Novel Intra-Operative Peripheral Nerve Agent for Fluorescence Guided Imaging

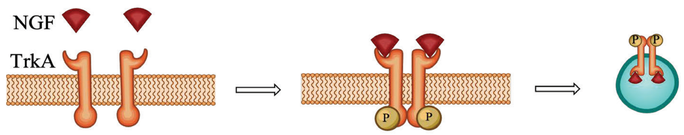
Xiang Liu[ab]*, Paul Lovell[a]*, Denzel Ryan Cruz[c], Lucas Houser[a], Chad A. LaGrange[bd], Aaron M. Mohs[a,b,e,f]

[a]Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE 68198, USA; [b]Fred and Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE 68198, USA; [c]University of Illinois at Urbana-Champaign, Champaign, IL 61820, USA; [d]Department of Urology, University of Nebraska Medical Center, Omaha, NE 68198, USA; [e]Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE 68198, USA; [f]Center for Drug Delivery and Nanomedicine, University of Nebraska Medical Center, Omaha, NE 68198, USA.

Abstract

Nerve injuries that significantly affect a patient's quality of life are a common complication of major surgeries[1]. Fluorescence-guided surgery (FGS) has become increasingly popular because it allows physicians to position their instruments precisely during surgeries to spare nerves, for example, in radical prostatectomies[2]. Visualization of nerves during oncological surgeries is an unmet clinical need that is under investigation. Here, we address this unmet need with a contrast agent that is selective for peripheral nerves. Our contrast agent combines an existing near infrared (NIR) dye that fluoresces in the 800 region with a naturally-occurring protein of the human nervous system, nerve growth factor (NGF) – a combination termed Nervelight™. Due to the fact that exogenously administered NGF localizes to the distal ends of nerves due to guidance by high affinity receptors, our contrast agent binds specifically to, then is endocytosed, and is transported up the nerve via retrograde axonal transport. In the clinical setting during nerve sparing surgeries, the area in question would be incised, and the surgeon could intra-operatively apply the agent to at-risk nerves before removing the tumor. In preliminary studies, after we directly applied the contrast agent to the nerve of interest, the targeted nerve was clearly labeled by this fluorescent imaging agent. In these experiments, visualization was obtained after 10 minutes. Other studies suggest that nerves may be seen for the duration of at least one hour and likely longer. These results suggest that Nervelight™ can serve as a fluorescence-guided surgical tool that will improve the visualization of at-risk nerves during radical prostatectomies, and possibly other oncological surgeries.

NGF-TrkA Signaling Complex



Tyrosine receptor kinase A (TrkA) is a membrane-bound receptor highly expressed in nerves and has a high binding affinity for the Nerve Growth Factor (NGF). The NGF-TrkA signaling transduction begins on the distal axon terminal of a nerve cell. It activates once NGF binds to the extracellular domain of TrkA, leading to dimerization and autophosphorylation on the tyrosine residues in the cytoplasmic domain of TrkA. With the help of different signaling intermediates, the NGF-TrkA complex is internalized into the nerve cell through endocytosis. Following formation of the signaling endosome, the NGF-TrkA complex recruits the motor protein dynein to promote retrograde transport towards the minus end of microtubules in the axon. Once it reaches the soma, past research suggests the signaling endosome can undergo lysosomal degradation or continue to travel along the dendrites to amplify the signal to other nerve cells. The NGF-TrkA signaling endosome triggers a number physiological functions essential for the growth and survival of peripheral nerves cells[3].

Results

Establish an Optimized Protocol for Nervelight™ by Utilizing Sciatic Nerves

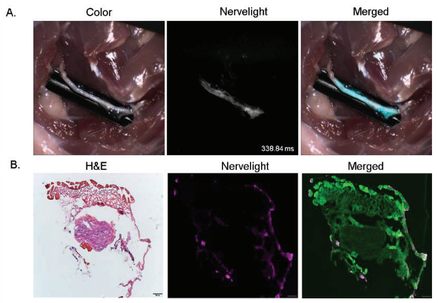


Figure 1. (A) Color, 800nm (NIR) channel, and merged images of exposed sciatic nerve tissue. Images were taken immediately following PBS washes.
(B). Histology confirmation of rat sciatic nerve tissue stained with H&E and NIR channel (800 nm) signal indicates Nervelight™ localization. Scale bars in each image represent 100 µm.

Cavernous Nerve Imaging Provided Profound Nerve Fluorescence

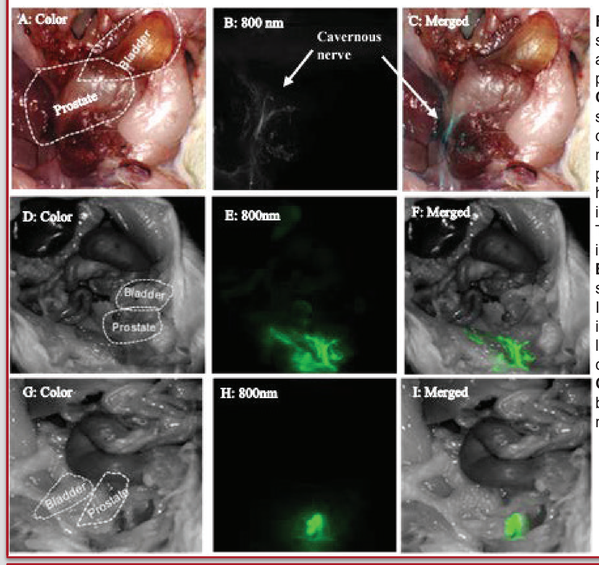


Figure 2. Nervelight™ imaged selectively in cavernous nerve after topical application to major pelvic ganglion (inset). **A, B, C: Curadel** system where **B and C** show robust visualization of cavernous nerves, where C is the merged image. **D:** Brightfield picture of the rat. The highlighted white dotted line area indicates Bladder and Prostate. The targeted nerve in this area is invisible macroscopically. **E: Green** indicates Nervelight™ signal in 800-channel of Pearl Image Studio System. **F:** Merged images of D and E show localization of Nervelight™ to the cavernous nerve. **G, H, I: unmodified control dye** brightfield, color image, and merged image.

