Corneal Confocal Microscopy Detects Early Nerve Regeneration After Pancreas Transplantation in Patients With Type 1 Diabetes

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OBJECTIVE — Corneal confocal microscopy (CCM) is a rapid, noninvasive, clinical examination technique that quantifies small nerve fiber pathology. We have used it to assess the neurological benefits of pancreas transplantation in type 1 diabetic patients.

RESEARCH DESIGN AND METHODS — In 20 patients with type 1 diabetes undergoing simultaneous pancreas and kidney transplantation (SPK) and 15 control subjects, corneal sensitivity was evaluated using noncontact corneal esthesiometry, and small nerve fiber morphology was assessed using CCM.

RESULTS — Corneal sensitivity (1.54 ± 0.28 vs. 0.77 ± 0.02, $P < 0.0001$), nerve fiber density (NFD) (13.8 ± 2.1 vs. 42 ± 3.2, $P < 0.0001$), nerve branch density (NBD) (4.04 ± 1.5 vs. 26.7 ± 2.5, $P < 0.0001$), and nerve fiber length (NFL) (2.23 ± 0.2 vs. 9.69 ± 0.7, $P < 0.0001$) were significantly reduced, and nerve fiber tortuosity (NFT) (15.7 ± 1.02 vs. 19.56 ± 1.34, $P = 0.04$) was increased in diabetic patients before pancreas transplantation. Six months after SPK, 15 patients underwent a second assessment and showed a significant improvement in NFD (18.04 ± 10.48 vs. 9.25 ± 1.87, $P = 0.001$) and NFL (3.60 ± 0.33 vs. 1.84 ± 0.33, $P = 0.002$) with no change in NBD (1.38 ± 0.74 vs. 1.38 ± 1.00, $P = 1.0$), NFT (15.58 ± 1.20 vs. 16.30 ± 1.19, $P = 0.67$), or corneal sensitivity (1.23 ± 0.39 vs. 1.54 ± 00.42, $P = 0.59$).

CONCLUSIONS — Despite marked nerve fiber damage in type 1 diabetic patients undergoing pancreas transplantation, small fiber repair can be detected within 6 months of pancreas transplantation using CCM. CCM is a novel noninvasive clinical technique to assess the benefits of therapeutic intervention in human diabetic neuropathy.

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Somatic polyneuropathy is one of the most common long-term complications of diabetes and is the main initiating factor for foot ulceration and lower extremity amputation (1,2). As 80% of amputations are preceded by foot ulceration, an effective means of detecting and treating peripheral neuropathy would have a major medical, social, and economic impact. With the exception of optimal glycemic control, there are currently no licensed treatments that prevent, slow, or arrest the development of neuropathy (1). The development of new treatments is of paramount importance, but they are hampered by a lack of clinically relevant surrogate end points favored by regulatory authorities (1). We have relied on tests that quantify predominantly large nerve fiber dysfunction, which were principally developed to aid diagnosis and not to assess nerve repair and hence a therapeutic response (3). Thus, nerve conduction studies are useful but only detect an abnormality in large myelinated nerve fibers, whereas thermal and pain thresholds assess thinly myelinated (Aδ) and unmyelinated (C) fiber function. Heart rate variability during respiratory stimuli indicates parasympathetic vagal efferent function, and blood pressure change during orthostatic manipulation evaluates sympathetic vasomotor efferents.

Although these tests correlate with axonal loss (4–7), there are major shortcomings when they are used to define therapeutic efficacy in clinical intervention trials (8). These tests do not target the specific fiber types that may benefit from the therapeutic intervention and demonstrate a limited ability to detect regeneration and repair. Only sural nerve biopsy with electron microscopy (9,10) and the assessment of intraepidermal nerve fiber density using skin-punch biopsies (11,12) directly assess nerve damage and repair; however, both are invasive procedures.

No available therapy (including glycemic control) has previously been shown to result in an improvement in diabetic neuropathy. Even in the most dramatic example of "curing" type 1 diabetes with pancreas transplantation, in 115 patients followed over 10 years, neurological function, nerve conduction studies, and autonomic function were only prevented from worsening and failed to show an improvement (13). This result is in keeping with the lack of improvement in heart rate variability 43 months after simultaneous pancreas and kidney transplantation (SPK) (14). Neuropathy is, of course, extremely severe at this stage, as evidenced recently by the demonstration of severe intraepidermal nerve fiber depletion in pancreas transplant recipients, suggesting that long-term follow-up is necessary to assess posttransplant nerve fiber regeneration (15).

