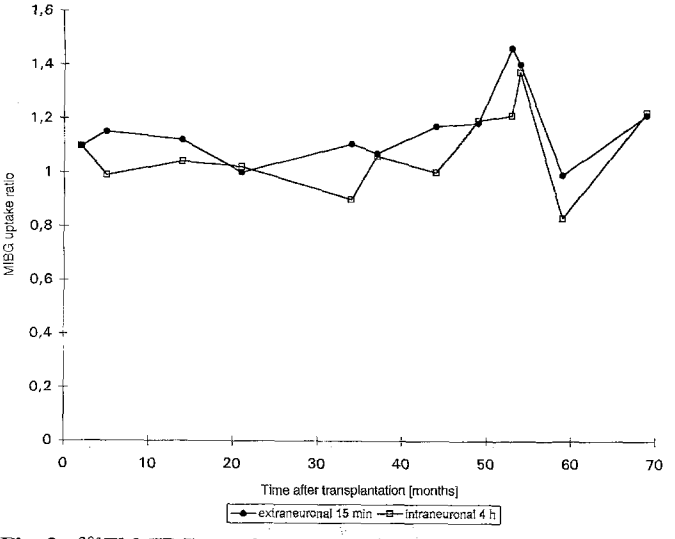

Fig. 2. ^{201}Tl-MIBG uptake ratios 15 min and 4 h p.i. plotted against the time after transplantation. As can be seen from this figure, there is a positive correlation of extraneuronal and intraneuronal ^{123}I-MIBG uptake indicating the specificity of ^{123}I-MIBG uptake (n=15). A slight increase in ^{123}I-MIBG uptake ratios is observed after 37 months. The low ^{123}I-MIBG uptake at 59 months is due to a non-re-innervated patient

Cross-over assessment

Mean cross-over from ^{201}Tl into the ^{123}I energy window was 11.5% (RP) and 11.1% (CP). Depending on attenuation in water (depth of water 0–5 cm), cross-over increased from 11.0% to 15.9% (RP) and from 11.0% to 15.8% (CP). Mean cross-over in 15 patients undergoing

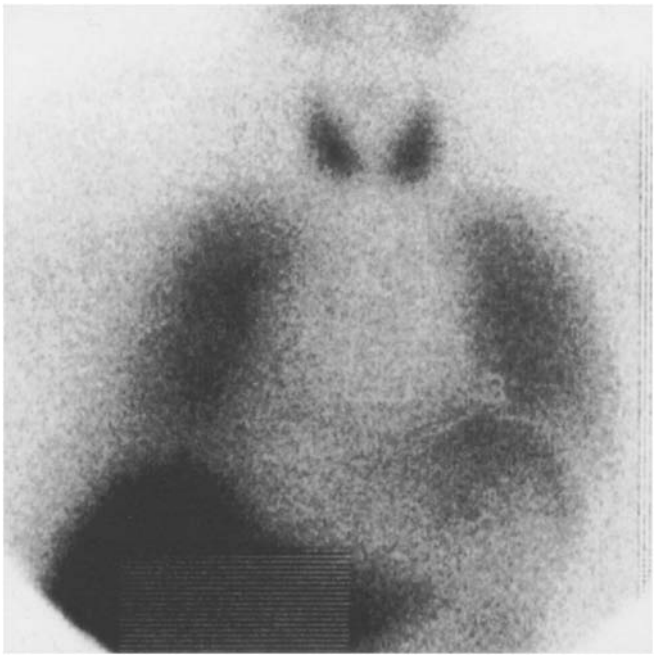

Fig. 3. Heart 54 months after transplantation. 4 h ^{123}I-MIBG uptake (1.37) showing re-innervation of the anterobasal myocardium

