

Review

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Review

Photodynamic Therapy for Thyroid Cancer

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Abstract: Background/Objectives: Thyroid cancer is the most common cancer of the endocrine system worldwide. Despite many available therapeutic options, the mortality rate of some subtypes, including anaplastic thyroid cancer, is still significant. Photodynamic therapy brings hope, which, through local activation of cell death pathways, as well as its effect on the immune system and vessels feeding the tumor, brings effects incomparable to classical methods. Photosensitizers particularly used in the case of thyroid cancer are hypericin, porphyrin, photophrin, radachlorin or 5-aminolevulinic acid. Even better effects are achieved by administering sulforaphene, carboplatin or genistein before therapy. **Methods:** For this research we review articles in regards to provide a critical summary of the existing literature on thyroid cancer to explain the current state of scientific evidence on this topic. **Conclusions:** Photodynamic therapy is undoubtedly a technique of the future, the main advantages of which are low invasiveness, the possibility of combining with other treatment methods, or the possibility of outpatient use.

Keywords: photophrine; photosensitizer; genistein; hypericin; carboplatin; 5-aminolevulinic acid; porphyrin; radachlorin; thyroid cancer; ROS; sulforaphene; thyroid; photodynamic therapy

1. Introduction

Thyroid cancer is the most common cancer of the endocrine system worldwide. Its incidence is estimated at about 6.7-9 cases per year, and the number of patients has been constantly growing for 30 years, with a constant number of deaths. [1–4] The peak incidence occurs between the ages of 51 and 60. [5] The prognosis of well-differentiated tumors detected at an early stage in the surgical stage is optimistic, in contrast to the prognosis of anaplastic cancer, which is most often diagnosed in the metastatic stage, and the median survival is about half a year. None of the previously used treatment methods, regardless of the way they are combined or intensified, is able to lead to long-term remission in most patients. Also, some clinical cases of other types of thyroid cancer are resistant to the proposed treatment and end in the patient's death. Photodynamic therapy is a hope, which, used alone or in combination with previously used chemotherapy, gave promising results in preclinical studies. [6–10]. The type of lesion determines the course of the disease, from slow-growing to aggressive, rapidly progressive forms. [1] Malignant changes derived from follicular cells are called differentiated thyroid cancer (DTC), and include papillary thyroid cancer (PTC, 70-80%), which has a 5-year survival rate of about 98% and recurrence of up to 7%, follicular thyroid cancer (FTC, 10-15%), and Hurthle cell carcinoma (HCC, 5%). [1,2,4,11–14] Together, they constitute 90-95% of thyroid cancers, and the 10-year survival rate is estimated at 98%. [1,2,5,15] The second type is medullary thyroid cancer (MTC), which arises from parafollicular C cells of the neural crest and accounts for approximately 1-10% of thyroid cancers. [1,2,15] The remaining 1% is anaplastic thyroid cancer

(ATC), an undifferentiated lesion of follicular cells. It is said to arise from follicular and papillary carcinomas as a result of genomic changes. [16] It is characterized by a median survival of several months, an aggressive course, rapid metastasis, in up to 50% of patients at the time of hospital admission, and invasion of surrounding tissues. [1,2,17] The average age of onset is 65-70 years, and the average survival of patients with ATC using currently used therapies is estimated at 4-6 months, with approximately 35% of patients achieving 6-month survival. [6,16,18].

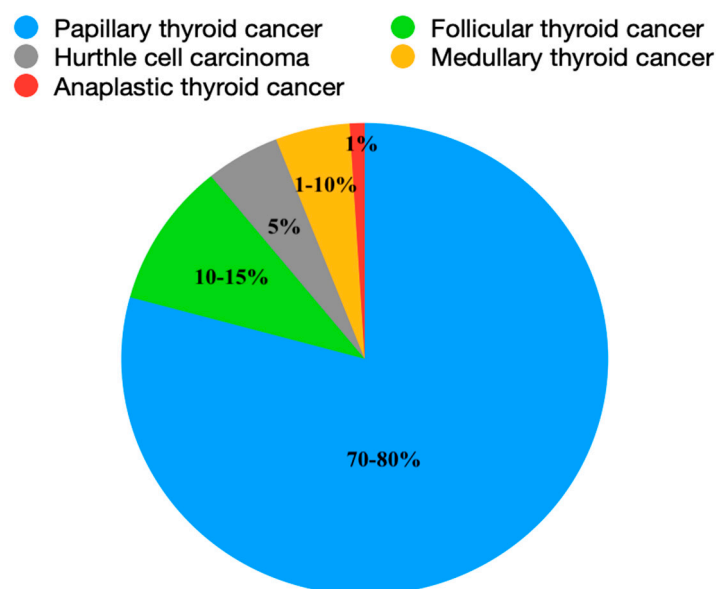


Figure 1. Symbolic representation of the relationship between the different types of thyroid cancer.

Risk factors, especially for differentiated thyroid cancer, include childhood exposure to thyroid radiation (treatment for another cancer, nuclear fallout, occupational exposure) and family history of thyroid cancer. [1,7,19] Subclinical disease is more frequently detected in females. [7,20]

2. Materials and Methods

This literature review summarizes the current information available in the Pubmed and Google Scholar databases regarding thyroid cancer, its incidence, clinical presentation, genetic background, histopathological features, diagnosis and treatment, and photodynamic therapy, particularly in the context of its potential use in the treatment of thyroid cancer. Due to the type of cells from which thyroid cancer develops, it can be divided into several types.

