ONCOLOGY

Accumulation of Porphyrins in Thyroid Tissue and Cells Induced by δ-Aminolevulinic Acid

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δ-Aminolevulinic acid and phenanthroline induced accumulation of metal-free porphyrins in homogenates of thyroid tissue and cells obtained after thyroidectomy. The rate of pigment synthesis considerably increased in specimens isolated from pathologically changed tissue, and the pigments were primarily located in cells. The results indicate that endogenous porphyrins can be used in the diagnosis and phototherapy of thyroid tumors.

Key Words: *δ-aminolevulinic acid; porphyrins; thyroid gland; pathological changes*

Selective accumulation of exogenous porphyrins in malignant cells is now widely used in the diagnosis and photodynamic therapy of tumors [6,8,12] due to bright fluorescence of porphyrins in the visible band of the spectrum and high photosensitizing activity of these pigments. Accumulation of endogenous porphyrins in animal cells and tissues can be induced by heme precursor δ-aminolevulinic acid (ALA) [11,13, 16]. This effect is most pronounced in cells with high proliferative activity and in transformed cells [5,9,14]. Endogenous porphyrins can be used in the diagnosis and photodynamic therapy of skin, mammary gland, bladder, lung tumors, and other neoplasms [10].

A drastic increase in the incidence of thyroid diseases in some regions, specifically, malignant tumors [1,3] necessitates search for new effective methods for their diagnosis and therapy. We have shown that human thyroid cells contain the main types of porphyrins: uroporphyrin (UP), coproporphyrin (CP), and protoporphyrin IX (PP) [2]. Protoporphyrin predominated and surpassed the content of CP 5-7-fold, and that of UP by an order of magnitude. In thyroid gland involved in pathological process and in isolated thyrocytes, the absolute and relative contents of individual porphyrins depend on the disease (goiter, adenoma, malignant tumor). However, the concentrations of endogenous pigments are very low, which impedes their analysis and utilization in the diagnosis or photodynamic therapy. We investigated ALA-induced accumulation of metal-free porphyrins in tissues and cells isolated from intact and pathologically changed thyroid.

MATERIALS AND METHODS

Tissue specimens (200 mg) obtained after thyroidectomy for thyroid adenoma or carcinoma were analyzed. Cells were isolated from tissue fragmented in a loose homogenizer. The suspension was incubated for 1.5-2 h at 37°C in medium 199 containing 5-10 mg/ml collagenase, filtered through a capron sieve, centrifuged for 5 min at 400*g*, and resuspended in phosphate- buffered normal saline.

Porphyrins were extracted as described previously [15]. Ethyl acetate:acetic acid (4:1) mixture was gradually added to tissue or cell homogenate to a final volume of 3 ml and left for 20 h in a refrigerator; UP, CP, and PP were isolated by successively transferring the extracts into 0.5, 0.1, and 1.5 N hydrochloric acid, respectively. The concentrations of pigments were measured by the intensity of fluorescence of the resultant hydrochloric solutions and calibration curves.

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Fluorescence was measured at excitation wavelengths of 405, 399.5, and 407 nm and emission wavelengths of 596, 594, and 601 nm for UP, CP, and PP, respectively.

The total content of porphyrins in cells and incubation media was evaluated by the intensity of fluorescence of precipitate and supernatant, respectively, after centrifugation of cell suspension. The rate of ALA-induced porphyrin synthesis in tissue homogenates was evaluated by the amount of UP, CP, and PP formed during 2 h after addition of ALA. For accumulation of endogenous porphyrins the specimens were incubated in buffered normal saline containing 1 mM ALA and/or 0.75 mM phenanthroline at 37°C. The measurements were carried out on a JY-3CS spectrofluorimeter (Jobin Ivon).

Coproporphyrin III (Sigma), PP IX dimethyl ether (Serva), UP III octamethyl ether (Sigma), ALA (Serva), and o-phenanthroline (Chemapol) were used. The results were statistically processed by parametrical methods [4].