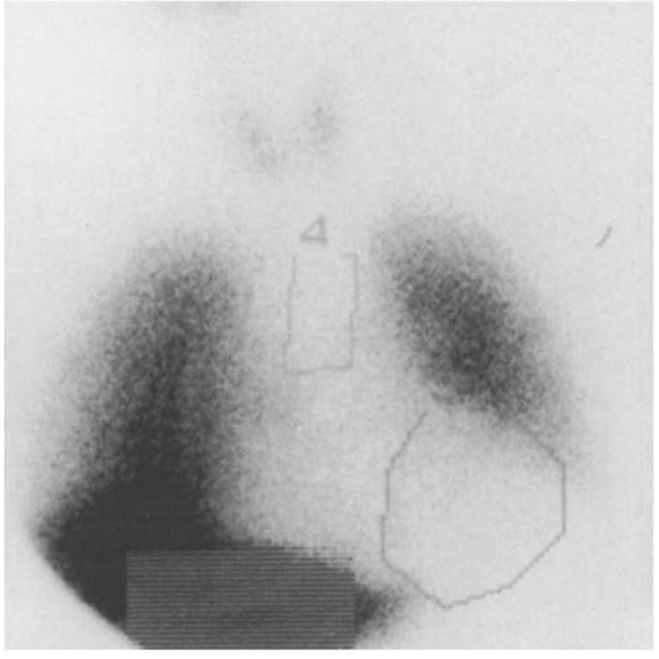

Fig. 4. Heart 5 months after transplantation. 4 h ^{123}I-MIBG uptake (0.99) without evidence of re-innervation

myocardial scintigraphy (only ^{201}Tl injection) and a simultaneous dual-window acquisition was 15.4% this being as high as the cross-over assessed in phanton studies taking into account attenuation and scatter caused by the chest wall. In order to reduce cross-over, ^{123}I activities were 6 times as high as ^{201}Tl activities. Relative cross-over from ^{201}Tl into the ^{123}I energy window was 3.0% (RP). The ^{123}I/^{201}Tl activity ratio was varied (8:1, 7:1, 6:1, 5:1, 4:1) (RP). Relative cross-over from ^{201}TI into the ^{123}I energy window ranged from 2.2% (8.1) to 4.4% (4:1). In three patients undergoing the modified protocol, relative cross-over from ^{201}Tl into the ^{123}I energy window was 3%.

Table 2. Immunohistochemical reaction to S100, NSE and anti-norepinephrine

Biopsy no.	Months after transplantation	S100	NSE	Anti-norepinephrine
1	1	–	–	–
2	1	–	+	–
3	72	+	+	–
4	9	+	–	–
5	2	–	–	–
6	45	+	+	–
7	5	–	+	–
8	7	–	–	–
9	19	+	–	–
10	4	+	–	–
11	41	+	+	–
12	13	+	+	–
13	1	–	+	–
14	8	+	+	–
15	47	–	+	–
16	2	+	+	–
17	8	+	–	–
18	17	–	+	–
19	47	+	+	–
20	48	+	+	–
21	6	+	–	–
22	39	+	+	–
23	27	–	–	–
24	56	+	+	–
25	19	n.a.	n.a.	–
26	16	n.a.	n.a.	–
27	75	n.a.	n.a.	–
28	12	n.a.	n.a.	–
29	72	–	–	–
30	70	n.a.	n.a.	–
31	88	+	+	–
32	29	+	–	–
33	52	+	+	–
34	56	+	+	–

n.a., Not assayed

Immunohistochemistry

Twenty-four biopsies 1–72 months (median 19 months) after transplantation revealed a positive reaction for either S100 or NSE (n=11) or for both (n=13). A positive reaction was found in interstitial cells, partly localized in close vicinity to cardiomyocytes. Five biopsies 1–72 months (median 7 months) after transplantation did not show any reaction to S100 or NSE (Table 2). Quantifica-

