Intraoperative Photodynamic Detection of Normal Parathyroid Glands Using 5-Aminolevulinic Acid

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Objective It is important to identify and save the normal parathyroid glands during head and neck surgery because of their role in regulating the blood calcium level, yet it is often difficult to localize normal parathyroid glands during surgery. Fluorescence-guided parathyroidectomy in patients with hyperparathyroidism has already proved useful. However, there are few reports of fluorescence-guided localization of normal parathyroid glands in humans. We investigated the utility of fluorescence-guided localization of normal parathyroid glands during thyroidectomy and completed a spectral fluorescence analysis of the accumulation of 5-aminolevulinic acid metabolites in the parathyroid glands.

Methods Eight patients with benign thyroid disease and five with malignant thyroid tumors were given 20 mg/kg body weight of 5-aminolevulinic acid orally 5 hours before surgery. After the posterior surface of the thyroid gland was exposed and the recurrent laryngeal nerve was identified, we illuminated the area with a violet-blue light of 405 nm. Tissues showing red fluorescence were biopsied to analyze the spectral fluorescence.

Results Under the violet-blue light, normal parathyroid glands showed red fluorescence, while the surrounding structures such as the thyroid gland, muscles, and fat remained nonfluorescent. The spectral peak was observed at 635 nm indicating 5-aminolevulinic acid metabolites. Histopathologically, the biopsied tissue corresponded to normal parathyroid glands.

Conclusions 5-Aminolevulinic acid is useful to localize the normal parathyroid glands during thyroid surgery in humans.

Key Words: 5-Aminolevulinic acid, parathyroid glands, photodynamic diagnosis, spectral fluorescence analysis, violet-blue light.

Level of Evidence: 3b.

INTRODUCTION

Identification of normal parathyroid glands during neck surgery can be difficult even for the experienced head and neck surgeon, because normal parathyroid glands are small, can be difficult to distinguish from normal fat tissue around the thyroid gland, and are variable in number (there may be more or less than four parathyroid glands in any individual), and their location varies. Injury or removal of parathyroid tissue can result in hypoparathyroidism, which may require life-long calcium supplementation. Identifying and preserving normal parathyroid glands is an important surgical goal.

Methylene blue has been used for over 40 years to localize pathologic parathyroid tissue because it selectively stains parathyroid tissue. But there are many reports showing that although pathological parathyroid tissue is easily stained with methylene blue, normal parathyroid tissue is not stained. Methylene blue also causes side effects such as nausea, vascular pain, thrombophlebitis, and staining of the skin and urine. Several recent studies reported neurologic toxicity and postoperative altered mental status such as toxic metabolic encephalopathy in patients who received methylene blue infusion. Due to the aforementioned problems surrounding the use of methylene blue, a more reliable and safer method is needed to identify and preserve normal parathyroid glands during surgery.

Fluorescence-guided detection of normal parathyroid glands using 5-aminolevulinic acid (5-ALA) has been reported recently in rats. However, there are few reports of fluorescence-guided localization of normal parathyroid glands in humans. In this study, we investigated the utility of fluorescence-guided human normal parathyroid glands localization using 5-ALA and analyzed its spectrum for the accumulation of 5-ALA metabolites in the parathyroid glands.

MATERIALS AND METHODS

Thirteen patients with thyroid disease (five cases had follicular adenoma, three cases had adenomatous goiter, and five cases had papillary carcinoma) were admitted for this...
investigation from September 2009 to June 2010. All patients gave their informed consent for participation in this study in writing. The protocol of this study was approved by the Ethics Committee of the National Hospital Organization Chiba Medical Center.

The patients were admitted into the hospital the day before the surgery. On the day of the operation and 5 hours before exposure of the parathyroid glands, they were orally administered 20 mg/kg body weight of 5-ALA hydrochloride (Cosmo Bio Co., Ltd., Tokyo, Japan) in white powder form dissolved in 10% glucose. The patients remained hospitalized until the day after the surgery to avoid direct exposure to sunlight. We checked them for the occurrence of side effects such as photosensitivity, symptoms of postoperative hypocalcemia, nausea, and vomiting, and increased serum transaminases before photosensitization and after the surgery.

During the operation, after a 5-cm collar incision, a midline vertical incision was made through the strap muscles that were retracted laterally to expose the thyroid gland. The middle thyroid vein was ligated and the thyroid lobe was rotated anteriorly and medially to expose the posterior surface of the thyroid. The room was then darkened and we illuminated the area with a violet-blue light of 405 nm to identify the parathyroid glands (photodynamic diagnosis: PDD). If no red

**RESULTS**

Under normal light conditions, the parathyroid glands could not be identified easily, but under the violet-blue light of 405 nm, clear red fluorescence illumination was detected in all 13 cases (Figs. 1–2). The surrounding structures such as the thyroid gland, muscles, and fat remained nonfluorescent and the red fluorescence area could be easily distinguished (Figs. 1B–3B). In 10 cases, two parathyroid glands were identified, in the other three cases, only one gland was detected. In these three cases, dissection for an additional parathyroid gland was limited because of the extent and location of malignant tumor. Fluorescence...
was observable on average at 5.4 ± 1.2 hours (range = 3.5–8 hours) after photosensitization with 5-ALA (Fig. 4). No false-positive parathyroid fluorescent areas were observed.

In the spectral emission analysis during the surgery, the apparent spectral peak of all the tissues showing red fluorescence was observed at 635 nm, which is the specific wavelength of 5-ALA metabolites (Fig. 5A). On the other hand, the peak of the specific wavelength was not detected on the surface of surrounding structures such as the thyroid gland, muscles, and fat (Fig. 5B). Furthermore, the surfaces of all dissected lymph nodes were nonfluorescent after the surgery. All the tissues that showed red fluorescence under the violet-blue light were histopathologically confirmed to correspond to normal parathyroid glands.

