Sexual Medicine

GDNF-Transduced Schwann Cell Grafts Enhance Regeneration of Erectile Nerves

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Abstract

Background: Schwann cell–seeded guidance tubes have been shown to promote cavernous nerve regeneration, and the local delivery of neurotrophic factors may additionally enhance nerve regenerative capacity. The present study evaluates whether the transplantation of GDNF-overexpressing Schwann cells may enhance regeneration of bilaterally transected erectile nerves in rats.

Methods: Silicon tubes seeded with either GDNF-overexpressing or GFP-expressing Schwann cells were implanted into the gaps between transected cavernous nerve endings. Six (10 study nerves) or 12 wk (20 study nerves) postoperatively, erectile function was evaluated by relaparotomy, electrical nerve stimulation, and intracavernous pressure recording, followed by ultrastructural evaluation of reconstructed nerves employing bright-field and electron microscopy. Additional animals were either sham-operated (positive control; 20 study nerves) or received bilateral nerve transection without nerve reconstruction (negative control; 20 study nerves).

Results: The combination of GDNF delivery and Schwann cell application promoted an intact erectile response in 90% (9 of 10) of grafted nerves after 6 wk and in 95% (19 of 20) after 12 wk, versus 50% (5 of 10) and 80% (16 of 20) of GFP-expressing Schwann cell grafts (p = 0.02). The functional recovery was paralleled by enhanced axonal regeneration in GDNF-overexpressing Schwann cell grafts, as indicated by larger cross-sectional areas and a significantly higher percentage of neural tissue compared with GFP-transduced controls.

Conclusions: These findings demonstrate that the time required to elicit functional recovery of erectile nerves can be reduced by local delivery of GDNF. In terms of clinical application, this enhanced nerve repair might be critical for timely reinnervation of the corpus cavernosum as a prerequisite for functional recovery in men.

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1. Introduction

Bilateral resection of cavernous nerve segments during radical prostatectomy for prostatic carcinoma eliminates spontaneous potency as seen prior to the development of nerve-sparing operations [1]. To date, clinical trials on cavernous nerve regeneration using autologous nerve grafts had only very limited success [2]. Thus, alternative strategies for erectile nerve repair are urgently needed.

Recently, we demonstrated that syngenic Schwann cell grafts seeded into artificial guidance tubes dramatically improve the regeneration of cavernous nerves in rats compared with autologous nerve grafts [3]. Within 12 wk after bilateral cavernous nerve ablation, 90% of animals that received Schwann cell–seeded guidance tubes regained erectile function compared with animals receiving nerve autografts with a recovery rate of 30%.

Neurotrophic factors have been extensively investigated in animal models of nerve injury to further enhance and accelerate the process of nerve regeneration and functional recovery [4]. Neurotrophic factors support the survival of axotomized neurons and enhance the intrinsic regenerative capacity after retrograde uptake and induction of specific signalling cascades. One member of the transforming growth factor superfamily, glial cell line–derived neurotrophic factor (GDNF), has been shown to promote the survival and function of several neuronal populations in the peripheral nervous system [5,6]. Penile expression of GDNF messenger RNA and retrograde transport of GDNF in penile parasympathetic and sensory neurons have previously been demonstrated, suggesting that GDNF may act as a specific target-derived neurotrophic factor for erectile nerves [7].

The application of neurotrophic factors can be combined with cell therapy most effectively by genetically modifying cells before transplantation to overexpress therapeutic transgenes. Several studies have shown that Schwann cells can be successfully genetically modified with the use of retroviral vectors to overexpress neurotrophic factors such as nerve growth factor (NGF), neurotrophin-3, or brain-derived neurotrophic factor [8–11]. The transplantation of neurotrophic factor–secreting Schwann cells provides a permissive cellular substrate for nerve regeneration and allows the local delivery of therapeutic transgene products at high concentrations without concomitant systemic side effects.

In the present study, we investigated whether genetic modification of syngenic Schwann cells to overexpress GDNF can enhance their capacity to promote structural and functional recovery in a rat model of bilateral cavernous nerve transection.

2. Materials and methods

2.1. Schwann cell cultures

Primary syngenic Schwann cell cultures were established from Fischer 344 rat sciatic nerve fragments as described previously [12]. Magnetic-activated cell separation of cells expressing p75 low-affinity nerve growth factor receptor (p75LNGFr) allows the reliable purification of Schwann cells within 9 d after biopsy by the use of direct selection of Schwann cells rather than fibroblast depletion assays.

