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Photosensitizer Radachlorin®: Skin cancer PDT phase II clinical trials☆

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Summary ''Radachlorin''®, also known in the EU as Bremachlorin, a composition of 3 chlorophyll *a* derivatives in an aqueous solution, was introduced into the Russian Pharmacopoeia. Its GMP (Good Manufacturing Practice) facility based manufacturing method was patented. Laboratory experiments and clinical phase I were performed.

Protocols were designed for PDT of basal cell carcinoma of the skin to result in GCP (Good Clinical Practice)-conformed randomized phase II clinical studies. ''Radachlorin''® solution for intravenous infusions 0.35% 10 mL in the doses of 0.5—0.6 and 1.0—1.2 mg/kg and a gel for topical application 0.1% 25 g in the dose of 0.1 g/cm^2 were photoactivated by 2.5W 662 nm semiconductor laser ''LAKHTA-MILON®'' (St. Petersburg, Russia) in light doses of 200, 300 (solution), 400, 600, 800 (gel) J/cm^2.

Safety study showed no side effects and a good tolerability of ''Radachlorin''® by patients. There was no normal skin/subdermal tissue damage after both laser and sun light exposure. The main part (98%) of the drug was excreted or metabolized in the first 48 h. Drug administration at a dose of 1.0—1.2 mg/kg and irradiation at 3 h with 662 ± 3 nm light at a dose of 300 J/cm^2 (solution) and 4 PDT sessions at an interval of 1 week with 3 h gel exposure, followed by 400 J/cm^2 light exposure (gel) were found to be the optimal treatment regimes.

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Having successfully passed clinical trials, ''Radachlorin''® achieved marketing authorization in Russia in 2009 and a conditional approval in South Korea in 2008. It is a candidate for phase III clinical trials in the EC and may be commercialized as a prospective second-generation photosensitizer.

Introduction

This paper represents the full report of a phase IIb clinical trials for the photosensitizer Radachlorin [1] in Russia (August 2003—August 2005), previously published partly [2—5], set forth in 2 different clinical trial Protocols (3 hospitals for ''Radachlorin''®, i.v. administration (RCS); 2 hospitals for ''Radachlorin''®, topical administration, RCG), aimed at obtaining confirmation of previous data [6—8] on applicability, safety and tolerability of Radachlorin-LAKHTA-MILON photodynamic therapy, as well as optimizing PDT regimes for improving photodynamic tumor destruction examining various drug doses, and light exposures. This followed laboratory studies (1999—2001) and clinical phase 0 and I trials (conducted in Russia in 3 hospitals in July 2001—March 2003, involving 67 patients with i.v. and 35 patients with topical administration of photosensitizer), for cutaneous basal cell carcinoma (BCC) treatment protocols.

Materials and methods

Photosensitizer

''Radachlorin''® active pharmaceutical ingredient (API) as well as its finished formulation ''Radachlorin''® gel for topical application 0.1% 25 g (RCG) were produced in a GMP-certified facility of RADA-PHARMA Co. Ltd. (Moscow, Russia).

''Radachlorin''® active pharmaceutical ingredient (API) is represented by the total sodium salts of chlorins (6.50/7.50 g), and purified water (up to 100.00 mL).

RCG is ''Radachlorin''® (1.43 g) (total sodium salts of chlorins—0.10 g), purified water (up to 100.00 mL), and additives: DMSO (10.00 g), carbopol CBPETD2001-989212 BFGoodrich, USA or analogous (0.60 g).

''Radachlorin''® solution for intravenous infusions 0.35% 10 mL (RCS),[6] also known in the EU as Bremachlorin, was made by Interhospital Pharmacy State Unitary Enterprise of the Medical Center of the Department of the President of the Russian Federation Affairs (Moscow, Russia).

RCS is ''Radachlorin''® (5.00 g) (total sodium salts of chlorins—0.35 g), water for injections (up to 100.00 mL), and additives: N-methyl-D-glucamine (0.20 g).

For photoactivation 3 W 662 ± 3 nm semiconductor lasers ''LAKHTA-MILON®'' (MILON Laser Co. Ltd., St. Petersburg, Russia) were employed.

[6] Here and further ''Radachlorin''® means active substance (API) having the non-proprietory name of ''Radahlorin'', while RCS, RCG, Bremachlorin relates to the finished formulations (pharmaceutical forms).

Photodynamic diagnosis

Photodynamic diagnosis (PDD) was performed using spectral diagnostic systems ''LESA-01-Biospec'' (BIOSPEC Laser Biospectroscopy Lab., Moscow, Russia) and ''SPECTR-CLUSTER'' (General Physics Institute of Russian Academy of Sciences, Cluster Co. Ltd., Moscow, Russia).