There were only some minor side effects. Four cases had nausea and two cases had vomiting that occurred within 1 hour of the surgery. There was no phototoxic reaction, no apparent tetany symptoms and no transient deterioration of liver function.

DISCUSSION

In the present study, we used fluorescence diagnosis, also known as PDD, employing 5-ALA to safely identify normal human parathyroid glands under violet-blue light. 5-ALA is a natural amino acid existing in our body and is incorporated into the structure of porphyrin. 5-ALA is produced in mitochondria by condensation of glycine and succinyl-CoA, which is an intermediate of the citric acid cycle. 5-ALA is metabolized into protoporphyrin IX (PpIX) in mitochondria.

Generally, PDD is a method to distinguish between normal and abnormal tissues by photosensitizer-induced fluorescence. Administered or metabolized agents, so-called photosensitizers, accumulate in abnormal tissues, and when irradiated by light of a specific wavelength, the photosensitizer emits fluorescence. In the case of 5-ALA, the fluorescence was observable on average at 5.4 ± 1.2 hours (range = 3.5–8 hours) after photosensitization with 5-ALA.
ALA, PpIX is induced by a defined wavelength (405 nm) and it emits a typical red fluorescence at about 635 nm (Fig. 5A). PDD with 5-ALA is used in many fields such as brain surgery,10 urologic surgery,11 and dermatologic procedures.12 It is not clear why normal parathyroid glands become fluorescent after the administration of 5-ALA. Because parathyroid glands have a large number of mitochondria compared with other tissues, it can be hypothesized that 5-ALA-induced PpIX accumulates in the parathyroid glands in large amounts and emits the typical red fluorescence under violet-blue light.13

Fluorescence diagnosis using 5-ALA has been described to identify, in rats, normal parathyroid glands as well as pathologic parathyroid.7–9 Furthermore, Prosst et al.14 demonstrated that the fluorescence intensity of the hyperplastic parathyroid glands was stronger than that of normal parathyroid glands in rats. In humans, some cases of pathologic parathyroid glands in primary and secondary hyperparathyroidism have been identified utilizing this technique.15–17 Prosst et al.18 have recently reported that the fluorescence behavior supported the identification of 92% of pathologic parathyroid glands in primary and secondary hyperparathyroidism patients. In cases of primary hyperparathyroidism, some of the atrophic parathyroid glands affected by disease have been shown to fluoresce.15–17 In the present report, we confirmed that it is possible to localize normal parathyroid glands using ALA-induced fluorescence in patients with thyroid disease.

Because of its distinct red color, ALA-induced fluorescence helped normal parathyroid glands to stand out in contrast to background soft tissue and the thyroid gland in all 13 cases. A previous study in rats using spectral analysis suggested that ALA-induced PpIX accumulated in both the thyroid and parathyroid glands.8 However, in our spectral study in humans, we did not detect a spectral peak on the surface of the thyroid gland, a spectral peak was only found on the surface of parathyroid tissue (Fig. 5). One potential explanation for the difference between human and rats might be because the rats were given more 5-ALA (50 mg/kg) than the humans (20 mg/kg).

Side effects such as skin sensitivity (phototoxicity), transient elevation of liver enzymes, nausea, and vomiting have been reported after PDD with 5-ALA.19 In the present study, there was no case of skin sensitivity and transient elevation of liver enzymes, but there were four cases of nausea and two cases of vomiting. As 5-ALA-induced PpIX is cleared within 24 hours from the body,20 there would be no risk for skin sensitivity if the patients remained hospitalized for at least 24 hours from the administration of 5-ALA.

In a pilot clinical study in which serial biopsies of oral normal and squamous cell carcinoma areas were taken over a subsequent period of 24 hours following oral administration of 5-ALA, fluorescence microscopy of the cancer specimens revealed maximum fluorescence emission between 4 and 6 hours.20 For fluorescence-guided parathyroidectomy, photosensitization was achieved with administration of 20–30 mg/kg 5-ALA 4 hours before the surgery to patients with primary and secondary hyperparathyroidism.13,15,17,18 In the present study, the patients were given 20 mg/kg 5-ALA 5 hours before exposure of the parathyroid glands. Although the parathyroid glands in all cases could be localized from 3.5 to 8 hours after the 5-ALA was given (Fig. 4), far more studies are needed to determine the timing of peak PpIX in normal parathyroid tissue.

In head and neck surgery with thyroid disease and lymph node dissection of the hypopharynx, larynx, and esophagus cancer, it is important to detect and save the parathyroid gland for the patient’s quality of life. This method would have made it possible to detect a normal parathyroid gland in a safer and more conclusive manner in this setting. In malignant head and neck surgery, there could be metastatic lymph nodes. In the present study, the metastatic lymph nodes in three cases had no fluorescent peak in the spectral analysis at the surface. Far more studies are required to determine whether the surface of metastatic lymph nodes have fluorescence or not in malignant surgery.

CONCLUSIONS
The present study confirmed that PDD for identifying normal parathyroid glands using 5-ALA during thyroidectomy is safe and useful in humans. 5-ALA taken 5 hours before the exposure of the parathyroid glands induced red fluorescence from the parathyroid glands under violet-blue light, making it easy to distinguish them from the surrounding tissue. PDD using 5-ALA may ultimately prove to preserve normal parathyroid glands during thyroid and other head and neck surgical procedures.

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BIBLIOGRAPHY


