—Original—

Identification of Pathological and Normal Parathyroid Tissue by Fluorescent Labeling with 5-aminolevulinic Acid during Endocrine Neck Surgery

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Abstract

Background: When performing parathyroid or thyroid surgery, surgeons must distinguish parathyroid tissue from the surrounding thyroid tissue, to preserve healthy parathyroid tissue while excising diseased thyroid tissue or to completely remove pathological parathyroid lesions. Here, we explored the feasibility of labeling the parathyroid glands for easy identification by administering 5-aminolevulinic acid (5-ALA) orally to patients undergoing endocrine neck surgery, because 5-ALA accumulates in the parathyroid and has a fluorescent metabolite, protoporphyrin IX.

Methods: Twenty-nine patients about to undergo endocrine (parathyroid or thyroid gland) neck surgery were orally given 5-ALA, a nontoxic substance that occurs naturally in the human body and has no known major side effects. During surgery, we used blue light to excite protoporphyrin IX, the fluorescent metabolite of 5-ALA, and viewed the resulting bright red fluorescence through an optical filter.

Results: In the majority of the patients, the parathyroid glands were defined by a clear fluorescence. In 23 patients with pathological parathyroid tissue, the fluorescence enabled us to identify and completely remove diseased parathyroid tissue. In 3 patients with thyroid disease, we were able to easily remove diseased thyroid tissue, and an accidentally removed parathyroid gland was autotransplanted during surgery.

Conclusions: In all but a few cases, 5-ALA clearly labeled parathyroid tissue, allowing for its clean removal or preservation according to the purpose of the surgery. This simple, benign technique is extremely useful for identifying parathyroid tissue, whether pathological or normal, during endocrine neck surgery.

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Key words: 5-aminolevulinic acid, parathyroid, fluorescent labeling, video-assisted neck surgery, video-assisted thoracic surgery

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Introduction

The chemical compound 5-aminolevulinic acid (5-ALA) is converted into the fluorescent substance protoporphyrin IX (PpIX) through the heme biosynthesis pathway. PpIX has been used as a photodynamic diagnostic tool in neurosurgery[1–6] (**Fig. 1**), where a positive fluorescent response in the pathological area enables surgeons to easily visualize and completely resect lesions. PpIX has also been used to visualize malignancies, such as lung, bladder, gastric, and colon cancers[7–10].

In 2011, Gahlen et al[11] found that 5-ALA, a precursor of PpIX, accumulates in the parathyroid gland. Surgeons performing endocrine neck surgery must be able to distinguish parathyroid and thyroid tissues macroscopically to cleanly remove pathological parathyroid lesions, or if removing thyroid tissue, to preserve normal parathyroid glands. Thus, we have used 5-ALA to label parathyroid tissue during endocrine neck surgery, because it occurs naturally in the human body, can be administered orally, and has no major side effects. Tissue containing PpIX fluoresces bright red when illuminated with blue light[8,12] and can easily be distinguished from surrounding tissue.

The aim of this study was to determine the feasibility of using 5-ALA to fluorescently label parathyroid tissue during surgery. We present both successful and unsuccessful cases—the latter including a peculiar reaction in pathological parathyroid tissue—and discuss the usefulness of this novel method and the possible reasons for slight discrepancies in the results.

Patients and Methods

Patients: This study involved 29 patients: 20 with primary hyperparathyroidism due to parathyroid tumors (PHP group), 6 with secondary hyperparathyroidism due to chronic renal failure (SHP group), and 3 with thyroid tumors and normal parathyroid glands (NP group). The patient characteristics, along with their preoperative and postoperative information, are shown for each group in **Tables 1 to 3**.

Administration of 5-ALA: PpIX begins to appear in tissues 1 hour after oral administration of 5-ALA, peaks at 4 to 6 hours, and then declines[5]. Therefore, 5-ALA should be administered at least 1 hour before the area of interest is to be illuminated and examined.