2.1. Genetic Background

Thyroid cancers may have a genetic background. The association is mainly observed with abnormal activation of the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAP, MAPK) pathways and with genes responsible for iodine metabolism in the thyroid encoding the sodium iodide symporter (NIS). [1,2,4] The PI3K pathway plays an important role in cellular mechanisms, including proliferation, survival, differentiation and apoptosis - its stimulation is the basis for uncontrolled multiplication of cancer cells leading to the development of cancer, especially in the head and neck region. This pathway is stimulated by the epidermal growth factor receptor (EGFR), and inhibited by the phosphatase and tensin homolog (PTEN). Overexpression of EGFR correlates with the clinical stage of ATC. [8] However, the loss of NIS expression in the basement membrane of thyroid cells, including cancer cells, is associated with impaired iodide transport to thyroid follicular cells, which results in the failure of RAI therapy (radioactive iodine therapy). Therefore, RAI is most effective in DTC. [4,21] It is estimated that in the case of PTC and FTC, the

familial occurrence is about 5%. [1,2] PTC is associated with a point mutation in the BRAF V600E gene in 29-69%, and in 0-12% in the case of anaplastic carcinoma associated with PTC. The follicular variant of PTC shows a mutation in the RAS proto-oncogene in 10-20%. RET/PTC translocation occurs in about 7% of PTC. PTC may also be associated with rarer mutations, such as TERT mutations, in highly aggressive cases, NTRK1/3 fusion, E1F1AX, NF1/2, TP53, ERCC5, ATM or CHEK2 mutations. [1,2,12] In the case of FTC, a correlation was noted with mutations in the RAS proto-oncogene in 40-50%, NRAS more often than HRAS and KRAS, with translocation in the peroxisome proliferator-activated receptor γ PAX8 in 30-35%, with PTEN mutation and with TERT mutation in 17%. BRAF mutation is almost never observed. [1,2,12] Approximately 60% of Hurthle cell thyroid cancer cases are characterized by mutations in mitochondrial DNA. In addition, mTor activation and extensive LOH can often be detected in multiple chromosomes. TP53 and TERT mutations can be found especially in more aggressive forms. BRAF and RAS mutations are more common in other types of differentiated thyroid cancer. [2] Mutations detected in MTC include germline mutations of the RET proto-oncogene in about 25-50% of sporadic forms, in about 95-98% of hereditary forms of MTC, and RAS mutations in 25% of all forms. In about 20%, no RAS and RAT mutations are found. In 20-25% of MTC, it is a component of hereditary cancer syndromes such as multiple endocrine neoplasia 2A (MEN2A) and 2B (MEN2B) and hereditary MTC (FMTC), in the remaining cases it occurs sporadically. [1,2,15,22] 50-80% of anaplastic thyroid cancers are characterized by early inactivating mutations and inactivating mutations of the tumor suppressor gene p53, 66% by mutations in the CTNNB1 gene, 20-40% by RAS mutations, 28-45% by BRAF mutation/fusion, 10-18% by PI3CA mutations, 9-15% by PTEN mutations, 6-73% by TERT promoter mutations, 4-9% by RB1 mutations, 10-21% by NF1/2 mutations, 0-12% by mismatch mutations, 4-8% by CDKN1/2 mutations, 0-6% by STKI1 mutations. [1,2,13,17]

2.2. Histopathological Picture

Each type of thyroid cancer has its own specific appearance under the microscope. PTC forms papillae, the fibrous core of which is surrounded by a layer of neoplastic cells, without the presence of hair follicles. It is characterized by the presence of Psammoma bodies and "Annie's orphan eyes" nuclei. FTC has a variable histological appearance. In addition to typical follicular changes, poorly differentiated changes can also be found, with nuclear atypia, aggressive growth, infiltration of the capsule or vessels, and lack of follicles. The presence of eosinophilic eosinophilic cells with prominent nucleoli and rich cytoplasm indicates Hurthle cell carcinoma. [1] MTC, as it originates from parafollicular C cells of the neural crest, is characterized by the production of a specific neoplastic marker, calcitonin, and several peptides, i.e. CEA, the level of which correlates with the mass of neoplastic cells (C cells) in well-differentiated MTC. [22] Multiple mitotic figures and atypical mitosis are common in anaplastic carcinoma.[1,2]

2.3. Diagnostics

It is estimated that 68% of thyroid nodules are detected during an ultrasound examination (USG), most often by accident. The initial assessment of a thyroid nodule for malignancy is performed by testing a thyroid function panel in the blood - changes associated with hyperthyroidism are usually at a lower risk of malignancy. In case of doubt, an additional radionuclide uptake test can be performed. Fine-needle biopsy is the test with the highest diagnostic accuracy in confirming malignant changes, at around 70-97%. [1,19,23]

Table 1. Summary of features suggestive of a malignant process in thyroid ultrasound.

