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INTRODUCTION

The 5-year survival rate of head and neck squamous cell carcinoma (HNSCC) patients is below 50% on average, although treatment modalities including surgery, radiation therapy, and chemotherapy are performed to treat the disease. HNSCC often invades adjacent tissues and metastasizes to cervical lymph nodes. Prognosis is poor when cervical lymph node metastasis is present; therefore, accurately determining the presence or absence of cervical lymph node metastasis is important to decide about cervical lymphadenectomy for high-risk patients and avoid unnecessary surgery for patients with less likely nodal metastasis. Avoidance of unnecessary surgery may also reduce morbidity associated with cervical lymphadenectomy such as lymph edema and shoulder dysfunction.

Sentinel lymph nodes are the first to receive lymphatic drainage from the primary tumor. They are the first locations where micrometastasis occurs. As a result, if no metastasis to the sentinel lymph nodes is present, the possibility of lymph node metastasis is not high and cervical lymphadenectomy can be avoided. Sentinel lymph node biopsy, which uses radioisotopes to identify and selectively remove sentinel lymph nodes, is already used for patients with HNSCC. However, radiation exposure is inevitable, and shine-through, characterized by the nature of head and neck cancers close to cervical lymph nodes, is an inevitable disadvantage of sentinel lymph node biopsy using radioisotopes. Indocyanine green (ICG) is a tracer for sentinel lymph node biopsy that is approved by the U.S. Food and Drug
Administration (FDA) and widely used in clinical practice. However, because ICG is a small, nontargeting particle, a disadvantage is that it is commonly found in the second and third lymph nodes outside sentinel lymph nodes. To overcome the limitations of radioisotopes and ICG, ICG:neomannosyl human serum albumin (MSA), which targets the macrophage mannose receptor CD206, was used to trace the lymphatic system in our study.5–7

Currently, robotic and endoscopic surgery are widely used for head and neck cancer surgery. Endoscopic and robotic surgery have advantages of low morbidity, rapid functional recovery after operation, and good cosmetic outcomes.5–7 A variety of endoscopic procedures have been reported for cervical lymphadenectomy.8,9 In response to this trend, studying sentinel lymph node surgery using endoscopic systems is necessary. The aim of this study was to identify the possibility of endoscopic sentinel lymph node biopsy of the head and neck region using ICG:MSA and a custom-made intraoperative color-and-fluorescence-merged imaging system (ICFIS).

MATERIALS AND METHODS

Animals

The animal studies were approved by the Korea University Medical Center Animal Experimentation Committee. Athymic BALB/c nude mice were used at 5 to 8 weeks of age (n = 9). For anesthesia, Zoletil (Virbac, Carros, France) and Rumpun (Bayer, Seoul, Korea) were injected intraperitoneally at 15 mg/kg. The depth of anesthesia was determined via toe pinch. The rabbit model was used for clinical situations of endoscopic sentinel lymph node biopsy. Three female rabbits weighing 2 kg each were used. Rabbits were anesthetized with 15 mg/kg Zoletil (Virbac) and Rumpun (Bayer) injected intravenously before initiation of procedures.

Fig. 1. Body distribution of ICG or ICG:MSA in intramuscular injected nude mice. ICG or ICG:MSA was intramuscularly injected in the lateral tongues of nude mice, and time-dependent distribution was investigated using an in vivo imaging system. ICG quickly spread throughout the body. ICG:MSA remained primarily in the ipsilateral cervical lymph node. White arrows: injection site; white circle: sentinel cervical LN; white dotted circle: contralateral cervical LN.

ICG = indocyanine green; LN = lymph node; MSA = neomannosyl human serum albumin.