Operative procedure: Patients under general anesthesia were placed in the supine position with the neck slightly hyperextended. For conventional surgeries, a collar incision was made on the anterior aspect of the neck, and a skin flap was created by dissecting the layer under the platysma. A central or lateral approach to the thyroid and parathyroid glands was selected depending on the location of the lesion(s). Endoscopic video-assisted thoracic surgery was used for patients 17 and 19 because of ectopic parathyroid tumors deep in the mediastinum, and endoscopic video-assisted neck surgery was performed for patient 20. Endoscopic parathyroidectomy was performed in almost the same manner as endoscopic video-assisted thyroidectomy, in which a thyroid lobe is moved to expose the posterior area of the thyroid. This procedure and its cosmetic benefits have been reported elsewhere[13–17]. For the video-assisted surgery for mediastinal parathyroid tumors, 3 ports were placed on the chest wall to visualize the lesion[18].

To excite accumulated PpIX, the operating room lights were dimmed, and the area of interest was illuminated with a blue light (D-Light, Karl Storz GmbH & Co. KG, Tuttlingen, Germany). Fluorescence was observed through an optical long-wave pass filter, with a cut-off wavelength of 460 nm to eliminate any reflected excitation light. In this way, the target tissue could be identified macroscopically via its bright red fluorescence. The fluorescence was quantified by comparing the spectrum from various tissues within a patient.

The brightness of the fluorescence was evaluated by 3 surgeons, who described the levels of reactivity as 0 (negative), 1+ (weakly positive), or 2+ (strongly positive). Positivity was displayed in various patterns, including diffuse, partial, or spotty.

The protocol was approved by the Ethics

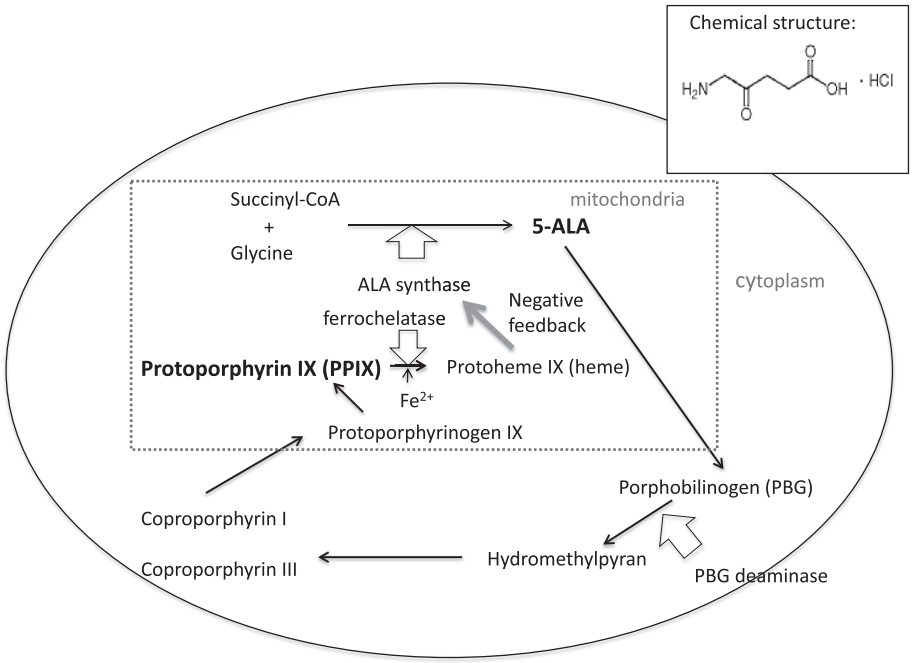

Fig. 1 The 5-ALA metabolic pathway and chemical structural formula. In the first step, 5-ALA is produced via the condensation of glycine and succinyl-CoA by ALA synthetase in the mitochondria. 5-ALA is transported via the cytoplasm. In the second step, porphobilinogen is created from ALA via ALA dehydratase. Porphobilinogen is converted into coproporphyrinogen I and Ⅲ. Coproporphyrin I and Ⅲ are converted via mitochondria. Protoporphyrin Ⅸ is synthesized from protoporphyrinogen Ⅸ via protoporphyrinoge oxidase activitivity, and heme is synthesized via the uptake of iron into the tetrapyrrole structure by ferrochelatase in the mitochondrial membrane.

Committee of Nippon Medical School, and informed consent was obtained from the patients and their families before surgery.

