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PART III

VITAL STRUCTURE IMAGING

Chapter 10

Intraoperative near-infrared fluorescence-guided identification of the ureters using low-dose methylene blue: a first-in-human experience

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ABSTRACT

Introduction

Near-infrared (NIR) fluorescence imaging is a promising technique that offers, real-time, visual information during surgery. The current study reports the first clinical results of ureter imaging using NIR fluorescence after a simple peripheral infusion of methylene blue (MB). Furthermore, optimal timing and dose of MB were assessed.

Materials and Methods

A total of 12 patients that underwent lower abdominal surgery were included in this prospective feasibility study. NIR fluorescence imaging was performed using the Mini-FLARE^{TM} imaging system. To determine optimal timing and dose, MB was injected intravenously at doses of 0.25, 0.5 or 1 mg/kg, after exposure of the ureters. Subsequently imaging was performed for up to 60 min following injection.

Results

In all patients both ureters could be clearly visualized within 10 minutes after infusion of MB. Signal lasted at least up to 60 minutes after injection. The mean signal-to-background ratio (SBR) of the ureter was 2.27 ± 1.22 (N = 4), 2.61 ± 1.88 (N = 4) and 3.58 ± 3.36 (N = 4) for the 0.25, 0.5 and 1 mg/kg groups, respectively. A mixed model analysis was used to compare SBRs between dose groups and time points and to assess the relation between dose and time. A significant difference between time points ($P < 0.001$) was found. However no difference between dose groups was observed ($P = 0.811$).

Conclusions

This study demonstrates the first successful use of NIR fluorescence using low-dose MB for the identification of the ureters during lower abdominal surgery.

INTRODUCTION

Iatrogenic ureteral injury is a rare, but serious complication of lower abdominal surgery, with a reported incidence rate varying from 0.7% up to 10%.[1-5] Early identification of ureteral damage permits direct repair and is of paramount importance to reduce morbidity and preservation of renal function.[2] Unfortunately, the diagnosis is often missed during surgery, which can result in severe complications, such as genitourinary fistula formation or severe renal dysfunction. Identification of the ureters can be challenging in the setting of pelvic tumors, inflammation or after radiation to the pelvic area.[6] Moreover, several anatomical variations can be present, which when unrecognized, increase the risk of ureteral damage by the surgeon.

To reduce the risk of injury, several preoperative imaging modalities have been developed such as: intravenous pyelography, retrograde pyelography, or urologic computed tomography.[7] However these techniques are not performed routinely prior to most abdominal surgical procedures since they are costly, expose patients to radiation, and are unable to provide real-time guidance during surgery.

If hampered intraoperative ureteral identification is anticipated, double-J stents can be placed in the ureter, which can be palpated during open surgery. Recently, lighted ureteral stents have become commercially available for laparoscopic surgery.[8] However, preoperative ureteral stent placement is an invasive procedure that harbors an increased risk of complications, such as ureteral perforation, urinary tract infection and acute renal failure.[8]

An ideal technique should be capable to detect the ureter in the acute setting using a noninvasive procedure that is available during both open and laparoscopic surgery. Recently, ureteral identification using an intravenously administered radiopharmaceutical agent has been described.[9] In this study, gamma probe localization was successfully performed after injection of 99mTc-DTPA in ten patients. However, this technique requires involvement of a nuclear physician, requires tissue contact, exposes patient and caregivers to ionizing radiation, and lacks visual information.

Intraoperative near-infrared (NIR) fluorescence imaging is a promising technique that offers, real-time, visual information during surgery.[10-13] This technique is based on the use of exogenous NIR fluorescent contrast agents that can be detected by intraoperative imaging systems to visualize specific tissues and/or anatomical structures. An advantage of this technique is that NIR fluorescent light has a relatively high tissue penetration of several millimeters. As such, it has wide applications, including sentinel lymph node mapping in several tumor types and visualization of bile ducts during laparoscopic surgery.[14-17] The clinically available dye methylene blue (MB) has the advantageous property that it becomes a moderate-strength fluorophore emitting at ≈ 700 nm when diluted to levels that are almost undetectable to the human eye.

MB is cleared renally and has previously been used for macroscopic identification of ureteral leakage and parathyroid glands in high dosages.[7,18,19] We have recently demonstrated feasibility of intraoperative visualization of the ureters by NIR fluorescence imaging using low-dose MB during both open and laparoscopic surgeries in Yorkshire pigs.[20] Moreover, NIR imaging has recently shown feasibility in diagnosing ureteropelvic junction obstruction in a swine model.[21] However, to date, no clinical data are available. The aim of the current study was to assess feasibility of this technique for detection of the ureters in patients undergoing lower abdominal surgery. Furthermore, optimal timing and dosage of MB administration for NIR fluorescence imaging were assessed.