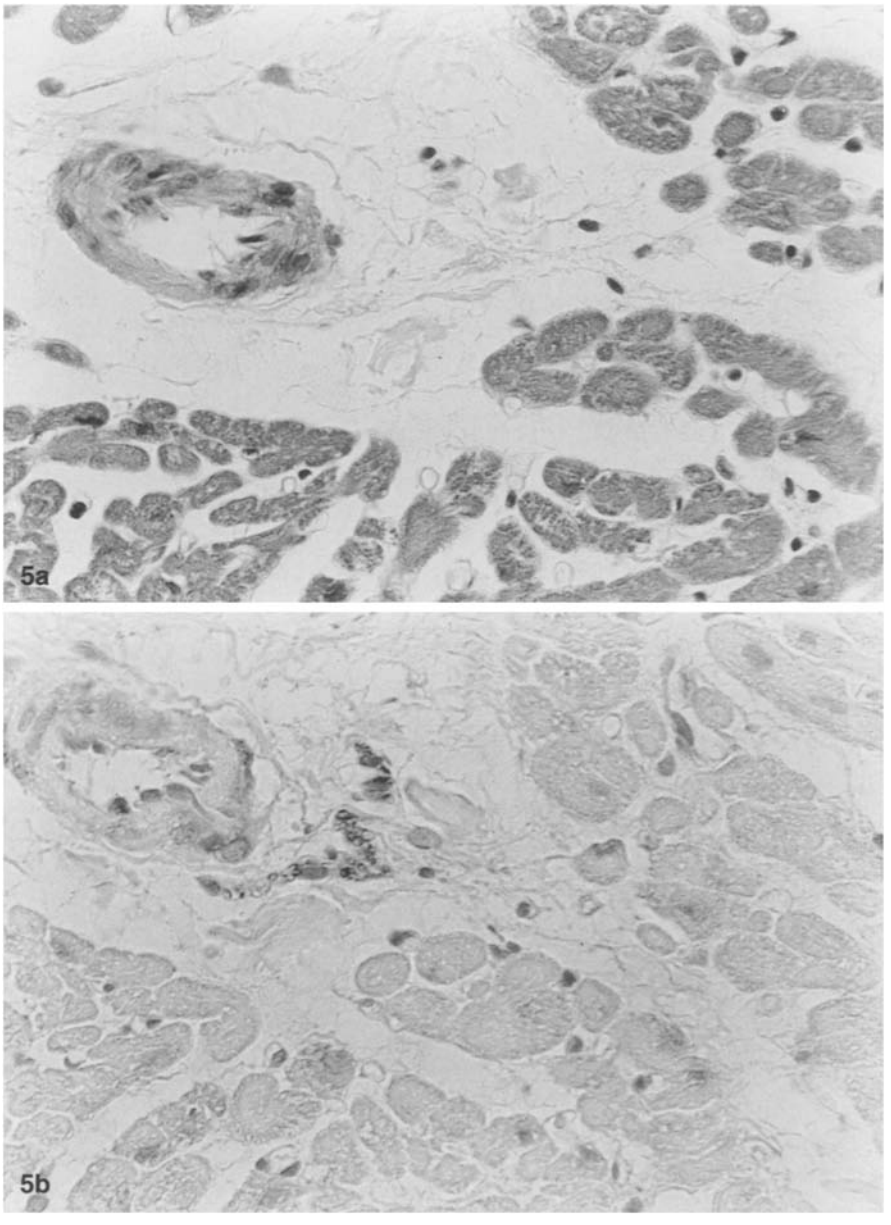

Fig. 5a, b. Heart biopsy 13 months after transplantation. **a** Small artery within a heart biopsy specimen. H&E, ×150. **b** Immunohistochemical demonstration of a nerve in the vicinity of the artery seen in **a**. NSE, ×150

tion of the number of S100 or NSE-positive cells seemed not to be useful due to the small and varying size of the biopsy specimens as well as to the known spatial heterogeneous pattern of re-innervation [38].

The antibody against norepinephrine showed no positive reaction in any biopsy specimen.

Routine haematoxylin and eosin staining performed additionally in two biopsies did not verify the nerve fibres that could be identified clearly by immunohistochemistry (Figs. 5, 6).

