Oral Oncology 49 (2013) 15–19

Contents lists available at SciVerse ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Near-infrared fluorescence sentinel lymph node mapping of the oral cavity in head and neck cancer patients

Joost R. van der Vorst [a], Boudewijn E. Schaafsma [a], Floris P.R. Verbeek [a], Stijn Keereweer [d], Jeroen C. Jansen [b], Lilly-Ann van der Velden [b], Antonius P.M. Langeveld [b], Merlijn Hutteman [a], Clemens W.G.M. Löwik [c], Cornelis J.H. van de Velde [a], John V. Frangioni [e,f], Alexander L. Vahrmeijer [a,*]

[a] Department of Surgery, Leiden University Medical Center, Leiden, The Netherlands
[b] Department of Otolaryngology and Head & Neck Surgery, Leiden University Medical Center, Leiden, The Netherlands
[c] Department of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands
[d] Department of Otorhinolaryngology Head and Neck Surgery, Erasmus Medical Center, Rotterdam, The Netherlands
[e] Department of Radiology, Beth Israel Deaconess Medical Center, Boston, MA 02215, United States
[f] Division of Hematology/Oncology, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA 02215, United States

ARTICLE INFO

Article history:
Received 6 March 2012
Received in revised form 10 May 2012
Accepted 26 July 2012
Available online 28 August 2012

Keywords:
Near-infrared fluorescence
Image-guided surgery
Oral cavity cancer
Oropharynx cancer
Sentinel lymph node
Indocyanine green

SUMMARY

Objectives: Elective neck dissection is frequently performed during surgery in head and neck cancer patients. The sentinel lymph node (SLN) procedure can prevent the morbidity of a neck dissection and improve lymph node staging by fine pathology. Near-infrared (NIR) fluorescence imaging is a promising technique to identify the sentinel lymph node (SLN) intraoperatively. This feasibility study explored the use of indocyanine green adsorbed to human serum albumin (ICG:HSA) for SLN mapping in head and neck cancer patients.

Materials and methods: A total of 10 consecutive patients with oral cavity or oropharyngeal cancer and a clinical N0 neck were included. After exposure of the neck, 1.6 mL of ICG:HSA (500 μM) was injected at four quadrants around the tumor. During the neck dissection, levels I–IV were measured for fluorescence using the Mini-FLARE imaging system.

Results: In all 10 patients, NIR fluorescence imaging enabled visualization of one or more SLNs. A total of 17 SLNs were identified. The mean contrast between the fluorescent signal of the lymph nodes and of the surrounding tissue was 8.7 ± 6.4. In 3 patients, of which 1 was false-negative, lymph node metastases were found. After administration of ICG:HSA, the average number of fluorescent lymph nodes significantly increased over time ($P < 0.001$).

Conclusion: This study demonstrated feasibility to detect draining lymph nodes in head and neck cancer patients using NIR fluorescence imaging. However, the fluorescent tracer quickly migrated beyond the SLN to higher tier nodes.

Introduction

In head and neck cancer patients, cervical lymph node involvement is the single most important prognostic factor.[1,2] To obtain adequate staging and local control of the cervical region, elective neck dissections are frequently performed, even in patients with clinical and radiological N0 stage. In approximately 25% of these patients, lymph node metastases are found.[3] Furthermore, micrometastases and isolated tumor cells are often missed during standard pathological workup.

To decrease the morbidity of neck dissection and to improve lymph node staging by fine pathology, the sentinel lymph node (SLN) procedure has been advocated. The SLN procedure is based on the theory that flow from a tumor travels sequentially to the first tier node (i.e. the sentinel node) and subsequently to the remaining lymph node basin. The SLN procedure is currently standard of care in breast cancer and melanoma in most centers. Although much work has been performed in head and neck cancer, the SLN procedure has not yet been established as standard of care.[4–7] As in breast cancer, the use of radiocolloids and a blue dye can be considered the gold standard to locate the SLN. However, the disadvantages of radiocolloids are the lack of real-time intraoperative visual information and the need for a nuclear physician; and disadvantages of blue dyes include limited depth penetration and blue staining of the surgical field.

* Corresponding author. Address: Albinusdreef 2, 2300 RC Leiden, The Netherlands. Tel.: +31 715262309; fax: +31 715266750.
E-mail address: a.l.vahrmeijer@lumc.nl (A.L. Vahrmeijer).

1368-8375/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.oraloncology.2012.07.017

Recently, the use of near-infrared (NIR) fluorescent light has been introduced to intraoperatively identify lymph nodes, tumors and vital structures.[8] NIR fluorescence using the fluorescent dye indocyanine green (ICG) has been successfully used for sentinel lymph node mapping in breast cancer, melanoma, cervical cancer, and vulvar cancer.[9–12] The concept of NIR fluorescence guided SLN mapping in oropharyngeal cancer has also been reported in humans.[13] However, in the study by Bredell et al.[13], the interval between injection of ICG and imaging varied between 5 and 30 min, which can result in identification of higher tier nodes. Preclinical work suggests that premixing ICG with human serum albumin (complex: ICG:HSA) could improve the fluorescent properties and improves retention in the SLN due to its increased hydrodynamic diameter.[14] Furthermore, the injected dose of ICG (without HSA) used by Bredell et al. was 10 mg per patient. Several clinical dose-finding studies have shown that a significant lower dose (0.5–1.0 mg ICG:HSA) can successfully be used for sentinel lymph node mapping in other indications.[10–12] The aim of the current study was to assess the feasibility of NIR fluorescence and ICG:HSA for SLN mapping in head and beck cancer using 1.6 ml of 500 μM ICG:HSA and the Mini-FLARE imaging system.