Localization of Nervelight™ to the Cavernous Nerve Confirmed by Histopathology and Immunohistochemistry

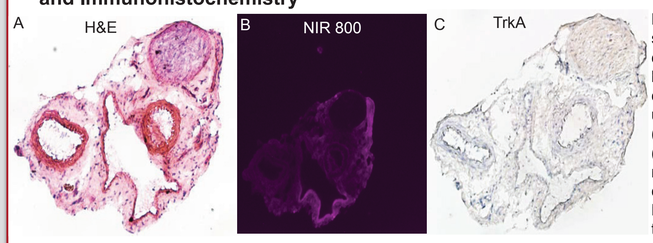


Figure 3. In a cross-section of frozen cavernous nerve tissue harvested from site comparing morphologies using **(A)** H&E staining, **(B)** NIR 800 signal, and **(C)** TrkA antibody. These micrographs shows co-localization, suggesting Nervelight™ has localized to the cavernous nerve.

Intra-operative Imaging of Nervelight™ on the Facial Nerve

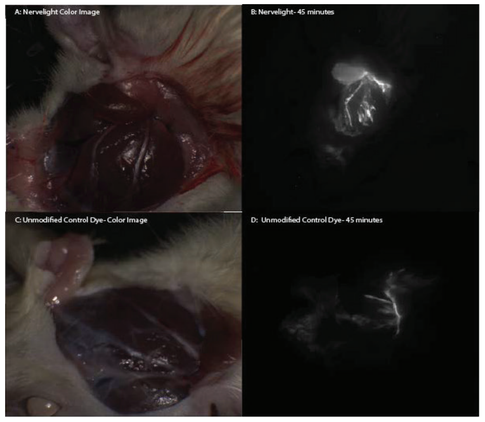


Figure 4: Facial nerve imaging after topical application via the Curadel Lab FLARE and the Fluoptics Fluobeam Fluorescence Imaging System. **A:** Non-fluorescent color image of Nervelight™ at 45 minutes. **B:** 45 minutes after topical application of Nervelight™.
C: Non-fluorescent color image of unmodified control dye at 45 minutes. **D:** 45 minutes after topical application of unmodified control dye.

Confirmation of Nervelight™ to the Facial Nerve via Histology and Immunohistochemistry

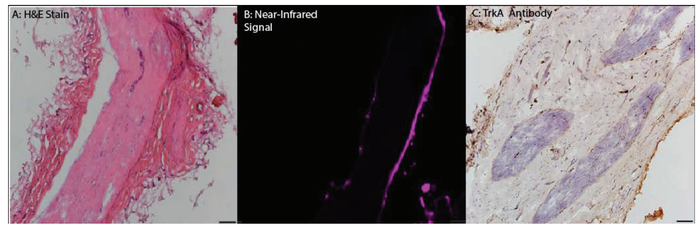


Figure 5. In a cross-section of frozen facial nerve harvested from the facial nerve. (**A**) H&E stain, (B) NIR Fluorescence (magenta along the edge of the nerve), and **(C)** TrkA antibody suggests co-localization of Nervelight™ has localized to the facial nerve.

Conclusions

- After direct application to the nerve of interest, the targeted nerve was clearly labeled by this fluorescent imaging agent.
- Topical application has its limitations, as the solution may move in between tissues and make qualitative and quantitative analysis from the images less accurate. More tests should evaluate other interactions between the fluorescent agent and non-nerve tissues.
- These results suggest that the nerve contrast agent can serve as a fluorescence imaging guided tool that will significantly improve the visualization of vital nerves during nerve sparing surgeries.

Future Directions

- Future evaluation will evaluate other interactions between the fluorescent agent and non-nerve tissues.
- We need to determine the lower and upper bounds of Nervelight dose when applied topically, and to assess rate of absorption (time).
- We will need to provide confirmation of safety for the non-GLP toxicology study as well as a future GLP toxicology study; we will need to assess the side effect of this substance applied on the rat topically.
- Investigate NGF-TrkA signaling endosome in the peripheral nerve cell culture using compartmentalized microfluidic platforms.

Acknowledgements

This work has been supported by the National Institutes of Health grant R44 CA180745-02. We would like to thank Constance McKee, Dr. Michael Olive, and Dr. Stephen Kahl, all of Manzanita Pharmaceuticals, Inc.; Dr. Jieyu Zhou (Abzena US); and the Lein laboratory, the University of California, Davis (Davis, CA) for their assistance and expertise. The University of Nebraska Medical Center Office of Comparative Medicine aided with protocol surgeries and training (Kristin Leland).

References

[1] Antoniadis, G., et al., "Iatrogenic Nerve Injuries: prevalence, diagnosis and treatment," Deutsches Aerzteblatt International 111(16), 273- 279 (2014).
[2] Walsh, E. M. et al., "Fluorescence Imaging of Nerves During Surgery," Annals of Surgery 270(1), 69-76 (2019).
[3] Marlin, M. and Li, G., "Biogenesis and Function of the NGF/TrkA Signaling Endosome," International Review of Cell and Molecular Biology 314, 239-257 (2015).