Features indicating a malignant process in an ultrasound examination of the thyroid gland
Hypoechogenicity
Irregular margins
Shape taller than wide
Central vascularization
Extension of the lesion beyond the thyroid gland
Solid structure

3. Treatment

The most commonly used methods, in various combinations, include surgical treatment, radioiodine ablation (RAI), thyroid hormone suppression, radiotherapy, and chemotherapy. [1,2,6] Alcohol, laser, and radiofrequency ablation (RFA) techniques are also an alternative, although they are not commonly used. [14,24] Factors influencing decisions regarding the actions taken include, among others, tumor size, tumor growth rate, tumor location, invasion of neighboring tissues, presence of metastases, involvement of lymph nodes, symptoms, patient preferences, comorbidities, and team experience. The TNM (tumor, nodules, metastases), ATA (American Thyroid Association), and AJCC (American Joint Committee on Cancer) scales are also helpful in stratifying treatment. [25] In the case of well-differentiated cancers, surgical treatment is considered first, in the form of hemithyroidectomy or thyroidectomy, with possible lymph node dissection. [1,26] Postoperative radioiodine ablation therapy (RAI) is used at least 4 weeks after the procedure to remove micrometastases from the remaining thyroid tissue or identified metastases, which in the case of DTC may affect up to 60% of patients. In most cases, it is also necessary to use thyroid hormone suppression therapy. [1,26] Its aim is to reduce the risk of cancer recurrence by suppressing TSH, which stimulates the growth of thyroid cells, including neoplastic cells, with supraphysiological doses of L-thyroxine. [26] In cases refractory to conventional treatment, radiotherapy or chemotherapy, including lenvatinib and sorafenib, multikinase inhibitors (MKI), may be considered. [1,2,7] Low-grade cancers, especially papillary microcancers, may also be candidates for RFA treatment.[24] The leading treatment method for MTC is total thyroidectomy with removal of regional and local metastases and prophylactic resection of central lymph nodes. [1] Chemotherapy also plays a role in the treatment of MTC. In cases with RET mutations, kinase inhibitors, vandetanib, cabozantinib, selpercatinib or pralsetinib are used. Due to the different origin of this type of cancer compared to other thyroid cancers, radioiodine ablation and thyroid hormone suppression are not used. [1,2] Radiotherapy, RFA, cryoablation or embolization are also used less frequently. [27] Resectable anaplastic cancers (up to stage IVa) are treated surgically with lymph node excision. In the remaining tumors (IVb and IVc) and as an adjunct to surgical treatment, cytotoxic chemotherapy with carboplatin and cisplatin and targeted radiotherapy are used. In patients with the BRAF V600E mutation, specific BRAF and MEK kinase inhibitors, such as dabrafenib and trametinib, can be additionally used as preparation for surgical treatment or radiotherapy. [1,13,16,17] The treatment of metastatic anaplastic thyroid cancer still poses therapeutic challenges. [13,16] Frequent complications of surgical treatment, especially in thyroidectomy combined with lymph node resection, include neurovascular damage, damage or paralysis of the recurrent laryngeal nerve, including hoarseness and respiratory failure, hypocalcaemia, hypoparathyroidism, visceral fistula, damage to the cervical motor nerve, hemorrhage, hematoma, postoperative fever, wound infection, thromboembolic

complications, or Horner's syndrome. [1,3,6,14] Untreated, advanced thyroid cancer may invade the surrounding tissues, including the mediastinum, esophagus, trachea, larynx, carotid artery, and in about 10% of DTC cases, produce distant metastases, most often to the lungs, bones, brain, and liver. [1,3,17–27]

Table 2. Podsumowanie metod leczenia w zależności od typu raka tarczycy.

Type of treatment	Type of cancer
Active supervision	DTC
Lobectomy	DTC
Thyroidectomy with/without nodulectomy	DTC, MTC, ATC
Chemotherapy	DTC, MTC, ATC
Radioterpia	DTC, MTC, ATC
Radioiodine ablation	DTC
Thyroid hormone suppression therapy	DTC
Radiofrequency ablation (RFA)	PTC, small recurrence, symptomatic in non-resectable cancers, MTC

None of the above-mentioned treatment methods are ideal. Each one differs in medical indications, side effect profile, availability, risk of complications. Moreover, despite maximizing their use and using different combinations, they are not always sufficient to achieve remission or at least stop the progression of the cancer, especially in the case of anaplastic thyroid cancer. [6]

3.1. Photodynamic Therapy (PDT)

Phototherapy, or the healing effects of light, has been known for hundreds of years. It has been used since ancient times to treat diseases such as skin cancer, vitiligo, psoriasis, swelling, paralysis, muscle weakness, rheumatism, scurvy, rickets, tuberculosis, and lupus vulgaris. [28,29] The term "photodynamic effect" was first used in 1907, when oxygen was recognized as an integral component of the process. Currently, it is used in many medical indications, the most important of which are oncological diseases of the urinary bladder, lungs, neck, head, esophagus, skin, as well as non-cancerous diseases including dermatological, ophthalmological, cardiovascular, neurological, gastroenterological, rheumatological, infectious, and dental.[28,29] The cytotoxic effect is caused by 3 components: light of a specific wavelength, photosensitizer (PS) and molecular oxygen.[28,30,31]