Our previous work suggested that

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Abbreviations: CCM, corneal confocal microscopy; NBD, nerve branch density; NFD, nerve fiber density; NFL, nerve fiber length; NFT, nerve fiber tortuosity; SPK, simultaneous pancreas and kidney transplantation.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

small nerve fibers are the earliest to undergo damage and retain the ability to repair longer than large fibers (9,10). More recently, we have used corneal confocal microscopy (CCM) to demonstrate that corneal nerve fiber damage is directly related to the severity of somatic neuropathy (16,17) and to intraepidermal nerve fiber pathology (18). These data led us to propose that CCM, a noninvasive and reiterative test might be an ideal surrogate end point for evaluating therapeutic efficacy in clinical trials of human diabetic neuropathy (19). To test this hypothesis, we have assessed corneal sensitivity and used CCM to evaluate corneal nerve fiber morphology at baseline and 6 months after SPK in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

— Twenty type 1 diabetic patients undergoing SPK and 15 nondiabetic healthy control subjects were studied at baseline, and 15 diabetic patients underwent repeat assessment 6 months after SPK transplantation.

Corneal sensitivity

The noncontact corneal esthesiometer uses a puff of air expressed through a bore of 0.5-mm diameter with an electronic pressure sensor, which displays the force exerted in millibars. The stimulus jet is mounted on a slit lamp positioned 1 cm from the eye and aligned to the center of the cornea. Corneal sensitivity was assessed using our established methodology (20).

Confocal microscopy

Patients underwent examination with a Tomey Confoscan corneal confocal microscope model P4 (Tomey, Erlangen, Germany). One eye of each subject was selected at random and anesthetized with 1 drop of 0.4% benoxinate hydrochloride (oxybuprocaine hydrochloride; Minims). The objective lens of the confocal microscope was disinfected (isopropyl alcohol 70% vol/vol, swabs), and 1 drop of Viscotears liquid gel (carbomer 940; Ciba Vision Ophthalmics) was applied onto the tip of the lens and advanced forward until the gel touched the cornea, allowing optical but not physical contact between the objective lens and corneal epithelium. The entire cornea was scanned in ~2 min and en face two-dimensional images (lateral resolution ~1–2 μm and final image size of 768 × 576 pixels) were acquired. Three to five high-quality images of Bowman's layer were examined as it contains the main nerve plexus. The investigator who examined the cornea and who performed morphometric measurements of the images was masked with respect to the identity of the patient. The following parameters were quantified to define corneal nerve fiber damage and repair: *1*) nerve fiber density (NFD), the total number of major nerves per square millimeter of corneal tissue; *2*) nerve fiber length (NFL), the total length of all nerve fibers and branches per square millimeter of corneal tissue; *3*) nerve branch density (NBD), the number of branches emanating from each nerve trunk per square millimeter of corneal tissue (16); and *4*) nerve fiber tortuosity (NFT), a parameter mathematically derived from the images (17). Measures 1 and 3 were determined using morphometric software incorporated within the Tomey instrument, measure 2 was determined using third-party image analysis software (Scion Image for Windows; Scion, Frederick, MD), and measure 4 was calculated using a MATLAB function (MATLAB version 6.5; MathWorks, Natick, MA) that was created for this purpose (17). Corneal nerve morphology was quantified fully in each patient in ~30 min. To estimate the error in measuring NFD, NFL, and NBD, we acquired images and determined each of these parameters for 15 subjects on two occasions separated by at least 48 h. The coefficients of variation of these parameters were 12% for NFD, 9% for NFL, and 24% for NBD.

Statistics

SPSS 11.05.0 for Windows was used to compute the results. The data are expressed as means ± SEM, and the analysis includes descriptive and frequency statistics. ANOVA with Scheffé post hoc tests was used to study differences between means. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline evaluation

Twenty type 1 diabetic patients aged 41 ± 1 years, with diabetes duration of 27 ± 2 years and A1C 8.9 ± 1.4% who were undergoing SPK, and 15 age-matched (46 ± 3 years) healthy control subjects were examined. Corneal sensitivity was significantly reduced in diabetic patients (1.54 ± 0.28 mbar) compared with control subjects (0.77 ± 0.02 mbar, $P < 0.0001$). Corneal NFD (13.8 ± 2.1 vs. 42 ± 3.2, $P < 0.0001$), NBD (4.04 ± 1.5 vs. 26.7 ± 2.5, $P < 0.0001$), and NFL (2.23 ± 0.2 vs. 9.69 ± 0.7, $P < 0.0001$) were significantly reduced, and NFT (15.7 ± 1.02 vs. 19.56 ± 1.34, $P = 0.04$) was increased in diabetic patients (Fig. 1*B*) undergoing pancreas transplantation compared with control subjects (Fig. 1*A* and Table 1).