Patients and methods

Preparation of indocyanine green adsorbed to human serum albumin

ICG (25 mg vials) was from Pulsion Medical Systems (Munich, Germany) and was resuspended in 10 cc of sterile water for injection to yield a 2.5-mg/ml (3.2-mM) stock solution. Of this solution, 7.8 cc was transferred to a 50-cc vial of Cealb (20% human serum albumin (HSA) solution; Sanquin, Amsterdam, The Netherlands) to yield ICG in HSA (ICG:HSA) at a final concentration of 500 μM.

Intraoperative NIR fluorescence imaging

SLN mapping was performed using the Mini-FLARE image-guided surgery system as described before.[11] Briefly, the system consists of 2 wavelength-isolated light sources: a "white" light source, generating 26,600 lx of 400–650-nm light and a "near-infrared" light source, generating 7.7-mW/cm^2 of 760-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 virtually anywhere over the surgical field, and at extreme angles. For intraoperative use, the imaging head and imaging system pole stand are wrapped in a sterile shield and drape (Medical Technique Inc., Tucson, AZ).

Clinical trial

The single-institution 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. A total of 10 consecutive patients with oral cavity or oropharyngeal cancer, and a clinical and radiological N0 neck were included. All patients underwent ultrasound guided cytology of lymph nodes in the neck region to assess nodal status. All patients provided informed consent and were anonymized. Exclusion criteria were pregnancy, lactation or an allergy to iodine, shellfish, or indocyanine green.

After exposure of the neck following subplatysmal flap elevation, 1.6-mL ICG:HSA (500 μM) was injected at 4 quadrants around the tumor using a 21G, 1½ in. needle. During the neck dissection, levels I–IV were measured for fluorescence using the Mini-FLARE imaging system after injection of ICG:HSA. To assess the occurrence of drainage to higher tier nodes and the optimal time of imaging after injection, measurements were performed at 5, 10, 15, 20, 25, 30, 45 and 60 min after injection. All first draining NIR fluorescent hotspots were considered SLNs and were marked using sutures. The neck dissections consisted of resection of level I, IIa, IIb and III and in some cases level IV. The resection of the primary tumor afterwards was performed following standard procedure. Afterwards, all resected lymph nodes were examined by routine histopathological analysis; lymph nodes were fixed in formalin and embedded in paraffin for routine hematoxylin and eosin staining. SLNs and non-SLNs were examined separately.

Statistical analysis

For statistical analysis and to generate graphs, GraphPad Prism Software (Version 5.01, La Jolla, CA) was used. Age, body mass index (BMI) and tumor size were reported as median and range. Signal-to-background ratio (SBR) was reported as mean and standard deviation. Difference in number of lymph nodes identified between time points was tested using a repeated measures ANOVA.

Results

Patient and tumor characteristics

Patient and tumor characteristics are detailed in Table 1. Ten patients with oral cavity or oropharyngeal tumors and a clinical and radiological stage N0 were included. Median patient age was 59.5 years (range 33–73 years), median BMI was 24 (range 19–35 kg/m^2), and median primary tumor size was 2.2 cm (range 0.3–5.2 cm). Location of primary tumor was the tongue in 7 patients, tonsil region in 2 patients, and retromolar trigone in 1 patient. Four patients underwent a hemiglossectomy, 4 patients underwent a commando resection, and 2 patients underwent a pull-through resection. Nine patients underwent a unilateral neck dissection and 1 patient a bilateral neck dissection. One patient was previously treated for a laryngeal cancer and developed a second primary tumor after a disease-free period of 10 years, for which the patient was treated and included in the current study. This patient was initially treated with surgical removal of the tumor and bilateral radiotherapy of the neck region. After histological examination, 9 patients were diagnosed with a squamous cell carcinoma and 1 patient with a basal cell adenocarcinoma.

Intraoperative NIR fluorescence imaging

In all patients ($N = 10$), NIR fluorescence imaging enabled identification of 1 or more SLNs. An example of the intraoperative identification of a SLN using NIR fluorescence is shown in Fig. 1. A total of 17 SLNs were detected. On average, 1.7 ± 0.8 SLNs per patient were detected and a total of 22.9 ± 9.7 lymph nodes were resected per patient (Table 2). The average contrast between fluorescent signal of the SLN and the surrounding tissue was 8.7 ± 6.4. In 3 patients, the identified SLNs were located in level I, in 5 patients in level IIA and in 2 patients in level III. No adverse reactions or complications occurred during the current study. Histological analysis showed that 3 out of 10 patients had metastatic disease, all in a single lymph node. In 2 cases, the tumor-positive lymph node was the NIR fluorescent SLN, and in 1 patient the tumor-positive lymph node was a non-SLN located in level 2A, which was also not NIR fluorescent. No adverse reactions occurred.