3.2. Translation

The main component involved in PDT is a photosensitizer (PS). Research is still underway to develop the ideal PS, which is characterized by, among others, low activity without exposure to light, light absorption in the range of 600 nm to 800 nm, good tissue penetration without energy losses, affinity and accumulation in the tumor, rapid elimination from the body, high quantum yield of singlet oxygen. PS is divided into three generations. The first generation includes derivatives of hematoporphyrin and sodium porphyrmer. Their main disadvantages are hydrophobicity, causing skin hypersensitivity, toxicity without a light source, low absorption in red light. The second generation PS, which differs in the range of activating light, lower skin phototoxicity, solubility in water, quantum yield of a single oxygen molecule and faster elimination from tissues, includes, among others, derivatives of phthalocyanines, bacteriochlorins and chlorides. Third-generation PSs

are characterized by a lack of solubility, selectivity and effectiveness limited to cancer tissue, thanks to the use of antibodies and carriers made of polymers, dendrimers, quantum dots, micelles, liposomes, gold or carbon and magnetic nanoparticles. [28,29] The second element needed to perform photodynamic therapy is light. Depending on the indication and the site undergoing therapy, the type and parameters of the light used may vary. [28,29] Laser light sources include: argon/dye lasers, metal vapor and solid-state lasers. Non-laser light sources include lamp light sources, light-emitting diodes, daylight or X-rays. [31] The wavelength used ranges from 600 nm to 1200 nm, which is the optical window of the tissue, while the optimal range, called the therapeutic window, is 600 nm to 850 nm. Shorter wavelengths - below 600 nm - penetrate the tissue less, focusing on the more superficial layers of the skin, which contributes to its hypersensitivity to light. On the other hand, wavelengths longer than 850 nm do not provide the amount of energy required to produce the appropriate amount of reactive oxygen species, and consequently - to induce the desired therapeutic effect. [28] The last but equally important element is molecular oxygen. Hypoxia of the tissue undergoing therapy causes reduced production of ROS and a decrease in the effectiveness of the treatment, which is particularly noticeable in solid tumors located deeper. It is important to select such light intensity that the rate of oxygen consumption is equal to the rate of oxygen penetration into the tissue undergoing therapy, and also that it does not cause occlusion of the blood vessels of the tumor tissue, which would deepen the hypoxia. [6,28]

3.3. Mechanism of Photodynamic Therapy

Administered locally, intravenously, orally, or intraperitoneally, PS accumulates in the target tissue. Absorbing light, it triggers numerous chemical reactions that result in the generation of reactive oxygen species and the transition of PS from the ground state to an unstable electronically excited singlet state. The singlet state can also be transformed into the triplet state by spin conversion of an electron located in a higher energy orbit. Due to its greater stability, the triplet state can transfer energy to molecular oxygen and enable the creation of singlet oxygen and the ground state of PS, which constitutes a type II reaction, which is the main mechanism of action of photodynamic therapy. Highly reactive singlet oxygen, through its ability to affect the nucleus, lysosomes, mitochondria, endoplasmic reticulum, and cell membrane, leads to cell death. Type I reaction involves direct interaction of excited PS with surrounding tissues, i.e. cell membrane or electron-rich molecule. The radical ions and free radicals generated in this mechanism react with molecular oxygen. By producing reactive superoxide anion, hydrogen peroxide or hydroxyl radical, they cause oxidative damage leading to biological modifications. [28–30] The ratio of type I and II reactions, and consequently the entire process of cell death, consists of many components, including PS affinity for the substrate, concentration, location relative to the cell, PS dose and type, light dose, oxygen concentration and availability, time elapsed between PS administration and exposure to light, location and degree of tumor oxygenation. Both reactions can be carried out at the same time. [28,29] Type I reactions in the case of hypoxia proceed better than type II reactions, but are associated with greater damage. [28–30] PDT can affect cancer tissue by three mechanisms, the combination of which gives the most durable, long-term effect. Cancer cell necrosis is a mechanism of unprogrammed cell death, occurring under the influence of large amounts of light. It leads to vacuolation of the cytoplasm, destruction of the cell membrane, the release of cytoplasmic content and proinflammatory mediators outside the cell and, as a consequence, local inflammation. Apoptosis is a process of programmed cell death, which occurs under the influence of small amounts of light. Its essence is a reduction in cell dimensions, wrinkling of membranes and production of apoptotic bodies, while maintaining the continuity of the cell membrane. In contrast to necrosis, toxic substances do not escape from the cell. The second mechanism is the destruction of blood vessels supplying cancer tissue, through the accumulation of PS in the vessel walls. This indirect mechanism of cancer tissue death is primarily important in the long-term effects of photodynamic therapy. A relatively recently discovered effect of PDT on cancer tissue is the generation of an immune response through the earlier induction of inflammation. Induction of necrosis and the effect on blood vessels causes a sudden release of inflammatory

mediators, which then affect the host's immune system. Macrophages phagocytizing destroyed cancer cells indirectly stimulate Tc lymphocytes, which provide immune defense against cancer cells for a long time. Local PDT probably has an immunosuppressive effect, while systemic PDT is immunostimulant. [28]