In the majority of patients ($n = 60$; 71%), the tumor borders determined by PDD were considerably wider than the tumor borders determined clinically, but in 2 patients received RCS at the doses of 1.2 and 0.6 mg/kg, respectively, fluorescence contrast was around 1.2:1.0, and in one female patient received RCS at the dose of 0.6 mg/kg the fluorescence in the tumor was lower than in healthy tissue; later, these three patients developed relapses [3].

Additional fluorescent foci were revealed in 8 patients with recurrent BCC (10% of the total number of patients); and in 62% of patients with primary multiple BCC, while the number of additional fluorescent foci ranged from 1 to 6, tumor being morphologically verified in 95% of them.

GCP (good clinical practice) protocols

Eighty-four patients took part in the present study of RCS, and 28 patients were enrolled in testing RCG.

Clinical trials were approved in Russia (Russian Healthcare Ministry permissions no. 120 of 04.07.02 and no. 221 of 18.08.03) and South Korea (KFDA Certificate of 17.12.2008). The Russian studies were sponsored by ''RADA-PHARMA'' Co. Ltd. (Moscow, Russia) and involved 4 clinical research bases accredited by the National Healthcare Authorities.

Photodynamic therapy

RCS was studied in the following regimes: drug doses of (a) 0.5—0.6 mg/kg or (b) 1.0—1.2 mg/kg and laser irradiation doses of 300 or 200 J/cm^2, correspondingly, involving the total of 84 patients randomized for 3 hospitals.

RCG trial was done using the following four schemes: (a) 1 h exposure of 0.1 g/cm^2 (2 sessions at an interval of 4 weeks, excesses of the gel were removed) with light dose of 600 J/cm^2; (b) 3 h exposure of 0.1 g/cm^2 (2 sessions at an interval of 4 weeks, excesses of the gel were removed) with light dose of 600 J/cm^2; (c) 3 h exposure of 0.1 g/cm^2 (4 sessions at an interval of 1 week, excesses of the gel were *not* removed) with light dose of 400 J/cm^2; (d) 3 h exposure of 0.1 g/cm^2 (3 sessions at an interval of 1 week, excesses of the gel were *not* removed) with light dose of 800 J/cm^2 in 2 hospitals.

During the PDT with RCG the optical irradiation power density (measured at the top of the fiber using an integrated sphere optical density meter) was 1.0/3.2 W/cm^2, as the light doses were rather high, its choice depending on the

intensity of the patient's pain reactions and the temperature in the treatment foci, that must not exceed 40 °C. The same value for PDT with RCS was considerably less—1.0 W/cm^2 for the light dose of 200 J/cm^2 and 1.5 W/cm^2 for the light dose of 300 J/cm^2. In case the size of the lesion was larger than 1 cm^2, laser power was increased up to 3 W (size up to 2—3 cm^2). When we treated areas bigger than 2—3 cm^2, we had to make the procedure longer than typical 20 min, that, of course, will not be considered feasible by practical clinicians.

Eighty-four patients with various forms of basal cell carcinoma of skin took part in the study of RCS, including:

- 38 patients with $T_{1-4}N_0M_0$ primary cancer;
- 24 patients with primary multiple cancer (number of foci ranged from 2 to 8); and
- 22 patients with recurrent basal cell carcinoma of skin.

All the 34 phase I—II patients treated with RCG had primary $T_{1-2}N_0M_0$ BCC.

Patients selection was carried out in complete accordance with protocols, with observation of the following conditions:

- any specific anti-tumor therapy had to be absent within 4 weeks before start of the study;
- result of pregnancy test carried out 1 week before start of the study had to be negative;
- patient's condition had to be satisfactory (ECOG performance status $\leq$2);
- the study had to be preceded by a 100-percent morphological verification of diagnosis;
- all the candidates for the study participation had to be informed about potential risks;
- informed consent was obtained from each patient
- the study protocols conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in their priori approvals by the institution's human research committees.

Efficacy of PDT was evaluated based on the following WHO criteria (UICC):

- complete remission: absence of any signs of disease after a 100-percent resorption of tumor foci 1 month after PDT proven with histologic biopsy; confirmation of treatment result was obtained 2, 6, 12 and 18 months after the end of treatment;
- partial remission: decrease in the total dimensions of tumor focus by not less than 50% with consecutive stabilization revealed 1 month after PDT and confirmed 2, 6 and 12 months after PDT;
- partial remission with stabilization: absence of any increase in tumor foci dimensions, absence of new foci or other signs of disease progression 2, 6 and 12 months after PDT;
- disease progression: increase in the total dimensions of tumor focus by not less than 25%, development of new foci.