Norepinephrine content in endomyocardial biopsies

Norepinephrine was determined in 31 endomyocardial biopsies obtained in 29 patients (Table 3). In 13 patients 1–88 months (median 13 months) after transplantation, the norepinephrine content range from 2.45 to 27.94 pg/mg wet weight (median 8.86), which corresponds to 0.02–0.19 pg/μg NCP (median 0.06). In 13 patients 1–75 months (median 17 months) after transplantation, norepinephrine could not be detected in the endomyocardial biopsy. In five patients the biopsies were not evaluable for technical reasons. There was no evidence of correlation between norepinephrine content and time since transplantation.

Discussion

Sympathetic re-innervation following heart transplantation has been shown in several animal models [9, 11, 20, 21]. As regards human heart transplantation, however, evidence of sympathetic re-innervation is still controver-

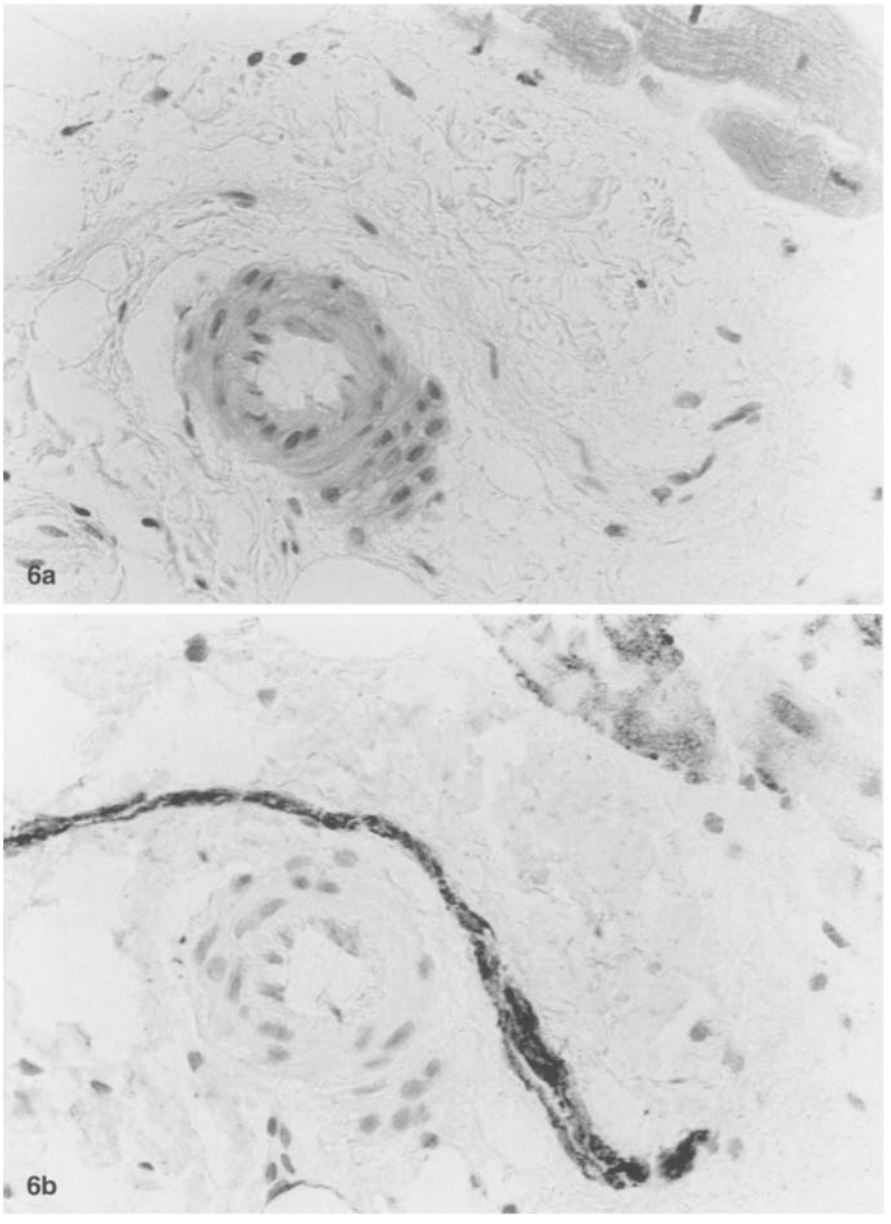

Fig. 6a, b. Heart biopsy 8 months after transplantation. **a** Heart biopsy containing a small artery within loose connective tissue. H&E, ×150. **b** Immunohistochemistry clearly detects a longitudinal section of a nerve in the neighbourhood of the artery seen in **a**. S100, ×150