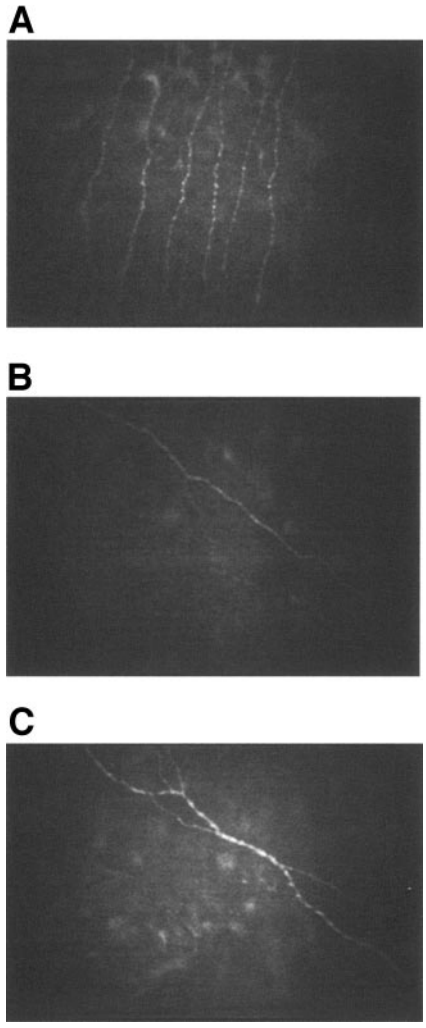

Figure 1—*Image of corneal nerves in Bowman's layer, showing six nerve fibers with a typical beaded appearance, mild tortuosity, and adequate branching in a control subject (*A*) compared with marked loss of nerve fibers with one nerve in a patient undergoing pancreas transplantation (*B*) and improvement in NFD with increased numbers of nerves, 6 months after transplantation (*C*).*

After transplantation

Fifteen patients underwent repeat assessment 6 months after SPK (Table 2). A1C fell significantly into the normal range (5.5 ± 0.1 vs. 8.6 ± 0.4%, $P = 0.007$), confirming successful pancreas transplantation. There was a trend for improvement in corneal sensitivity (1.23 ±

Table 1 —*Corneal confocal nerve fiber morphology in control subjects and in type 1 diabetic patients undergoing SPK*

	Age (years)	NCCA (mbar)	NFD (n/mm^2)	NBD (n/mm^2)	NFL (mm/mm^2)	NFT (Tc)
Control (n = 15)	46 ± 3	0.77 ± 0.02	42.04 ± 3.2	26.73 ± 2.5	9.69 ± 0.7	19.56 ± 1.34
Before SPK (n = 20)	41 ± 1.8	1.54 ± 0.28	13.88 ± 2.1	4.04 ± 1.5	2.23 ± 0.28	15.76 ± 1.02
P	NS	<0.0001	<0.0001	<0.0001	<0.0001	0.04

Data are means ± SEM with significant difference. NCCA, noncontact corneal esthesiometer; Tc, tortuosity coefficient.

0.39 vs. 1.54 ± 00.42, P = 0.59). Corneal NFD (18.04 ± 10.48 vs. 9.25 ± 1.87, P = 0.001) (Fig. 2*A*) and NFL (3.60 ± 0.33 vs. 1.84 ± 0.33, P = 0.002) (Fig. 2*B*) improved significantly. No change was observed for either NBD (1.38 ± 0.74 vs. 1.38 ± 1.00, P = 1.0) or NFT (15.58 ± 1.20 vs. 16.30 ± 1.19, P = 0.67).

CONCLUSIONS — The natural history of nerve damage in patients with type 1 diabetes is not entirely clear. Longitudinal data from the Rochester cohort support the contention that the duration and severity of exposure to hyperglycemia are related to the progression and hence severity of neuropathy rather than its onset (21). A recent study of patients with type 1 diabetes showed a high prevalence of diabetic neuropathy, which progressed in a significant proportion of patients and was related not only to glycemic control but also to conventional cardiovascular risk factors such as hypertension and lipids (22).