Results

Safety study showed no side effects and a good tolerability of RCS by patients, save for moderate pain, depending on individual sensitivity, tumor localization and irradiation field. There was no normal skin/subdermal tissue damage after both laser and sunlight exposure. The main part (98%) of the drug was excreted or metabolized in the first 48 h.

A low dark toxicity, 48 h clearance of RCS from the human's body and a low affinity to the skin helped to avoid the skin photosensitivity to daylight [2].

Using RCG no side effects were observed.

Evaluation of side effects was done with the use of standard methods in accordance with protocol in the course of the first 2 months after treatment using RCS.

(1) The patients had no reactions during, immediately after or 3 h after administration of the drug according to the following criteria:
During drug administration:
- evaluation of patient's general condition, body temperature, hemodynamic parameters (blood pressure, pulse) and external respiration function (respiration rate);
- evaluation of the presence of pain, allergic or toxic reactions by oncologist.

Immediately after drug administration:
the same*Three hours after drug administration*:
- evaluation of patient's feeling and general condition, body temperature, hemodynamic parameters (blood pressure, pulse) and external respiration function (respiration rate).

(2) According to monitoring of the data, no hemato-, nephro- or hepatotoxic effects were present as evaluated before, 1 h, 3 h after the i.v. administration; during PDT, 1 h, 24 h after it:
- *before PDT* patient's evaluation was carried out by physical, laboratory and special examinations in accordance with the following plan:
- disease history,
- physical examination (hemodynamic parameters (blood pressure, pulse), external respiration function (respiration rate), body temperature),
- complete blood count (erythrocytes, Hb, coloring, leukocytes (stab neutrophil, segmentonuclear, eosiniphiles, basophils, limphicytes, monocytes), thrombocytes, ESR),
- urinalysis (coloring, protein, sugar, pH reaction, urobilin (±), specific gravity, hepatogenous pigments (±), luekocytes, erythrocytes, epithelium),
- blood chemistry (glucose, urea, creatinine, bilirubin, ALT, AST and alkaline phosphatase),
- ECG and X-ray examination of the lungs—for all patients,
- and endoscopic and ultrasonic examinations—in accordance with indications.

after PDT tolerability and toxicity of RCS and PDT procedure were evaluated based on patients' follow-up using clinical observation and tests including:
- examination of patient's general condition,

- physical examination (hemodynamic parameters (blood pressure, pulse), external respiration function (respiration rate), body temperature),
- appearance of irradiated area (presence of edema, paleness, reddening of the skin or other local reactions),
- appearance of allergic, pain or toxic gastrointestinal manifestations (nausea, vomiting),
- urinalysis (color, protein, sugar, pH, urobilin (±), specific gravity, bile pigments (±), leukocytes, erythrocytes and squamous epithelium),
- complete blood count (erythrocytes, Hb, color index, leukocytes (stab neutrophils, segmented neutrophils, eosinophils, basophils, lymphocytes, monocytes), platelets, ESR),
- blood chemistry (total protein, albumin, albumin/globulin index, bilirubin (total and direct), transaminase activity (ALT and AST), urea, creatinine, glucose).

(3) Most of the patients (67 patients, 80%), especially those who had demonstrated low peripheral blood leukocytes at baseline, demonstrated an increase in the absolute count of peripheral blood leukocytes, while the fluctuations of leukocyte values from pre-PDT baseline did not exceed 50%. However, 24 patients (29%) showed a 50—125% increase in these values 24 h after PDT as compared to baseline. Additionally, 56 patients (67%), with all of them being among those above who demonstrated an increase in the absolute count of peripheral blood leukocytes, showed some increase (less than 20%) in the count of granulocytes (segmented neutrophils), and the rest 28 patients (33%)—an increase of 20—35%. Seven days after the treatment, the parameters studied decreased back to baseline levels in all the patients. Taking into consideration that the changes in these complete blood count parameters appeared simultaneously on the peak of edema and inflammatory changes in tissues in the zones of PDT, as well as bearing in mind that the most expressed changes in complete blood count were observed in patients with multiple tumor foci or foci of larger size that had the maximum area of photochemical reactions, the following conclusion can be drawn: increase in leukocyte count due to peripheral blood granulocytes was a manifestation of natural inflammatory reaction in the treated zones and cannot be considered as a complication of Radachlorin-based PDT.