2.2. Construction of retroviral vectors and cellular transduction

Vectors were generated that expressed either the full-length coding sequence of rat GDNF or, in control subjects, the reporter gene green fluorescent protein (GFP; Clontech, Palo Alto, CA, USA), as previously described [13,14]. Primary Schwann cells harvested from Fisher 344 rats were transduced in vitro with retroviral vectors derived from Moloney leukemia virus and expressing GDNF or GFP, respectively, and a neomycin-resistant gene. Successful transduction in vitro was confirmed with the use of a GDNF-specific enzyme-linked immunosorbent assay (ELISA; Promega, Madison, WI, USA).

Successful and stable in vivo GDNF production was examined by immunohistochemical analysis: Respective animals were transcardially perfused with a 0.9% saline solution followed by 4% paraformaldehyde in phosphate-buffered saline (PBS) at room temperature. The reproductive organs were removed, postfixed overnight in 4% paraformaldehyde in PBS, and were cryoprotected by incubation in PBS containing 30% sucrose. Sagittal 20-μm-thick sections of the nerve conduit were cut with a cryostat and mounted immediately on superfrost glass slides. The sections on the coverslips were washed with TRIS-buffered saline (TBS), blocked in TBS + 1% bovine serum albumin + 0.2% fish skin gelatin + 0.1% Triton-X (TBS+; all chemicals from Sigma), and incubated with the rabbit–anti-GDNF antibody (D-20, Santa Cruz Biotechnology, Heidelberg, Germany) at 1/2000 in TBS+ overnight at 4°C. The following day, sections were washed in TBS, incubated with the biotinylated donkey–anti-rabbit immunoglobulin G (Jackson, Hamburg, Germany) at 1/1000 in TBS+. Sections were incubated with avidin-biotinylated-peroxidase complex (Vector Elite kit; Linaris, Wertheim, Germany) followed by development for 3–15 min in a 0.05% solution of 3,3′-diaminobenzidine (DAB), 0.01% hydrogen peroxide, and 0.04% nickel chloride in TBS yielding a brown-black reaction product. Sections were air-dried, dehydrated, and coverslipped with Neo Mount (Merck, Darmstadt, Germany). The histological analysis was performed with the use of a Leica DMR microscope equipped with a Spot CCD camera, model 2.2.1 (Diagnostic Instruments, Michigan, USA).
## 2.3. Preparation of Schwann cells for transplantation

Nonbiodegradable, nonimmunogenic, silastic surgical tubing (internal diameter, 0.51 mm; external diameter, 0.94 mm; Aromando Medizintechnik GmbH, Duesseldorf, Germany) was selected for nerve conduits. Prior to seeding, the silastic tubes (tubing length, 5 mm) were sterilized by autoclaving at 121 °C. Cultured GFP- and GDNF-transduced Schwann cells were dissociated by trypsinization, counted with trypan blue, and resuspended to 25,000 cells per milliliter in Schwann cell medium (Dulbecco modified Eagle medium with 10% fetal calf serum). One milliliter of the cell suspension was drawn into Eppendorf pipettes. After incubation for 3 d at culture conditions, the tubes were washed with PBS before implantation.

## 2.4. Animals and experimental groups

Adult male Fischer 344 rats, weighing 250–350 g, were used. All institutional and national guidelines for animal safety and comfort were strictly followed. In 30 animals (60 study nerves), 5-mm sections of the cavernous nerve were excised bilaterally, followed by immediate bilateral microsurgical reconstruction: The animals were randomly divided into two groups. In 15 animals (30 study nerves), reconstruction was performed by interposition of silicon tubes seeded with GDNF-transduced Schwann cells. Another 15 animals serving as controls received GFP-expressing Schwann cells seeded into silicon tubes. Postoperatively, erectile function was evaluated after 6 (5 animals per group, 10 study nerves) or 12 wk (10 animals per group, 20 study nerves) by relaparotomy and electrical nerve stimulation, as previously described [3]. Afterwards, all grafts were explanted for histological and morphometrical analysis. An additional 20 animals either underwent a sham operation (positive control; 10 animals, 20 study nerves) or received bilateral nerve transaction without nerve reconstruction (negative control; 10 animals, 20 study nerves).

