Encapsulated Cell-Based Delivery of CNTF Reduces Photoreceptor Degeneration in Animal Models of Retinitis Pigmentosa

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Purpose. The objective of the present study was to evaluate the therapeutic efficacy of ciliary neurotrophic factor (CNTF) delivered through encapsulated cells directly into the vitreous of the eye in an *rcd1* canine model of retinitis pigmentosa. The dose–range effect of the treatment was also investigated.

Methods. Polymer membrane capsules (1.0 cm in length and 1.0 mm in diameter) were loaded with mammalian cells that were genetically engineered to secrete CNTF. The cell-containing capsules were then surgically implanted into the vitreous of one eye of *rcd1* dogs at 7 weeks of age, when retinal degeneration is in progress but not complete. The contralateral eyes were not treated. The capsules remained in the eyes for 7 weeks. At the end of the studies, the capsules were explanted, and CNTF output and cell viability were evaluated. The eyes were processed for histologic evaluation.

Results. In each animal, the number of rows of photoreceptor nuclei in the outer nuclear layer (ONL) was significantly higher in the eye that received a CNTF-secreting implant than in the untreated contralateral eye. No adverse effects were observed on the retina in the treated eyes. The explanted capsules produced a low level of CNTF. The cells in the capsules remained viable and densely distributed throughout.

Conclusions. CNTF delivered through encapsulated cells directly into the vitreous of the eye protects photoreceptors in the *PDE6B*-deficient *rcd1* canine model. Furthermore, sparing of photoreceptors appeared dose-dependent with minimum protection observed at CNTF doses of 0.2 to 1.0 ng/d. Incrementally greater protection was achieved at higher doses. The surgically implanted, cell-containing capsules were well tolerated, and the cells within the capsule remained viable for the 7-week implantation interval. These results suggest that encapsulated cell therapy may provide a safe and effective strategy for treating retinal disorders in humans. (*Invest Ophthalmol Vis Sci.* 2002;43:3292–3298)

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Ophthalmic disorders are a group of diseases with a rapidly increasing frequency that is associated with an increase in the aged population.[1–6] Patients with potentially blinding diseases have become one of the largest segments of the healthcare field with more than 50 million patients in the United States alone. Their sight is threatened by age-related macular degeneration,[7–9] diabetic retinopathy,[10–14] glaucoma,[15–18] or retinitis pigmentosa (RP).[19–22] Few effective treatments for these disorders are available to date, due in part to a lack of effective delivery of therapeutic molecules to the retina. We have developed an encapsulated cell therapy (ECT) device, specifically designed for intraocular implantation. Cells genetically engineered to secrete therapeutic factors are responsible for controlled, continuous, long-term delivery of therapeutics, including a wide variety of novel proteins, directly to the inner eye. In addition, the implants can be retrieved, providing an added level of safety.

Transplantation of encapsulated mammalian cells can deliver therapeutic agents to the target site in the central nervous system and produce therapeutic effects at lower doses than are required with other means of delivery.[23] Therapeutic efficacy of growth factors delivered by ECT has been well demonstrated in several animal models of neurodegenerative diseases, including CNTF in the rodent and primate models of Huntington's disease,[24,25] glial cell line–derived neurotrophic factor (GDNF) in rat model of Parkinson's disease,[26,27] and nerve growth factor (NGF) in rodent and primate models of Alzheimer's disease.[28–30] Furthermore, previous studies have shown that mammalian-cell–produced growth factor, synthesized de novo, is more potent than purified, *Escherichia coli*–produced growth factor.[23,31] However, ECT-mediated therapeutic delivery has not been tested in any models of ocular diseases. With limited distribution volume and easy access, eyes are an ideal target site for ECT-mediated therapeutic delivery.

Although ECT can be applied to treat several human ocular diseases, we chose RP for the following reasons: In many instances the cause and pathogenesis of the disease are well defined[32]; credible animal models that are molecular homologues of the human disease[33–39] are available; and a number of neurotrophic factors[40–43] have shown protective effects against photoreceptor degeneration.

Prior studies have demonstrated the promise of growth factors, neurotrophic factors, and cytokines as potential therapeutics for RP by intravitreal injection in short-term animal experiments.[40,41,43,44] Among these, ciliary neurotrophic factor (CNTF) has been shown to be effective.[43] Unfortunately, the chronic nature of the disease and the adverse effects associated with repeated short-duration intraocular administration militates against the use of CNTF by this delivery method and has prevented the initiation of clinical trials and its further development.