(4) The treatment was associated with a pain syndrome that was noted throughout the PDT session and for a period of up to 1 h (or up to 24 h, according to another report [2]) after PDT, and intensified after 6—8 h, according to the same report [2]. The expression of the pain syndrome varied depending on the extent of the pathologic process and individual sensitivity of patients; in case of necessity, non-narcotic analgesics were used (analgin, promedol, keturol, ketonal). A moderate burning sensation or discomfort lasted for 2—4 h post-PDT [3,7]. This analgesia needed for 20% patients.

(5) In 1 h after PDT, an expressed edema of the soft tissues in the irradiation zone developed, accompanied with some hyperemia of that area, involving the surrounding soft tissues as well, which could last up to 2—3 days [3]. Edema of the surrounding soft tissues of the head could last for 2—7 days. Similar edema had been noted also in cases of other PSs.

(6) In one female patient with Type I diabetes mellitus, the course of necrosis rejection was complicated by suppuration, which was stopped in the course of one week by local application of antiseptic solutions.

(7) In one female patient, irradiation was followed by some increase in skin itching that had been also noted before the treatment.

Light fields were to overlap the lesions with a 0.5—1 cm margin. In most cases the real margines were found and proven using fluorescence before PDT.

As it was earlier indicated, PDT was accompanied with some pain, depending on individual sensitivity, tumor localization and irradiation field. A moderate burning sensation or discomfort lasted for 2—4 h. For 1 h after PDT, there was an expressed swelling of the soft tissue in the irradiation zone, accompanied with the hyperemia of this area, and the surrounding soft tissues as well. For stopping pain sensations, local anesthetics, non-steroid anti-inflammatory drugs or premedication with analgesics was recommended to be used.

According to data of the study, necrosis formation started 2—4 days, and eschar rejection took place 2—8 weeks, after the treatment. Treatment effect was registered 1 month after the treatment. Confirmation of treatment effect was obtained 2 months after the treatment.

During laser exposure, physician and patient had to use protective goggles with a light filter absorbing 662 ± 3 nm light.

Pharmacodynamics data

RCS's effect is based on its ability to selectively accumulate in skin tumor after its intravenous administration and, during exposure to light at a wavelength corresponding to one of the absorption peaks of the drug (i.e., 406, 506, 536, 608 or 662 nm), generate singlet oxygen that causes toxic effect on tumor cells and modifies their plasmatic membranes.

Three stages of RCS-based PDT effect development can be distinguished:

- 1st stage: specific reaction to light exposure during PDT manifested by varying degrees of edema and hyperemia in the irradiated zone;
- 2nd stage: tumor necrosis that forms 2—4 days after PDT session;
- 3rd stage: necrotic tissue rejection and wound epithelialization 2—8 weeks after PDT depending on tumor size.

Though a significant amount of ''Radachlorin''® was accumulated on the F1-offspring in the experiments *in vivo*, it caused no damage to the DNA of normal cells [9].

Pharmacokinetics data

After intravenous administration of a single dose of RCS of 0.5—0.6, 1.0—1.2 or 2.0—2.4 mg/kg, the drug distributed between blood and tissues in the course of 0.5—5 h. Max-

imum serum concentration of RCS after administration at the dose of 0.5 mg/kg was reached in 15—30 min and quickly decreased to 10, 5 and 1 μg/L by 1, 3 and 24 h, respectively.

During PDT, a photobleaching effect (considerable decrease in fluorescence intensity in the zone of irradiation) was noted; by the end of the treatment session, intensity of fluorescent signal approached zero. This can serve as an additional criterium for selecting the dose of light for a PDT session, because it is useless to increase the dose of light in conditions of drug absence in tumor tissue. One day after drug administration, fluorescence intensity decreased uniformly in healthy skin and mucous membranes outside the PDT fields respectively 2.2- and 2.3-fold. In case of eschar formation, the form of spectrum in the zone of PDT changed to a so-called necrotic one. Traces of the PS were detected in healthy skin and mucous membrane of patients for a period of up to 5—6 days after its administration.

The PS's concentration in tumor reached its maximum 1 h after administration (10—20 μg/mL); however, its elimination from healthy tissues surrounding the tumor going quicker, maximum therapeutic index (contrast index) was observed 3 h after administration of the drug. The PS's concentration in tumor tissue was, on an average, 3—6 times higher than in the surrounding healthy tissues, depending on tumor morphology, and varied from 2 to 10 μg/mL [3,7,8].

Quick elimination of the PS from blood, skin and mucous membranes, as well as its high contrast index, exclude damage to healthy organs and tissues and skin hypersensitivity to daylight, so that there is no special recommendation of the Healthcare Authorities for the patients to protect their eyes and skin against sunlight after ''Radachlorin''® PDT.

Table 1 Overall phase II (RCS) and phase I—II (RCG) study results with ''Radachlorin''® finished formulations.

