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Event: SPIE BiOS, 2021, Online Only

An intravenous fluorophore for enhanced intraoperative nerve visualization

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ABSTRACT

Iatrogenic nerve injury is a risk in many surgical procedures. We present our experience with a novel fluorescent agent to enhance the visualization of at-risk nerves during a prostatectomy in a canine model.

Illuminare-1 is an intravenously administered myelin-binding fluorophore. After IRB approval and successfully visualizing target nerves in murine and porcine tests, we undertook a robotically-assisted prostatectomy on a dog, which has comparable genitourinary anatomy to a human. Dogs were positioned supine, anesthetized, the abdomen was insufflated, and the peri-prostatic and obturator nerves were exposed with a DaVinci SI Surgical System. A modified FDA-approved laparoscope with white and blue (370 – 425nm wave length) light settings was positioned via an assistant port to illuminate these nerves. A bolus of 1mg/kg of Illuminare-1 was administered intravenously with uninterrupted visualization of the presumed nerves. Fluorescent structures were resected for histological assessment.

With 1mg/kg of Illuminare-1, nerves rapidly fluoresced under blue light, displaying a distinct hue. Fluorescence was seen within 90 seconds of administration and sustained for over three hours in the obturator and smaller peri-prostatic nerves. Tiny linear structures that were not initially seen under white light conditions were clearly identified as fluorescent tissue after injection with Illuminare-1. Seven fluorescent peri-prostatic structures were resected and all were histologically confirmed to be myelinated nerves. The cross-sectional nerve fiber diameters ranged from 64 – 247nm.

Illuminare-1 enhanced the visualization of the neurovascular bundle in a dog. Phase-1 in-human trials with Illuminare-1 will follow to address the unmet need to reduce unintended surgical morbidity.

Keywords: fluorescence; prostatectomy; laparoscopy; nerve sparing; robotic surgery; nerve injury; visualization; myelination

1. INTRODUCTION

Intraoperative nerve injury is an important potential complication affecting many procedures including pelvic, head and neck and limb surgeries [1]. Unfortunately, due to anatomical variation, suboptimal visualization of nerves relative to surrounding tissues, and close proximity of nerves to key structures, nerve-sparing outcomes are not always achieved. As nerve injuries often portend chronic and disabling morbidity, there remains an ongoing need to improve real time intraoperative nerve visualization and nerve-sparing outcomes.

Currently there are no adjunct visualization tools that are routinely used in surgery and therefore surgeons rely on the naked eye and magnification alone to identify nerves. Illuminare-1 is a novel myelin-binding fluorophore, designed to improve intraoperative nerve visualization. The agent is administered intravenously and fluoresces in real-time under blue light (370 – 425nm wave lengths). While Illuminare-1 has demonstrated promising results fluorescing a target nerve in both murine and porcine experiments[2], the agent had not been tested on different caliber nerves or in

Molecular-Guided Surgery: Molecules, Devices, and Applications VII, edited by Sylvain Gioux, Summer L. Gibbs, Brian W. Pogue, Proc. of SPIE Vol. 11625, 116250N · © 2021
SPIE · CCC code: 1605-7422/21/$21 · doi: 10.1117/12.2577710

clinically applicable scenario. Therefore, we use a canine model to evaluate whether Illuminare-1 can enhance the visualization of the peri-prostatic and obturator nerves, both of which are routinely encountered during an extended robotically-assisted prostatectomy[3]. Herein we present our findings with this novel technology.

2. METHODS

A mature four-year-old 47.2kg male grey hound was selected for the study. The dog was cared for under the direction of an experienced veterinary team from Intuitive Surgical Laboratories (Sunnyvale, California, USA). For the procedure, nerve visualization is achieved with the Karl Storz Photodynamic Diagnostic D-Light C (PDD) rigid system (Karl Storz, Tuttlingen, Germany). The PDD system for the experiment comprises of a Tricam PDD Camera Head attached to a Hopkins II 10mm, 30-degree laparoscope with an added eyepiece containing a Fluorescein blue optical filter. The laparoscope is attached to a D-Light C Light Source via a 10cm Fluid Light Cable. The surgical dissection was performed with a DaVinci SI Surgical System (Intuitive Surgical Systems, Sunnyvale, California, USA), with the Storz PDD laparoscope inserted through an assistant port. The Storz laparoscope is able to be toggled between white light and blue light, with the camera output embedded into the DaVinci SI picture as a dual-display. The blue light setting is familiar to urologists as it is used for improved visualization of bladder tumors during select cystoscopies[4].

The dog was placed under general anesthetic without complication and kept in a supine position for the duration of the procedure. Abdominal insufflation was performed through a midline port and five additional port sites were placed under vision. The DaVinci robot was docked, mounting four ports to the robot, and leaving two available assistant ports. An experienced urologist systematically exposed the prostatic and bilateral obturator nerves with minimal bleeding and cautery. As in humans, the canine's obturator nerves are larger caliber and easier to identify than the peri-prostatic nerves. To assess the penetrance of Illuminare-1, tissue surrounding the right obturator nerve was thoroughly skeletonized, while the left obturator nerve was deliberately only partially exposed.

