The First Experience of Photodithazine Clinical Application For Photodynamic Therapy of Malignant Tumors

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ABSTRACT

In 1998 a new second-generation photosensitizer was introduced in Russia - a chlorin E6 derivative named Photodithazine - water-soluble preparation with absorption peak at 668 nm. This novel preparation has passed pre-clinical *in vitro* and *in vivo* studies, in 1999 a limited series of clinical testing of photodynamic therapy with photodithazine has been performed in the State Research Center for Laser Medicine of Russian Ministry of Public Health.

In comparison to hematoporphyrin derivative (Photofrin, Photosan, Photoheme etc.) and aluminum phthalocyanine (Photosense) used in clinical practice, Photodithazine has a number of special properties. Most significant of them is rapid tissue accumulation and clearance from the organism within 48 - 72 hours.

Photodithazine was used as a photosensitizer in 44 patients with malignant tumors of skin, oral cavity, larynx, bronchi, esophagus, stomach, vulva and other locations.

The dose of photodithazine ranged from 0.8 to 1.2 mg/kg of body weight. Laser irradiation has been performed on a solid-state laser device "Poljus-2" with wavelength 670 nm and output power 1000 mW. Energy density ranged from 100 to 400 J/cm^2. Laser irradiation of the inner organ was performed via flexible endoscope.

PDT with Photodithazine was not accompanied with systemic and local complications. The treatment was well-tolerated by the patients, including those with severe accompanying diseases. There were no complications associated with possible high skin photosensitivity in all treated group. Therapeutic effect took place in all cases, including complete tumor regression in 22 cases (53,7%) and partial regression in 19 cases (46,3%).

By present time clinical PDT with Photodithazine is in the initial state. Photodithazine-based PDT appears to be a very promising technique, but analysis of long-term follow-up results is necessary to confirm its therapeutic efficacy. Further research is required to estimate adequate doses of drug and light for various types of tumors.

Keywords: cancer, tumor, drug, photodynamic therapy, photosensitizer, chlorin E6, clinical use

INTRODUCTION

Until the end of the last year only two photosensitizers had been clinically used in Russia. One of them is known to be hematoporphyrin derivative named Photoheme (λ_{max} 630 nm, $E^{1\%}_{1\ cm}$ = 45, borate buffer, pH 9.2; Photofrin II analogue), and the other - sulfonated aluminum phthalocyanine named Photosense (λ_{max} 672 nm, $E^{1\%}_{1\ cm}$ = 1360, borate buffer, pH 9.2). They both have specific photodynamic activity, but the main disadvantage of them both is long-term skin phototoxiciry. Photodithazine is a promising second-generation photosensitizer for photodynamic therapy of tumors (PDT).

Comparing to Photoheme, Photodithazine possesses an intensive absorption band in the long-wave red part of the spectrum (λ_{max} 668 nm, $\varepsilon = 4.8*10^4$ M" cm^{-1}) where biological tissues are transparent to considerable extent as well as fluorescence at 660-680 nm (half-width of the band).

The most well-known chlorin photosensitizers are derivatives of chlorin E6. It has already been shown[1, 2, 3] that some of them favor the tumor-to-normal ratio 10:1 in experiments with tumor-bearing animals after i.v. injections of 5-200 mg/kg doses. Easily obtained from pheophorbide a in alkaline conditions[4], chlorin E6 was by itself well biologically studied [5, 6, 7]. It has been known to be suitable for PDT for long. However, to our knowledge, there were no reports about clinical applications of chlorin e6, mainly because of difficulty of its high-scale production from biological substrates. There is a patented procedure at our disposal now allowing for preparation of a stable, well water soluble and filtrating form of chlorin E6 - "Photodimazine"[8].

MATERIALS AND METHODS

In the State Research Center for Laser Medicine there had been performed a limited series of clinical testing of PDT with Photodimazine in volunteer patients. All patients had been informed about the search character of the treatment and signed in a printed form of agreement. Tumor locations, hystological types, number of patients and number of tumors are presented in table 1.