PDT efficacy[a]	RCS		RCG	
	Patients	%	Patients	%
CR	71	**84.5**	28	**82.4**
PR	12	14.3	4	11.8
S	1	1.20	2	5.80
P	—	—	—	—
Totally	84	100	34	100

Bold values indicate the mean complete response rates, being important proofs of efficiency.
[a] Proven by biopsy.

Three hours after RCS administration, its highest levels are reached in liver, kidneys and tumor tissue [6,12].

About 70—80% of ''Radachlorin''® are metabolized in the liver to biladiens (linear tetrapyrrols that are also produced as a result of hem metabolism). Fecal and urinary excretions of unchanged drug were respectively 15 and 3%. Cumulative fecal and urinary excretion of the PS in the first 12 h was, on an average, 15—20% of the administered dose. The main part (98%) of the PS was excreted or metabolized in the first 48 h.

Efficacy data obtained in the course of 2-month observation

The clinical studies have shown that RCS-based PDT had high anti-tumor activity with respect to all the specified forms of

Table 2 The results of phase II clinical trials of RCS.

No.	Category		Absolute value, people	%
1	Patients classified by BCC form:			
	Primary, one lesion ($T_{1-4}N_0M_0$)		38	45.3
	Multiple lesion		24	28.5
	Recurrent		22	26.2
	Totally		84	100.0
2	Resulting efficiency depending on PDT protocol:			
	0.5—0.6 mg/kg—300 J/cm^2	CR	22	**78.6**
		PR	6	21.4
		S	—	—
		P	—	—
	1.0—1.2 mg/kg—200 J/cm^2	CR	24	**85.7**
		PR	3	10.7
		S	1	3.6
		P	—	—
	1.0—1.2 mg/kg—300 J/cm^2	CR	28	**100.0**
		PR	—	—
		S	—	—
		P	—	—
3	Overall phase II clinical trial efficiency:			
	Complete response (CR)		71	**84.5**
	Partial response (PR)		12	14.3
	Stabilization (S)		1	1.2
	Progression (P)		—	—
	Totally		84	100.0

Bold values indicate the mean complete response rates, being important proofs of efficiency.

Table 3 The results of phase I—II clinical trials of RCG.

No.	Category		Absolute value, people	%
1	Patients classified by BCC form:			
	Primary, one lesion ($T_{1-2}N_0M_0$)		34	100.0
	Totally		34	100.0
2	Resulting efficiency depending on PDT protocol:			
	a. Gel exposure 1 h	CR	—	—
	—Light exposure 600 J/cm^2	PR	1	—
		S	2	—
	2 sessions at an interval of 4 weeks, excesses of the gel were removed	P	—	—
	b. Gel exposure 3 h	CR	1	—
	—Light exposure 600 J/cm^2	PR	2	—
		S	-	—
	2 sessions at an interval of 4 weeks, excesses of the gel were removed	P	—	—
	c. Gel exposure 3 h	CR	14	**100.0**
	—Light exposure 400 J/cm^2	PR	—	—
		S	—	—
	4 sessions at an interval of 1 week, excesses of the gel were NOT removed	P	—	—
	d. Gel exposure 3 h	CR	13	92.9
	—Light exposure 800 J/cm^2	PR	1	7.14
		S	—	—
	3 sessions at an interval of 1 week, excesses of the gel were NOT removed	P	—	—
3	Overall phase I—II clinical trial efficiency:			
	Complete response (CR)		28	**82.4**
	Partial response (PR)		4	11.8
	Stabilization (S)		2	5.80
	Progression (P)		—	—
	Totally		34	100.0

Bold values indicate the mean complete response rates, being important proofs of efficiency.

basal cell carcinoma of skin (Table 1). This conclusion was confirmed morphologically.

Efficacy data obtained in the course of observation during the period from month 2 to month 18

The follow-up lasting for 1.5 years after the end of a 2-month period set by protocol revealed the preservation of partial regression and stabilization in all of the patients (15.5% treated with RCS, and 17.6% treated with RCG). One year after the treatment with RCS, CR was preserved in 92.8% patients who had CR as evaluated after 2 months (Fig. 2).

One year later, for the benefit of the patients with incomplete effect, the repeated PDT (not reflected in reports) or treatment with other methods was carried out to reach complete regression of tumor.

Use of RCG is mainly limited to T1-T2 superficial and infiltrative BCC lesions (Fig. 3).

