

Photodynamic Therapy with Boronated Chlorin as a Photosensitizer

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Abstract

The purpose of this work was to study the efficiency of photodynamic therapy with boronated chlorin as a photosensitizer for treating experimental tumors such as M-1 sarcoma and B-16 melanoma. PDT of M-1 sarcoma with laser radiation energy density of 300 J/cm² and power density of 0.42 W/cm² resulted in complete tumor regression at all three doses of the photosensitizer. Photodynamic therapy of B-16 melanoma appeared to be the most effective when boronated chlorin at a dose of 10.0 mg/kg and laser radiation energy density of 150 J/cm² and power density of 0.25 W/cm² were used. A satisfactory effect of PDT on the tumor was achieved when a dose of 5.0 mg/kg, energy density of 300 J/cm² and power density of 0.25 W/cm² were used. At 10 - 21 days after PDT with use of other studied parameters, some animals showed continued tumor growth. Nevertheless, by the end of observation (at day 21), no lung metastases were found in animals. In control, they were detected in up to 43.4 % of cases.

Keywords: Photodynamic therapy; Laser; Photosensitizer; Boronated chlorin; Metastases

Abbreviations: PDT: Photodynamic therapy; MTS: Metastases

Introduction

Progress in PDT of tumors, especially malignant ones, is associated with advance in creating effective photosensitizers providing the maximum therapeutic effect on tumor while minimizing the damage of surrounding healthy tissues without general toxicity.

In recent years, researchers have paid special attention to synthesizing photosensitizers with a wide range of their potentialities. In particular, the creation of boronated porphyrins and chlorins enables to carry out simultaneously both neutron capture therapy and photodynamic therapy [1-4], which essentially improves the efficiency of each of the specified methods allowing to use their advantages and minimize disadvantages.

Our study was aimed at investigating the antitumor activity of PDT on two models of malignant tumors with use of boronated chlorin as a photosensitizer synthesized by our co-authors from A.N. Nesmeyanov Institute of Elementoorganic Compounds of the Russian Academy of Sciences.

Material and Methods

Experiments were performed on 67 outbred rats weighting 180-200 g and on 72 F-1 hybrid mice (CBA x C₅₇BL₆) weighting 20 ± 3g. The animals had been delivered from the vivarium of the Medical Radiological Research Center of the Russian Academy of Medical Sciences and kept under standard conditions. As tumor models, M-1 sarcoma and B-16 melanoma were used. Both strains had been delivered from the Bank of Tumor Strains of the laboratory of combination therapy of tumors at the Russian Oncological Research Center named after N.N. Blokhin of the Russian Academy of Medical Sciences.

Depending on tumor, the animals in our experiment were divided into two series:

Series I included rats with inoculated M-1 sarcoma;

Series II included mice with inoculated B-16 melanoma.

Each series was subdivided into groups which differed by a dose of boronated chlorin and conditions of laser irradiation.

Rats with M-1 sarcoma (series I)

In group 1, PDT was performed using boronated chlorin at a dose of 10.0 mg/kg of animal's body weight, laser radiation power density of 0.42 W/cm² and energy density of 300 J/cm².

In group 2, boronated chlorin was administered at a dose of 5.0 mg/kg with laser radiation power density of 0.42 W/cm² and energy density of 300 J/cm².

In group 3, boronated chlorin was administered at a dose of 2.5 mg/kg with laser radiation power density of 0.42 W/cm² and energy density of 300 J/cm².

In group 4, boronated chlorin was administered at a dose of 2.5 mg/kg with laser radiation power density of 0.25 W/cm² and energy density of 150 J/cm².

Untreated animals with tumors inoculated at the same time served as control.

Mice with B-16 melanoma (series II)

In group 1 of this series, boronated chlorin was administered at a dose of 5.0 mg/kg with laser radiation power density of 0.25 W/cm² and energy density of 150 J/cm².

In group 2, boronated chlorin was administered at a dose of 5.0 mg/kg with laser radiation power density of 0.44 W/cm² and energy density of 150 J/cm².

In group 3, boronated chlorin was injected at a dose of 10.0 mg/kg using laser radiation of 0.25 W/cm² and 150 J/cm².

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In group 4, boronated chlorin was administered at a dose of 10.0 mg/kg with power density of 0.44 W/cm² and energy density of 150 J/cm².

In group 5, the mice received treatment with the photosensitizer at a dose of 5.0 mg/kg, power density of 0.25 W/cm² and energy density of 300 J/cm².

In group 6, the photosensitizer was administered at a dose of 5.0 mg/kg with power density of 0.44 W/cm² and energy density of 300 J/cm².

Untreated animals with tumors inoculated at the same time served as control.