Results

The patients were given 5-ALA (20 mg/kg) orally at various times, ranging from 45 minutes to 5 hours 20 minutes, before the start of the operation.

In the PHP group, pathological examination revealed 15 adenomas, 3 cases of hyperplasia, 1 case of hyperplasia or adenoma, and 1 carcinoma (**Table 1**). In the SHP group, pathological examination revealed hyperplasia in all cases (**Table 2**). In the NP group, thyroid diseases included papillary carcinoma in 2 cases and adenomatous goiter in 1 case (**Table 3**). Patients were given 5-ALA from 45 minutes to 5 hours 20 minutes before the start of surgery. For all but 1 patient, the interval between the initial skin incision and blue-light illumination of the target area was less than 1 hour and well within the 6-hour window after which fluorescent reactivity begins to decline (**Table 3**).

In patients with parathyroid disease (the PHP and SHP groups) (**Tables 1 and 2**), the 5-ALA reaction in the parathyroid had a brightness of 1+ to 2+. In the PHP group, fluorescence was stronger at the cut surface than at the tumor surface (**Table 1**).

Although the levels of intact parathyroid hormone (iPTH) returned to normal after surgery for most patients in the PHP group, the iPTH level in patient 20 remained elevated after surgery owing to a rare double adenoma. The second adenoma, diagnosed postoperatively, was removed 6 months later, and the iPTH level returned to normal.

Although iPTH levels did not return to normal in the SHP group, the serum calcium levels were improved after surgery in patients 22 and 26. For patient 22, 1 of the 4 resected parathyroid glands was small (0.1 g) and negative for 5-ALA, indicating

Table 1 Cases of primary hyperparathyroidism due to parathyroid tumor (PHP group)

Patient		Serum levels before/after surgery		Resected specimen		Pathologic change	Interval from 5-ALA administration to surgery	Reactivity (Surgeon 1/2/3)	
No.	Age (years)/Sex	Calcium (mg/dL)	iPTH (pg/mL)	Size (mm)	Weight (g)			External surface	Cut surface
1	72/F	11.6/9.8	100/11	17×12	0.68	adenoma	3 h 15 min	1+/1+/1+	2+/2+/2+
2	59/M	15.4/7.9	1,200/57	35×30	14.11 (including thyroid)	adenoma	Unknown	1+/1+/1+	2+/2+/2+
3	69/M	11.7/9.3	120/32	19×11	0.55	hyperplasia or adenoma	2 h 0 min	1+/0/1+	2+/1+/2+
4	78/M	15.8/7.9	1,100/7	48×38	2.65	adenoma	2 h 30 min	1+/2+/2+	2+/2+/2+
5	77/F	11.1/9.1	127.4/10.2	5×7	0.56	adenoma	3 h 10 min	1+/1+/2+	2+/2+/2+
6	60/F	11.0/8.3	182.4/28.0	35×13	2.45	adenoma	3 h 0 min	1+/2+/1+	1+/2+/2+
7	75/F	12.4/8.5	512.1/12.4	31×17	4.74	hyperplasia	3 h 0 min	2+/2+/2+	2+/2+/2+
8	67/F	10.6/8.2	228.6/52.6	18×9	0.42	adenoma	3 h 0 min	2+/2+/1+	2+/2+/2+
9	63/F	11.1/9.0	148.0/51.5	18×9	0.23	adenoma	3 h 0 min	1+/1+/2+	2+/2+/2+
10	40/M	12.1/5.5	1,621/10	95×45	4.89	adenoma	1 h 30 min	1+/1+/1+	2+/2+/2+
11	58/F	11.6/8.2	148/40	48×15	2.28	adenoma	1 h 30 min	2+/2+/1+	2+/2+/2+
12	70/F	10.0/7.8	369/30	14×6	0.51	adenoma	1 h 30 min	1+/2+/1+	2+/2+/2+
13	58/F	11.8/8.6	180/50	11×7	1.00	adenoma	3 h 0 min	0/0/0	1+/1+/1+
14	67/F	12.8/7.4	780/30	40×25	4.72	adenoma	1 h 30 min	1+/1+/2+	2+/1+/2+
15	66/F	11.3/8.9	110/32	14×10	0.72	adenoma	1 h 30 min	2+/2+/1+	2+/2+/2+
16	35/F	11.1/7.4	127/21	30×15	1.12	adenoma	2 h 10 min	1+/2+/1+	2+/2+/2+
17	44/F	12.3/8.9	550/16	30×10	2.42	hyperplasia	2 h 0 min	1+/2+/1+	2+/2+/2+
18	71/F	10.4/7.9	184/16	11×6	0.41	hyperplasia	0 h 45 min	0/0/0	0/0/0
19	38/M	13/9.7	871/7	38×28	15.79	carcinoma	2 h 25 min	0/1+/1+	1+/1+/1+
20	59/F	11.8/10.0	213.8/112	35×28	10.96	adenoma	2 h 10 min	1+/2+/1+	2+/2+/2+