Table 1: Photodynamic therapy of malignant tumors with Photoditazine

Hystological type and location of tumor	Number of patients	Number of tumors
Basal-cell skin cancer	24	111
Squamous-cell skin cancer	2	2
Primary skin melanoma	1	1
Intradermal melanoma metastases	1	63
Squamous-cell oral cavity cancer	2	2
Squamous-cell oesophagus cancer	2	2
Stomach adenocarcinoma	2	2
Squamous-cell larynx cancer	5	5
Squamous-cell lung cancer	2	2
Squamous-cell vulva cancer	1	1
Transional-cell bladder cancer	1	1
Non-differentiated parotid gland cancer	1	1
TOTAL:	44	193

In vivo studies of Photodithazine revealed its very fast accumulation in tissues of the organism and rapid elimination. The peak of tissue accumulation was found to be on 1-2 hours after the injection of Photodithazine. Tumor to normal ratio was of about 10:1. In 24 hours after the drug injection *ex vivo* studies showed, that only 6% of the administered dose of Photodithazine remained in tissues and blood; 48 hours after the drug administration its concentration decreased to 2%. Taking these data in consideration we performed Photodithazine-based clinical PDT at the following parameters:

Photodithazine was injected intravenously in doses of 0.8-1.2 mg/kg. In the majority of cases a dose of 1.0 mg/kg was used. Quick peak of accumulation in tumors allowed for the light to be delivered in 1-2 hours after the drug injection.

Light doses ranged from 150 in superficial skin tumors to 400 J/cm^2 in cancer of oral cavity at

fluency rates from 100 to 500 mW/cm^2, using solid-state therapeutic laser "Poljus" with wavelength 670 nm and output power of about 1 W. For irradiation of superficial tumors bare-tip fibers or fibers with microlens were used. For laser irradiation of the inner organ (larynx, esophagus, stomach) via endoscopic devices we used fibers with 5-, 10-, 20- and 40-mm length diffusing tips.

Most of the patients (35) were treated by a single course PDT. Repeated PDT courses were performed in 9 cases: 6 patients underwent 2 courses of PDT with Photodithazine, 2 patients received 3 courses. A patient with multiple intradermal metastases of melanoma underwent 5 repeated courses of PDT. Minimal interval between courses was 4 weeks, maximal - 3 months.

PDT was applied for recanalization of hollow organ in 5 cases: obturative cancer of esophagus (2), cancer of bronchi (2) and obturative cancer of cardia (1). In these cases aim of PDT was completely palliative. In all patients blood tests (clinical and biochemical analysis) were performed before treatment, in 1, 7 and 14 (partially) days after PDT. Arterial blood pressure was controlled daily. Blood tests yielded normal results. In one patient with unstable arterial hypertension a peak of blood pressure (up to 220/110 mm Hg) was observed on the first day after PDT, it has been controlled by typical hypotensive drugs. All patients were treated in the in-patient clinic with hospitalization in sunlight-protected rooms. Two days after PDT the first three of our patients were asked to perform a simple sunlight sensitivity test. They exposed their hands to direct sunlight for 10 and 15 minutes. None of those patients complained of pain, heating or other subjective symptoms of phototoxicity. Sunburns, redness, hyperemia and other objective signs of skin phototoxicity were not mentioned. Nevertheless we recommended all patients to avoid direct sunlight for at least one week after PDT.

RESULTS

Photodithazine injections did not affect anyhow the state of health of the patients. It has been found not to cause any late complications. The skin sensitivity to sunlight ceased in 1-2 days. There were no complications associated with possible high skin photosensitivity in all treated patients.

Local photodynamic reaction started immediately on the 3-5 minute of irradiation in all cases. Light local edema was the first sign of PDT affection on the tumor. Some patients, especially those with ulcerated tumors of the skin and vulva, felt mild pain in the irradiation zone. There was no necessity of analgesics administration. By the end of laser irradiation session tumor surface in most cases became wet with exudate, dark, with visible signs of hemorrhagic nesrosis. In those ulcerated lesions, which had initial slight hemorrhage, complete hemostasis took place. Tumor site and surrounding 1.5-2 cm zone became anemic. Edema and hyperemia of the surrounding tissues appeared in 30-60 minutes after PDT session, involving about 3 cm of surrounding skin. In cases of oral mucose tumors edema of cheeks took place.