The replacement of functioning islet β-cells by pancreas transplantation has been considered to be the most logical treatment for patients with type 1 diabetes to normalize blood glucose and ameliorate long-term complications. Although pancreas transplantation takes ~5 years to prevent progression and 10 years to reverse the lesions of diabetic nephropathy (23), a recent study has demonstrated an improvement and/or stabilization of diabetic retinopathy after a median follow-up of only 17 months (24). For diabetic neuropathy, the largest and longest follow-up series to date has shown that pancreas transplantation improved both motor and sensory nerve conduction as well as sudomotor function in the hand and foot within 1 year, which was maintained throughout follow-up for 10 years (13,25). However, autonomic function did not improve (13), as confirmed by another recent study (14). At this stage, most patients receiving transplantation will have severe nerve fiber damage as evidenced by marked depletion of intraepidermal nerve fibers (15). In addition, concomitant uremia, which often coexists in these patients, may contribute to nerve damage and limit repair, although renal transplantation in patients with diabetic nephropathy has not been shown to halt progression of neuropathy (26), and there was no difference in outcomes when pancreas alone transplantation was compared with combined pancreas and renal transplantation (13).

To determine a therapeutic response to an intervention in diabetic neuropathy, a standardized set of end points, which include clinical and neurophysiological evaluation combined with quantitative sensory and autonomic function testing, have been recommended (1,27,28). Pancreas transplantation has previously been shown to improve large nerve fiber conduction and, in particular, upper limb motor and sensory action potentials as well as sudomotor function within 1 year (13), but no impact on autonomic function was seen (13,14). Our studies show no relationship between quantitative sensory tests evaluating small fiber function and unmyelinated fiber pathology (9). Thus, although nerve conduction studies and quantitative sensory testing are useful and well-validated measures to help diagnose and assess progression of diabetic neuropathy, their utility in evaluating a therapeutic response may be limited (29).

Although more detailed and reproducible measures that accurately quantify small fiber neuropathy such as skin or nerve biopsy will reduce this variability, they are invasive (9–12). Our recent work using the corneal confocal microscope suggests that this noninvasive and reiterative test might be an ideal surrogate end point for use in clinical trials of human diabetic neuropathy and therefore may be appropriate for assessing the benefits of pancreas transplantation (16–18). The cornea is richly innervated by the ophthalmic division of the trigeminal nerve via the anterior ciliary nerves. Corneal nerves have been studied by detailed light and electron microscopy (30,31) and immunohistology (32,33). CCM provides a novel approach to study corneal nerve morphology (34). The main advantage of this technique is that it enables a noninvasive in vivo evaluation of the human cornea at 700× magnification, with excellent resolution and contrast. Initial studies were descriptive, and hence their usefulness for the purposes of interpreting small fiber degeneration and regeneration is limited. However, a study from our group in control subjects refined and significantly improved the quantification of C and A-δ corneal nerve fibers (35), showing high concordance with previous histological studies (30).

One may argue that corneal innervation has little relevance to diabetic somatic neuropathy, characterized by a distal loss of nerve fibers innervating the lower limbs, and therefore has limited application as a measure of peripheral neu-

Table 2 —*A1C and corneal confocal nerve fiber morphology at baseline and 6 months after SPK in 15 patients with type 1 diabetes*

	A1C	NCCA (mbar)	NFD (n/mm^2)	NBD (n/mm^2)	NFL (mm/mm^2)	NFT (Tc)
Before SPK	8.6 ± 0.4	1.54 ± 0.42	9.25 ± 1.87	1.38 ± 0.74	1.84 ± 0.33	16.30 ± 1.19
6 months after SPK	5.5 ± 0.1	1.23 ± 0.39	18.04 ± 1.48	1.38 ± 1.0	3.60 ± 0.33	15.58 ± 1.20
P	0.007	0.59	0.001	1.0	0.002	0.67

Data are means ± SEM with significant difference. NCCA, noncontact corneal esthesiometer; Tc, tortuosity coefficient.

