Stereotactic Striatal Injection of a Regulated GDNF Expression System: Preclinical Testing in a Primate Model

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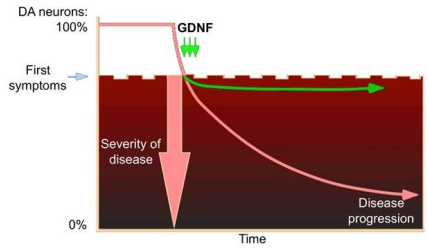
Introduction

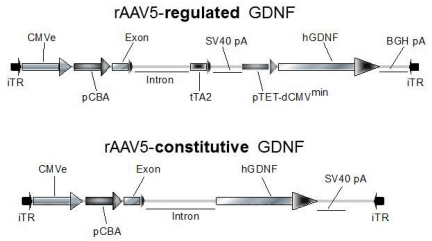
Glial derived neurotrophic factor (GDNF) is a trophic factor for dopaminergic (DA) neurons, which is neuroprotective for these neurons in animal models. GDNF is considered a potential treatment of Parkinson's disease (PD) to slow disease progression. However, initial trials of direct delivery of GDNF protein to the brain for PD failed to generate positive clinical results (Gill et al., 2003; Nutt et al., 2003). This lack of observed clinical efficacy is most probably due to an unsuccessful delivery method and/or the patient selection process rather than a failure of GDNF activity. We have thus developed a doxycycline-regulated viral vector capable of expressing more homogeneous levels of GDNF, which demonstrates therapeutic benefit in rodent models of PD. We tested the safety and efficacy of striatal vector injection in primates as a precursor to potential human trials.

Clinical Rationale: virally delivered GDNF within neostriatum of PD patients could slow or halt the progressive loss of nigrostriatal DA neurons



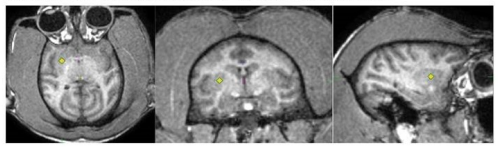
Methods

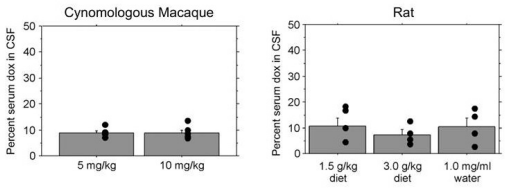
We performed bilateral stereotactic viral vector injections into the anterior putamen of six cynomolgus macaques. Each animal received both constitutively active and regulated vectors in separate hemispheres. Half of the animals received oral doxycycline (5 mg/kg) after injection. After a 21-day incubation period animals were sacrificed to permit tissue analysis, including quantitation of GDNF expression and tyrosine hydroxylase (TH) staining using a LiCor Odyssey infrared imaging system.



Surgical Procedure

Under general anesthesia, each animal was placed in a stereotactic CRW headframe. Stereotactic targeting of the postcommisural putamen was performed using a T1 weighted MRI performed on a single animal, then scaled to fit the others based on skull size. The animals were registered in the frame using facial surface landmarks. Bilateral burr holes were performed and 15 ml of vector was stereotactically injected over 30 min on each side using a Hamilton syringe and a programmable infusion pump. For each animal, constitutively active vector was injected into the left hemisphere and regulated vector was injected in the right hemisphere. The needle was withdrawn after a 10 min diffusion time.

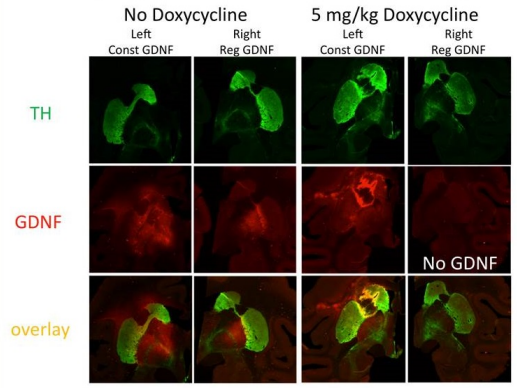




Doxycycline crosses the blood brain barrier in primates. Both Cynomologous Macaques and Rats were fed a diet including doxycycline at the doses indicated. Lumbar punctures were subsequently performed for CSF analysis. The relative CSF concentration of doxycycline compared to serum levels was consistent in both primates and rodents at the doses tested.

Results

LiCor Odyssey infrared immunohistochemical staining



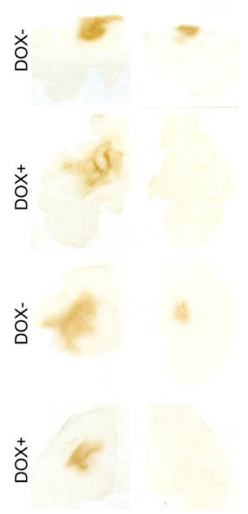
Reproducible GDNF expression was observed at the vector injection site. Expression of the regulated GDNF expression vector was fully suppressed by low dose doxycyline without evidence of leakiness.

Regulated GDNF expression was reduced relative to expression from the constitutively active vector. Of note, this same pattern has been observed in rodents. Notably, the reduced level of expression of GDNF by the regulated vector was still sufficient to observe clinical benefit in rodent models of PD.

There was minor inaccuracy in targeting in the dorsal direction that correlated with degree of size difference between the subject and the animal that was imaged for targeting.

No surgical complications were observed

GDNF Diaminobenzidine immunoperoxidase labeling



animal	$GDNF_{const}$	$GDNF_{reg}$	% $GDNF_{const}$
50	2309.0	--	--
150	2755.0	--	--
89	904.8	375.0	41.4
51	4962.0	1321.0	26.6
126	4545.7	1420.0	31.2

Average striatum GDNF immunostaining intensity (represented with arbitrary units) expressed by the regulated GDNF vector ($GDNF_{reg}$) was reduced relative to expression from the constitutively active vector ($GDNF_{const}$).

animal	L TH -GDNF	R TH -GDNF	L TH +GDNF	R TH +GDNF	L %TH_{-GDNF}	R %TH_{-GDNF}
50	930.1	--	1328.2	--	142.8	--
150	145.5	--	385.0	--	264.6	--
89	1339.3	743.1	1531.0	1514.1	114.3	203.8
51	2478.7	4594.0	2683.7	8209.0	108.3	178.7
126	970.7	687.4	1581.4	864.1	162.9	125.7

In all animals, TH immunostaining was increased in striatal regions that were induced to overexpress GDNF via viral injection

Conclusions and Future Directions

In primates, striatal transduction of a drug-responsive GDNF expression system results in focused GDNF production that can be fully suppressed with doxycycline. Doxycycline reliably crosses the primate blood-brain barrier at low doses that are capable of regulating the vector. We plan to further evaluate the astrocytic and migroglial response to both constitutive and regulated viral vector injection. The results of this pilot study indicate that this regulated viral GDNF expression system is appropriate for further preclinical testing in primate models of PD, and potentially human clinical trials.

Aknowledgements

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References

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Nutt et al. Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. Neurology. 2003 Jan 14;60(1):69-73.