sial. In spite of scintigraphically proved anterobasal myocardial uptake of norepinephrine analogues such as ^{123}I-MIBG using single-photon emission tomography (SPET) 1 year after transplantation [22] and ^{11}C-HED using PET more than 2 years after transplantation [12], no measurable norepinephrine content could be detected in endomyocardial biopsies in patients up to 5 years after transplantation [23]. Surprisingly, some patients undergoing retransplantation showed increased norepinephrine levels in the left and the right ventricle, indicative of sympathetic re-innervation [24]. Other investigators found that transplanted human hearts did not respond to the denervation with an upregulation in β-adrenergic receptors, which indicates that the denervation supersensitivity is exclusively presynaptic in origin [43]. Indirect signs of sympathetic re-innervation were found in tyramine-induced norepinephrine release in patients more than 5 months after transplantation [25]. An investigation with angiographically documented severe coronary artery stenoses in cardiac transplants showed that two patients with tyramine-induced norepinephrine release suffered from classical angina pectoris symptoms while three patients without chest pain had minimal or no norepinephrine release in response to administration of tyramine. On the basis of the aforementioned results, it was concluded that sensory re-innervation of sympathetic nerves can occur in cardiac transplants in humans [26]. Clinical studies of late cardiac transplants showing improvement in heart rate response to exercise also endorse sympathetic re-innervation [27].

Our results from scintigraphic, biochemical and immunohistochemical investigations are, however, conflicting.

There seemed to be intraneural ^{123}I-MIBG uptake in

Table 3. Norepinephrine content in endomyocardial biopsies

Biopsy no.	Months after transplantation	Biopsy (mg wet weight)	Norepinephrine, total (pg)	Norepinephrine (pg/mg wet weight)
1	1	1.27	b.d.t	b.d.t
2	1	2.12	b.d.t	b.d.t
3	72	2.25	Drop out	Droup out
4	9	1.26	9.0	7.0
5	2	1.05	16.96	16.15
6	45	1.00	27.94	27.94
7	5	0.63	3.65	5.79
8	7	0.72	b.d.t	b.d.t
9	19	2.03	b.d.t	b.d.t
10	4	1.16	Drop out	Drop out
11	41	1.88	Drop out	Drop out
12	13	2.33	24.87	10.67
13	1	0.89	24.70	27.76
14	8	1.01	Drop out	Drop out
15	47	0.94	Drop out	Drop out
16	2	3.01	4.67	1.55
17	8	1.88	4.60	2.45
18	17	1.64	b.d.t	b.d.t
19	47	0.93	7.15	7.69
20	48	0.94	3.41	3.62
21	6	1.41	b.d.t	b.d.t
22	39	1.66	b.d.t	b.d.t
23	27	2.70	b.d.t	b.d.t
24	56	4.22	b.d.t	b.d.t
25	19	2.64	b.d.t	b.d.t
26	16	2.21	b.d.t	b.d.t
27	75	4.66	b.d.t	b.d.t
28	12	1.86	6.17	3.32
29	72	0.28	2.71	9.68
30	70	1.71	15.14	8.86
31	88	2.86	60.96	21.31
32	29	n.a.	n.a.	n.a.
33	52	n.a.	n.a.	n.a.
34	56	n.a.	n.a.	n.a.