Fig. 2. (A) Mouse model of tongue cancer. White arrow: tongue cancer. (B) Peritumoral injection of indocyanine green-neomannosyl human serum albumin in tongue cancer mouse model. (C) Image of a custom-made intraoperative color-and-fluorescence-merged imaging system.
Preparation of Indocyanine Green and Indocyanine Green-Neomannosyl Human Serum Albumin

Human serum albumin (SK Chemical, Seoul, Korea) and α-D-mannopyranosylphenyl isothiocyanate (Sigma-Aldrich, Seoul, Korea) were incubated for 40 hours at room temperature in 0.1M sodium carbonate buffer (pH 9.5). Bound material was purified using normal saline and PD-10 desalting columns (GE Healthcare, Uppsala, Sweden) and stored at -70°C. ICG was dissolved in 10mL sterile water to 2.5mg/mL. ICG and MSA were mixed at a molar ratio of 1:10 to generate ICG:MSA.

Custom-Made Intraoperative Color-and-Fluorescence-Merged Imaging System

The custom ICFIS was described previously. A commercially available, 30-degree rigid endoscope was connected to the ICFIS using an adaptor. A 750-nm diode laser was delivered through a fiber inside the endoscope. Fluorescence intensity was detected by a near infrared (NIR) camera and modified with contrast-control software. Contrast of fluorescence images was controlled manually to optimize image quality. Near infrared images were overlaid on color images, and real-time operative views of operative files were displayed on a monitor.
During procedures, surgeons performed operations using the operative field view displayed on the monitor.

**Body Distribution of Indocyanine Green and Indocyanine Green-Neomannosyl Human Serum Albumin in Mouse Tongue Cancer Model**

For in vivo experiments, 8-week-old female BALB/c nude mice were used. The FaDu cell line (2 × 10⁶ cells) was injected into the lateral part of the mouse tongue for the animal tongue cancer model. The experiment was performed after 4 weeks. ICG was injected into the tongue of three mice and ICG:MSA was injected into the tongue of three mice. Lymphatic flow was observed as NIR images. Fluorescence was observed using the ICFIS at 15, 30, 1, 2, 3, and 6 hours after injection.

**Sentinel Lymph Node Detection Using Indocyanine Green-Neomannosyl Human Serum Albumin and Intraoperative Color-and-Fluorescence-Merged Imaging System in Mouse Tongue Cancer Model**

In the mouse model of tongue cancer (n = 3), sentinel lymph node biopsies were performed with ICG:MSA and ICFIS. One hour after peritumoral injection of ICG:MSA, the position of sentinel lymph nodes was visualized and identified via ICFIS. Cervical lymph nodes were resected and evaluated for histopathological findings.

**RESULTS**

ICG and ICG:MSA were injected into lateral tongues of mice, and fluorescence was continuously detected by ICFIS (Fig. 1). In ICG-injected mice, fluorescence was observed in the ipsilateral and contralateral cervical lymph nodes at 15 minutes. Fluorescence had spread rapidly throughout the entire lymphatic system, including the liver, about 30 minutes later. ICG:MSA was localized to the cervical lymph node on the ipsilateral side for up to 30 minutes, and fluorescence was detected only in ipsilateral cervical lymph nodes for 1 hour. It did not spread to the opposite side cervical lymph nodes or the liver. Fluorescence of contralateral cervical lymph nodes was detected 2 hours after injection of ICG:MSA.

Detection of sentinel lymph nodes was investigated after injecting ICG:MSA into the mouse tongue cancer model. ICG:MSA (3 µL) was peritumorally injected around the tongue cancer; after 1 hour, sentinel lymph nodes were confirmed using ICFIS (Fig. 2). Fluorescence was strongly expressed in the tongue area injected with ICG:MSA. During the experiment, lymph nodes were easily identified and resected using ICFIS without the aid of other magnifying tools (Fig. 3) (Supporting Video 1). Fluorescence of removed lymph nodes was confirmed with no other fluorescence in the surgical site. After finishing experiments, tongues were resected for pathologic examination. Removed lymph nodes were also examined by histologic evaluation (Fig. 4).