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In the present study, the therapeutic efficacy and safety of prolonged intraocular delivery of CNTF through encapsulated cells in RP animal models were investigated.

Materials and Methods

Cell Lines

CNTF-secreting cell lines were generated by transfection of NTC-200 cells (derived from human retina) with a CNTF gene containing plasmid using a transfection reagent (Fugene 6; Roche, Indianapolis, IN) according to the manufacturer's protocol. Polyclonal stable cell lines were selected by using geneticin (G418; Gibco-BRL, Gaithersburg, MD) at a concentration of 1.0 mg/mL and maintained at 0.25 mg/mL. Stable CNTF-secreting cell lines were established and redesignated as NTC-201. Cell lines were maintained in DMEM-F12 supplemented with 10% FBS (Gibco-BRL, Gaithersburg, MD) in a 5% CO_2, 95% humidity, 37°C incubator.

Expression of CNTF

Output of CNTF was determined by CNTF ELISA (R&D Systems, Minneapolis, MN). The expression of CNTF was also monitored by Western blot analysis, using polyclonal goat anti-human CNTF antibodies (R&D Systems) followed by secondary biotin-conjugated rabbit anti-goat antibody and streptavidin-horse-radish peroxidase (both from Jackson ImmunoResearch Laboratories, West Grove, PA).

Devices

The nonfilled device is composed of a scaffold, providing cell attachment within a sealed, hollow-fiber membrane, and a retrieval loop at one end for surgical manipulation. Before cell encapsulation, devices were assembled in a controlled environment, packaged, and e-beam sterilized (25 kGy with exposure time of 18 seconds; Titan Scan, Lima, OH). The semipermeable hollow-fiber membrane is composed of polyether sulfone (PES, >99%), caprolactame (plasticizer) and polyvinyl pyrrolidone (antifouling). PES is considered a stable polymer with decades-long stability. The membrane has a breaking strength of 200 g and an elongation of 50%. The membrane's inner diameter is 845 to 880 μm and wall thickness ranges from 90 to 110 μm. Although the 90% molecular weight cutoff was calculated to be less than 20 kDa (using multidispersion dextran cocktail), we have found empirically that under normal conditions CNTF (~25 kDa) can readily diffuse across the membrane, but larger molecules such as antibodies (150 kDa) cannot.

It is important to note that molecular weight is not the only parameter that determines the diffusive property of the molecule. In addition to molecular weight, the shape of the molecule, its flexibility, and tertiary structure, all play important roles in its transport.

The substratum matrix is created by the extrusion of poly(ethylene terephthalate) into monofilament yarns. The monofilament yarns are s-twisted into 30-filament strands with a denier (thickness) of 150 dtex. There are 180 monofilament strands of yarn per device. There was no exogenous biological matrix (e.g., collagen) added to the substratum matrix at encapsulation. Polymer membrane capsules approximately 1.0 cm in length and 1.0 mm in diameter were manufactured with hollow-fiber membrane and poly(ethylene terephthalate) yarn. Device ends were secured with medical-grade methacrylate glue with a titanium loop incorporated into one end of the capsule.

NTC-201 cells harvested on the day of encapsulation were suspended in Endothelial-SFM growth media (Gibco-BRL, Gaithersburg, MD) at a density of 66,000 cells/μL. Devices were loaded with approximately 400,000 cells instilled through a syringe (Hamilton, Reno, NV), sealed, and maintained in Endothelial SFM. Before surgical implantation, devices were held for 14 days after encapsulation in growth medium to assure sterility. Different CNTF doses were achieved by either encapsulating populations of cells that had different levels of CNTF output, or mixing the nontransfected cell line (NTC-200) with the CNTF-secreting cell line (NTC-201) to maintain consistent cell density.

CNTF Release Kinetics

CNTF release from cell-filled devices was measured before surgical implantation and immediately after surgical retrieval from the *rcd1* dog by CNTF ELISA (R&D Systems). Devices were incubated in 1.0 mL Endothelial SFM for 6 hours (preimplant) or 24 hours (postexplant) in 12-well tissue culture plates (Falcon Labware; BD Biosciences, Bedford, MA) in a 5% CO_2, 95% humidity, 37°C incubator, after which time CNTF was quantified in the conditioned medium. For devices implanted in vivo, release of CNTF was evaluated before implantation (2 weeks after encapsulation), after 4 weeks in vivo (6 weeks after encapsulation) and after 7 weeks in vivo (9 weeks after encapsulation). Similarly, CNTF release for in vitro cohort devices (nonimplanted) was evaluated at these time points for the duration of the study (9 weeks). The preimplantation CNTF output by the device represents device performance under the optimal culture condition during the manufacturing process, whereas postexplantation CNTF output represents performance of the device in in vivo conditions.

