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The History of Development of Fluorescence Diagnosis and Photodynamic Therapy and Their Capabilities in Oncology

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Abstract—The main steps of development of fluorescence diagnosis and photodynamic therapy were overviewed. Brief historical data were presented about Photohem, Photosens, Alasens, Radachlorin, and Photoditazine photosensitizers, along with their corresponding pharmacokinetic properties, mechanisms of action, and specific features of use. Key aspects of development of fluorescence diagnosis and photodynamic therapy in Gertsen Moscow Research Oncological Institute were described. The main indications for photodynamic therapy and its efficiency in patients with oral, tongue, stomach, esophageal, skin, cervical, and central lung cancer and vulval lesions were demonstrated. It was shown that photodynamic therapy is effective for adjuvant or intraoperative treatment in patients with high risk of local recurrence after surgical treatment, and palliative photodynamic therapy increases the quality of life and survival rates in the most challenging group of cancer patients. The results of photodynamic therapy for cervical pre-cancer and cancer associated with human papillomavirus were reported. The main trends of intraoperative "fluorescence guidance" with Alasens were covered. Clinical experience allows rating photodynamic therapy among the most encouraging methods for the current medicine.

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Cancer control remains among the top priorities for medicine. The World Health Organization predicts that, in 1999–2020, the cancer morbidity and mortality will double worldwide, so the development and introduction of new high-technology methods of early diagnosis and treatment of cancer constitute a crucial task faced by modern medicine. Examples of such methods can be found, in particular, in fluorescence diagnosis and photodynamic therapy whose application has become possible only in the last few decades, after the invention and development of laser technology. However, the development of the very methods utilizing the photochemical properties of medicinal drugs dates back to more than one century.

Exogenous or endogenous photosensitizers are applied in photochemotherapy as initiators of the chemical reaction proceeding in the biological tissue. The photochemotherapy concept is not new; it was employed centuries ago in treatment of vitiligo in India, Egypt, and China. The first detailed description of the effect of chemical photosensitization of biological tissue was given in the early XX century. It is commonly believed that the modern scientific and experimental approach to photosensitization studies has its reference point in the work by O. Raab, published in 1900 [1]. When doing his medical student's research work at the University of Munich as supervised by Prof. H. Tappeiner, Raab found that, at low concentrations, acridine and other series dyes, chemically inert in the dark, led to rapid death of paramecia under sunlight irradiation. That finding received a high opinion from Tappeiner who suggested that this effect would find medicinal application and introduced the term "photodynamic effect" (to avoid confusion with similar photochemical effects involved in photographic practice), which is still widely used today [1, 2].

In 1903, jointly with H. Jesionek from the Munich Dermatological Clinic, Tappeiner published the results from clinical application of eosin dye and light in

treatment of herpes, psoriasis, and skin cancer [3]. After a while, in 1905, fluorescein, along with eosin, was used as photosensitizer by those researchers.

Subsequent study of the photodynamic effect had culminated in the development of a new direction in medicine, photodynamic therapy of malignant tumors.

In the 1920s, it was found that, along with other numerous properties, malignant tumors exhibit an ability to accumulate porphyrins exhibiting fluorescent properties under UV radiation exposure. An important step towards development of the methods of fluorescence diagnosis and photodynamic therapy of cancer was made in 1924 by A. Policard who revealed accumulation in animal tumors of endogenous porphyrins able to fluoresce under UV irradiation [4]. In 1942, H. Auler and G. Banzer from the University of Berlin recorded red fluorescence in the primary tumor and metastases in rats subjected to subcutaneous and intramuscular administration of hematoporphyrin [5].

The modern stage of development of fluorescence diagnosis and photodynamic therapy began in the 1960s, when R. Lipson et al. (the United States) revealed the possibility to record the fluorescence of porphyrin in tumors of cancer patients who were administered the hematoporphyrin derivative prepared by acetylation and reduction of a porphyrin mixture enriched in hydrophobic oligomers [6, 7]. It is commonly accepted that wide clinical use of photodynamic therapy of cancer began in 1978, when T. Dougherty et al. reported the results from application of this method for treatment of 25 patients with 113 primary, recurrent, and metastatic skin tumors [8].