MATERIALS AND METHODS

Intraoperative Near-Infrared Imaging System (Mini-FLARE)

Imaging procedures were performed using the Mini-Fluorescence-Assisted Resection and Exploration (Mini-FLARE) (figure 3) image-guided surgery system, as described previously.[22] Briefly, the system consists of 2 wavelength isolated light sources: a "white" light source, generating 26,600 lx of 400 to 650 nm light, and a "near-infrared" light source, generating 1.08 mW/cm^2 of ≈ 670 nm light. Color video and NIR fluorescence images are simultaneously acquired and displayed in real time using custom optics and software that separate the color video and NIR fluorescence images. A pseudo-colored (lime green) merged image of the color video and NIR fluorescence images is also displayed. The imaging head is attached to a flexible gooseneck arm, which permits positioning of the imaging head at extreme angles virtually anywhere over the surgical field. For intraoperative use, the imaging head and imaging system pole stand are wrapped in a sterile shield and drape (Medical Technique Inc., Tucson, AZ). Subsequently, the camera head is positioned 30 cm above the surgical field during image acquisition. Fluorescence intensity was calculated using the mini-FLARE software, which allows quantitative measurements.

Clinical Trial

This prospective clinical trial was approved by the Medical Ethics Committee of the Leiden University Medical Center and was performed in accordance with the ethical standards of the Helsinki Declaration of 1975. Patients, from the department of Gynecology and Urology, were included between December 2010 and April 2012. All patients scheduled to undergo open surgery in the lower abdomen in which exposing the ureters was part of the procedure were eligible for participation in the trial. Exclusion criteria were pregnancy or lactation, the use of serotonin reuptake inhibitors, serotonin and noradrenalin reuptake inhibitors and/or tricyclic antidepressants, severe renal failure, G6PD-deficiency, or a known allergy to MB. All patients gave informed consent and were anonymized. After standard midline laparotomy and exposure of the surgical field, the ureters were identified and isolated from surrounding tissue. Subsequently, the Mini-FLARE imaging system was positioned above the surgical field and 0.25, 0.5 or 1 mg/kg of MB (concentration 10 mg/ml) was infused intravenously over 5 min. NIR fluorescence imaging of each exposed ureter was performed at 0-5, 15, 25, 35, 45 and 60 minutes after injection.

Statistical Analysis

For statistical analysis, SPSS statistical software package (Version 16.0, Chicago, IL) was used. Patient age and body mass index (BMI) were reported in median and range and signal-to-background was reported in mean and standard deviation. To compare patient characteristics, the one-way ANOVA and chi-square tests were used. To compare signal-to-background ratios (SBR) between dose groups and time points, and to assess the relation between dose and time, a mixed model analysis was used. When a significant difference was detected, a one-way ANOVA was used to post-test for differences between separate dose groups and/or time points. The one-way ANOVA was corrected using the Bonferroni correction. $P < 0.05$ was considered significant.

RESULTS

Study subjects

A total of 12 patients were included in this study. Patient characteristics are listed in Table 1. Median patient age was 48 years (range 29-75 years), median BMI was 23 kg/m^2 (range 21-28) and median eGFR was 91 (range 66-125). No patient suffered from severe renal impairment. Eight patients were planned for surgery for cervical cancer, of which 4 underwent a radical hysterectomy with pelvic lymphadenectomy and 4 underwent a radical trachelectomy with pelvic lymphadenectomy. Three patients underwent a cystectomy with uretero-ileocutanostomy or Indiana pouch. One patient underwent cytoreduction surgery for advanced stage ovarian cancer.

Table 1 – Patient Characteristics and Ureter Identification Results

	Total		0.25 mg/kg MB (N = 4)		0.5 mg/kg MB (N = 4)		1 mg/kg MB (N = 4)		*P*
Characteristic	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	
Age (mean, range)	49 (27-75)		53 (27 – 69)		52 (37-75)		42 (29 - 60)		0.70
Body mass index (mean, range)	24 (20-28)		25 (24 - 28)		24 (21-28)		24 (20 - 25)		0.30
eGFR (ml/min/1.73m2)* (mean, range)	94 (66-125)		90 (66-125)		92 (68-120)		101 (90-107)		0.72
Procedure									
Cystectomy	3	25	1	25	2	50	0	0	0.37
Uterus extirpation/ trachelectomy	8	67	3	75	2	50	3	75	
Debulking	1	8					1	25	
Intraoperative NIR fluorescent ureters	12	100	4	100	4	100	4	100	1

*: eGFR: estimated Glomerular Filtration Rate, calculated using the MDRD GFR equation.