Table 2 Cases of secondary hyperparathyroidism due to chronic renal failure (SHP group)

Patient		Serum levels before/after surgery		Resected specimen		Pathologic change	Interval from 5-ALA administration to surgery	Reactivity (Surgeon 1/2/3)		
No.	Age (years)/Sex	Calcium (mg/dL)	iPTH (pg/mL)	Size (mm)	Weight (g)			External Surface	Cut surface	
21	64/F	10.5/8.2	1,100/14	Not measured	1.48, 0.4, 2.24, 0.34	hyperplasia	3 h 30 min	2+/1+/2+	2+/2+/2+	
22	54/M	10.5/7.8	920/120	Not measured	0.1, 0.82, 0.60, 1.01	hyperplasia	2 h 50 min	0/1+/1+	1+/1+/1+	
23	54/M	10.6/8.7	660/17	14×10	11.17 (including thyroid)	hyperplasia	3 h 0 min	0+/0+/0+	2+/2+/1+	located in the normal thyroid
24	54/M	11.1/7.7	1,000/21	5, 15, 25, 35	0.06, 1.14, 3.26, 2.21	hyperplasia	3 h 0 min	1+/1+/1+	1+/2+/1+	
25	67/F	10.3/9.0	659/38	10, 8, 12, 8	0.30, 0.05, 0.43, 0.10	hyperplasia	3 h 0 min	1+/1+/1?	2+/2+/2+	
26	45/F	9.5/8.8	140/120	7×4	7.07 (including thyroid)	hyperplasia	2 h 18 min	1+/1+/1+	1+/1+/2+	

Table 3 Cases of normal parathyroid found at thyroid surgery (NP group)

Patient		Serum levels before/after surgery		Resected specimen		Pathologic change	Interval from 5-ALA administration to surgery	Reactivity, external surface of parathyroid (Surgeon 1/2/3)	Pathologic diagnosis
No.	Age (years)/Sex	Calcium (mg/dL)	iPTH (pg/mL)	Size	Weight (g)				
27	19/F	9.2/8.9	Not measured/31	Not measured	Not measured	normal	5 h 20 min	1+/1+/1+	Papillary carcinoma of thyroid
28	72/F	9.5/8.2	Not measured/27	Not measured	0.08	normal	3 h 0 min	2+/1+/1+	Papillary carcinoma of thyroid
29	55/F	9.7/9.6	Not measured/32	Not measured	Not measured	normal	1 h 40 min	1+/1+/2+	Adenomatous goiter

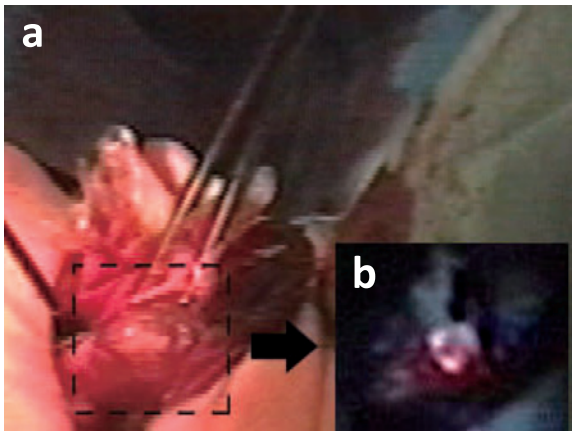

Fig. 2 Identification of parathyroid adenoma (patient 1, PHP group, Table 1). The operative field (a) was illuminated with blue light and observed through an optical filter. The parathyroid tumor is easily located via the red fluorescence (inset, b).

that this lesion may not have been parathyroid tissue. Efforts to identify a fourth parathyroid gland were unsuccessful. For patient 26, 3 weakly fluorescent parathyroid glands were resected. We were unable to locate a fourth parathyroid gland.