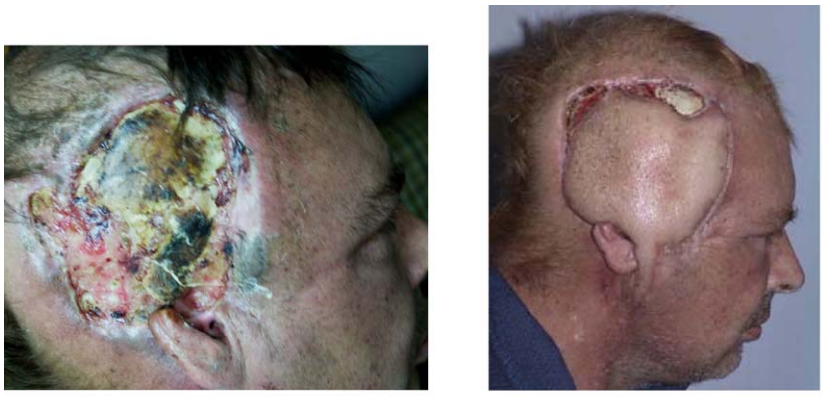
The PDT efficacy data are presented in Tables 1—3, Figs. 1—3.

The CR was proven, according to phase I and phase I—II GCP clinical research protocols, by histologic biopsy in all the cases.

Response rates for patients having multiple and recurrent lesions were reported by just two of three clinical research bases: (hospital 1) among 28 patients, 9 had recurrences and 13 primary multiple lesions, objective anti-tumor response corresponded to 100%, however, the number of CR in these groups was lower, respectively, 6 patients (67%) and 9 patients (69%); (hospital 2) among 28 patients, 7 had recurrences, and PDT resulted in CR for 2 of 3 patients with 0.5—0.6 mg/kg—300 J/cm^2, while when using the other two regimens, CR was reached for all the 4 patients. So, one could conclude that treating recurrences CR could be expected using 1.0—1.2 mg/kg—300 J/cm^2 or 1.0—1.2 mg/kg—200 J/cm^2 in some 80—85% cases, and 0.5—0.6 mg/kg—300 J/cm^2 would result in CR in about 65-70% cases. When treating primary multiple lesions, using all the treatment regimens, around 70% of CR could be expected.

Discussion

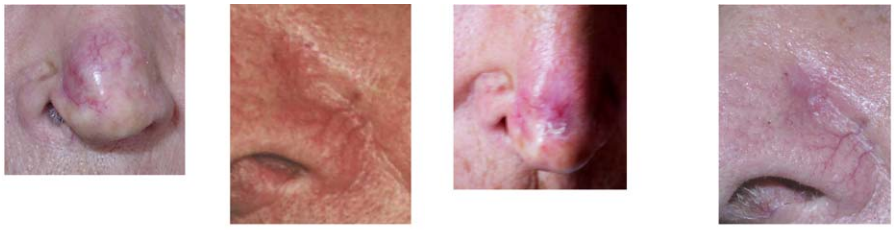
''Radachlorin''® active substance is chemically stable in solutions for 1.5 years at 0 + 8 °C in the dark. When introduced to embryocarcinoma T36 bearing mice, it had maximal tumor uptake within 0.5—5 h post-injection with tumour-to-skin ratio around 14 by 3 h post-injection and



(a) before - vast defect of soft tussues of parietotemporal region and auricle with baring of skull and spreading of the process to the external acoustic meatus.

(b) 5 months after PDT, 1 month after plastic surgery - complete rejection of necrotized tissues with formation of clean granulating wound.

Figure 1 PDT using RCS for a repeated recurrence of parietotemporal BCC after surgical and combined treatments. RCS dose 1.0 mg/kg, light dose 300 J/cm^2. Light-drug interval 3 h.



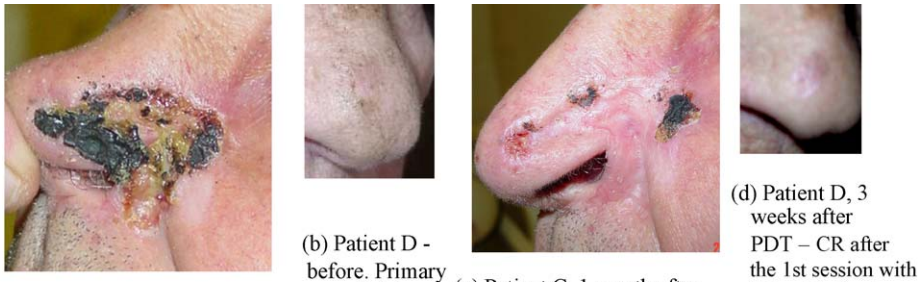
(a) Patient A - before. Recurrence after beam and combined treatment. Phase II protocol. RCS dose 1.0mg/kg, light dose 300J/cm^2. Light-drug interval 3h.