In Russia, photodynamic therapy of tumors has been the subject of experimental research for many years, but it was not until 1992 that it received development in clinical practice, when the first dosage form of the domestic photosensitizer Photohem from a group of hematoporphyrin derivatives was created [Prof. A.F. Mironov, Lomonosov Moscow Institute of Fine Chemical Technologies (now, Lomonosov Moscow State University of Fine Chemical Technologies)]. Successful clinical trials were conducted at Gertsen Moscow Research Oncological Institute, Ministry of Health Care and Social Development of the Russian Federation, Federal State Budgetary Institution (MNIOI), and the State Scientific Center of Laser Medicine, Federal Medicobiological Agency, Ministry of Health Care and Social Development of the Russian Federation, Federal State Institution.

After two years (in 1994), clinical trials of second-generation photosensitizer Photosens (sulfonated aluminum phthalocyanine), developed at the Research Institute of Organic Intermediates and Dyes, State Scientific Center, Federal State Unitary Enterprise (FSUE SSC "NIOPIK") (Corresponding Member of RAS Prof. G.N. Vorozhtsov, Prof. E.A. Luk'yanets) were started. In 1999, clinical application of Alasens, a 5-aminolevulinic acid-based drug (GHTs NIOPIK, Corresponding Member of RAS Prof. G.N. Vorozhtsov, Prof. E.A. Luk'yanets) and in 2002 and 2004, that of drugs prepared from e_6 chlorin, Radachlorin [Radafarma, Limited Liability Company, A.V. Reshetnikov, Cand. Sci. (Chem.)], and Photoditazine (VETA–GRAND, Limited Liability Company, Prof. G.V. Ponomarev), respectively, were begun.

In MNIOI, experimental research on fluorescence diagnosis and photodynamic therapy were started about 30 years ago, and their first results were reported at the IV All-Union Conference on Chemistry and Application of Porphyrins in 1984 [9]. Clinical trials have been underway since 1992. By now, techniques based on the use of these of drugs were developed, as well as methodological guidelines and manuals for medical practitioners and training programs for specialists.

The introduction of new treatment methods in Russia was facilitated by the development of appropriate domestic diagnostic and therapeutic equipment. The available instrumental base and domestic photosensitizers make photodynamic therapy not only a highly effective but also an economically feasible method.

The photodynamic therapy method is suitable for treatment of tumors in virtually all major locations, both independently and in combination with traditional therapies (surgery, radiotherapy, and chemotherapy). Combination therapies aim to improve the radical and palliative treatment of the most challenging groups of cancer patients. For enhancing the effectiveness of photodynamic therapy, different versions of laser irradiation procedure (single- and multiple-point, invasive and noninvasive, interstitial, etc.) were developed for different stages, types, and shapes of tumors.

Currently, the techniques developed are being applied in MNIOI for diagnosis and treatment of patients with tumors in various stages and in various locations.

The fluorescence diagnosis and photodynamic therapy methods are based, essentially, in administration by a patient of photosensitizers that are selectively

accumulated in the tumor tissue and, under light (in particular laser light) exposure, depending on the wavelength and the irradiation mode, either emit a light quantum (whereby their fluorescence can be recorded) or stimulate the formation of cytotoxic agents, above all singlet oxygen $^1O_2$ and reactive radicals, whose accumulation leads to destruction and death of the vital structures of the tumor cells. Along with direct phototoxic effect exerted on the tumor cells, certain role in the destruction mechanism is played by circulatory disorders in the tumor tissue due to endothelial damage and thrombosis of blood vessels, as well as by cytokine responses caused by stimulation of production of tumor necrosis factor and interleukins and by macrophage and leukocyte activation.

In recent years, studies into immunomodifying and stimulating effects of photodynamic therapy have received increased attention. The experience of MNIOI shows that photodynamic therapy exerts a stimulating effect on neutrophils and some other immunity indicators.

Thus, the fluorescence diagnosis and photodynamic therapy sessions constitute a multistage procedure giving rise to multicomponent responses to therapy. This creates the need for new approaches to clinical trials of photosensitizers for the purpose of development of medical techniques.

With a view to optimizing the diagnosis and therapy regimes for domestic photosensitizers from different classes, the "Program for Clinical Trials of New Photosensitizers" was developed in MNIOI [10]. In accordance with the clinical trial stages, studies of photosensitizers from different groups were conducted. In particular, the tissue distribution kinetics of these drugs in cancer patients was examined, which allowed optimizing the fluorescence diagnosis and photodynamic therapy techniques. Also, the interstitial distribution kinetics of photosensitizers was examined for the purpose of identifying biological targets, since the implementation of therapeutic effects is directly dependent on the structures of the tumor node, which act as the photosensitizer accumulation sites during a photodynamic therapy session. The presence of a photosensitizer in the tumor cells causes direct injury to them, and its accumulation in the vessel-rich tumor stroma leads to ischemic necrosis due to blood vessel thrombosis and destruction. Therefore, a correct planning procedure for implementation of efficacious photodynamic therapy techniques requires that the photosensitizer accumulation and distribution data be available for tumor and normal cells and tissues. Obtaining these data was specifically the aim of this study.