Animals

All studies were performed according the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All experimental procedures were performed according to written protocols approved in advance by the Animal Care and Use Committee, and the studies were conducted in compliance with the American Association for Accreditation of Laboratory Animal Care.

Transgenic Rat Model for Retinal Degeneration

A rapid retinal degeneration transgenic rat RP model, S334ter-3,[38] was used to investigate photoreceptor protection of CNTF delivered through unencapsulated NTC-201 cells. Heterozygous S334ter-3 rats carrying the rhodopsin mutation S334ter were produced by mating homozygous breeders (kindly provided by Matthew M. LaVail, University of California, San Francisco, CA) with wild-type Sprague-Dawley rats. The S334ter-3 transgenic rats were treated with either NTC-200 (parental cell line, $n = 6$) or NTC-201 (CNTF-secreting cell line, $n = 6$) through intravitreous injection into one eye on postnatal day (P)9, when retinal degeneration has already begun.[38] Approximately 10^5 NTC-201 cells (in vitro CNTF output at 100 ng/million cells per day) in 2 μL phosphate-buffered saline (PBS) were injected into the vitreous of the left eye of S334ter-3 rats ($n = 6$) through a 32-gauge needle. Control animals were injected with untransfected parental cells ($n = 6$). Contralateral eyes were not treated. For the CNTF bolus injections, 1 μg CNTF (R&D Systems) in 1 μL PBS was injected into the vitreous at P9. Eyes were collected at P20 and processed for histologic evaluation. Plastic-embedded sections of 1 μm thickness stained with toluidine blue were examined by light microscopy.

Rcd1 Canine Model for Retinal Degeneration

The *rcd1*-affected dogs were provided by the Retinal Disease Studies Facility (Kennett Square, PA) which is a resource maintained by the National Eye Institute (National Institutes of Health, Bethesda, MD) and the Foundation Fighting Blindness (Hunt Valley, MD). The *rcd1* dogs carry a mutation of the *PDE6B* gene, and retinal degeneration in this model has been well characterized.[39,45] Encapsulated NTC-201 devices were surgically implanted into one eye of each *rcd1* dog at 7 weeks of age, a point at which 40% to 50% of photoreceptors have already been lost because of degeneration[39,45] (i.e., five to six layers of outer nuclear layer [ONL] remain). This age is 3 weeks after weaning and the earliest time-point that the surgical procedure can be performed without disruption of the retina (the eyes of younger dogs would be too small to accommodate a 1-cm device). As a control, the contralateral eye was not treated. Devices were explanted at 14 weeks of age (7 weeks after

implantation), after which time, in untreated eyes, an additional 50% of photoreceptors are expected to be lost, leaving only two to three layers of ONL.[39,45] At the end of the study, devices were explanted and evaluated for output of CNTF by ELISA and for cell viability by histologic analysis. The eyes were enucleated and processed for histologic analysis.

Histology

ECT devices were fixed in 4% paraformaldehyde and processed for glycidylmethacrylate (GMA) embedding, sectioned, and stained for histologic evaluation using hematoxylin and eosin (H-E). Each device was reviewed to determine cell density and viability.

Enucleated eyes were fixed in Bouin's solution, embedded in paraffin, and sectioned at 6 μm. Vertical sections through the optic nerve and pupil were stained with H-E and examined by light microscopy. Clinical monitoring of potential adverse effects was conducted under the guidance of a board-certified ophthalmologist. Histopathology evaluation of the eyes was performed by a board-certified veterinarian pathologist.

Photoreceptor Evaluation

The rows of nuclei in ONL were counted within three areas each of the superior retina (S1–S3) and inferior retina (I1–I3). S1 is defined as within two 10× fields peripheral to the optic nerve, S2 as the midpoint between optic nerve edge and ora serrata, and S3 as within two 10× fields from the ora serrata. I1 is defined as within one 10× field from the optic nerve, I2 as the midpoint between the optic nerve edge and ora serrata, and I3 as within one 10× field from ora serrata. Three count samples were taken from each area. For area-specific protection, the average number of layers of photoreceptors in ONL for each area of treated eye were compared with the corresponding area of untreated eye for each animal. For overall protection, all 18 ONL count samples from the treated eye were compared with that of untreated eye for each animal, to determine efficacy. Statistical analysis was performed with the paired *t*-test.