(b) Patient B - before. Primary lesion. Phase I protocol. RCS dose 1.2mg/kg, light dose 300J/cm^2. Light-drug interval 3h.

(c) Patient A, 2 months after PDT – CR with epithelisation and formation of a barely visible scar.

(d) Patient B, 3 weeks after PDT – CR with epithelisation and excellent cosmetic effect.

Figure 2 PDT using RCS for a nodular BCC of nose skin.



(a). Patient C - before. Recurrent infiltrative BCC lesion after repeated X-ray therapy. Phase II trial: 3h exposure of 0.1g/cm^2 gel (4 sessions at an interval of 1 week, excesses of the gel were not removed), light dose 400J/cm^2.

(b) Patient D - before. Primary superficial lesion. Phase I trial: >3h exposure of 0.2g/cm^2 gel (2 session, excesses of the gel were not removed), light dose 600J/cm^2).

(c) Patient C, 1 month after the last PDT sessions – CR with partial epithelisation.

(d) Patient D, 3 weeks after PDT – CR after the 1st session with full epithelisation.

Figure 3 PDT using RCG for superficial and infiltrative BCC lesions of nose skin.

Table 4 The results in comparison with some approved PDT treatments (ALA-derivatives).

Pharmaceutical agent	Can PDT of the nodular BCC give 60—80% rates of CR and a 2-year and longer remissions?	Is PDT very painful?	Can the pain during PDT procedure be considerably reduced using analgetics?	Therapeutic ratio?
The precursors of the endogenous PPIX (Alasense, Laevulan-Kerastik, MAL-Methvix)	No	Yes	No, hardly	<3/1 at 3 h after topical application, with PP-IX retention period of more than 4 weeks in the normal tissues
RCS	Yes	No, moderate pains for 1 h in 20% cases at power densities less than 1 W/cm^2	Yes, efficiently: local use (lidocain) or premedication (ketonal, promedol) at more than 1 W/cm^2	(2 through 4)/1 (for the 0.6 mg/kg dose) (4 through 6)/1 (for the 1.2 mg/kg dose) at 3 h p.i. with retention period of less than 6 days in the tissues around tumor and 98% clearance from the body in 2 days.
RCG	No	No, moderate pains during the first minutes of laser irradiation	N/A	N/A
			Analgetics are not required	Cannot be measured
Comment	PP-IX:	Less affinity of the components of RC to the nerv endings.	Due to reallocation of endogenously generated PP IX: a non-specific action of the ROS on the nerv endings inside the skin.	PP-IX stronger binds to proteins and lipoproteins than RC
	1. little $\lambda_{630-635\,nm}$ light penetration to tissues (vs. $\lambda_{662\,nm}$ for RC), 2. 10-time lower maximum of the excitation band (chlorin e_6 in RC at 662 nm in the serum has $\varepsilon = 34{,}200\,M^{-1}\,cm^{-1}$); 3. Lower ROS production: interconversion quantum yield of 40% due to aggregation vs. 96% for RC.			

Figure 4 Videofluorescence examination 3 h after i.v. administration of 1.2 mg/kg RCS (BCC recurrecy after X-ray therapy, courtesy from E.I.Volkov, M.D., H&N Department of Sverdlovskiy regional oncological dispancery, Ekaterinburg, Russia).

clearance period about 24 h. In the animal PDT experiments ''Radachlorin''®, active substance showed an expressed specific PDT activity, causing an intensive but bearable by animals necrotic action to the tumors [10—14].

''Radachlorin''®, active substance, represents a composition of 3 chlorophyll derivatives differing by their lipophilicity and carrying different numbers of negative charges, will achieve various localizations in tumor. This is a distinguishing feature of Radachlorin as compared to its major component and chemical precursor, chlorin e_6. For example, ''Radachlorin''® has a quicker cellular kinetics. Using fluorescence microscopy it was shown that, even if ''Radachlorin''® quickly crossed a human colonic cancer line HT29 cell membrane, cellular distribution evolved from a diffuse cytoplasmic repartition 1 h after RCS addition to a delimited localisation into organelles all around the nucleus. ''Radachlorin''® intracellular fluorescence decreased after 4 h, whereas a decrease of chlorin e_6 intracellular fluorescence was not observed for times up to 24 h. Therefore, during *in vivo* experiments on mice grafted with human lung carcinoma A549, maximum ratios with «Radachlorin»® (1.45 ± 0.14 for tumor-to-skin and 1.95 ± 0.29 tumor-to-muscle) were observed 7 h after injection. With chlorin e_6 the best tumor-to-muscle ratio (2.56 ± 0.97) was reached 8 h after injection, fluorescence in skin being always at least equivalent to tumor fluorescence [15].