3.4. Advantages

The main advantages of PDT include low therapy costs compared to other methods in oncological treatment, short therapy time, no long-term side effects, low invasiveness, possibility of use in outpatient settings, high effectiveness in killing cancer cells, also affecting the vascularization of the tumor, possibility of repeated use, no interaction with other treatment methods, leaving small scars or no scars at all. [6,28,30] Cytotoxic specificity, which distinguishes PDT from other methods, e.g. ablation, is a combination of PS molecules predilection for the tumor, focusing the low-power laser only on the tumor and local action, while generating ROS, not dehydration or temperature change. [14]

3.5. Defects

The main disadvantage of PDT is related to ROS, which causes pain by affecting nociceptors. One of the probable mechanisms is the stimulation of N-methyl-D phosphorylation, which is involved in pain mechanisms. Additionally, ROS stimulates the release of proinflammatory cytokines, including tumor necrosis factor alpha or interleukin 1 and 6 by affecting the transient receptor potential of ankyrin 1, transient receptor potential of vanilloid type 1 and aspartate receptors. [30] Other disadvantages of PDT reported in the literature include possible skin hypersensitivity to light after the procedure, lack of application in the case of disseminated metastases due to the selectivity of action and in poorly oxygenated tumors, dependence on the precision of irradiation of pathological tissue. [28] Another problem may be the difficulty in preparing advanced targeting probes used during PDT, as well as control of their quality. The costs associated with the maintenance of the required, modern equipment, as well as its preparation and transport, must also be taken into account. [6]

PDT is also associated with the occurrence of adverse effects. The literature reports skin phototoxicity, liver damage, nausea, edema, and skin reactions. [10]

Table 3. Summary of the most important advantages and disadvantages of PDT.

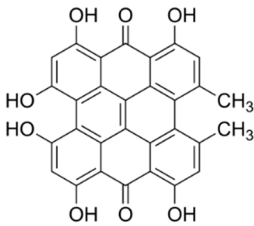
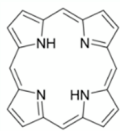
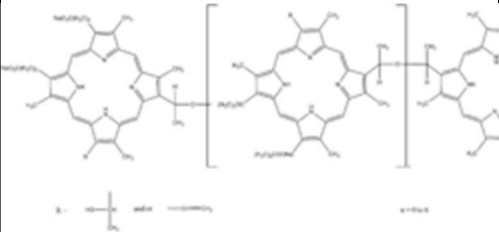
Advantages of PDT	Disadvantages of PDT
Low invasiveness	It causes pain
Low costs compared to other oncological treatment methods	Dependence on the accuracy of pathological tissue irradiation
Possibility of outpatient use	High costs of preparation, transportation, and maintenance of equipment needed for PDT
Protection of surrounding tissues	Lack of specific standards and quality control
No long-term side effects	No use in case of disseminated cancers
Leaves minor scars or no scars at all.	Not applicable in the case of deeper, less oxygenated tumors
High effectiveness	

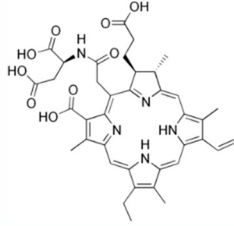
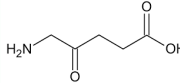
Also affecting the vascularization of the tumor - long-term effects	
Short therapy time	
Can be used multiple times and combined with other methods	

4. Application of Photodynamic Therapy in Thyroid Cancer

There are limited studies available on the use of PDT in the treatment of thyroid lesions. The main therapeutic problem is anaplastic carcinoma, which, as mentioned above, is characterized by high mortality and resistance to the proposed treatment. PDT, due to its concentration on pathological cells and the possibility of combining with other therapeutic methods, offers a chance to improve the prognosis of patients with this disease. [6] Moreover, it has been shown that the combination of PDT with chemotherapy inhibits tumor growth and induces cell apoptosis to a greater extent than chemotherapy alone, probably by increasing the internalization of anticancer drugs and combating resistance to chemotherapy. [6,10] The most commonly used PS in PDT for the treatment of thyroid cancers include hypericin, porphyrin, photophrin, radachlorin, and 5-aminolevulinic acid (ALA). [8–10,14,32]

Table 4. Summary of the molecular and structural formulas of the most commonly used PSs in PDT for thyroid cancer.

Name	Formula	Ref.	
Hipericin	C30H16O8	33	
Porphyrin	C44H34N8	34, 35, 36	
Photofrin	C68H74N8O11	37, 38	

