Epidermal Innervation in Type 1 Diabetic Patients

A 2.5-year prospective study after simultaneous pancreas/kidney transplantation

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OBJECTIVE — To assess the effect of normoglycemia following simultaneous pancreas/kidney transplantation (SPK) on neurological function and intraepidermal nerve fiber density (IENFD) in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS — We performed vibration perception threshold (VPT) testing and autonomic function testing (AFT) and assessed IENFD in skin biopsies from the lower thigh and upper calf in 14 healthy control subjects and 18 patients with type 1 diabetes at the time of and at 21–40 (median 29) months post SPK.

RESULTS — At baseline, significantly increased VPTs, pathological AFT results, and severe reduction in IENFD were present in SPK recipients. After SPK, an increase of IENFD in the thigh of more than one epidermal nerve fiber per millimeter was noted in three patients (median 4.1, range 1.9–10.2), but changes were not significant for the group as a whole.

CONCLUSIONS — We conclude that either irreversible nerve damage might be present in some SPK recipients or that longer periods of normoglycemia might be needed to allow nerve regeneration.

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Diabetic neuropathy is a common diabetes complication that may result in serious consequences such as pain, foot ulcers, and amputations. Although optimal glycemic control is considered an effective preventive measure, intervention studies in advanced stages of diabetic neuropathy have been almost uniformly unsuccessful (1). Only arrest of progression of diabetic neuropathy could be achieved in patients after pancreas transplantation (2). To assess nerve regeneration following pancreas transplantation, Kennedy et al. (3) proposed the use of skin biopsies with quantification of intraepidermal nerve fiber density (IENFD). Previously, we documented severe IENFD reduction in lower-limb skin biopsies performed at the time of pancreas transplantation (4). Here we present assessment of IENFD following a mean of 2.5 years of normoglycemia.

RESEARCH DESIGN AND METHODS

— A total of 22 patients with type 1 diabetes undergoing simultaneous pancreas/kidney transplantation (SPK) and 14 healthy control subjects participated in the study. For details of the procedure and study subjects, please see the online appendix (available at http://dx.doi.org/10.2337/dc07-2409). The study was approved by the local ethics committee, and informed consent was obtained from all subjects.

Skin biopsies were performed using a 3-mm punch (Stiefel Laboratories, Sligo, Ireland) from the distal thigh (two samples at a distance of 1 cm, one assessed in Prague and the other in Würzburg) and the proximal calf (one sample, assessed in Prague) at the time of SPK and at 30 ± 5 (mean ± SD) months post transplant. Biopsies from control subjects were taken from corresponding regions. After fixation (4% paraformaldehyde for 3 h at 4°C, then cryoprotection with 10% sucrose in 0.1 mol/l PBS) and freezing (in isopentane cooled by liquid nitrogen), 40-μm sections were immunoreacted with a rabbit polyclonal antibody to the panaxonal marker protein gene product (PGP) 9.5 (DakoCytomation, Glostrup, Denmark), followed by mouse anti-rabbit IgG conjugated with rhodamine or Cy3 (Jackson Immuno Research, West Grove, PA). Samples were imaged with an Olympus microscope BX 51 (Olympus Optical, Hamburg, Germany) in Prague and with a Zeiss Axiophot 2 (Carl Zeiss, Göttingen, Germany) in Würzburg. Three sections per patient were examined. The mean number of intraepidermal nerve fibers (IENFs) per millimeter epidermis was derived using the software Olympus DP-SOFT (Software Imaging Systems, Münster, Germany) and Image Pro Plus 4.0 (Media Cybernetics, Leiden, Netherlands), respectively. Established counting rules were followed (5). Changes >1 IENF/mm were considered meaningful. In addition, the subepidermal nerve plexus was classified semiquantitatively in Würzburg as "normal," "reduced," or "absent." Clinical neuropathy evaluation in the patients included vibration perception threshold (VPT) tests (Bio-Thesiometer; Bio-Medical Instrument, Newbury, OH) and autonomic function testing (AFT) (VariaPulse TF3; Sima Media, Olomouc, Czech Republic) (6). The Mann-Whitney *U* test and Wilcoxon's signed-rank test were used for inter- and intra-group comparisons, respectively.