In all other cases in the PHP and SHP groups, the parathyroid gland was reactive for 5-ALA, and surgery was completed successfully. The degree of 5-ALA reactivity and responses, some peculiar, are shown in **Tables 1 and 2**.

In the NP group, normal parathyroid glands were investigated at the resected thyroid lobe; these parathyroid glands were also reactive for 5-ALA, with a brightness grade of 1+ or 2+ at the surface (see **Table 3**).

There were several interesting cases in this study. **Figure 2** shows a parathyroid tumor in patient 1 which preoperative ultrasonography and scintigraphy had suggested to be present. The excised parathyroid tumor was 17×12 mm and weighed 680 mg. Notably, the iPTH level in this patient was 100 pg/mg preoperatively but had decreased to as low as 11 pg/mL by 32 days after surgery, and the serum calcium returned to a normal level of 9.8 mg/dL.

Figure 3a shows normal thyroid tissue **(Fig. 3a, right)** and a cross section of 1 of 4 parathyroid glands excised from patient 23 of the SHP group **(Table 2)**. The parathyroid gland was hyperplastic, and the thyroid tissue was adjacent to the parathyroid tumor. **Figure 3b** shows the same specimens under excitation light. The cut surface of the resected hyperplastic parathyroid gland was brightly fluorescent (2+) **(Fig. 3b left)**, whereas the normal thyroid tissue did not fluoresce (0) **(Fig. 3b right)**. The operative field was examined with the excitation light to confirm that there was no residual fluorescent tissue.

Figure 4 shows parathyroid tissue, easily located by its fluorescence, in patient 28 of the NP group **(Table 3)**. The fluorescent parathyroid tissue was preserved in situ during an operation to treat micropapillary carcinoma of the thyroid. The images in **Figure 2 to 4** have been previously published[19].

Figure 5 shows a typical strongly positive

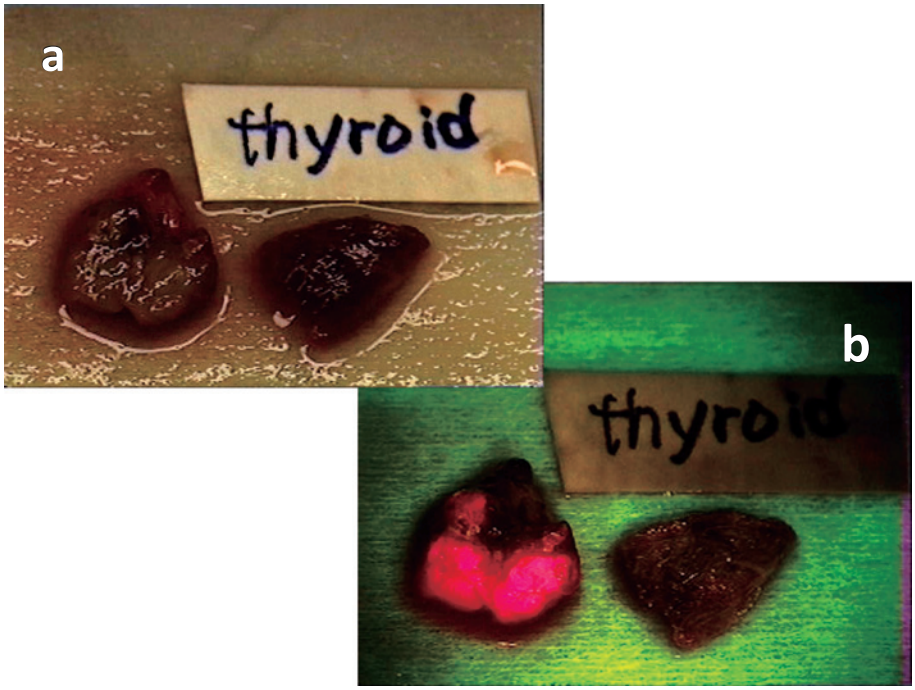

Fig. 3 One of the resected hyperplastic parathyroid glands (left) and normal thyroid (right) in patient 23 in the SHP group (Table 2). (a) A resected parathyroid, which was located in normal thyroid (left), and normal thyroid tissue that was removed from the area close to the resected parathyroid gland (right). (b) The same specimens photographed through the optical filter, with the excitation light illuminating the tissue. Only the parathyroid tissue emitted red fluorescence (left); the thyroid did not (right).