Radachlorin	C38H41N5O9	39, 40	
5-Aminolevulinic acid (ALA)	C5H9NO3	38, 41	

Hypericin (HYP) is a potent PS isolated from St. John's wort. Previous studies have shown its efficacy in the treatment of anaplastic thyroid cancer, mainly by stimulating cancer cell apoptosis and mitochondrial damage. [6,9] In vitro studies conducted by Hyejin Kim and colleagues using HYP as PS showed that the absorption peak of HYP was 593 nm, and the degree of absorption was directly proportional to the HYP concentration, making it a suitable potential PS. The viability of anaplastic cancer cell lines under the influence of PDT using HYP decreased significantly, in an amount dependent on the HYP concentration, and this correlation was more pronounced up to a HYP concentration of 10 µg/ml. The study group, i.e. subjected to PDT using HYP, compared to the control groups, i.e. exposed to HYP only, laser only or no treatment at all, was characterized by a large number of induced cell death, significantly increased intracellular ROS and significantly decreased mitochondrial membrane potential. In the mouse study group, tumor weight and volume were almost completely reduced. The tumors in the control groups gradually increased. On day 4 of the study, necrosis was observed in the skin that was exposed to HYP-PDT, while the skin in the control group using only laser remained intact. Studies are ongoing on the combination of HYP with nanocarriers such as iron oxide, polyacrylamide, polyethylene glycol, polylactic acid, in order to increase their efficacy as PS in PDT. [9] In DTC, the PS used is mainly porphyrin-HDL nanoparticle (PLP). Its advantages include safety, low invasiveness, enabling complete ablation of pathological tissue while leaving normal thyroid tissue and the recurrent laryngeal nerve intact. [6] Studies conducted by Nidal Muhanna et al. on mouse and rabbit models have shown high efficacy in the treatment of PTC using PLP as a PS in PDT, assessing cellular toxicity, the function of the recurrent laryngeal nerve by endoscopic assessment of vocal cord mobility, and trachea and thyroid histology. After 24 h, in vitro PDT using light at a wavelength of 671 nm at 10 J/cm² was associated with significant destruction of cancer cells, with preserved viability of cells from PLP not exposed to light. It was confirmed by fluorescence imaging that PLP administered to mice systemically at a dose of 4 mg/kg accumulated in pathological tissue to a high degree, compared to healthy tissue, which showed minimal fluorescence. PLP labeled with ⁶⁴Cu after 24 hours of injection showed a significant predilection for tumor, compared to trachea, thyroid and muscles. Additionally, the TUNEL staining test revealed a significant level of apoptosis of tumor cells after PLP-PDT compared to the control group (75.7% vs. 10.6%). Studies conducted on rabbits confirmed a significantly higher accumulation of PLP in pathological tissue, compared to surrounding muscles or nervous tissue. The fluorescence signal after PLP administration also proved helpful for tumor resection together with the surrounding tissues. Also in this case, significant efficacy of PLP-PDT in inducing apoptosis of cancer cells was confirmed, compared to the preservation of normal structure of healthy cells, as confirmed by hematoxylin and eosin (H&E) staining and TUNEL test. Moreover, 24 hours after systemic administration of PLP, the rabbit recurrent laryngeal nerve was subjected to PDT and its structure was assessed histologically in relation to the non-PDT-treated recurrent laryngeal nerve. Both preparations showed minimal apoptosis and normal morphology, which indicates the safety of PDT in relation to the nervous tissue. Long-term effects of PLP-PDT also showed satisfactory results. In comparison to the control groups of rabbits with thyroid tumors left untreated and exposed only to

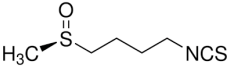
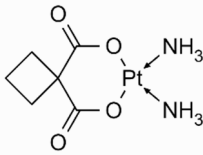
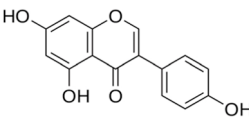
laser, in which significant tumor progression occurred, the study group was characterized by the absence of tumor cells in the histological examination of the thyroid after the 53rd day of the study, as well as a normal image of the neck region in the computed tomography scan. The absence of soft tissue pathology was also confirmed by comparing the functional state of the recurrent laryngeal nerve with the state before the study, 24 hours after and 7 days after the treatment. [14]

Looking at the promising results of studies confirming the high efficacy of 5-aminolevulinic acid in the treatment of actinic keratosis of the skin, Al-Watban et al. examined the effect of PDT using 5-ALA as PS and a 633 nm diode laser in the treatment of undifferentiated thyroid cancer. Compared to the control group, the study group was characterized by a significant reduction in tumor size already on the 7th day after treatment, and the percentages of tumor growth delay were approximately 48% after 7 days, approximately 55% after 14 days, and approximately 51% after 21 days, respectively. In 20% of the mice examined, tumor necrosis occurred. [42] The influence of PDT therapy with photophrin on anaplastic thyroid cancer cells was studied by, among others, Seung Hoon Woo and his colleagues. They proved that the degree of cytotoxicity correlated with the concentration of the photosensitizer, in this case photophrin. Cellular cytotoxicity was most pronounced in the group of cells exposed to photophrin-PDT at a photophrin concentration of 3.2 µg/ml, compared to photophrin-PDT at a photophrin concentration of 1.6 µg/ml and using only photophrin without a laser. In addition, after 24 hours, this group also showed the most marked indicators of cell death, including necrosis, apoptosis, and increased expression of caspase 3, caspase 9, and PARP. Caspase 8 expression was similar to the control group. By examining the fluorescence signal, they found that photophrin accumulated mainly in the mitochondria of anaplastic cancer cells, indicating that cell death was most likely mediated by mitochondria. [43]