Results

Intraocular Device Design

An intraocular implantable encapsulated cell device prototype for prolonged delivery of therapeutic agents was developed for treating ophthalmic disorders (Fig. 1). The device consists of genetically modified cells packaged in a hollow-fiber, semipermeable membrane. The hollow-fiber membrane prevents immune molecules (e.g., antibodies) and host immune cells from entering the device, whereas it allows nutrients and therapeutic molecules to diffuse freely across the membrane. The encapsulated cells, continuously secreting therapeutic agents, are maintained in a proprietary matrix material (Fig. 1A) and derive nourishment from the host milieu. The device is anchored to the sclera at the pars plana by a small titanium wire loop (Fig. 1B). The active intravitreous portion of the device measures approximately 1 mm in diameter and 10 mm in length. It is fixed outside the visual axis. The surgical procedure for its placement requires approximately 15 minutes and involves a small incision in the pars plana.

Characterization of NTC-201, CNTF-Expressing Cell Lines

Human cell lines were established that were stably transfected to express CNTF. Conditioned media collected from transfected cells were evaluated by ELISA for expression of CNTF. The cell lines that secreted a consistent amount of CNTF

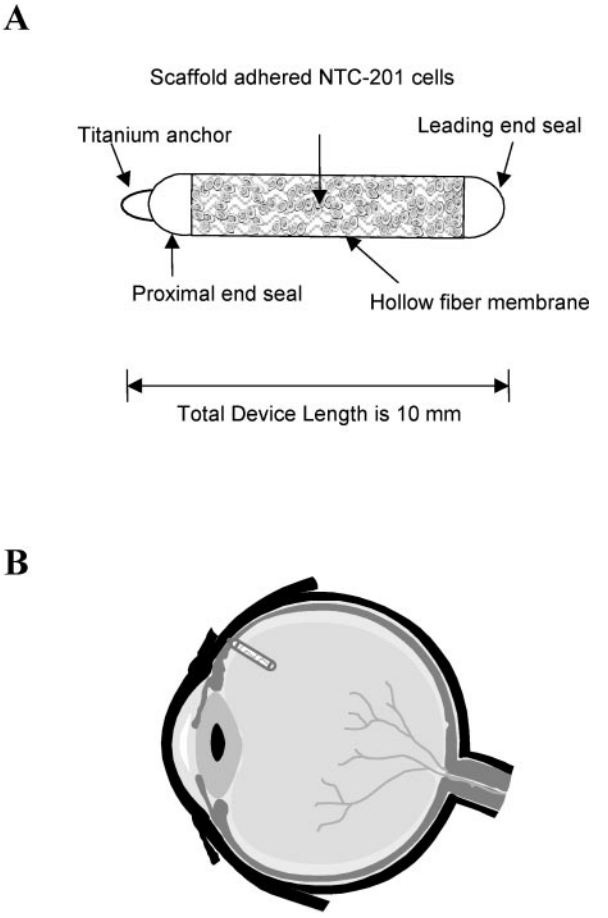

Figure 1. ECT delivery device (Neurotech USA, Inc., Lincoln, RI). Encapsulated cell implants consist of living cells encapsulated within semipermeable polymer membranes and supportive matrices. (**A**) Longitudinal view of the device, containing cells. (**B**) Intraocular placement of an ECT device.

(>100 ng/10^6 cells/24 h) for a period longer than 6 months were designated NTC-201. To analyze the CNTF released from these cells, conditioned media were subjected to Western blot analysis. The secreted protein migrated as a doublet of 27 and 29 kDa, which is somewhat larger than the molecular weight of *E. coli*–derived CNTF (23 kDa; Fig. 2). The presence of two bands of CNTF is not surprising, because two active forms of native CNTF have been isolated from several species.[46–49]

Sustained Release of CNTF by Encapsulated NTC-201

CNTF release kinetics of encapsulated NTC-201 cells was evaluated after different in vitro holding and intraocular implantation (in vivo) intervals (Table 1). Encapsulated NTC-201 devices, held in Endothelial-SFM, secreted CNTF for the entire holding period (9 weeks) with CNTF levels ranging from 33.6 to 12.4 ng/d, with relative stability achieved at 4 weeks after encapsulation. In contrast to devices held under optimal tissue culture conditions in vitro, there was a decrease in CNTF output for the explanted devices after intraocular implantation. Because vitreous humor contains much fewer nutrients than Endothelial-SFM, the observed decrease may reflect the function of encapsulated NTC-201 cells under much more stringent in vivo conditions. After the initial decrease, the in vivo release of CNTF from encapsulated NTC-201 cells was stabilized at approximately 1.5 ng/d, measured at 4 and 7 weeks after implantation.