b.d.t., Below detection threshold; drop out, not evaluable for technical reasons; n.a., not assayed

the anterior basal region in six patients 37–69 months after transplantation. Since intraneuronal ^{123}I-MIBG uptake can only be documented in intact sympathetic neurons [15, 28, 29], partial sympathetic re-innervation of the anterior basal region can be postulated. In our patients the re-innervation occurred at a later time than in other studies [12, 22]. Several factors such as rejection episodes, interstitial fibrosis caused by immunosuppression [30, 31] and anatomical circumstances, e.g. available nerve fibre basal lamina sheaths that provide the guidance for re-innervation [32–34], may affect the re-innervation capacity in patients after heart transplantation. This might also explain the lacking I-MIBG uptake in one patient 59 months after transplantation. Even though in our study plasma catecholamines could not be determined, the absence of cardiac ^{123}I-MIBG uptake because of high circulating catecholamines due to a competitive uptake mechanism could be ruled out [35]. In spite of a possible increase in plasma catecholamine levels during long-term cyclosporine treatment, it could be shown that plasma catecholamine levels normalize after transplantation [36].

The neuronal uptake of norepinephrine has two compartments. In one compartment there is rapid uptake with long retention, referred to in the older literature as uptake-1, which represents true intravesicular localization. A second compartment with much more rapid washout, referred to as uptake-2, represents the extraneuronal localization of norepinephrine. Analogous compartments are observed with ^{123}I-MIBG. Rapid washout of extraneuronal ^{123}I-MIBG occurs, with a plateau of intraneuronal ^{123}I-MIBG approximately 4 h after administration in both animals and man, this consequently being the optimum time for imaging [28]. ^{123}I-MIBG represents the norepinephrine distribution of the sympathetic nerve endings in the uptake and storage process of the vesicles [28].

In accordance with new findings on the ^{123}I-MIBG

uptake, there was a positive correlation between extra- and intraneuronal ^{123}I-MIBG uptake, with only slightly higher extraneuronal than intraneuronal uptake ratios in our patients. It is postulated that the ^{123}I-MIBG uptake in humans is solely mediated through a human norepinephrine transporter protein (hNET) which is located at the plasma membrane [37]. The higher extraneuronal ^{123}I-MIBG uptake is explained by a non-specific membrane binding effect [37]. Since the non-specific cardiac uptake of ^{123}I-MIBG is considered a transient phenomenon with rapid clearance of approximately 15 min after injection [37], it seems obvious that the determined ^{123}I-MIBG uptake ratios of 15 min and at 4 h after injection did not differ significantly. In accordance with other studies [9] we did not see extraneuronal ^{123}I-MIBG uptake in transplanted hearts without re-innervation. This finding emphasizes the specificity of the ^{123}I-MIBG uptake depending on intact sympathetic neurons and the presence of human norepinephrine transporter protein.

Another argument for sympathetic re-innervation is the positive immunohistochemical reaction of antibodies to Schwann cell-associated S100 protein and to NSE in 83% of our patients 1–72 months after transplantation. The immunohistochemical investigations were based on both tests (S100 and NSE) because antibodies to Schwann cells detect only Schwann cells and not axons. Thus it remains unknown whether these immunohistochemically positive reacting nerve fibres belong to postganglionic parasympathetic nerves or to regenerated sympathetic axons. In correspondence with the scintigraphic results, they are suggestive of sympathetic re-innervation. Evidence merely of a parasympathetic origin of nerve fibres was provided by three biopsies 1–2 months after transplantation because sympathetic re-innervation is not likely to occur even in animals before 10 weeks after transplantation [3]. The lack of nerve fibres in five biopsies 1–72 months after transplantation might have been due to the small size of endomyocardial biopsies and the known spatial heterogeneity of re-innervation [38].

Despite the scintigraphically and immunohistochemically proven existence of intact neurons, no significant norepinephrine content could be detected in the endomyocardial biopsies. In 13 patients 1–75 months after transplantation the norepinephrine content was below the detection threshold of 0.01 pg/μg NCP. In 13 patients 1–8 months after transplantation the median norepinephrine content was 0.06 pg/μg NCP, which is just above the detection threshold. Using a comparable biochemical method in ten healthy control subjects, the normal right ventricular norepinephrine content was determined to be 10.9±2.9 pg/μg NCP [19]. Compared to normal right ventricular norepinephrine content our biopsies contained less than one-hundredth of the normal myocardial norepinephrine content. This finding is emphasized by the negative immunohistochemical reaction of antibodies against norepinephrine in all patients.