The use of sulforaphane (SFE) - 4-methylsulfinyl-3-butenyl isothiocyanate is also being studied in PDT. Its main advantages are its low production cost, purity and natural origin. It works by arresting the cell cycle of cancer cells in the G0/G1 phase. Saswata Chatterjee and colleagues have shown that treatment of anaplastic cancer cell lines with SFE, before photophrin PS, and then exposing them to an LED diode is associated with a reduction in cell viability, greater than when using photophrin alone. Cells subjected to combined therapy, especially with a higher dose of photophrin (6.25 µg/ml vs. 1.5 µg/ml) were characterized by a significantly higher number of apoptotic bodies and the intensity of cell death, increased ROS production in cancer cells due to impaired mitochondrial potential, reduced expression of RAS, MEK (1/2) and ERK (1/2) proteins in cells with RAS mutations and inhibited activation of phosphorylated p38. [16] PDT may also have an impact on the inhibition of cancer tissue development by reducing the expression of EGFR and PI3K pathways. It is assumed that the therapeutic effect of carboplatin is enhanced by treatment methods acting as PI3K inhibitors, therefore the effect of combining carboplatin with PDT on the effect of anaplastic thyroid cancer treatment was examined. [8] In one of the first studies by Biswas et al. on this subject, it was shown that the percentage of cell viability of anaplastic cancer lines was inversely proportional to the increase in the concentration of radachlorin as PS in PDT, the increase in the concentration of carboplatin and the incubation time after laser irradiation. A synergistic effect in combined treatment could be observed at a concentration of radachlorin of at least 0.8 µg/ml and it was then gradually increased. Treating cells only with radachlorin did not result in significant changes in the percentage of cell viability. The use of combined therapy was associated with a much better effect than that obtained after the use of individual therapies. A significant decrease in cell viability, an increased percentage of cells showing signs of early and late apoptosis, a significant increase in the percentage of sub-G1 cells, a significant decrease in the percentage of cells in G0/G1, a significant increase in the depolarization of the mitochondrial membrane potential, a significantly increased expression of Bax, cytochrome c, caspase 3, caspase 9, caspase 12, poly ADP-ribose polymerase (PARP) and apoptosis-inducing factor (AIF) proteins, and a significant decrease in Bcl-2 expression were observed, and the values correlated with the concentration of radachlorin and carboplatin. The changes were much more marked in the case of combined treatment. [10] Further, extended studies conducted by the same group of researchers on anaplastic cancer cell lines similarly

showed that the number of live cells decreased in direct proportion to the concentration of carboplatin. In the combination of PDT with radachlorin as PS and carboplatin, the percentage of live cells decreased very significantly, to about 30%, compared to the control groups using only carboplatin (about 89%) or only PDT with radachlorin (about 80%). The combination of carboplatin and PDT with radachlorin completely inhibited the cancer cell line at concentrations of 0.05 mg/ml carboplatin and 3.12 ng/ml radachlorin. The combined therapy was characterized by apoptotic cells with irregular outlines, disruption of nuclear membranes, chromatin condensation in histopathological examination. Cell motility also decreased most in the case of combination therapy, and in the case of single therapies, this decrease was greater in the case of PDT than carboplatin. As for EGFR expression, it was unchanged in the case of carboplatin therapy and slightly decreased in the case of PDT and combination therapy. It was shown that in the case of PDT, the number of cells with increased ROS was 81.6%, compared to the combination therapy, where it was 87.1%. Mitochondrial membrane potential was moderately decreased after the treatment of cell lines with carboplatin (14.5%), a noticeable decrease in PDT (49.3%), and in the combination therapy this decrease was significant (80.6%). Cytochrome c expression, which is a marker of mitochondrial membrane depolarization and cell apoptosis associated with mitochondrial dysfunction, increased with PDT and then increased slightly in combination therapy. A relationship between PDT and combination therapy and the expression of proteins involved in the mitochondrial pathway, caspase-3 and caspase-9, was also demonstrated. Their activity increased in both cases, with a greater increase in combination therapy. Combination therapy has a significant effect on the decrease in PI3K expression, with no significant decrease in single therapies. In the case of PTEN expression, PDT showed no change, carboplatin a slight one, and combination therapy a significant one. Combination therapy was the only one to have a significant effect on the change in PI3K and PTEN expression. The size of the tumor was also compared depending on the therapy used after 3, 7, and 14 days. After 7 days, tumor volume was comparable in each of the 3 treatment groups, but inhibited compared to the control group. After 14 days, the decrease in tumor volume was most pronounced in the group of tumors treated with combination therapy, less pronounced with PDT, and least in the group using carboplatin, but the decrease was still significant compared to the control group. Additionally, in the case of tumors treated with combination therapy, significantly increased expression of caspase-3, caspase-9, and PTEN proteins, decreased expression of EGFR and PI3K, and proteolytic cleavage to the active form of poly(ADP-ribose) polymerase (PARP) were observed compared to tumors treated with single therapies. The above reports confirm that the therapeutic effect of combined carboplatin and PDT with radachlorin is due to the effect on the mitochondrial pathway and the EGFR-PI3K pathway. In addition, PDT can increase the internalization of chemotherapy drugs, which may be helpful in chemotherapy resistance, which results from reduced endocytosis of the tumor cell. [8] Jin-Chul Ahn and his colleagues conducted a study on anaplastic cancer cell lines using photophrin as PS in combination with genistein, a chemotherapeutic drug. It was shown that treatment of cells with genistein, regardless of the time interval (3, 6 and 24 h) and concentration (25, 50, 100 mM) did not result in changes in cell viability. Treatment of the cell line with PDT using photophrin at concentrations of 0.8 and 1.6 mg/ml reduced cell viability, to a greater extent at higher photophrin concentrations. However, cell viability was significantly inhibited in the combination therapy. Additionally, in the case of combined therapy compared to individual therapies, after 6 h the following changes were observed: a greater number of apoptotic cells, covering almost all cells, a greater degree of mitochondrial membrane depolarization, increased fluorescence intensity, increased expression of caspase 3, 8, 9, 12, PARP, CHOP, Bax, cytochrome c, AIF and a higher level of ROS, and the above changes were more pronounced when using higher concentrations of photophrin. [32]

Table 5. Summary of information on sulforaphane, carboplatin and genistein - substances used to enhance PDT.