## 2.5. Surgical procedures

The animals were anesthetized with isoflurane followed by a combination of intramuscular fentanyl (0.005 mg/kg body weight), midazolam (2.0 mg/kg body weight), and medetomidin (0.15 mg/kg body weight). A lower abdominal midline incision was made from the symphysis pubis to the midabdomen. The endopelvic fascia overlying the cavernous nerve was incised and nerve ablation performed by sharply excising a segment of the cavernous nerve between the major pelvic ganglion and the symphysis pubis, leaving a 5-mm nerve gap. Collateral fibers to the cavernous nerves were also carefully dissected. The procedure was repeated on the contralateral side. Rostral and caudal nerve stumps were adapted by fixation inside the proximal and distal ends of the silicon tubes with 10-0 nylon sutures. In the surgical ablation group, the cavernous nerves were excised bilaterally without reconstruction. In sham control animals, the cavernous nerves were bilaterally exposed but not transected.

## 2.6. Assessment of erectile function

Functional tests were performed after postoperative intervals of 6 or 12 wk. Each animal underwent relaparotomy and functional testing only once. An intact erectile response was defined as a clear visible erection upon direct electrical stimulation to the proximal cavernous regenerated nerves. Systemic blood pressure was recorded during electrostimulation. Electrical stimulation was undertaken with the use of an HAMEG programmable function generator (HM 8131-2; Villejuif, France), bipolar platinum wire electrodes, and stimulus parameters of 6 V, 20 Hz, and 15 ms. The animals were reexplored through the previous midline incision. Upon identification of the reconstructed nerves, electrical stimulation was applied as described above. For monitoring of arterial blood pressure, a catheter was inserted into the tail artery. Intracavernous pressure was measured by inserting a silicone tube (0.4-mm internal and 0.8-mm external diameter) into one corpus cavernosum. The tube was attached to tubing filled with heparinized saline (25 U/ml) and connected to an Ellipse pressure transducer (Andromeda Medizinische Systeme, GmbH, Munich, Germany).

## 2.7. Morphological analysis

Experimental rats were scarified at 6 and 12 wk postoperatively via lethal doses of pentobarbital sodium (2 ml/kg body weight) before both silicon tubes containing the regenerated nerves were dissected out. Probes were fixed in 2.5% glutaraldehyde and processed for semithin histology. Histological preparations were evaluated to assess the overall nerve architecture, quantity and quality of nerve regeneration, and degree of myelination. For morphometrical analysis, photographs of whole cross sections were taken by a Zeiss Axiosvert 100 light microscope equipped with a Power Shot G5 digital camera (Canon). After manual outlining of the outer perineurial border and identification of neural compartments within digital images, these areas were measured automatically by LEICA- and QWIN-software Routine Nersm.q5r.

## 2.8. Data analysis

Data are expressed as mean ± standard error of the mean. Comparisons between groups were made with the chi-square test and Fisher exact test. Comparisons of intracavernous pressure between groups were made with the Mann-Whitney U test. Systemic blood pressure was analyzed with nonparametric Kruskal-Wallis and Mann-Whitney U tests; p < 0.05 was considered significant.

## 3. Results

### 3.1. In vitro production of GDNF

Successful transduction of primary Schwann cells was confirmed by ELISA analysis of cell culture supernatants: GDNF-transduced Schwann cells produced 111 (± 9.6) × 10^6 ng/ml of GDNF cells per day.
compared with 0.07 (± 0.025) ng × 10^6 ng/ml of GDNF cells per day in control, GFP-transduced Schwann cells.

3.2. In vivo production of GDNF

GDNF could be detected in the peripheral nerve conduits coated with GDNF-transduced Schwann cells at 12 wk post-transplantation by immunohistochemistry, indicating that stable GDNF expression could be achieved in vivo (Fig. 1A). In the peripheral nerve conduits that were coated with GFP-transduced Schwann cells, no GDNF expression could be detected (Fig. 1B). Cerebellar purkinje cells served as positive controls (Fig. 1C) [15].