Tumors of the hollow organ responded in the same manner, but the edema was not so manifesting. We never met edematous obturation of esophagus or bronchi. Neither we met hemorrhage after PDT.

The peak of local reaction took place one day after PDT. Tumors developed complete hemorrhagic necrosis. Color of skin tumors changed to dark-violet or black, line of demarcation appeared, splitting necrotic tissue and surrounding skin. Tissue blood circulation restored at least partially -anemia was not observed. Edema of the surrounding tissues was maximal by the first day after PDT and gradually decreased in 3-5 days. Tumors of oral cavity also became dark in color. Layer of fibrin appeared on the surface. Response of visceral tumors was similar. None of the patients complained on pains. Fever or other signs of intoxication were not observed.

One week after PDT local changes were only visible in tumor site. For skin tumors formation of solid crust was completed by that time. Tumors of oral mucosa, esophagus, stomach were completely covered with a thick layer of fibrin. Surrounding tissues returned to initial state, no signs ofedema, hyperemia were observed.

Healing of the sites took place in terms from 2 weeks to 1 month, depending on initial tumor size. Only in one case complete healing was delayed until 1.5 months - in a patient with massive (3.5 x 3.0 x 0.7 cm) infiltrating tumor of the cheek skin.

Clinical response was evaluated 6 weeks after PDT according to the following parameters:

- Complete regression (CR) was stated when there was no visual and palpated lesion confirmed by negative results of the hystological or cytological examination.
- Partial regression (PR) was stated when reduction of maximal size of the malignant node was by 50%, as well as when there was visual absence of the tumor but malignant cells were revealed by morphological investigations (in such a way some recurrences after PDT were found).
- Tumor reduction by less then half size or status idem of the tumor were considered as no response (NR).

The results of PDT with Photodithazine are presented in table 2.

Table 2: Results of photodynamic therapy of malignant tumors with Photoditazine

Hystological type and location of tumor	Tumor regression		Not assessed	TOTAL
	Complete	Partial		
Basal-cell skin cancer	12	9	3	24
Squamous-cell skin cancer	1	1		2
Primary skin melanoma	1			1
Intradermal melanoma metastases		1		1
Squamous-cell oral cavity cancer	2			2
Squamous-cell oesophagus cancer		2*		2
Stomach adenocarcinoma	1	1*		2
Squamous-cell larynx cancer	4	1		5
Squamous-cell lung cancer		2*		2
Squamous-cell vulva cancer	1			1
Transional-cell bladder cancer		1		1
Non-differentiated parotid gland cancer		1		1
TOTAL:	22	19	3	44

* - palliative PDT for lumen reopenning

Clinical testing showed high efficiency (100% response) of PDT with Photodithazine in the treatment of cancer of various locations and melanoma. Complete regression of tumors was achieved in 53.7% of cases; in 46.3% of cases partial regression was stated. Unfortunately, three patients receiving PDT for basal cell skin cancer were lost from the follow-up due to non-medical causes. In 5 patients palliative PDT for recanalization of hollow organ led to satisfactory result. Although complete tumor regression was not achieved, the quality of patients' living was

significantly improved.

The data presented are yet only short-term results. All patients are being followed-up and the long-term results will be presented in further publications.

DISCUSSION

Since 1992 clinical research of PDT with first- and second-generation photosensitizers is being carried out in the State Research Center for Laser Medicine. Our team has gained certain experience in both Photoheme- and Photosense-based PDT [9, 10, 11]. Taking in consideration the results of pre-clinical tests of Photodithazine we were curious if its clinical application would be successful and the results comparable to those with known first- and second-generation photosensitizers.

