BDNF as targeting platform to deliver small molecules to the optic nerve: anti-inflammatory drugs to treat blast-induced traumatic optic neuropathy (b-iTON)

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Intracisternal Administration of BDNF-SMCs for Intraneuronal Delivery to the Optic Nerve

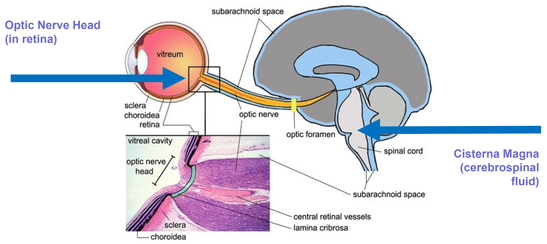


Figure 1. Axoplasmic Flow Originates in Cisterna Magna (source: Wostyn 2015)

The ocular nerve structure, including globes, optic chiasm and post-chiasmal sections, originates in the cisterna magna. Axoplasmic flow goes from the distal ends, at the base of the skull, to the proximal ends terminating in the optic nerve heads (Quigley 2000). Others have shown delivery to the retina after intracisternal injection, but not using BDNF (Rennels 1985; Yang 2013: Mathieu 2017). It is established that NGF (BDNF) binds selectively to tropomyosin kinase A receptors (TrkA) found on the cornea (Roberti 2014); BDNF binds to TrkB expressed in optic nerve heads, in Retinal Ganglion Cells (Fournier 1997; Pease 2000; Quigley 2000; Lindqvist 2010; Mysona 2014).

Hypothesis

We attached a Near InfraRed dye that fluoresces in the 800 region (MW ~1000 + BDNF MW kDa 26) to recombinant human BDNF (800-rhBDNF). We hypothesized that intracisternally injected 800-rhBDNF would be absorbed by TrkB receptors expressed at the at the distal ends (base of the skull) and moved via retrograde axonal transport via the optic chiasm to the optic nerve heads.

In Vitro Bioassay Confirms 800-rhBDNF Retains TrkB Binding Activity

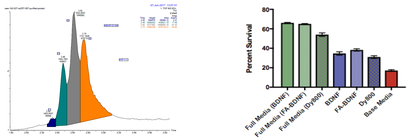


Figure 2. (L) Analytics of 800-rhBDNF; (R) Bioassay in RGC cultures

(LEFT) Analytics of 800-rhBDNF. Number of 800 dye molecules attached per BDNF monomer (Abzena US). **(RIGHT) Bioassay in primary neuronal cultures of Retinal Ganglion Cells.** We plated study probe in bioassays of Retinal Ganglion Cells (Barres 1988; Winzeler and Wang 2013). If 800-rhBDNF or FA-rhBDNF support neuronal survival, then TrkB binding is intact after synthetic manipulation. Work was done in the former Barres lab, Stanford University.

In Vivo Proof-of-Concept: intracisternal 800-rhBDNF reaches optic nerve heads

Group	Dose	Rat	Baseline	15 min	6 h	Brain tissue 6 h
800-rhBDNF	5 µL	17				
800-rhBDNF	10 µL	18				
800-rhBDNF	30 µL	19				
Unmodified dye	10 µL	64				

Figure 3. BDNF localizes 800 to optic nerve in dose-dependent, time-dependent effect

Figure 3 is a pilot study in naïve rats, in which we tested 800-rhBDNF at 3 doses: 5 µl, 10 µl, and 30 µl. The control article was the carboxylic acid form of unmodified dye. We injected each dose mid-line, a single intracisternal injection. (We thank Tony Yaksh PhD, University of California, San Diego, who provided training to Absorption Systems, San Diego for intracisternal injection). Figure 3 shows fundus, in-life imaging at baseline prior to treatment, 15 min and 6 h after treatment. Retinal images were taken in-life with Spectralis. Post-euthanasia, whole brain was also imaged using Spectralis. Results showed that 800 in the 800-rhBDNF construct was localized to nerve structures in the brain. By contrast, the control of unmodified dye was not localized, but diffused throughout the brain.

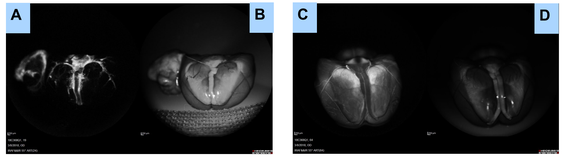


Figure 4. Post-euthanasia images of whole brain show localization of 800-rhBDNF in nerve structure resembling optic nerve and optic chiasm

Figure 4 is a close-up of **30 µL dose,** post-euthanasia images obtained 6 h after dosing **(IRAF&IR) Spectralis® > 6h (brain tissue post dissection).**

Left images A, C appear to show NIR 800 in optic chiasm (**red circle**), but 800-rhBDNF (A) localizes to structures resembling nerve tissue, whereas unmodified dye (C) is diffuse nonspecific uptake. **Right images B, D** photo show orientation of sample (bottom of image is optic chiasm at front of brain).