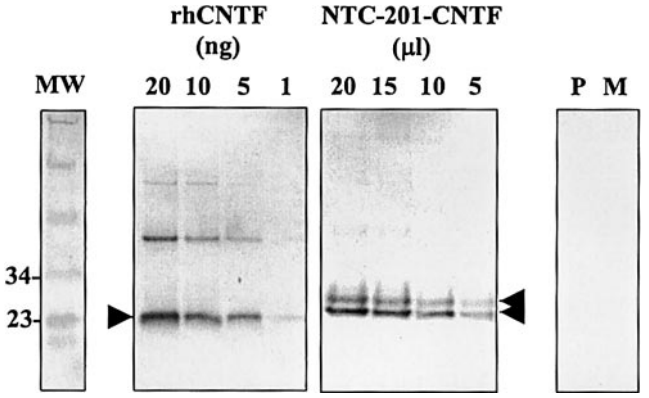

FIGURE 2. Western blot analysis of NTC-201–secreted CNTF. Conditioned media were subjected to SDS-PAGE on a 10% gel under denaturing conditions. RhCNTF, purified recombinant CNTF derived from *E. coli*; NTC-201-CNTF, conditioned medium from stable CNTF-secreting cell line; P, parental NTC-200; M, media; MW, molecular weight in kDa.

Protective Effect of NTC-201 in a Transgenic Rat Model of RP

In untreated eyes of S334ter-3 transgenic rats, severe photoreceptor degeneration was observed by P20, and examination of the ONL showed only one row of nuclei remaining (Fig. 3A). The NTC-201–injected eyes had five to six rows of nuclei in the ONL (Fig. 3C), whereas in the control eyes that were injected with nontransfected cells (NTC-200), only one to two rows of nuclei remained (Fig. 3B). Furthermore, no evidence of retinal inflammation was observed in any of the treated or control eyes. In animals treated with a single intravitreous injection of purified human recombinant CNTF, the ONL had two to three rows of nuclei (Fig. 4). It is important to point out that the total amount of CNTF delivered through cells (~100 ng) was approximately 10 times less than that delivered through bolus injection (1 μg) for the duration of the treatment. These results clearly demonstrate that continuous delivery of CNTF through mammalian cells afforded better protection against retinal degeneration in this model. Intravitreous cell survival was confirmed for the duration of the study with injection of green fluorescent protein (GFP)–transfected NTC-200 cells (data not shown).

Protective Effect of the Encapsulated NTC-201 Device on Photoreceptors in the *rcd1* Canine RP Model

As expected, the ONL in untreated eyes was approximately two to three layers thick. In contrast, the ONL in the ECT-CNTF–treated eyes had five to six layers remaining. This represents the rows of nuclei present at the time when the treatment began (Table 2 and Fig. 5). Moreover, the protection of photoreceptors was evenly distributed throughout the retina (Table 2) and was not localized near the site of implant. The observed protection is statistically significant ($P < 0.0001$). Again, there were no apparent adverse effects in the retina. All explanted devices secreted CNTF (Table 1) and contained viable cells (Fig. 6).

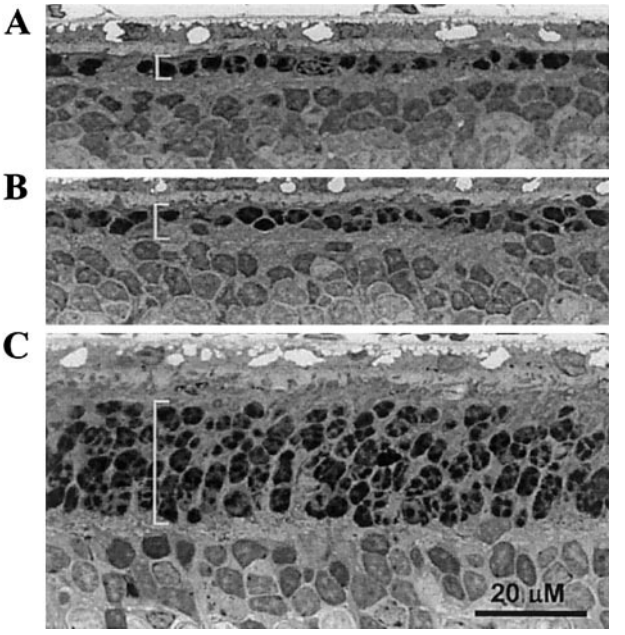

FIGURE 3. Retinal photomicrographs of transgenic rats carrying the rhodopsin mutation S334ter. (**A**) S334ter untreated eye, (**B**) NTC-200 parental cell–treated eye, and (**C**) NTC-201 cell–treated eye. The cells were injected on P9 and the experiment was terminated on P20. *Brackets* denote ONL.