The photodynamic therapy sessions with Photohem showed that this drug accumulates predominantly in the tumor tissue, with the fluorescence contrast ratio tumor/norm reaching a maximum 24–48 h after intravenous administration. In this case, ischemic necrosis prevails because Photohem accumulates in the vessel-rich tumor stroma in a larger amount than in the tumor cells. A not very high content of the drug in the wall of the unaltered vessels may indicate a low risk of ischemic injury of unaltered tissues across the laser-exposed zone for the standard irradiation regimes.

In the case of Photosens, ischemic necrosis of the tumor dominates. The maximal fluorescence levels of the drug in the tumor structures were recorded 2–8 h after intravenous administration, during which period the maximum injury was induced to the tumor. The presence of a significant amount of Photosens in the tumor structures within 1 week after intravenous administration allows conducting photodynamic therapy sessions in this period after single injection of the drug. Identical amounts of Photosens were revealed in the tumor structures and in the wall of the adjacent unaltered vessels after 1–2 h, which may be responsible for blood vessel injuries and ischemic changes in tissues across the laser-irradiated zone.

With chlorin e_6-based photosensitizers, the prevailing effect is ischemic necrosis of the tumor. The optimal interval between intravenous administration of the drug and the therapy session is 3–8 h, during which period the content of chlorin e_6 photosensitizers in the tumor structures is at a maximum. Virtually identical amounts of the photosensitizer are contained in the tumor structures and in the walls of the unaltered vessels 3–4 h after intravenous administration of the drug. This can lead to injury of normal vessels across the laser-irradiated zone and to ischemic changes in healthy tissues during this period in the case of laser irradiation at high doses.

Alasens-induced protoporphyrin IX (PPIX) is the only photosensitizer that preferentially accumulates in the tumor cells, whereby direct cytotoxic effect is exerted during the photodynamic therapy. Studies of the tissue distribution kinetics for Alasens-induced PPIX in tumors with different locations and in the unaltered tissues showed that, depending on whether Alasens is administered locally or systemically, the optimal interval between the drug administration and

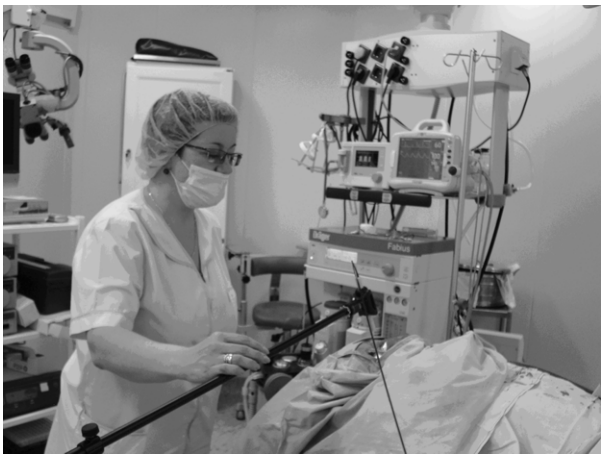

Fig. 1. An intraoperative photodynamic therapy session.

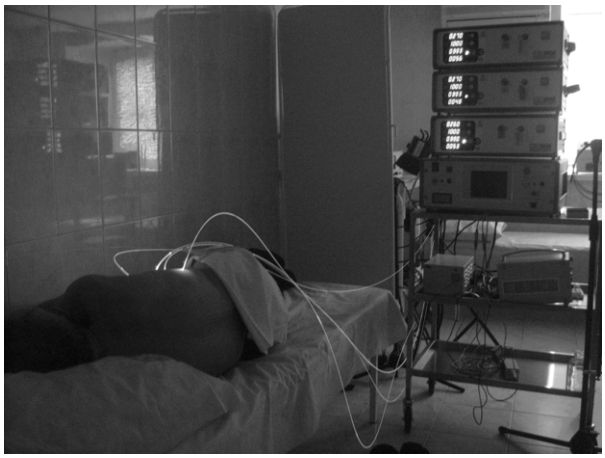

Fig. 2. An intrapleural photodynamic therapy session.

the diagnosis and therapy session is 1–3 or 3–6 h, respectively.