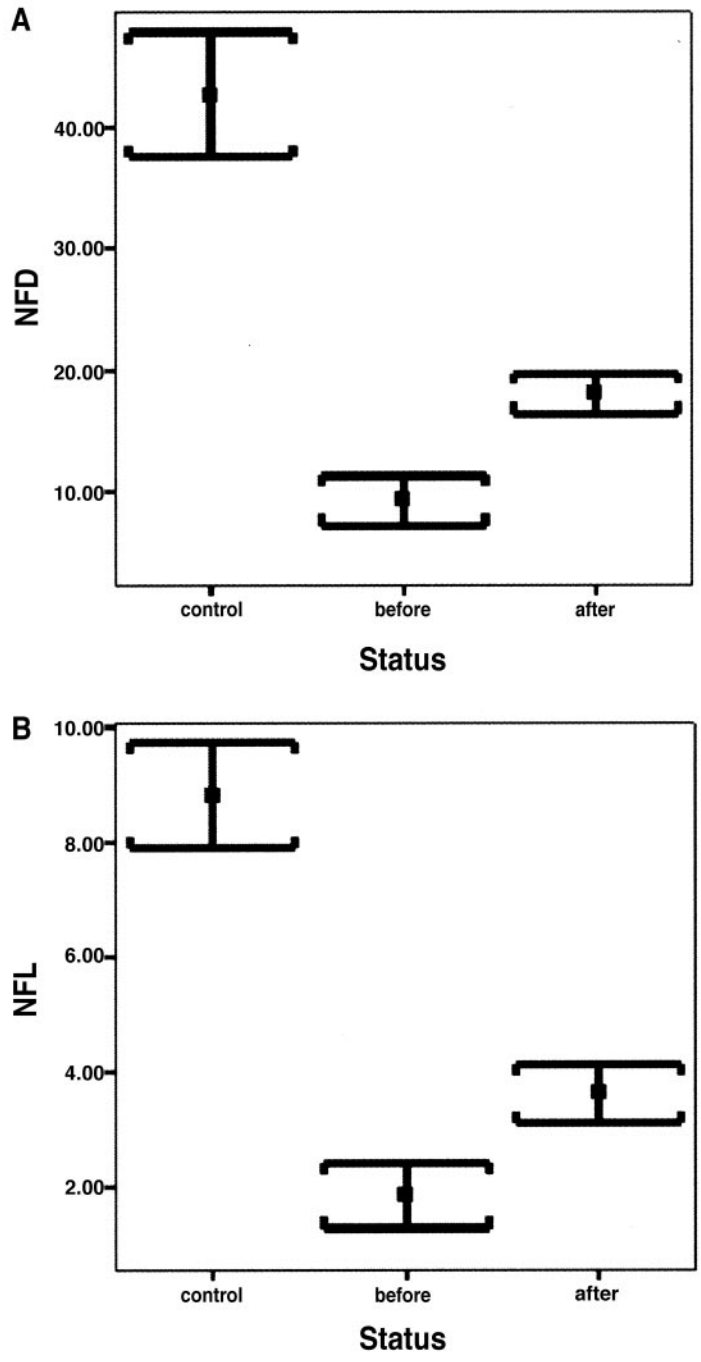

Figure 2—*NFD in diabetic patients before SPK at baseline compared with that of age-matched nondiabetic control subjects* ($P < 0.0001$) *with a significant improvement after SPK* ($P = 0.001$) (A)*, and NFL in diabetic patients before SPK at baseline compared with that of age-matched nondiabetic control subjects* ($P < 0.0001$) *with a significant improvement after SPK* ($P = 0.002$) (B)*. Results are means ± SEM.*

ropathy in diabetic patients. However, diabetic patients have a reduction in corneal sensitivity and a reduction in corneal nerve fiber bundles, which correlate with the Michigan Neuropathy Screening Instrument, a quantitative measure of somatic neuropathy (36). We have also shown a progressive reduction in corneal sensitivity (20) and significant corneal nerve pathology, which relates to the severity of neuropathy assessed using neurophysiology, quantitative sensory testing (16,17), and, in particular, intraepidermal nerve fiber density (18).

In the present study, we have demonstrated a highly significant loss of corneal nerve fibers in type 1 diabetic patients undergoing pancreas transplantation, which confirms previous studies demonstrating severe neuropathy in patients undergoing pancreas transplantation (13–15). However, despite this considerable baseline damage, we have shown a significant improvement in corneal NFD and NFL within 6 months of transplantation, indicating an early repair process with the restoration of euglycemia. These findings are in contrast to previous studies in diabetic nephropathy (23), retinopathy (24), and particularly neuropathy (13,14,25), in which at best a prevention of progression in nerve damage was shown only after several years of euglycemia. However, these latter studies focused heavily on electrophysiology and quantitative sensory assessment, which predominantly measure large fiber function and, to a lesser extent, small fiber function. When small fiber function was assessed in the form of sudomotor function, it is of relevance that significant improvement was demonstrated within 1 year of SPK (13).

Our study using CCM focused on detailed pathology as opposed to function of the small fibers and demonstrated repair despite significant baseline damage. These observations support the view that in clinical intervention trials for diabetic neuropathy, perhaps the focus should be on assessment of small fiber damage and repair (1,8). Until recently, this could only be provided by costly, time-consuming, and, most importantly, invasive procedures such as nerve (10) and skin biopsy (11,12). We now show that CCM, a noninvasive and hence reiterative test, might be an ideal surrogate end point for assessing the benefits of pancreas transplantation and indeed for assessing therapeutic efficacy of other therapies in clinical trials of human diabetic neuropathy.

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