Photoreceptor Protection in the *rcd1* Canine RP Model through Encapsulated NTC-201 Clinical Devices Is Dose-Dependent

In the *rcd1* mutant canine RP model, NTC-201 devices significantly protected photoreceptors from degeneration in a dose-dependent manner (Fig. 7). Complete protection was achieved at the highest dose (5–15 ng/d CNTF), and minimal but statistically significant protection was observed at levels as low as 0.2 to 1 ng/d of CNTF. CNTF delivered below 0.1 ng/d had no protective effect, indicating that the observed protective effect was due to the presence of CNTF and not the ECT device itself.

Histologic evaluation indicated that all devices contained healthy, viable cells throughout (Fig. 6). No cellular evidence of an immune reaction, inflammation, or damage to the retina was observed by histologic evaluation. In clinical and histologic examination of the eye, focal areas of opacity of the lens,

TABLE 1. ECT-CNTF Secretion Kinetics

Time Course CNTF Release of In Vitro Hold Devices		Time Course CNTF Release of Explanted Devices	
Time after Encapsulation (wk)	**Device CNTF Output (ng/day)**	**Time after Implantation (wk)**	**Device CNTF Output (ng/day)**
1	33.6 ± 1.42	—	—
2	22.8 ± 2.0	2 *in vitro preimplant*	22.8 ± 2.0
4	14.8 ± 2.78	4 *in vivo*	1.5 ± 0.2
9	12.4 ± 2.59	7 *in vivo*	1.6 ± 0.4

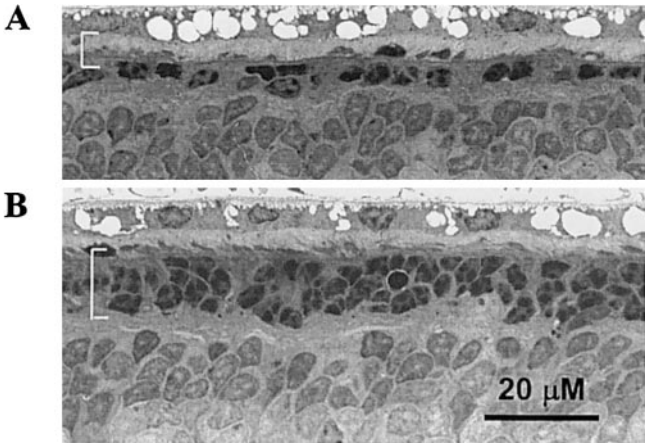

FIGURE 4. Retinal photomicrographs of transgenic rats carrying the rhodopsin mutation S334ter. (**A**) S334ter untreated eye and (**B**) eye treated by bolus intravitreous injection of CNTF. The CNTF was injected on P9, and the experiment was terminated on P20. *Brackets* denote ONL.

in most cases located adjacent to the placement site of the device, were observed in some animals. The CNTF dosage received by the animal was not correlated with the incidence or severity of these lens changes.

DISCUSSION

We have shown that encapsulated NTC-201 cells enabled sustained release of CNTF in vitro and in vivo (Table 1). Furthermore, intraocular implantation of an encapsulated cell device that releases CNTF directly into the vitreous gel protected photoreceptors in the *rcd1* mutant canine model of RP in a dose-dependent manner, and both the surgical procedure and device were well tolerated.