After adequate exposure was achieved, the Storz camera system was inserted via the right assistant port and orientated towards the right neurovascular bundle of the prostate prior to administration of Illuminare-1. The camera was fixed in position and the primary display in the monitors was switched from the DaVinci camera to the Storz camera.

1.0mg/kg of Illuminare-1 was injected intravenously as a slow bolus over 5 minutes. The peri-prostatic nerves were monitored in real-time incrementally alternating between blue light fluorescence and white light (standard vision). This was continued until the uptake of Illuminare-1 appeared to stabilize. After the fluorescence from Illuminare-1 plateaued, a systematic exploration was done to identify partially submerged nerves and possibly also previously unseen nerves. Resection was only performed in white light conditions, with toggles to blue light used to identify and/or confirm presumed nerve structures.

Fluorescent structures of interest were resected and stored in formalin for independent external pathological evaluation. The study continued for two and half hours after the time of injection, after which the dog was humanely euthanized and the port sites were closed.

3. RESULTS

Upon injection of Illuminare-1, nerves rapidly fluoresced under blue light. These nerves displayed a distinct hue that was evident within the first five minutes of administration and plateaued around 20-30 minutes post-injection (Figure 1). After the injection of Illuminare-1, fat, muscle, blood vessels and fascia also responded differently under blue light conditions: there were significant color changes which enabled the nerves to be clearly differentiated from other structures. The differences in tissue types can be hard to discern under ordinary white light conditions.

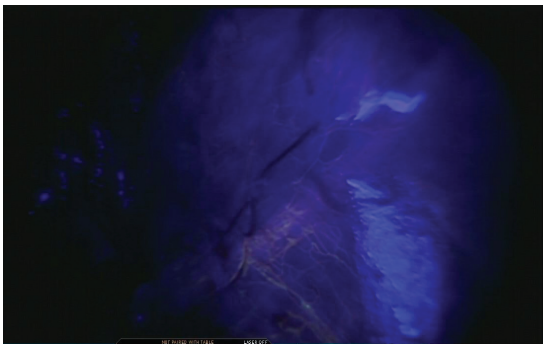

Figure 1. Two minutes post injection of Illuminare-1, demonstrating rapid drug uptake & fluorescence of the peri-prostatic nerves (*under magnification*)

Both the small peri-prostatic and the larger obturator nerves fluoresced (Figure 2). This fluorescence was consistent and sustained for the duration of the study. The dog did not exhibit any detectable changes throughout the routine intraoperative monitoring.

Some artifact was detected, most notably the blue light from the scope could reflect on peritoneal fluid. Cauterized tissue also sometimes had vivid fluorescence. However, when combined with clinical knowledge, neither of these artifacts appeared to resemble nerve structures and indeed the reason for their fluorescence was easily confirmed under white light.

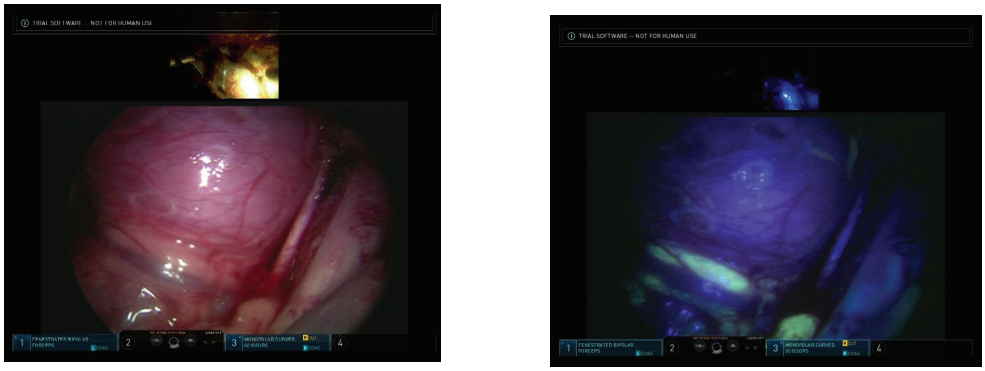

Figure 2. Twenty minutes post injection of Illuminare-1, demonstrating a) white light visualization of the obturator nerve and b) blue light fluorescence of the obturator nerve.

Structures that were not initially seen or that were equivocal as nerves were clearly identified as fluorescent tissue after injection of Illuminare-1 (Figure 3). Seven fluorescent peri-prostatic structures were resected, with histology confirming that all were myelinated nerves (Table 1). The cross-sectional nerve fiber diameters ranged from 64 – 247 microns.