3.3. Restoration of erectile function

After bilateral interposition grafting of silicon tubes filled with either GDNF- or GFP-transduced Schwann cells, functional assessment (erectile function after electrical stimulation of the proximal cavernous nerve) was undertaken in animals at either 6 or 12 wk postoperatively. After a postoperative interval of 6 wk, electrical stimulation of the proximal cavernous nerves resulted in an intact erectile response in 90% (9 of 10) of animals grafted with GDNF-transduced Schwann cells compared with 50% (5 of 10) in animals grafted with GFP-transduced control cells (Fig. 2A). After 12 wk, an intact erectile response was demonstrated in 95% (19 of 20) of animals with conduits filled with GDNF-hypersecreting Schwann cells compared with 80% (16 of 20) in animals with GFP-transduced conduits. Statistical comparison indicated significant differences between GDNF-transduced Schwann cell grafts and GFP-transduced controls (p = 0.02 for both 6 and 12 wk). None of the nerve-ablated untreated animals showed spontaneous recovery of erectile function 3 mo postoperatively, whereas all sham-operated control animals revealed an erectile response, confirming the reliability of the lesion and testing paradigm. The mean inducible intracavernous pressure divided by arterial blood pressure in animals with GDNF- and GFP-transduced Schwann cell grafts, after 6 and 12 wk in both, was comparable to that in nonablated, sham-operated rats (Fig. 2B). In contrast, the pressure in nerve-ablated untreated animals was significantly lower. The pressure increase in GDNF- and GFP-transduced Schwann cell grafts did not differ significantly.

3.4. Structural restoration

Histological analysis using transverse azur II methylene-blue/safranin or p-phenylenediamine (myelin visualization) staining of transverse semithin sections and specific visualization of NADPH-diaphorase in longitudinal sections for light microscopy or electron microscopy was performed midway through the silicon tube at 12 wk postoperatively. In tubes filled with GDNF- or GFP-Schwann cells, regenerated nerves were usually confined to the center of the conduit and surrounded by an acellular

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**Fig. 1 – (A–C) In vivo overexpression of GDNF by GDNF-transduced Schwann cells.** (A) GDNF-overexpressing Schwann cells (arrowheads) could be detected in the Schwann cell-coated peripheral nerve conduits at 12 wk post-transplantation by immunohistochemistry, indicating that stable GDNF expression could be achieved in vivo. (B) In the peripheral nerve conduits that were coated with GFP-transduced Schwann cells, no GDNF expression could be detected. (C) Cerebellar purkinje cells functioned as positive controls (arrowheads) for the immunohistochemical GDNF detection method [15]. Bright-field micrographs; scale bar: 50 μm. GDNF, glial cell line–derived neurotrophic factor; GFP, green fluorescent protein.
matrix filling the gap between the regenerated nerve and the inner wall of the silicon tube (Fig. 3). The extracellular matrix surrounding the regenerated nerve normally was lost in the course of histological processing.

Reconstructions of electron micrographs showed thin layers of perineurium forming around nerve fascicles, which were well vascularized (Fig. 4). Nerve regeneration within GDNF-transduced Schwann cells appeared superior to tubes filled with GFP-expressing Schwann cells, with larger regenerating nerve fascicles containing abundant unmyelinated and myelinated regenerating axons, sparse extracellular matrix, and a tight oligolamellar perineurium.

Comparing the overall cross-surface areas of regenerated nerves at midlevel, after removal of the extracellular matrix, revealed that tubes filled with GDNF-overexpressing Schwann cells contained thicker nerve cables (38.200 ± 31.300 μm²) compared with GFP-expressing Schwann cells (7.300 ± 4.200 μm², p = 0.09; Fig. 5A). Specific quantification of the area within these regenerated nerves occupied by axonal profiles (Fig. 5B) indicated that the degree of axonal regeneration was more pronounced with GDNF-overexpressing Schwann cells (17.100 ± 13.900 μm²) compared with GFP-transduced Schwann cells (1.400 ± 1.300 μm², p = 0.04). Consistently, mean percentage of neural area per total area was significantly
higher compared with GFP controls (42.1% vs. 19.9%; \( p = 0.02 \)).

The course of functional recovery over time was paralleled by morphological changes at different time points. Ultrastructural analysis using electron microscopy revealed that axon regeneration into and through GDNF-transduced Schwann cell grafts improved dramatically between 6 and 12 wk postoperatively (Fig. 6): At 6 wk, numerous myelinated and unmyelinated axon profiles were found at midlevel through the silicon tube, with highly variable diameters however (Fig. 6A). At 12 wk, axon diameters became very similar in size, indicating further maturation of regenerated nerves, partially recapitulating the tissue architecture of intact peripheral nerves (Fig. 6B).