Thus, methodological approaches to the development of medical techniques were determined. Specifically, the optimal time for fluorescence diagnosis and photodynamic therapy with each photosensitizer was identified; it was shown that a number of photosensitizers (Photohem, chlorin e_6-based drugs, and Alasens-induced PPIX) are rapidly eliminated from the tumor tissue, so these drugs can be used for single and multiple courses of therapy. At the same time, other drugs (Photosens) are retained in tumors for prolonged time, which allows conducting repeated therapy sessions after single injection of the drug (prolonged photodynamic therapy). Studies showed which of the effects, direct destruction of the tumor cells or ischemic necrosis due to destruction of the vessel-rich stroma of tumor, results from therapy sessions with different photosensitizers. These findings allowed development of fluorescence diagnosis and photodynamic therapy techniques which underlie the medical techniques of interest (Fig. 1).

The results from application of these techniques indicated their high efficiency. In treatment of precancer and cancer stage T1N0M0, complete tumor regression with long-term disease-free state was achieved in patients with oral mucosa and tongue cancer (64.4% cases), gastric cancer (72.6%), esophageal cancer (77.1%), central lung cancer (86.5%), skin cancer (99.6–100%), and cervical cancer (84–100%), as well as in patients with vulva pathology (92.5%).

When used for adjuvant or intraoperative treatment, photodynamic therapy is effective in patients with high risk of local recurrence of tumor after surgical treatment. For example, in a group of patients with superficial urinary bladder cancer, treated with adjuvant photodynamic therapy in combination with mitomycin C after transurethral resection, the recurrence-free survival in the follow-up period of up to 24 months was 100% (in the control group, recurrence was detected in 50% of the patients); in patients with metastatic brain tumors after surgery with intraoperative fluorescence diagnosis and photodynamic therapy, the continued tumor growth in a period of 1 to 6 months was diagnosed in 4.2% of cases (in the control group, 30.3%); in patients with nonorgan retroperitoneal tumors the rate of recurrence after surgery with intraoperative photodynamic therapy was 12% (against 50–80% after surgical treatment).

The use of palliative photodynamic therapy techniques led to improved quality of life and increased survival rates in the most challenging groups of cancer patients (Fig. 2). In treatment of intradermal metastases of breast cancer and melanoma after prolonged photodynamic therapy, complete tumor regression was achieved in 39.3 and 38% of the patients, respectively, and partial tumor regression, in 46 and 52.4% of the patients, respectively. Multiple-course photodynamic therapy allowed restoring the natural diet in 100% of patients with stenosing esophageal cancer, and prolonged intrapleural photodynamic therapy led to stable termination of intrapleural exudation in 92% of the patients with mesothelioma and metastatic pleural lesions over the follow-up period of up to 3.5 years.

In recent years, a correlation between the development of cancer and a viral infection was established for a number of locations. Hence, the impact of photodynamic therapy on virus-associated