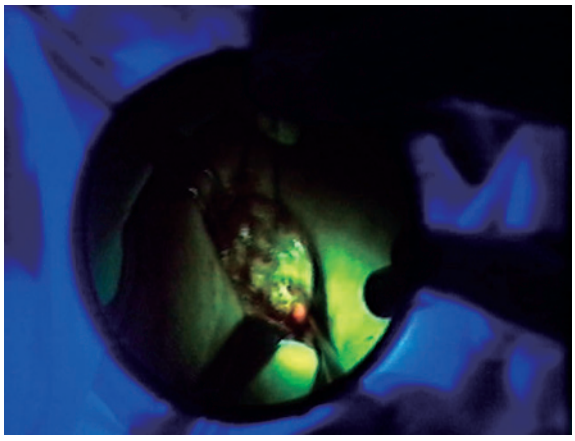

Fig. 4 Identification of normal parathyroid tissue during thyroid surgery in patient 27 of the NP group (Table 3). The normal parathyroid tissue was easily detected as the fluorescence-positive tissue behind the thyroid tumor. Head is to the left.

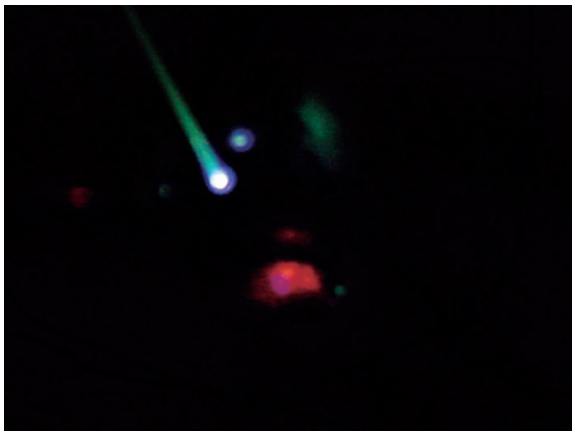

Fig. 5 The diffuse strong reaction (2+) was confirmed as red brightness indicating pathological parathyroid in patient 8 of the PHP group (Table 1).

fluorescent response in the PHP group; images were obtained during surgery in patient 8 (**Table 1**). A bright red, diffuse strong reaction (2+) indicated pathological parathyroid tissue.

The parathyroid adenoma of patient 13 of the PHP group (**Table 1**) was unusual in that it showed no fluorescence on its exterior surface but did show a fine, spotty reaction on its cut surface (**Fig. 6**). The

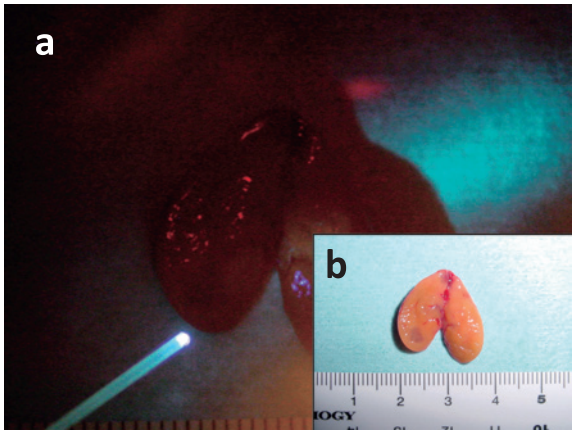

Fig. 6 A peculiar fine, spotty reaction on the cut surface of a parathyroid adenoma, in patient 13 of the PHP group (Table 1). (a). The pathological finding was adenoma, indicated by swelling in the resected parathyroid (b).