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Table 1—*A1C, VPT, and IENFD (number of IENF per millimeter) in healthy control subjects and in type 1 diabetic patients at baseline and at 21–40 months (median 29) of normoglycemia following SPK*

	Control subjects	Diabetic subjects at SPK	Diabetic subjects post-SPK
n	14	18	18
A1C (%)	5.6 ± 0.3*	8.2 ± 1.4†	5.4 ± 0.5
VPT (volts)	16 ± 5*‡	34 ± 11	35 ± 11
T_P (IENFD)	11.4 ± 4.2*‡	0.8 ± 1.3	1.6 ± 2.5
T_W (IENFD)	8.9 ± 1.8*‡	0.8 ± 1.5	1.5 ± 3.2
C_P (IENFD)	8.0 ± 3.0*‡	0.4 ± 1.1	0.4 ± 0.8

Data are means ± SD. T_P, thigh (Prague); T_W, thigh (Würzburg); C_P, calf (Prague). *$P < 0.001$ for control vs. diabetic subjects at SPK; †$P < 0.001$ for diabetic subjects at SPK vs. diabetic subjects post-SPK; ‡$P < 0.001$ for control subjects vs. diabetic subjects post-SPK.

RESULTS — Normoglycemia with insulin independence and satisfactory renal graft function was achieved in 18 patients (male/female 10/8, aged 47 ± 10 years, with diabetes duration 29 ± 9 years and P-creatinine 1.3 ± 0.4 mg/dl at follow-up; online appendix Table A1). At baseline, significantly increased VPTs, reduced AFT results (online appendix Table A2), and severe reduction in IENFD in both regions were present in SPK recipients (Table 1 and online appendix Figure A1). At follow-up 21–40 months (median 29) after SPK, increases in IENFD of the thigh samples were seen in three patients, with results verified in both Prague and Würzburg (median 4.1, range 1.9–10.2 IENF/mm). The subepidermal plexus was reduced or absent in all but one patient. A change in category from "reduced" to "normal" occurred in two patients with improvement of IENFD but in none of the other patients. No significant changes occurred in neurological function or IENFD of the transplanted group as a whole.

CONCLUSIONS — Previous reports of neuropathy follow-up in pancreas or islet transplant recipients were mostly based on clinical examination, electrophysiology, and AFT. Most recently, stabilization of electrophysiological parameters could be shown over a 6-year period in 18 patients with islet transplantation after kidney transplantation (7). An innovative noninvasive approach, corneal confocal microscopy, was proposed by researchers from Manchester (8). Using this method, a significant improvement of corneal nerve fiber density and length was detected within 6 months of SPK (9).

We did not encounter a similarly significant early regenerative response of lower-limb nerve fibers after SPK. While a type II error cannot be excluded and more advanced diabetic neuropathy could have been present, other reasons may be also responsible. The length-related pattern of diabetic neuropathy and varying regenerative capacity of nerve fibers from different body regions could play a role. Moreover, the subepidermal plexus from which epidermal reinnervation should occur was reduced in most patients. We observed some improvement of nerve fiber counts in the biopsies from the more proximal lower-thigh area in three patients. While this subgroup did not differ in clinical characteristics including time from SPK, a still longer period of normoglycemia might be needed to achieve nerve fiber regeneration in the lower limbs of the remaining patients. Of note, in the case of diabetic nephropathy, reversal of renal lesions was seen after more than 5 years of normoglycemia following pancreas transplantation (10).

Irreparable damage of lower-limb nerves might also be present in some advanced cases. Although generally producing an immense improvement of the recipient's clinical condition and long-term prognosis, SPK does not eliminate risks connected with diabetic neuropathy. Matricali et al. (11) recently reported on a high rate of Charcot foot complications at a mean of 1.8 years posttransplant. Foot ulcers and gangrene, while often co-initiated by vascular disease and infection, are not uncommon throughout the postoperative period. Such complications have occurred in 62 of 200 pancreas transplantation recipients at our center since 1994.

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