RESULTS

In animal cells ALA is produced in the mitochondria and transformed into uro- and then into coproporphyrinogen in the cytoplasm. Coproporphyrin then enters the mitochondria, where it is oxidized to PP, which chelates iron to form the heme regulating the rate of ALA synthesis via a feedback mechanism. Normally the rate of porphyrinogen transformation allows just negligible accumulation of free UP and CP in cells. Exogenous ALA disturbs the feedback regulation, and the excess of porphyrinogens distributed between the mitochondria, cytoplasm, and extracellular space is separated from the corresponding oxidases, which promotes their nonenzymatic transformation into porphyrins [7]. Phenanthroline inhibits ferrochelatase activity and suppresses iron chelation by PP,

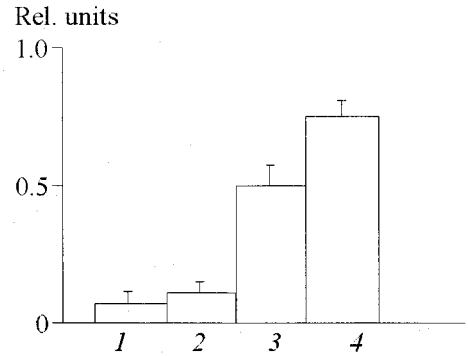

Fig. 1. Fluorescence intensity of normal thyroid tissue homogenates incubated at 37°C for 2 h in buffered normal saline in the absence (*1*) and presence of 0.75 mM phenanthroline (*2*), 1 mM δ-aminolevulinic acid (*3*), and both agents (*4*).

which can also contributes to accumulation of pigments in the cells.

Addition of ALA to thyroid tissue homogenates in our experiments induced accumulation of porphyrins, which manifested by increased fluorescence of hydrochloric tissue extracts. Fluorescence increased for more than 10 h and during the first 2-6 h this increase linearly depended on the incubation time. The rate and duration of the process depended on substrate concentration. The kinetic curve reached the plateau at ALA concentrations above 0.8 mM. Phenanthroline also increased porphyrin fluorescence in test samples. The rate of this process attains the maximum at concentrations of 0.50-0.75 mM. Addition of ALA and phenanthroline together into incubation medium showed that their effects were not additive, and the amount of pigments formed in tissue far surpassed the summary level of porphyrins accumulated in the presence of both test agents (Fig. 1).

Incubation of homogenates of normal and pathologically changed thyroid tissue for 2 h at 37°C without modifiers caused no appreciable accumulation of pigments. The levels of UP, CP, and PP increased in

TABLE 1. Content of Metal-Free Porphyrins (pmol/g tissue) in Thyroid Tissue Homogenates Incubated for 2 h at 37°C with and without 1 mM ALA and 0.75 mM Phenanthroline (*M*±*m*)

Porphyrin	Modifiers	Thyroid tissue sample		
		normal	malignant tumor	adenoma
PP	—	24.4±2.8 (9)	88.9±23.6 (6)*	38.2±9.6 (8)
	+	114.6±21.7 (10)	241.4±62.4 (5)*	409.5±284.0 (4)
CP	—	3.7±0.8 (9)	3.0±1.1 (6)	5.0±2.0 (8)
	+	84.6±15.4 (10)	87.1±21.9 (5)	125.1±18.7 (4)*
UP	—	1.8±0.5 (9)	1.7±0.7 (6)	1.6±0.8 (8)
	+	21.4±4.0 (10)	20.0±7.1 (5)	23.6±9.4 (4)

Note. *$p \leq 0.05$ *vs.* normal tissue. Number of experiments is shown in parentheses.

normal tissue homogenates incubated with ALA and phenanthroline (Table 1). In tumor homogenates, the rate of UP accumulation increased 1.7-fold, while the rate of CP and PP accumulation did not differ from normal. In homogenates of adenomatous tissue, the rate of CP formation increased 1.5-fold and the rate of PP production sharply (3-5-fold) increased in many specimens. Thus, the rates of ALA-induced accumulation of metal-free porphyrins are different in homogenates of normal and pathologically changed thyroid tissue, which prompts the use of pigment fluorescence for detecting the pathological focus in the organ.

Photodynamic therapy of tumors is possible if photosensitizer (porphyrin in our case) is located intracellulary. To elucidate possible release of pigments formed in the presence of exogenous ALA from thyrocytes, we compared the fluorescence of cells and media after centrifugation of cell suspensions incubated in the presence of ALA and phenanthroline. The study of ALA-induced fluorescence showed that isolated cells from pathologically changed thyroid tissue produce much more pigments than cells from intact tissue. The fluorescence intensities in suspensions of malignant cells and cells from adenomatous foci 7.2- and 3.5-fold surpassed fluorescence intensity of normal cells, respectively. Porphyrin fluorescence was present in both the cells (precipitate) and incubation medium (supernatant). Prolongation of thyrocyte incubation with ALA led to an increase in the relative content of porphyrins in the medium. The excitation and emission spectra of supernatants indicate that cells release mainly PP.

The results suggest that normal and transformed thyroid cells produce porphyrins in response to exogenous ALA. Marked selective accumulation of intensely fluorescing photodynamically active pigments in pathologically changed tissues and thyrocytes opens vistas for the development of a highly sensitive fluorescent methods for diagnosis and effective therapy of thyroid tumors.

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