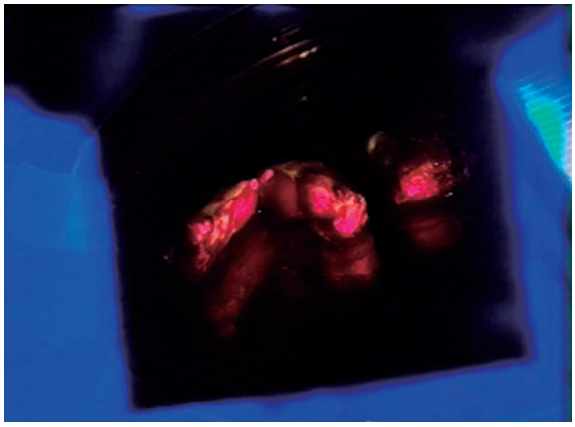

Fig. 7 Fluorescence due to 5-ALA of a resected thyroid papillary carcinoma in patient 27 of the NP group (Table 3).

pathological diagnosis of adenoma was indicated by marked swelling in the parathyroid (b). The levels of iPTH and serum calcium returned to normal after surgery. Furthermore, interestingly, the papillary carcinoma of the thyroid in patient 27 of the NP group (**Table 3**) was clearly positive for 5-ALA fluorescence (**Fig. 7**).

Quantitative analysis of fluorescence (**Fig. 8**) in a case of pathological parathyroid tissue (patient 5 in the PHP group) and a case of thyroid tissue with papillary carcinoma (patient 27 in the NP group), both of which were positive for fluorescent 5-ALA reactivity with no distinguishable difference in brightness, showed that they differed in the strength of fluorescence at 650 nm. Normal thyroid was quantitatively negative for 5-ALA reactivity.

Discussion

Various procedures can be used to locate parathyroid tissue, whether normal or pathological, both before and during surgery[20–25]. However, diseased parathyroid tissue cannot always be definitively identified, and waiting for the results of PTH measurements during surgery wastes time and prolongs surgery. Identifying normal parathyroid tissue macroscopically is difficult during thyroid surgery, especially a second thyroid surgery. However, 5-ALA fluorescence can be used to reliably identify pathological and normal parathyroid tissue in real time, shortens the operating time, and allows the surgeon to perform minimally invasive surgery with confidence. Our results show that 5-ALA-induced fluorescence allows the parathyroid to be clearly identified during endocrine neck surgery and is of great help to endocrine surgeons.

At least 2 previous studies have used 5-ALA-induced fluorescence to indentify parathyroid glands[26,27]. However, our present report involved a greater number of cases and includes normal and pathological parathyroid tissues and papillary carcinoma of the thyroid. The use of 5-ALA-induced fluorescence addresses 2 important issues in endocrine neck surgery. The first is the complete removal of pathological parathyroid tissue when treating hyperparathyroidism; if any of this tissue remains, the surgery can be considered to have failed because excessive secretion of PTH will continue. The second issue is the preservation of normal parathyroid tissue during thyroid surgery; removing the parathyroid causes hypocalcemia, which necessitates life-long calcium supplementation to avoid the complications of persistent hypoparathyroidism. Normal parathyroid glands that have been accidentally injured or removed can be autotransplanted if they are recognized in time.

In the past, primary hyperparathyroidism was treated with bilateral surgical exploration[28] to examine the 4 parathyroid glands. As imaging techniques improved, bilateral exploration was

