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Near infrared fluorescence imaging of rabbit thyroid and parathyroid glands

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ABSTRACT

Background: Near infrared fluorescence imaging using intravenous methylene blue (MB) is a novel technique that has potential to aid the parathyroid gland (PG) localization during thyroid and parathyroid surgery. The aim of this study was to examine MB fluorescence in the rabbit neck and determine the influence of MB dose and time following administration on fluorescence from thyroid and PGs.

Methods: Thyroid and external PGs were exposed in six New Zealand white rabbits under anesthesia. Varying doses of MB (0.025–3 mg/kg) were injected through the marginal ear vein. Near infrared fluorescence from exposed tissues was recorded at different time intervals (10–74 min) using Fluobeam 700. Specimens of identified glands were then resected for histologic assessment.

Results: Histology confirmed accurate identification of all excised thyroid and PGs; these were the only neck structures to demonstrate significant fluorescence. The parathyroid demonstrated lower fluorescence intensities and reduced washout times at all MB doses compared with the thyroid gland. A dose of 0.1 mg/kg MB was adequate to identify fluorescence; this also delineated the blood supply of the external PGs.

Conclusions: The study demonstrates that near infrared fluorescence with intravenous MB helps differentiate between thyroid and PGs in the rabbit. This has potential to improve outcomes in thyroid and parathyroid surgery by increasing the accuracy of parathyroid identification; however, the findings require replication in human surgery. The use of low doses of MB may also avoid the side effects associated with currently used doses in humans (3–7 mg/kg).

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1. Background

Thyroid surgery is the most frequently performed endocrine surgical procedure. Hypocalcaemia is the most common postoperative complication following thyroidectomy, with rates of transient and permanent hypocalcaemia ranging from 2%–50% [1–3] and 0.4%–13.8% [4–6], respectively. Symptoms and signs of hypocalcaemia include circumoral paraesthesia, tetany, laryngospasm, and electrocardiogram changes [7]. Although hypocalcaemia can be treated effectively with calcium and/or vitamin-D supplements, there may be persistent long-term morbidity due to adverse effects on bones and kidneys [7–9]. Primary hyperparathyroidism is the main indication for parathyroid surgery, and postoperative morbidity can include failure to cure due to undertreatment and the likelihood of hypocalcaemia with overtreatment [10].

Methylene blue (MB) is a dye that localizes in both thyroid and parathyroid tissue on intravenous administration. Its use in parathyroid surgery was first described in 1971 to aid the "naked-eye" visualization of enlarged parathyroid glands (PGs) [11,12] and subsequently used at doses ranging from 3 to 7.5 mg/kg depending on local practice [13]. Side effects and serious neurotoxicity have been reported, especially in patients on selective serotonin reuptake inhibitors [13–15]. MB also exhibits fluorescent properties in the near-infrared (NIR) range (light outside the visible spectrum) at significantly reduced concentrations than those used in clinical practice [16] and has the potential to reduce the adverse effects of MB, while improving the accuracy of parathyroid identification.

Intraoperative NIR fluorescence imaging is a new technology that allows real-time visualization of normal and abnormal tissues. In surgical oncology, combined with appropriate fluorescent agents, the technique has been found beneficial in the assessment of cancer resection margins [17,18], image-guided lymph node mapping [19–22], and lymph node mapping in gynecologic cancer surgery [23] and to confirm the patency of biliary anastomoses in pancreaticoduodenal biliary surgery [24]. In thyroid and parathyroid surgery, the technique has the potential to differentiate PGs from thyroid nodules and lymph nodes and subsequently reduce the incidence of inadvertent damage and devascularization of normal glands, improving patient outcomes.

The aim of the current *in vivo* experiment was to establish the pattern of MB-induced fluorescence detected by Fluobeam 700 from the soft tissue structures in the rabbit neck. This animal model was chosen as it has an extra-thyroidal "external" PG on each side of the neck [25]. The specific objectives were to:

1. determine the lowest systemic MB dose that allows detection of fluorescence in the neck tissues
2. identify and study patterns of fluorescence (onset, peak intensity, and duration) in thyroid and PGs.

2. Methodology

2.1. Animals

Two New Zealand white rabbits were obtained from Highgate Farm in Sheffield, UK (Home Office authorized supplier), and held in the animal facility at University of Sheffield field laboratories 3 months before experimentation. Animals were housed in a humidity- and temperature-controlled environment and allowed access to water and food *ad libitum*. Procedures were performed in accordance with UK Home Office Animal Procedures Act (1986), under Project License number 40/3531 (N.J.B.). Following terminal anesthesia, the neck was dissected for the researchers to become familiar with the anatomy of rabbit thyroid and PGs.

Further experiments were performed under general anesthesia on six New Zealand white rabbits at INRA (Institute of National Agronomic Research) Animal Institute, Tours, France. Rabbits were obtained from HYPHARM (Roussay, France) and held in the animal facility at the "Platform for Experimental Infectious Diseases" (INRA, Nouzilly, France) for 6 days before experimentation. Experiments were conducted in accordance with the European directive 2010/63/EU on the protection of animals used for scientific purposes and approved by the regional ethics committee (CEEA VdL) and in accordance to the UK animal welfare act 2006 [26].

2.2. Experimental protocol

Two rabbits were dissected post mortem in Sheffield to determine the anatomy of the neck central compartment with specific reference to the thyroid and PGs. The tissues were then resected and processed for histologic evaluation.

At INRA, anesthesia was induced with intramuscular injection of ketamine (35 mg/kg), xylazine (5 mg/kg), and morphine (2 mg). Inhalational isoflurane 1.5% was used for maintenance of anesthesia. The central neck compartment was exposed via a vertical midline incision over the ventral aspect of the neck. Care was taken to preserve the parathyroid vascular supply during dissection.

Details of each experiment were recorded using a standard proforma. MB was administered as bolus injections into the marginal ear vein at doses ranging from 0.025 mg/kg to 3 mg/kg and flushed with an infusion of normal saline. Volumes of MB injections ranged from 0.29 mL to 2.16 mL, with varying dilutions, appropriate for an approximate circulatory volume of 56 mL/kg body weight [27–29]. NIR fluorescent images of the exposed neck soft tissues (thyroid, external PGs, and skeletal muscle) were recorded using Fluobeam 700 (Fluoptics, Grenoble, France), with black and white images of the operating field before and after each intravenous MB bolus (Figs. 1A and B).

Oxygen saturation and heart rate were monitored throughout surgery to identify whether the circulation was compromised, as this could affect MB delivery to the soft tissues of interest. At the end of each experiment, the rabbit was euthanized using intravenous thiopental (350 mg). Specimens from both external PGs and the thyroid were retrieved for histopathologic confirmation.

ImageJ software, developed by the National Institute [30,31], was used for quantification of fluorescent intensity emitted from the thyroid, PGs, and the strap muscles. Fluorescent intensities were recorded as numerical values on an excel spreadsheet (Microsoft Office 2007; Microsoft Corporation), and displayed in tables and charts to demonstrate the evolution of MB fluorescence. Ratios, median values, and ranges are also reported.