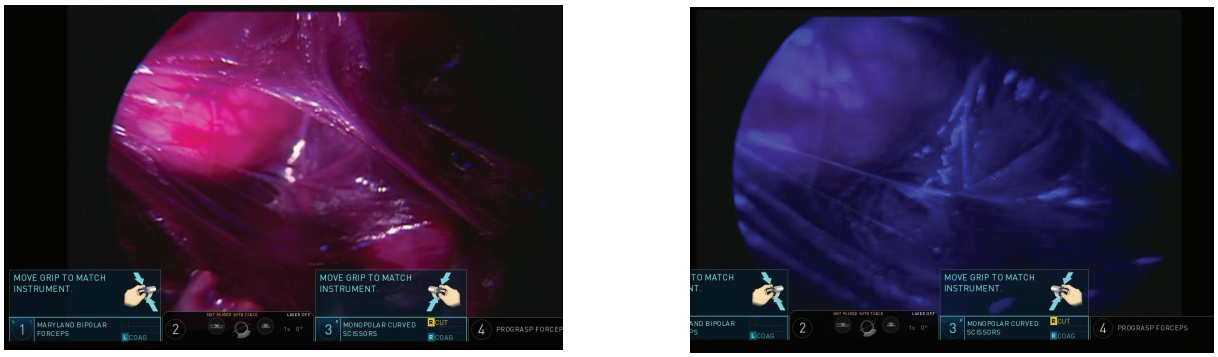

Figure 3. 2.5 hours post injection of Illuminare-1, demonstrating a) multiple possible nerves under white light and b) fluorescent midline vertical curvilinear structure (confirmed as a 180 micron nerve).

Table 1. Histopathology for fluorescent structures presumed to be nerves that were resected intraoperatively

Label	Laterality	*Ex vivo* diameter (microns)	Final pathology[#]	Additional tissue present[##]
A	Left	247	Myelinated nerve	Fibroadipose and vascular tissue
B	Left	87	Myelinated nerve	Fibroadipose and vascular tissue
C	Right	64	Myelinated nerve	Blood vessel and vascular tissue
D	Right	102	Myelinated nerve	Scant fibrous tissue
E	Right	84	Myelinated nerve	Scant skeletal muscle
F	Right	86	Myelinated nerve	Scant fibrous tissue
G	Right	188	Myelinated nerve	Fibrous and skeletal muscle tissue
[#] None of the nerves were unmyelinated				
[##] Resection of small quantities of other surrounding structures is normal in surgery				

4. DISCUSSION

This experiment demonstrated that very small nerves in the neurovascular bundle of the canine prostate fluoresced with Illuminare-1. The uptake and fluorescence of Illuminare-1 was rapid and sustained. Importantly, as the experiment was extended for the duration of a typical human robotically-assisted radical prostatectomy, fluorescence was sustained throughout for the length of the study. Therefore, the duration of fluorescence should be sufficient to ensure clinical utility. By focusing on both the obturator and peri-prostatic nerves, this experiment was the first time that a single dose of Illuminare-1 was demonstrated to fluoresce both small and large caliber nerves.

The prostatectomy was selected for the experiment as it helped to evaluate whether key nerves could be identified in a common procedure, which is clinically applicable as many of the key complications are related to intraoperative nerve damage. In addition, as the there is considerable dissecting required prior to resection of the prostate, the prostatectomy introduces a number of factors that may alter the effectiveness of fluorescence including different anatomical layers, bleeding in the operative field, peritoneal fluid, saline and diathermy causing artifact. Despite the potential for these factors to interfere with the operating field, all tissue resected was confirmed to be myelinated nerve. However, careful dissection was required to identify these structures and the decision to resect a particular structure was made as a combination of the presence of fluorescence and the surgeon's understanding of prostatic anatomy. While the large obturator nerve and smaller resected nerves all unequivocally fluoresced, we do not know whether other smaller myelinated nerves did not fluoresce and therefore were not seen.

Many questions regarding clinical application will be addressed in the forthcoming human trials where *in vivo* and *ex vivo* evaluation of pathological specimens will occur. Given the distinct colors seen under blue light with Illuminare-1, depending on the type of tissue, there is potentially a role for further image optimization. Indeed, the DaVinci System already incorporates this for identifying structures with near infrared light[5].

There are limitations to our findings. While Illuminare-1 has now been demonstrated to be effective in porcine, canine and murine models, human studies are required to definitively determine whether a similar response will be seen in clinical practice. Furthermore, as Illuminare-1 is a myelin-binding fluorophore, demyelinating diseases may reduce the effectiveness of Illuminare-1 to help identify nerves. Finally, 1.0mg/kg was effective in porcine studies and therefore was chosen as dose for this experiment, however, more rigorous evaluation is required to identify and select the optimal human dose.

5. CONCLUSION

Illuminare-1 enhances the visualization of large and small pelvic nerves in a dog. We are eagerly awaiting in-human Phase-1 trials, anticipated for early-2021, and are optimistic that Illuminare-1 will improve the intraoperative visualization of nerves and thereby help to reduce and potentially prevent surgical morbidity.

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