There are two explanations for the discrepancy between (a) scintigraphic evidence of sympathetic re-innervation and immunohistochemically verified nerve fibres and (b) lack of norepinephrine content. Endomyocardial biopsies were obtained from the right ventricular septum and the ^{123}I-MIBG uptake was shown in the anterobasal region. Since the sympathetic neurons run in epicardial layers along the vessels from base to apex [38], the septum is an area which is likely to be re-innervated very late and therefore may not contain sympathetic nerve fibres that store norepinephrine. In this case the immunohistochemically verified nerves would be of parasympathetic origin. The other explanation is based on findings of central noradrenergic nerve endings showing that the development of granular storage mechanism lags far behind the development of the specific membrane mechanism for uptake of norepinephrine [39]. Experiments in denervated canine hearts demonstrated that in spite of functional re-innervation and a return of norepinenephrine uptake to approximately 57% of normal, the myocardial norepinephrine content remained at 13% of control indices [10, 38]. It could be demonstrated that after cardiac denervation the catecholamine content of several myocardial regions did not correspond to the extent of functional response. That is why it was postulated that large functional responses may be mediated by a very small number of neural connections [3]. Obviously the norepinephrine content determined in endomyocardial biopsies correlates poorly with the degree of re-innervation while the norepinephrine uptake seems to be good indicator of sympathetic re-innervation [10]. On this basis and in view of the histological evidence of vital nerve fibres, it may be concluded that the ^{123}I-MIBG uptake indicates sympathetic re-innervation even if norepinephrine content is not detectable.

Dual-isotope studies using ^{123}I-MIBG and ^{201}Tl improve the accuracy of the assessment of sympathetic re-innervation in patients following heart transplantation due to better myocardial delineation. The activity ratio is of great importance in dual-isotope studies [17, 40, 41]. The ^{123}I/^{201}Tl activity ratio determines the relative cross-over from ^{201}Tl into the ^{123}I energy window. ^{201}Tl images were only used for myocardial landmarking and did not sever for semiquantitative ratio calculations. Therefore increased ^{123}I cross-over in the ^{201}Tl images due to increased activity ratio did not introduce a significant error. Dual-isotope studies represent a frequently used procedure in nuclear medicine investigations [17, 41, 44–46]. Most of the gamma cameras today provide digitized acquisition modalites and allow dual-energy acquisitions [40, 47, 48] or even multiple simultaneous energy window acquisitions [49]. However, dual-isotope studies are still controversial due to limitations mainly related to physical parameters such as cross-over, choice of an optimal isotope activity ratio, assessment of the influence of cross-over on the accuracy of the method and repositioning problems [40, 50–52]. In this study, cross-over was quantified in phantom and patient studies. The optimal ^{123}I/^{201}Tl activity ratio is between 4:1 and 6:1, thus

allowing precise delineation of the myocardium and causing an acceptable overestimation of the ^{123}I-MIBG uptake ratio in the order of 3%–5%. Repositioning was not necessary in our study since the dual-isotope studies were performed simultaneously. Relative cross-over from ^{201}Tl in the ^{123}I energy window was in the order of about 3% and thus would have led only to a moderate and statistically irrelevant overestimation of the myocardial ^{123}I-MIBG uptake ratio.

Limitations

One limitation of this investigation is that it was not a longitudinal study designed to demonstrate the appearance of re-innervation. For logistic reasons it was not possible to undertake a complete examination consisting of a scintigraphic, an immunohistochemical and a pharmacological part in all patients.

It would have been of special interest to compare the anterobasal ^{123}I-MIBG uptake with endomyocardial biopsies of the same region. But as a matter of routine investigation only right heart catheterizations were performed and therefore only biopsies of the right ventricular septum could be obtained.

In order to prove functional re-innervation of the nerve fibres it would have been an elegant approach to measure the enzymes necessary for norepinephrine synthesis, but this was impossible because the study was limited to a SPET system and because of the lack of available tracers.

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