4. Discussion

Syngenic Schwann cells seeded into silicon guidance tubes significantly promote nerve regeneration after cavernous nerve transection in rats and are far superior to autologous nerve grafts [3]. In the current study, we provide evidence that the time required to elicit functional recovery can be significantly reduced by genetically modifying Schwann cells to overexpress GDNF before trans-
Fig. 5 – (A,B) Total cross-sectional area (A) and neural area (B) measured at the guidance channel midpoint 12 wk after interposition grafting (box-and-whisker plots). GDNF-transduced Schwann cell grafts led to larger regenerates and improved regeneration of neural tissue compared with GFP-transduced controls. GDNF, glial cell line–derived neurotrophic factor; GFP, green fluorescent protein.

Fig. 6 – (A,B) Ultrastructural analysis using electron microscopy revealed that axon regeneration into and through Schwann cell-filled silicone tubes improves dramatically between 6 and 12 wk postoperatively. At 6 wk, numerous myelinated and unmyelinated axon profiles were found (A); however, myelinated fibers present with a larger scatter in diameter than on postoperative week 12 (B) (see box-whisker-plots of fiber area). On week 12, axon diameter values become narrower, indicating maturation of regenerated nerves.
injection or in a viral vector injected at the site of nerve injury when administered as a penile related compounds are neuroprotective after caver-

The prolonged restoration of erectile function, which is dependent on the relatively slow axonal regeneration process, represents a crucial clinical issue: Extended denervation of the penis leads to irreversible degeneration of the smooth muscle, which is associated with irreversible loss of potency [16]. Even with current refinements in surgical nerve-sparing techniques, recovery of erectile function takes 18–24 mo in most patients. For these reasons, the recovery of erectile function remains either incomplete or absent in many patients after radical prostatectomy. Therefore, the acceleration of nerve repair is important in not only the context of regaining function as soon as possible; moreover, it might dramatically increase the likelihood that regeneration will occur in time to reinnervate an intact corpus cavernosum, a prerequisite for functional recovery. Preclinical and clinical trials have demonstrated that gene therapy strategies may be feasible for these purposes [17].

Recent findings have shown that GDNF and related compounds are neuroprotective after cavernous nerve injury when administered as a penile injection [18] or in a viral vector injected at the site of nerve injury [19]. Which GDNF-mediated mechanism induces the observed enhanced recovery of erectile function remains to be assessed. Morpho-

studies, which used the same retroviral vector containing the coding sequences for GDNF, reported long-term expression in vivo after trans-

planted Schwann cells. Whether local application of GDNF alone (ie, without Schwann cell grafting) is as efficient in promoting restoration of erectile function as the combined approach with Schwann cell grafting needs to be addressed in future studies.

Previous studies, which used the same retroviral vector containing the coding sequences for GDNF, reported long-term expression in vivo after trans-

planted GDNF-overexpressing fibroblasts into the adult rat central nervous system for at least 3 mo [14]. In Schwann cells, stable transgene expression (GDNF) was shown for 3 mo in our experiments and can be maintained (NGF overexpression) for at least 6 mo after transplantation into the rat spinal cord [9]. The fact that functional recovery can be achieved as soon as 6 wk after cavernous nerve transection in rats calls for appropriate measures to shut down the production of therapeutic transgenes such as GDNF once regeneration is completed. As a prerequisite for potential clinical studies, future gene therapy–based approaches need to implement regulatable systems (eg, driven by tetracycline promoters), which have been demonstrated to effectively control transgene expression in vivo [20].

Taken together, data from this preclinical study provide proof of concept that the regenerative effects of cell-based therapeutic approaches can be further enhanced by the co-application of neurotrophic factors such as GDNF. Furthermore, our data provide the rationale for future studies to develop alternative methods for the controlled local application of nerve growth–promoting substances. This issue is especially important, because the retroviral vector derived from the Moloney leukemia virus used in this study has been shown to have oncogenic properties [21].

Notably, in humans a regeneration distance of at least 40 mm versus 5 mm in rats has to be crossed by regenerating axons. Enhanced regeneration by local application of GDNF could be crucial in reinnervating the corpora cavernosa just in time before denervation has induced irreversible atrophy. However, before this strategy can be considered for clinical studies that aim to restore erectile function after cavernous nerve lesions, several issues have to be addressed. In particular, the risk of GDNF stimulating the growth of residual prostatic cancer cells should be examined in preclinical studies. Second, the regulated and revers-

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References