Intraoperative NIR Fluorescence Imaging

Intraoperative NIR fluorescence imaging was performed using the Mini-FLARE imaging system directly after exposure of the ureters and MB infusion. In all patients, both ureters could be clearly identified using NIR fluorescence (Fig. 1). In all cases adequate signal of the ureter was obtained within 10 minutes after injection. Signal lasted up to 60 minutes after injection, even in the lowest dose group.

A second objective of this trial was to determine the optimal dose of injected MB for NIR fluorescence imaging, expressed as SBR. To assess the effect of the dose of injected NIR fluorescent dye on the SBR, patients were allocated to three dose groups ranging from 0.25 to 1 mg/kg MB. SBR ratios were calculated by dividing the fluorescence intensity of a large region of interest of the ureter by the tissue directly surrounding the ureter and SBRs were taken from the same part of the ureter over time. The mean signal-to-background ratio of the ureter was 2.27 ± 1.22 (N = 4), 2.61 ± 1.88 (N = 4) and 3.58 ± 3.36 (N = 4) for the 0.25, 0.5 and 1 mg/kg groups, respectively. The calculated overall coefficient of variation was 0.85. A mixed model analysis showed a significant difference between time points ($P < 0.001$) and no significant difference between dose groups ($P = 0.811$). Furthermore, no significant relation between dose groups and time points was found ($P = 0.614$). The highest SBRs were observed at 45 minutes after injection (Fig. 2). Overall, the average signal-to-background ratio (SBR) on this time point was 4.59 ± 1.68. However, post-tests between SBRs of different dose groups on the 45 minutes time point using a one-way

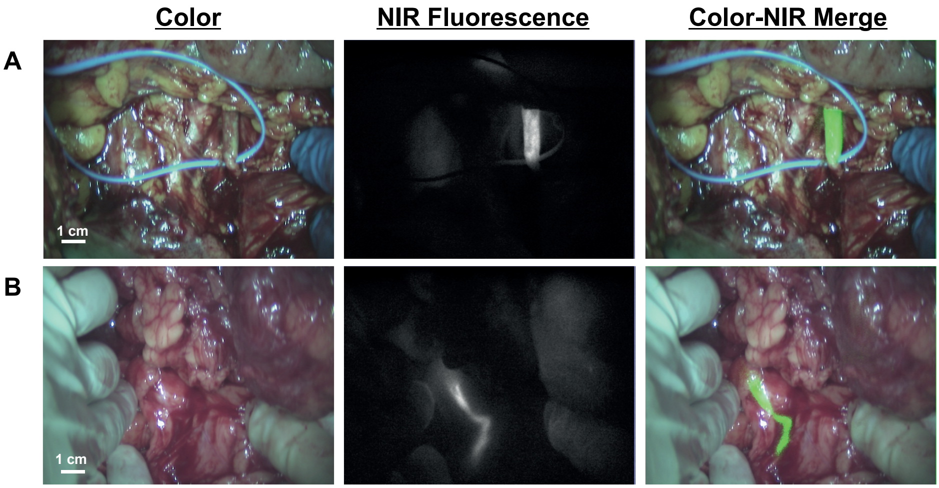

Figure 1 – NIR fluorescence imaging of the ureter during lower abdominal surgery
Color video (left panel), NIR fluorescence (middle panel), and a color-NIR overlay (right panel) of intraoperative imaging of the ureters. The upper row (A) shows a clear identification of the left ureter, which was exposed 45 min after administration of 1 mg/kg Methylene Blue. The lower row (B) shows clear identification of the right ureter with overlying blood and tissue, 15 min after administration of 0.5 mg/kg Methylene Blue.

ANOVA corrected with the Bonferroni correction method, showed no significant differences ($P = 0.35$). No differences in overall fluorescence signal between left and right ureter were found (*p = 0.80*). No patient suffered from renal impairment prior or during surgery. No adverse reactions associated with the use of MB or the Mini-FLARE™ intraoperative NIR fluorescence camera were observed.

DISCUSSION

This study describes a novel, non-invasive, technique for ureteral identification within 10 minutes after infusing of low dose MB. Previously, MB has been used extensively in high dosages to macroscopically identify ureteral injury by blue color. The primary objective of the current study was to test the feasibility to identify the ureters using dilute MB and NIR fluorescence. The potential advantages of using NIR fluorescence imaging are the increased tissue penetration of light at 700 nm and the fact that MB can be administered via a simple peripheral vein on the operating room in significantly lower doses, thereby reducing the risk for adverse events.[23] A clear identification of the ureters using NIR fluorescence was found in all patients within 10 minutes after injection, and as shown in figure 1, the ureters could be detected even if covered superficially by the peritoneum. Patients were allocated to three