In the rabbit tongue cancer model, ICFIS equipped with a 30-degree endoscope was used to determine the feasibility of endoscopic sentinel lymph node biopsy. Sentinel lymph node biopsies were performed by using ICFIS equipped with a 30-degree endoscope (Fig. 5). The procedure was as follows: 1) The cervical skin was incised with a number 15 scalpel blade; 2) the strap muscle was separated at the midline with scissors; 3) a surgeon checked the monitor of the ICFIS system to locate sentinel lymph nodes; and 4) the sternoclavicular muscle and fibrofatty tissue were dissected to remove sentinel lymph nodes, guided by our imaging system (Fig. 6). We detected and dissected sentinel lymph nodes without using other detection tools in the rabbit model.
DISCUSSION

ICG, a NIR fluorescence tracer, is the only drug approved by the FDA that is widely used to find sentinel lymph nodes during cancer surgery. Due to the small hydrodynamic diameter of ICG, it easily flows to upper lymph nodes. It is problematic because the fluorescence images are easily contaminated by room light. An optimal tracer would be localized in sentinel lymph nodes within a short time after implantation and would stay in the first-echelon lymph node without moving to the upper lymph node. Wehrhan et al. reported macrophage depolarization and expression of surface markers such as CD206 in regional lymph nodes of oral squamous cell carcinoma. Combining MSA targeting CD206 of macrophage with ICG can overcome the disadvantages of existing ICG. In this study, specific targeting ICG:MSA was localized to the first echelon of lymph nodes in the neck for more than 30 minutes compared to the conventional ICG. A custom-made optical imaging system for nude mouse and rabbit animal models was effective in identifying sentinel lymph nodes.

Robotic and endoscopic procedures have been introduced for head and neck surgery recently. For surgical treatment of tumors in the head and neck area, incisions should be made in the skin of the face or neck. This has the disadvantage of visible scars on the face or neck after surgery. To avoid this, surgical approaches using robots or endoscopes have been developed and are reported to have superior cosmetic outcomes and comparable oncologic outcomes to conventional surgical methods. HNSCC is usually spread by cervical lymph node metastasis and is known to have a worse prognosis in patients with cervical lymph node metastasis. Therefore, cervical lymphadenectomy may significantly affect outcomes and prognosis. Because of morbidity related to the operation, cervical lymphadenectomy should be performed selectively in high-risk patients considering the benefits and complication of the procedure. Sentinel lymph node biopsy using conventional radioisotopes is widely used to avoid unnecessary surgery in low-risk HNSS patients. This procedure has satisfactory sensitivity and specificity for detection of micrometastasis to cervical lymph nodes but has the disadvantages of leaving visible scars on the neck and radiation exposure. These disadvantages are why endoscopic sentinel lymph node biopsy with advanced endoscopic equipment and new tracers are needed for head and neck surgery. This preclinical animal study confirmed the feasibility of endoscopic sentinel lymph node biopsy using our ICFIS system equipped with an endoscope and ICG:MSA. The efficacy and safety of our surgical technique will be investigated in large-animal studies before future use with patients with head and neck cancer.

CONCLUSION

In this study, we confirmed the usefulness of sentinel lymph node biopsy using a NIR fluorescence technique and endoscopic system. Avoidance of radiation exposure and shine-through phenomenon, which are problems of sentinel lymph node biopsy using conventional radioisotopes, are advantages of our surgical technique. Also, by using an endoscopic system for sentinel lymph node biopsy, we avoided visible scars in the neck, unlike conventional sentinel lymph node biopsy without visual assistance. If this technique of endoscopic sentinel lymph node biopsy is applied to HNSCC patients who undergo transoral robotic surgery or transoral videolaryngoscopic surgery, visible scars might be completely

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Fig. 6. Endoscope-assisted sentinel lymph node biopsy of rabbit tongue cancer model. (A) Cervical skin incision. (B) Separation of strap muscle at midline. (C) Dissection of sentinel lymph nodes. (D) Fluorescence image of sentinel lymph node during procedure under near infrared. (E) Operative view of sentinel lymph biopsy under white light. (F) Merged image of operative view under near infrared and white light. Sentinel lymph node biopsy performed using merged image of operative field without other detection method.
avoided, leading to excellent cosmetic outcomes with comparable oncologic outcomes.15

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Y.M.P. and Y.H.Q. contributed equally to the article as first author. H.K.K. and J.-J.S. contributed equally to the article as corresponding author.

BIBLIOGRAPHY