Although several appropriate animal models are available, we selected RP to demonstrate retinal neuroprotection using an ECT device to deliver a therapeutic protein. Not only is the disease pathogenesis for RP in several cases well defined,[32] but also there are valuable precedents: Therapeutic factors have already shown clear efficacy in photoreceptor protection.[40–43]

TABLE 2. Protective Effect of ECT-CNTF on Photoreceptors of *rcd1* Dogs

		Photoreceptor Number (ONL)		
Animal	Retina Area	ECT-CNTF Treated	Not Treated	*P*
1485	S1	5.5	3.0	
	S2	5.8	2.7	
	S3	6.0	4.0	
	I1	4.0	2.5	
	I2	3.8	2.3	
	I3	4.2	2.7	
	Average	4.8 ± 0.23	2.9 ± 0.15	<0.0001
1489	S1	5.3	3.5	
	S2	7.5	3.3	
	S3	7.5	4.0	
	I1	5.5	3.7	
	I2	4.5	3.2	
	I3	5.3	2.7	
	Average	5.9 ± 0.29	3.4 ± 0.14	<0.0001

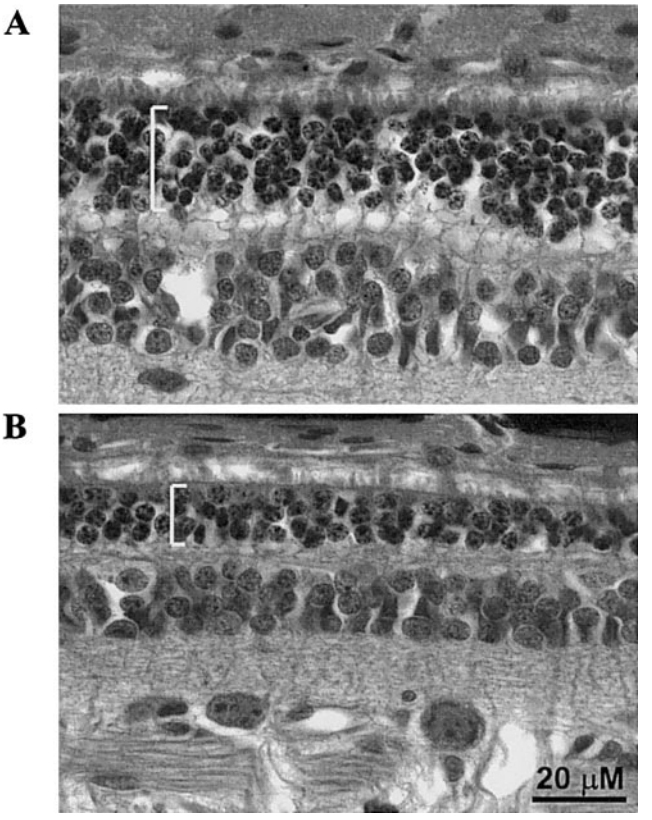

FIGURE 5. Retinal photomicrographs of *rcd1* canine model of RP. Comparison of ONLs in (**A**) ECT-CNTF–treated versus (**B**) nontreated eyes. The device was implanted into one eye at 7 weeks of age and explanted at 14 weeks of age. The contralateral eye was not treated.

Unfortunately, their use has been limited because of the risks involved in frequent intravitreous injections. CNTF delivered through an unencapsulated NTC-201 cell line, which was injected intraocularly, prevented severe photoreceptor loss in a S334ter-3 transgenic rat model of RP. Because of the rapid photoreceptor degeneration time course of this model, protective effects could be evaluated within the first 3 weeks of life.[38] We found that NTC-201 cells protected photoreceptors from rapid degeneration (Fig. 3). The results also suggest that a sustained release of CNTF from the cells achieved better protection of photoreceptors than a bolus injection of purified CNTF protein (Fig. 4). Similar observations have been reported with adenovirus-mediated CNTF gene transfer in the *rd* or *rds* mouse model of RP.[50,51]

Although the protection in the rat RP model with a very rapid retinal degeneration is encouraging, most types of human RP have a much slower time course, because photoreceptor degeneration often takes place over many decades. It was therefore important, to the degree practical, to evaluate the protective effect of CNTF in a chronic RP animal model that more closely resembles the human disease. For an intermediate term study we evaluated ECT in the *rcd1* canine model of RP. In this model, retinal degeneration begins at 3.5 to 4.0 weeks of age and continues gradually over the course of a year, with 50% of photoreceptor loss at 7 weeks and 70% to 80% loss at 14 weeks.[45]

Because the canine eye is similar in size to the human eye, a device configuration identical in size and composition with a human clinical intraocular device was used. For those eyes that received CNTF-secreting ECT devices, significant photoreceptor protection was observed (Table 2, Fig. 5). In addition, there was a dose-dependent protection of photoreceptors (Fig. 7)