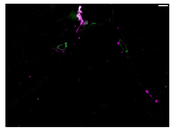
Histopathology: DY-800 detectable in optic chiasm after intracisternal injection

Imaging methods

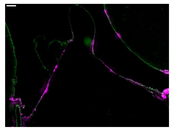
All studies were done in naïve rats. To image 800 in the 800-rhBDNF complex, we used the Heidelberg Spectralis at two settings: (i) infrared autofluorescence (IRAF) used to detect ICG, indocyanine green, an NIR dye in clinical use since the 1950s uses a diode laser with a wavelength of 790 nm in the NIR spectrum; and (ii) Infrared Reflectance (IR), which excites at the 820 nm wavelength.

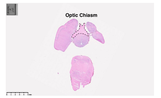
After dissection, from frozen tissue we produced 2 directly contiguous sets of slides from 2 sections of optic nerve, optic chiasm from brain tissue, and optic nerve heads. We applied hematoxylin and eosin (HE) stain to 1 slide set to reveal nerve tissue. We read the second set at the University of Nebraska Medical Center (UNMC) using an Olympus IX73 inverted 2-deck microscope with motorized filter cube turret. NIR images are acquired in two channels at 4X in the FITC channel (detects auto-fluorescence) and ICG channel **(purple)**. To confirm that NIR 800 had localized to in nerve tissue, we matched the morphology of HE stain **(pink)** to FITC+NIR images.

Low dose:
5 µL 800-rhBDNF
Rat 17 FITC+NIR
800 dye barely detectable in optic chiasm

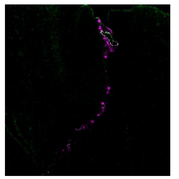


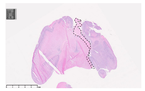
Medium dose:
10 µL 800-rhBDNF
Rat 18 FITC+NIR
800 dye localizes to optic chiasm)



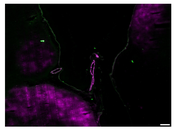


High dose:
30 µL 800-rhBDNF
Rat 19
FITC+NIR
800 dye localizes to optic chiasm





Control:
10 µL unmodified DY-800 dye (carboxylic acid form).
Rat 64 FITC+NIR
Dye diffuse throughout brain



Conclusions & Next Steps: Test FA-rhNGF and FA-rhBDNF in blast-ITON model

Conclusions

We conclude that BDNF injected intracisternally offers a receptor-mediated delivery platform, moving a NIR dye 800 into the optic nerve, for retrograde axonal transport to optic nerve heads in the retinas. The same approach may also prove clinically useful for BDNF-small molecule drugs, repurposed and in development, applied topically as eye drops or injected intracisternally.

Clinical choices: eye drops or intracisternal - or both?

There are no drugs approved to treat acute blast-induced Traumatic Optic Neuropathy. Recombinant human NGF is now approved as eye drops to treat neurotrophic keratitis. Since rhNGF accumulates in the optic nerve (Lambiase 2005), eye drops using NGF as localizing moiety are feasible. Intrathecal BDNF was studied in humans (BDNF Study Group 1999; BDNF Study Group 2000). Intracisternal injection is a type of intrathecal administration; intrathecal administration was standard clinical practice in the 1960s. An NGF- or BDNF-targeted conjugate may be clinically feasible if given intracisternally.

Next Steps

We have previously shown the anti-inflammatory activity of two soluble study drugs in which we conjugate the potent glucocorticoid, fluocinolone acetonide (FA) using a heterobifunctional linker either to Nerve Growth Factor (FA-rhBDNF) or BDNF (FA-rhBDNF) (DoD Grant Log 32173014).

Our next step is to formulate two study drugs as eye drops and test them in a mouse model that incurs inflammatory processes in a mouse model of ocular blast-induced Traumatic Optic Neuropathy (b-iTON) developed in the Rex laboratory (Bernardo-Colón 2018). Recently, the Rex laboratory examined histopathology in the b-TON model and confirmed the targets IL-1α and IL-1β (Bernardo-Colón 2019). It is known that glucocorticoids act on IL-1β.

The ocular topical form would be field-deployable and could be given by a non-expert. A supporting scenario is that after evacuation to a medical field hospital, eye drops could be combined with intracisternal administration, or used as maintenance therapy post-deployment.

Commercial goals

Goal #1: develop study drugs as eye drops. Our next goal is to test the anti-inflammatory effect of FA-rhNGF or FA-rhBDNF formulated as eye drops in the mouse model of b-iTON. We hope to collaborate with the Rex Laboratory at Vanderbilt University (Hines-Beard 2012; Bricker-Anthony 2014; Bernardo-Colón 2018). If successful, we can advance drugs immediately into development by (i) optimizing effect, to explore combining the 2 study drugs; (ii) non-GLP dose range-finding; and (iii) GLP IND-enabling studies.

Goal #2: out-license BDNF-linker technology. We can out-license proprietary BDNF-linker technology now by making an affordable license available to all third parties developing small molecules for b-ITON.

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