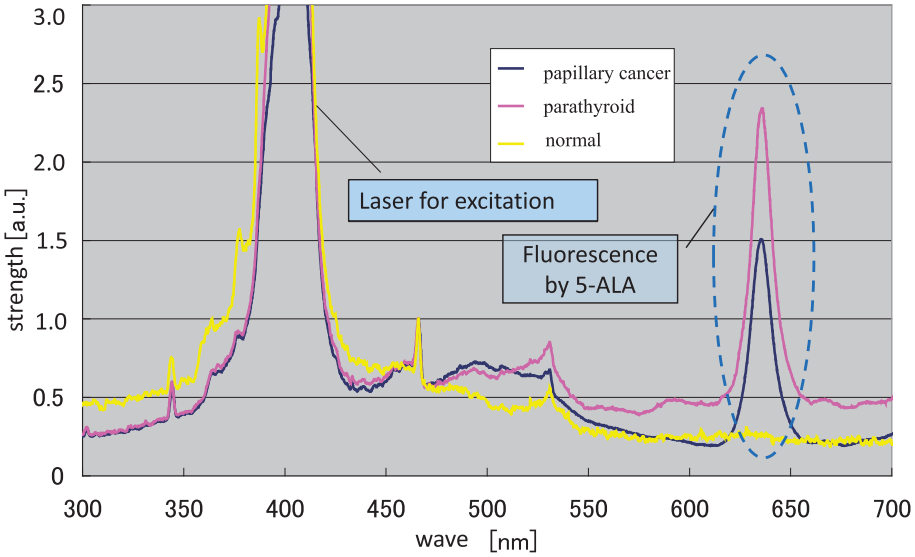

Fig. 8 Spectrum from various tissues: Quantitative analysis of the fluorescence in pathological parathyroid (patient 5 of the PHP group, Table 1) and papillary carcinoma of the thyroid (patient 28 of the NP group, Table 3). The strength of reactivity was determined by the fluorescence intensity at 650 nm, which differed between parathyroid tissue and papillary carcinoma of the thyroid. Normal thyroid 5-ALA reactivity was quantitatively negative.

replace by unilateral exploration[29], in which the pathological parathyroid glands and normal thyroid gland on the same side were examined concurrently. With further improvements in imaging methods and equipment, "focused parathyroidectomy"[30–34] has become widely accepted. Focused parathyroidectomy is less invasive because only 1 pathological parathyroid gland is identified and excised, but accurately locating the parathyroid lesions during surgery is extremely important. In parathyroid hyperplasia, including secondary hyperparathyroidism, if the PTH level does not decrease after all 4 parathyroid glands have been completely removed, the existence of a supernumerary gland might require re-exploration. Thus, it is crucial to identify and excise all pathological parathyroid tissue.

We have been performing parathyroid surgery with radio-guided navigation using a mobile scintigram[32–34]. A quick PTH assay[30] during surgery enables the surgeon to determine whether the focus has been excised. Our new method of intraoperative fluorescence detection with 5-ALA can be used with these procedures. Our method will increase the reliability of endocrine neck surgery, especially when combined with the minimally invasive video-assisted endoscopic thyroid and parathyroid surgery, which we developed in 1998[13] and have performed in more than 650 cases to date[14–17].

The present study is, to our knowledge, the first large clinical study of the use of 5-ALA-induced fluorescence for parathyroid identification during endocrine neck surgery. Our method has many advantages: 5-ALA occurs naturally in the human body and can be administered orally. Our method is also easy to use, because both the excitation wavelength and the fluorescence are in the normal visible spectrum and no additional invasive, complicated methods or equipment is needed.

However, several unclear results of our study should be clarified. In this series, some pathological parathyroid specimens were negative or only weakly positive for 5-ALA reactivity. In addition, several relationships remain to be clarified, such as the timing of 5-ALA administration before surgery and 5-ALA reactivity, the appropriate timing of illumination, and the effects of pathological findings, such as differences in vascularity, tumor size,

thickness of the tumor capsule, and inflammation of the surrounding tissue. In parathyroid tumors, the grade of positive reactivity depended on the thickness of the tumor capsule: the thicker the capsule, the weaker the fluorescence. In addition, the reactivity of the tumor was stronger on its cut surface than on its exterior surface.

Hence, 5-ALA-induced fluorescence is a highly promising method for clearly distinguishing pathological and normal parathyroid tissues during thyroid surgery and, thus, for shortening the operating time. Further basic research is needed to understand the physiological mechanism by which 5-ALA accumulates in pathological and normal parathyroid tissues but not in the normal thyroid or other surrounding tissues. This phenomenon invites further investigation and may help us analyze the 5-ALA pathway, which is unknown. The strong reactivity of papillary carcinoma of the thyroid motivates us to further investigate the mechanism of the metabolic pathway of 5-ALA. Further clinical studies, including the quantitative observation of fluorescence and elucidation of the relationship between fluorescence and tissue type, are also expected to be forthcoming. This method of 5-ALA-induced fluorescence may also provide a useful marker for accurately identifying papillary carcinoma of the thyroid during surgery.

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Conflict of Interest: The authors declare no conflict of interest.

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