As shown by the preceeding studies, ''Radachlorin''® active substance, is not aggregated in biological media, favors good spectral, pharmacokinetic, toxicological characteristics, and an excellent capacity to generate Reactive Oxygen Species (ROS) [11,14,15].

Comparing to protoporphyrin IX (metabolite of δ-aminolevulinic acid and its esters), HPD, ''Photofrin II'', ''Radachlorin''® has an intensive absorption band in the medium red part of the spectrum (662—664 nm), where biological tissues are transparent to a considerable extent (Table 4). It also gives an intensive fluorescence peak at 668 nm, that is helpful for fluorescent diagnosis (PDD). A comparatively higher therapeutic ratio and ROS production rate are observed for ''Radachlorin''®, as well as PDT with this substance is less painful, and the pain can easier be coped with using analgetics. ''Radachlorin''® PDT is also more applicable for very big BCC lesions (Fig. 1), and as well for the areas where due to surface relief the light spot is not homogenous or where there are many nerv endings (ears, around eyes, nose) (Figs. 2b and 3a).

In general, one can expect some benefits from ''Radachlorin''® PDT vs. protoporphyrin-IX PDT owing to better pharmaceutical, spectral, pharmacokinetic, toxicological, and energy performance of the former.

It is expedient to recommend for further wide use in clinical practice the RCS-based PDT at a dose of 0.5—0.6 mg/kg (for patients with superficial basal cell carcinoma of skin) or 1.0—1.2 mg/kg (for patients with nodular forms of tumor) in combination with light irradiation at a dose of 200—300 J/cm^2.

However, the best treatment pattern was 1.0—1.2 mg/kg/300 J/cm^2 as the highest light and drug doses (it ensured a 1-year preservation of the effect of complete regression in 100% of patients).

It is worth mentioning, that in one out of three studies [5] 3 patients from 32 receiving this very treatment pattern developed recurrences in 8—12 months. Since in all these cases the recurrences of tumor growth were marked on periphery of the primary tumors, we suppose that the recurrences were not connected with fluence and drug doses, but with an insufficient area of laser irradiation. Repeated PDT procedures were applied to these patients. That resulted in complete regress of tumors in all these cases, with the remission period from 2 to 3.5 years.

For the light doses of 300 J/cm^2 the increase of drug doses from 0.5 to 1 mg/kg did not result in an adequate growth of PDT efficiency (it was already high enough at 0.5 mg/kg). Similarly, at the drug dose of 1.2 mg/kg no significant efficiency increase resulted from light doses higher than 200 J/cm^2. But going lower than 200 J/cm^2 decreased distinctly the PDT efficiency in spite of large drug doses.

Thus, for patients with partial effect or stabilization, retreatment with 1.0—1.2 mg/kg/200 J/cm^2 is successful.

A 0.5—0.6 mg/kg/300J/cm^2 regime (drug economical) can also be used (objective anti-tumor effect—100%), provided that repeat treatment is available for patients with partial effect or stabilization. The use of this regime was associated with the highest frequency of recurrences (14.3%).

It was noted that continuation of tumor growth and recurrences were more frequent in patients with primary multiple or recurrent basal cell carcinoma of skin. In general, the use

of other methods of treatment (radiation therapy, surgery, cryodestruction, laser destruction, electrocoagulation) in these groups of patients is also characterized by lower efficacy.

Some patients receiving RCS-based PDT showed an increase in absolute peripheral blood leukocyte count with elevated granulocyte count most probably, due to general inflammatory reaction of the body in the treated zone (the zone of necrosis). Thus, there was a certain general response of the immune system to the local PDT procedure.

High-quality long-term cosmetic result of treatment was obtained in all cases.

For detecting additional tumor foci and obtaining more precise data about tumor spread, it is recommended to combine RCS administration with fluorescence diagnosis—for example, by using ELAN complex for photodynamic therapy and fluorescence diagnosis (Qualitech Co. Ltd., Moscow, Russia) (Fig. 4), or by using a Spectr-Cluster or a LESA-01-Biospec spectrofluorimeters [7]. Fluorescence intensity reached its maximum 3 h after drug administration and was considerably higher for the 1.2 mg/kg dose. Fluorescence ratio ''tumor/norm value'' ranged from (2 through 4)/1 (for the 0.6 mg/kg dose) to (4 through 6)/1 (for the 1.2 mg/kg dose).

''Radachlorin''® finished formulations are potential candidates for phase III clinical trials and can be commercialized as a prospective second-generation photosensitizers.

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