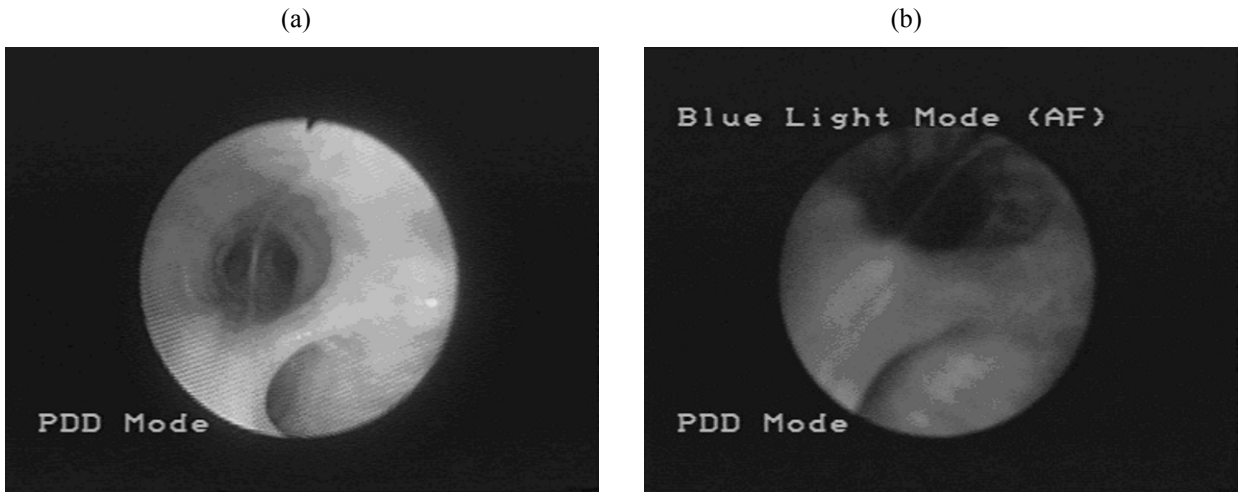

Fig. 3. A fluorescence bronchoscopy session: (a) white light inspection and (b) fluorescence examination (red fluorescence from a hidden focus of III stage dysplasia at the spur of a segmental bronchus).

tumors in different locations constitutes a very topical issue. Studies conducted in MNIOI showed that, along with getting high oncological results, photodynamic therapy of cervical pre-cancer and early cancer associated with human papillomavirus (HPV) allowed achieving complete eradication (destruction) of HPV in 92.3% of the patients. The activity of HPV was reduced in 4.7%, and no effect against HPV was revealed in 3% of the patients. In treatment of pre-cancer and early cancer against background of virus-associated laryngeal papillomatosis, complete tumor regression was achieved in 82.9%, and partial regression, in 17.1% of the patients; total eradication of HPV was revealed in 70%, reduced HPV activity, in 11%, and no effect against HPV, in 19% of the patients; the interrecurrent period increased threefold (from 4 to 12 months).

The Alasens-based fluorescence diagnosis techniques developed allow the tumor boundaries to be more precisely identified when planning surgical treatment and photodynamic therapy, as well as hidden foci of early primary and superficial recurrent skin and hollow (tubular) organ mucosa cancer to be effectively revealed (Fig. 3).

In the studies performed, the sensitivity and specificity of fluorescence diagnosis with Alasens in patients with different tumors were as follows: 100 and 88%, respectively, for upper respiratory tract tumors, 96 and 98% for upper gastrointestinal tract tumors, 87.5 and 95.7% for colon tumors, 98.4 and 76.6% for bladder tumors, 100 and 97.9% for endometrial tumors, 89.1 and 88.4% for pleural tumors, and 87.5 and 76% for peritoneum tumors. These studies diagnosed hidden foci of pre-cancer, early cancer, and superficial recurrences of skin cancer in 25.5% of the patients and in 19.4% of the patients with upper respiratory tract cancer, as well as hidden foci of metastatic lesions of pleura in 57.2% of the patients, and those of peritoneum, in 15.5% of the patients. The intraoperative fluorescence diagnosis technique developed allows prompt detection of lymph node metastases at the first level with the sensitivity of 87.2% and specificity of 94.8%.

Over the last years, intraoperative "fluorescence guidance" with Alasens has been developed. Depending on the task set, the application of this technique has two directions: improving the surgical radicalism in order to completely remove the tumor under fluorescence control or, alternatively, identifying the resection boundaries in order to prevent accidental trauma or removal of healthy tissues. An example of the first direction can be found in widespread surgical interventions aimed to remove the tumor, e.g., brain or bladder tumor, under fluorescence control. Using the "fluorescence guidance" technique it is possible to visualize and remove the fluorescent foci of residual tumor tissue at the tumor resection bed (in the case of surgical treatment of brain tumors) and/or diagnose and remove hidden cancer foci on bladder mucosa (whose changes were not visible under white light) during fluorescence-guided transurethral resection. An example of the second direction of "fluorescence guidance" is identification of parathyroid glands aimed to preserve them during surgery of thyroid gland tumors.

The fluorescence diagnosis and photodynamic therapy techniques developed in MNIOI are demanded by medical institutions. A program for specialists training on fluorescence diagnosis and photodynamic therapy techniques was developed, and over the 1995–2011 period training was received in MNIOI by 192 medical practitioners from Russia, and by 20, from neighboring (3) and far (17) countries.

Further improvement of the photodynamic therapy technique requires finding new photosensitizers having higher photoactivity, possessing better tumor-tropic properties, and excitable in the near-infrared spectral region, as well as designing highly sensitive and reliable diagnostic and therapeutic equipment. Clinical experience with photodynamic therapy allows rating this method among the most promising directions in modern clinical oncology.

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