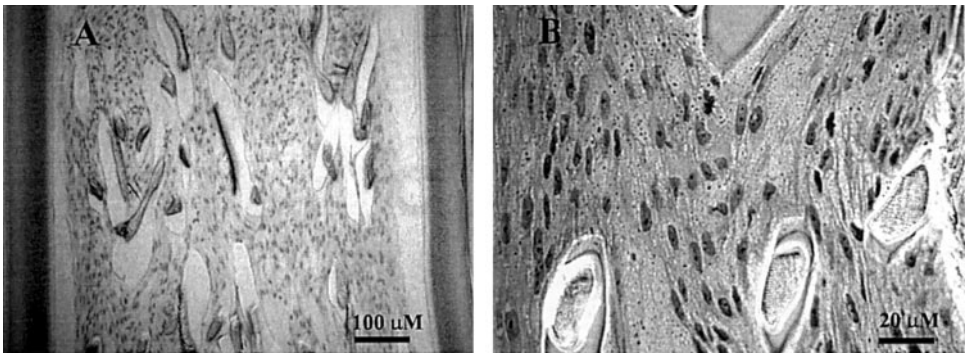

FIGURE 6. Representative cell viability in the device after 7 weeks in vivo. GMA-embedded sections, 4 μm thick, stained with H-E, were examined under light microscope. Sections of poly(ethylene terephthalate) yarn scaffold along with cells were shown. Magnifications: (**A**) ×10; (**B**) ×20.

with minimum protection attained at 0.2 to 1 ng/d of CNTF. Incrementally greater protection was achieved at higher doses (Fig. 7). These results, and the fact that no protective effect was observed when CNTF output was less than 0.1 ng/d, suggest that encapsulated cell-based delivery of CNTF is effective in photoreceptor protection for the duration studied, and the observed protection of photoreceptors is due to the effect of CNTF and not the presence of the device or the implantation procedure. Furthermore, unlike repeated intravitreous injections of recombinant CNTF (Pearce-Kelling S, Acland G, Aguirre G, et al., manuscript in preparation), no severe adverse effects associated with either CNTF or ECT were observed during the study period.

With an increase in aging populations in developed countries, the number of patients who have potentially blinding retinal diseases is growing rapidly. However, because of difficulties associated with delivery of potential intraocular therapeutics, few effective treatments are available to date. Implantation of encapsulated, genetically engineered cells that secrete a therapeutic factor (Fig. 1) offers an alternative to frequent intravitreous administration. To this end, ECT provides a site-directed, continuous, low-dose of de novo synthesized therapeutic molecules to the target site (Table 1), therefore avoiding the additive risks commonly associated with repeated bolus injection or systemic delivery. In addition, that the ECT devices can be easily retrieved and replaced makes the treatment reversible and renewable, providing a safer alternative to in vivo gene therapy. The small intraocular device design, large number of potential therapeutic candidates, relatively simple surgical procedure for implantation and removal, and minimal side effects all contribute to the promise of this technology.

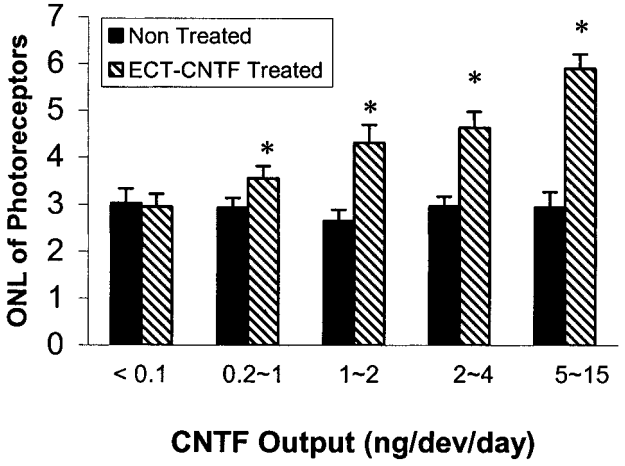

FIGURE 7. Dose–response protection of photoreceptors in *rcd1* canine model of RP. Comparison of ONLs in ECT-CNTF–treated eyes with those in nontreated eyes (mean ± SEM). *$P < 0.05$.

In summary, we have successfully developed a human CNTF-producing cell line that demonstrates protective efficacy in animal models of RP. Perhaps more significantly, with an intraocular device designed for the human eye, we have demonstrated therapeutic efficacy of encapsulated CNTF-secreting cell therapy in a mutant canine model of RP. Our data confirm that sustained delivery of protein therapeutics is more effective than bolus injection, while avoiding the additive risks of frequent intraocular injections.[50,51] The availability of a delivery system for treatment of ocular diseases can accelerate the development of therapies for treating RP and other ophthalmic disorders, for which no effective therapies are currently available.

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