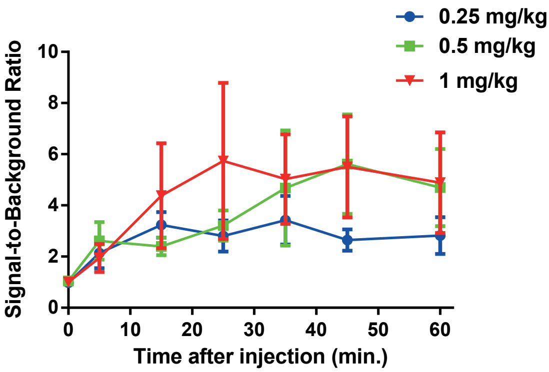

Figure 2 – Differences between dose groups over time
Signal-to-background ratios of the dose groups (mean ± S.D.) are plotted as a function over time after injection of 0.25/0.5/1 mg/kg Methylene blue. No differences between dose groups were found.

different dose groups to determine optimal dosage and timing of MB, however no significant differences in SBR between groups were found. Therefore, based on this study, the lowest dose of 0.25mg/kg MB seems optimal based on logistical and safety preferences.

Intraoperative identification of the ureters remains challenging in some cases, in particular in patients with severe inflammation or a history of radiation of the lower abdomen. Not surprisingly, injury to the ureter is a common complication in these cases. When hampered ureteral identification is anticipated, preoperative placed ureteral stents can assist in identifying the ureter by palpation. However stent placement comes with a certain risk of complication and can be challenging during abdominal surgery. If extra ureteral guidance during surgery is necessary, NIR fluorescence imaging using MB has the potential to be a effective, non-invasive, addition to ureteral stents.

Due to the inability to palpate the retroperitoneum during laparoscopy surgery, NIR fluorescence could also be of value to identify the ureters during laparoscopy. Fluorescence guided laparoscopy has already been introduced in the clinic for various indications and systems are becoming more and more available.[24-26]

A potential pitfall using the present technology is that MB can only be used in patients with adequate renal function. Moreover, as the urine flow in the ureter is not continuous but pulsatile and the fluorescent signal is related to urine flow, the fluorescent signal in both ureters varies over time. However a preclinical study using this technique showed that a diuretic, which increased the flux of urine through the ureters, did not increase NIR fluorescence.[20] In the present study, the ureter was exposed prior to injection of MB for dose and timing optimization. During normal use, the ureter will be located retroperitoneally and will therefore be covered by

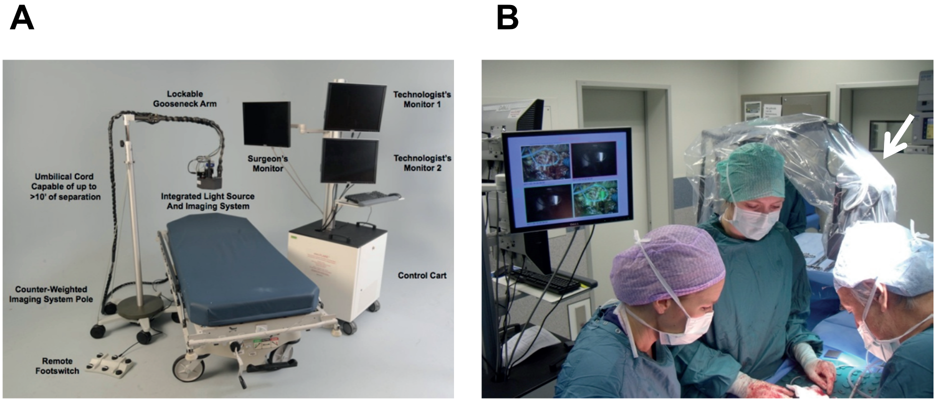

Figure 3 – Mini-FLARE imaging system
The mini-FLARE near-infrared fluorescence imaging system. A setup of the imaging system, including imaging head, flexible gooseneck arm and monitor chart. B Imaging system on the operation room. The camera head (indicated with arrow) is covered in a sterile drape.

peritoneum. Since the penetration depth of NIR fluorescence imaging using 700 nm light and MB has a maximum of approximately 3-5 mm, identification of the ureter may still be challenging under some circumstances. Improved contrast agents with 800 nm fluorescence, a higher extinction coefficient, and a higher quantum yield would help in this regard. It is expected that these contrast agents will become widely available during the next years.

CONCLUSIONS

The current study is the first clinical study to show that low-dose MB and NIR fluorescence can be used for the intraoperative detection of the ureters after a simple peripheral infusion. As NIR imaging is extremely sensitive, this technique has the potential to decrease the risk of injury during complicated lower abdominal surgery and thereby reducing operation time and avoiding the need for reoperation. This technique can provide valuable additional information during surgery in a safe manner, provided that proper exclusion criteria are followed. As in the lowest dose group the SBR was amply sufficient for early identification of the ureter, we would recommend injecting 0.25mg/kg MB intravenous several minutes before ureter visualisation is desirable.

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