Name	Formula	Name	Structure	Ref
Sulforaphane	C ₆ H ₉ NOS ₂	4-methylsulfinyl-3-butenyl isothiocyanate		16, 44, 45
Carboplatin	C ₆ H ₁₂ N ₂ O ₂ Pt	(SP-4-2)-diamino[cyclobutane-1,1-dicarboxylate(2-)-O,O']-platinum		46
Genisteina	C ₁₅ H ₁₀ O ₅	5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one		47

5. Future Directions

Currently conducted research aimed at increasing the efficiency and effectiveness of PDT focuses on improving the stimulation, biocompatibility, localization, inactivation, singlet oxygen capture and reducing toxicity to the surrounding tissues. [29,35,48] The solution may be the use of nanotechnology achievements, such as combining PS with antibodies, magnetic nanoparticles or molecules with another, specific structure. [35,49,50] So far, the effectiveness has been demonstrated by using the folic acid receptor or the epidermal growth factor receptor for this purpose. [49] Current research also focuses on modifying PS so that it absorbs in the NIR spectrum, and thus - improves tissue penetration. [35] Self-luminescent systems are beginning to be used, including chemiluminescence or bioluminescence. [36] The solution may also be the use of X-ray induced photodynamic therapy. [51] Another challenge is the measurement of oxygen in pathological tissue before and during PDT, and increasing the oxygenation of the tissue undergoing therapy. Research is ongoing to increase oxygen production at the site of the lesion and to deliver oxygen to the site of the lesion using a carrier. [6,28] Another problem is the generation of anti-tumor immunity, which can be solved by using anti-tumor preparations, such as cancer cells subjected to PDT or vaccines in the form of cancer cell lysates. [49] Research is also ongoing to improve the effect of PDT on gene expression and immune reactions. [48,51]

Summary

Thyroid cancer is one of the most common endocrine cancers worldwide. It is divided into differentiated thyroid cancers, including papillary, follicular and Hurthe cell; medullary and anaplastic cancer. In addition to histological differences, they also show differences in terms of occurrence, prognosis, genetic background, diagnostics and therapeutic options. Currently, the most commonly used treatment methods - surgical treatment, chemotherapy, radiotherapy, radioactive iodine ablation, thyroid hormone suppression - are effective in lower advanced cancers, while in the case of lower differentiated, disseminated, recurrent cancers, they are not sufficient. Photodynamic therapy brings hope, which due to its minimally invasiveness, the possibility of outpatient use and combination with other techniques, the possibility of repeated use, leaving a small scar or no scar at all, is superior to classical methods. Studies on the effect of PDT with hypericin, porphyrin, photophrin, radachlorin or 5-aminolevulinic acid as PS on thyroid cancer have shown promising effects, including a decrease in cell viability, a high percentage of apoptotic cells, an increased percentage of cells showing features of early and late apoptosis, a significant increase in the

percentage of sub-G1 cells, a significant decrease in the percentage of cells in G0/G1, a high degree of mitochondrial membrane depolarization, an increase in fluorescence intensity, increased expression of caspase 3, 9, 12, PARP, CHOP, Bax, cytochrome c, AIF, a significant decrease in Bcl-2 expression, a decrease in tumor mass, a decreased expression of EGFR and PI3K, proteolytic cleavage to the active form of poly(ADP-ribose) polymerase and a high level of ROS. The changes were particularly marked in higher concentrations of the photosensitizer or in combination with sulforaphene, carboplatin, or genistein.

Photodynamic therapy is undoubtedly a method of the future, but requires further studies to validate and standardize its use.

List of Abbreviations

5-ALA	5-aminolevulinic acid
AIF	apoptosis-inducing factor
AJCC	American Joint Committee on Cancer
ATA	American Thyroid Association
ATC	anaplastic thyroid cancer
BACC	fine-needle aspiration biopsy
DTC	differentiated thyroid cancer
EGFR	epidermal growth factor receptor
FTC	follicular thyroid cancer
H&E	hematoxylin and eosin
HCC	Hurthle cell carcinoma
HYP	hypericin
MAP, MAPK	mitogen-activated protein kinase
MEN	multiple endocrine neoplasia
MKI	multikinase inhibitors
MTC	medullary thyroid cancer
NIS	sodium-iodine symporter
PARP	poly(ADP-ribose) polymerase
PDT	photodynamic therapy
PI3K	3-kinase phosphoinositide
PLP	porphyrin-HDL
PLP-PDT	PDT using PLP as PS
PS	photosensitizer
PTC	papillary thyroid cancer
PTEN	phosphatase and tensin homologue
RAI	radioactive iodine therapy
RFI	radiofrequency ablation
ROS	reactive oxygen species
SFE	sulforaphene
TNM	tumor, nodules, metastases
TSH	thyroid-stimulating hormone
USG	ultrasound examination

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