Typical scheme of Photoheme-based PDT presumes laser irradiation in 48 hours after photosensitizer injection. Photosense in general was being injected 24-48 hours before irradiation. Rapid tissue uptake and elimination of Photodithazine allowed us to perform laser irradiation in 1-1.5 hours after injection. In these conditions local changes were clinically close to those with Photoheme and Photosense, although not completely similar.

Unlike in Photoheme-based PDT, necrotic changes in tumor by the end of irradiation were visible with Photodithazine in the majority of cases. This clinical picture was close to that with Photosense, but in Photosense-PDT tumor necrosis volume did not significantly change in the following days. In several cases of PDT with Photodithazine necrotic changes in tumor were visible already during the procedure, but in the first 3 days the border of necrotic zone widened significantly. This was, to our opinion, due to the vascular effect, so-called "ischemic necrosis". Immediate tumor changes are related to the photosensitizer's direct cytotoxic activity and make up the following sequence: *Photodithazine=Photosense>Photoheme*.

Local edema appeared during the irradiation session in all cases. Terms of edema occurrence were the same for all photosensitizers. Degree of manifest of edema was not the same and unlike Photoheme, Photodithazine caused massive edema of the surrounding tissues. Like Photosense, Photodithazine PDT was often followed with formation of anemic zone, very rarely met with Photoheme. Edema, hyperemia and especially anemia of tissues are, to our opinion, symptoms of high vascular PDT activity of Photodithazine. Vascular activity assessment makes the following sequence: *Pholosense>Photodithazine>Photoheme*. Terms of disappearance of mentioned symptoms did not differ.

Pain and sense of heating during and in first hours after the procedure were mild and even in such painful areas as oral cavity and vulva were controlled by oral analgesics. Compared to other photosensitizers pain test would be as follows: *Photosense>Photodithazine>Photoheme*. It is necessary to mention that pain is not an objective, but subjective criterion; nevertheless we found it possible to report on some difference in this symptom.

Two days after PDT the patients were not sensitive to sunlight. This is a very important result. All photosensitizers used before caused long-term skin photosensitivity up to 6-8 weeks. The possibility of application of effective photosensitizing agent with rapid elimination from the organism brings up the new stage of clinical photodynamic therapy. In many cases long-term photosensitivity was a cause of patients' and physicians' refuse from PDT. Introduction of Photodithazine completely solves this problem.

One week after PDT changes were only visible in the irradiated site. Surrounding tissues returned to initial state. Same as in PDT with Photoheme and Photosense irradiated sites were

covered with crust. Terms of wound healing did not differ between photosensitizers and mostly depended on the size of rumors. Local outcome of Photodithazine-based PDT was not significantly different from Photoheme and Photosense. We found good and excellent cosmetic results in the majority of cases.

CONCLUSIONS

A novel photosensitizer of chlorin E6 group has appeared in Russia - Photodithazine. This preparation has been for the first time used in clinic for treatment of 44 patients with malignant tumors of various locations.

Photodynamic therapy with Photodithazine leads to photodynamic tumor damage, characterized with the same local reactions as photodynamic therapy with other photosensitizers.

Therapeutic effect was achieved in 100% of cases, including complete regression of tumors in 53.7% and partial - in 46.3%.

PDT with Photodithazine was not accompanied with systemic and local complications. The treatment was well-tolerated by the patients, including those with severe accompanying diseases.

One of the main advantages of PDT with Photodithazine is a short-term skin phototoxicity - about three days. In many cases long-term photosensitivity associated with known photosensitizers was a cause of patients' and physicians' refuse from PDT. Introduction of Photodithazine completely solves this problem.

The use of Photodithazine as a photosensitizer for PDT allows one to complete the treatment procedure in 2-3 hours. This is especially important for those patients, who can be treated in an out-patient clinic.

By present time clinical PDT with Photodithazine is in the initial state. Photodithazine-based PDT appears to be a very promising technique, but analysis of long-term follow-up results is necessary to confirm its therapeutic efficacy. Further research is required to estimate adequate doses of drug and light for various types of tumors.

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