Abstract

**Purpose.** To determine whether a combination of contrast-enhanced ultrasound-guided methods and dye-guided methods can identify sentinel lymph nodes in animals.

**Methods.** Seven pigs were put under general anesthesia and injected subcutaneously in the neck: three with 2ml saline and four with 2ml fluid comprising 0.4ml 5% patent blue violet solution and 1.6ml of hydroxyethylated starch (Salinhes) solution (PB + HS). The regional lymph nodes were observed by ultrasound; blue-stained regional lymph nodes found after the skin was cut were situated as ultrasound had shown they would be.

**Results.** The regional lymph nodes of the pigs given saline were unchanged, but in the pigs receiving PB + HS, the echo level in the lymph nodes nearest the injection site was altered, producing a clear contrast with the surrounding tissues. The area of the relevant regional lymph node in each PB + HS-injected pig increased significantly (t-test, \( P < 0.01 \); from 25.7, 39.6, 9.36, 70.2mm², and mean, 36.2mm²; to 50.7, 65.5, 21.1, 98.3mm², and mean, 58.9mm², respectively). These enlarged regional lymph nodes were easily found by contrast-enhanced ultrasonography. When excised under ultrasound guidance, all were stained blue, indicating that they were sentinel lymph nodes.

**Conclusion.** These results suggest that this combination of contrast-enhanced ultrasound-guided and dye-guided methods warrants use as a quick, simple procedure for detecting sentinel lymph nodes.

**Keywords** contrast-enhanced ultrasound-guided method · dye-guided method · ultrasonography · sentinel lymph node · animal model · hydroxyethylated starch (Salinhes)

Introduction

Considerable attention has been given recently to detecting sentinel lymph nodes (SLNs) and diagnosing metastasis to regional lymph nodes (LNs) in preoperative examinations in cases of breast cancer. SLNs are the LNs first reached by cancer cells carried by the lymph flow from a primary tumor. Histopathologic detection of cancer cells in an SLN suggests that metastasis has occurred to the regional LNs as a group. The absence of such cells indicates an absence of metastasis.

The most common SLN identification techniques are currently the dye-guided method\(^1\)\(^–\)\(^3\) and the gamma probe-guided method.\(^4\)\(^–\)\(^6\) The dyes used in the dye-guided method are inexpensive and easy to use, but carrying out the requisite dissection without causing extraneous damage to lymph vessels and nodes requires a high level of skill.

For the gamma probe-guided method, on the other hand, the minimum requirements are, first, the expensive gamma probe itself for identifying SLNs in which the radioisotope (RI) accumulates; and second, a facility capable of handling radioactive isotopes. Although the method itself offers objectivity and accuracy, the difficulties raised by these requirements hinder its rapid spread.

To overcome these drawbacks, we investigated a simpler, easier, and yet reliable and accurate method for identifying SLNs. We evaluated the SLN detection capability offered by contrast-enhanced ultrasound (CE-US) images obtained transdermally using a mixture of sulphan blue (patent blue violet) and hydroxyethylated starch (PB + HS) injected subcutaneously in an animal experiment. This method revealed enlarged, contrast-enhanced LNs. Then, through an incision, and using ultrasound images for guidance, the LNs were located and seen to be stained blue, demonstrating that they were the SLNs.

We report on the capability of this new procedure combining contrast-enhanced ultrasound to detect SLNs using ultrasonography transdermally, and blue staining to identify SLNs directly.
Materials and methods

We examined the cervical LNs of seven pigs aged 3 to 7 weeks, weighing 8 to 21 kg, which were placed under general anesthesia. The study was conducted in accordance with the Jichi Medical University Guide for Laboratory Animals.

First, we used ultrasound to determine the locations, sizes, and numbers of cervical LNs. We injected 2 ml of physiological saline (PS) solution subcutaneously into the necks of three pigs (group 1), and 2.0 ml of a mixture consisting of 0.4 ml 5% sulphan blue (patent blue violet; Wako Pure Chemical Industries, Osaka, Japan) and 1.6 ml hydroxyethylated starch (Salinhes; Kyorin Pharmaceutical, Tokyo, Japan) (PB + HS) into the necks of four pigs (group 2). Thus, effectively, the patent blue violet was a 1% solution.

The subcutaneous injections were administered with a 23G needle 5–7 cm from the neck LNs closest to the snout and on the snout side of the nodes (Fig. 1). Group 1 pigs were injected with PS (pig 1: 5 weeks old, 14 kg; pig 2: 4 weeks old, 13 kg; pig 3: 5 weeks old, 16 kg); group 2 pigs received PB + HS solution injections (pig 4: 6 weeks old, 15 kg; pig 5: 7 weeks old, 18 kg; pig 6: 6 weeks old, 21 kg; pig 7: 5 weeks old, 15 kg).

After the injections, the area was massaged for 10 s, and the regional LNs were observed over time with B-mode ultrasound, using an Acuson Sequoia 512 Ultrasound System (Acuson, Mountain View, CA, USA) with an 8.0-MHz linear array transducer. As the transducer was moved smoothly across the skin of the pigs, changes in the LN, that is, changes in contrast enhancement and size (length, width, and area), were observed. The gain setting of the ultrasound apparatus was maintained uniform from before the injection; image magnification was adjusted, based on the pre-injection screen image, to facilitate LN examination.

To make sure that no LN that had undergone change was missed, the skin was incised and all LNs that were stained blue were sought by examining the ultrasound images. We verified that the LNs that exhibited changes in the ultrasound images were identical to the blue-stained LNs. The animals were then promptly killed and the LNs that had changed were carefully excised and fixed in formalin. Histologic specimens were prepared, and were microscopically examined for any uptake of blue dye.

Results

In the necks of pigs 1–3 (group 1 pigs), which had received PS injections, one or two LNs were 5–22 mm in diameter before injection. After the subcutaneous injection of PS, the regional LNs were examined intermittently for 10 min. The ultrasound images showed changes in the LN echo level attributable to the injection. In determining the size of an LN, the area in mm² occupied by the LN was assumed to be elliptical, and was calculated approximately as long diameter (mm) × short diameter (mm) × 0.78.

Respective long diameters, short diameters, and areas of the LNs before injection were: pig 1: 12 and 5 mm, and 47 mm²; pig 2: 15 and 5 mm, and 59 mm²; and pig 3: 20 and 7 mm, and 109 mm². Respective dimensions after injection were: pig 1: 12 and 5 mm, and 47 mm²; pig 2: 16 and 5 mm, and 42 mm²; and pig 3: 20 and 7 mm, and 109 mm². Thus, almost no change occurred, and no significant change in dimensions was found after injection (Fig. 2). As an example, pre-injection and post-injection ultrasound images of the LN closest to the snout in the lateral part of the neck of pig 1 are shown in Fig. 3a and Fig. 3b, respectively.