Tumors were inoculated subcutaneously in the femur area. A sarcoma was inoculated to the rats in the form of pieces sized approximately 0.1 mm², melanoma was inoculated to the mice in the form of suspension in a volume of 0.10 – 0.15 ml. A day before the tumor inoculation, hair in the femur area was depilated. The rats were involved in the experiment as soon as the palpable tumor achieved 0.5-0.7 cm in diameter (at 8-10 days after transplantation), the mice - at 4-5 days when the tumor sized 3-5 mm.

At 1.5 hours after the injection, the tumor was irradiated by laser. As a photosensitizer, boronated chlorin was administered intraperitoneally at doses of 2.5, 5.0 and 10.0 mg/kg.

As a source of laser radiation served the semi-conductor laser device ATKUS-2 made by the Joint-Stock Company «Semi-Conductor Devices» (St.-Petersburg, Russian Federation). The radiation wavelength was 661±1nm. To deliver radiation to the tumor, a quartz mono-fiber light guide with a lens at the end was used. The light spot was 1.5 cm in diameter for rats, and 1.0 cm in diameter for mice, falling radiation energy density was 150 or 300 J/cm², power density was 0.25, 0.42 or 0.44 W/cm². The time of irradiation was calculated by the formula:

$$T = (D^2 \cdot E \cdot 13.09) / P \quad (1)$$

Where: T is the time (min) of irradiation,

D is the biggest tumor diameter (cm),

E is the density of absorbed light energy (J/cm²),

P is the power (mW) of emitted light.

Prior to laser irradiation, the animals were anaesthetized using thiopental sodium administered intraperitoneally: (rats received 2.5%-solution at a dose of 0.15 ml/100 g of animal's body weight; mice received 1.25%-solution at a dose of 0.03 ml/10g of animal's body weight).

The animals were kept under observation for 21 days. Tumor volume was determined at different stages: before treatment and at 3, 7, 10, 14 and 21 days after treatment.

The efficiency of PDT was evaluated by:

1. Coefficient of absolute tumor growth (C). For this purpose, preliminary diameters of tumors were measured and volumes were calculated by the formula:

$$V = \frac{1}{6} \pi \cdot d_1 \cdot d_2 \cdot d_3 \quad (2)$$

Where: d₁, d₂, d₃ are three mutually perpendicular tumor diameters,

V is tumor volume in cm³.

The coefficient of absolute tumor growth was calculated by the formula:

$$\bar{N} = \frac{V_t - V_0}{V_0} \quad (3)$$

Where: V₀ is tumor volume before PDT,

V_t is tumor volume at different stages of observation.

2. Percentage of complete tumor regression (PR %) and percentage of the inhibition of tumor growth (ITG %) versus control. ITG was calculated by the formula:

$$ITG = \frac{V_k - V_o}{V_k} \cdot 100\% \quad (4)$$

Where: V_k is average tumor volume in control,

V_o is average tumor volume in experiment. The absence of the palpable tumor was considered to be complete tumor regression. When the tumor appeared at the margin of the irradiation field, we considered it continued tumor growth.

Moreover, we determined the percentage of the mice with lung metastases (mts). At the end of the observation period (21 days), the animals were killed by chloroform narcosis in order to detect mts. The excised lungs were rinsed and cut into lobes. Mts were counted using MBS-1 microscope (12 x magnification). In some animals without palpable tumors, histological slices were stained with hematoxylin-eosin.

Statistical analysis of our findings was performed using the computer program STATISTICA 6.0 by non-parametric methods for independent samples: descriptive statistics, the Mann-Whitney U test (Z, p). The minimum level of the significance was p < 0.05.

Results

Series I (rats, tumor: M-1 sarcoma)

PDT with boronated chlorin administered at doses of 10.0, 5.0 and 2.5 mg/kg (groups 1, 2, 3 and 4, respectively), but with the same laser radiation power density (0.25 and 0.42 W/cm²) and energy density (150 and 300 J/cm²) resulted in a similar tumor response (Figure 1). During the whole period of observation, all the animals showed complete tumor regression, whereas in corresponding control group, progressive tumor growth was noted. Differences between the experimental and control groups were statistically significant to a high degree (from p < 0.002 to < 0.006). In all four groups, no animal mortality occurred.

Series II (mice, tumor: B-16 melanoma)

The results indicated that the effectiveness of PDT with boronated chlorin for treating melanoma was different depending on the dose of the photosensitizer and laser irradiation parameters. In group 1, PDT caused a violent swelling of the whole paw at day 3 after irradiation. Therefore, it was difficult to identify the presence of a tumor. However, 7 days later, it was obvious that complete tumor regression was achieved in 100 % of mice (not palpable). ITG was 100% (p < 0.001). Such a picture lasted 21 days. However, in that period, all the mice had tumor growth outside the irradiation field, but the coefficient of absolute tumors growth was only 7.15 ± 1.85 (p < 0.001). In control,

it was 80.44 ± 8.51 ; ITG was 90 % (Figure 2). No mice mortality was noted, whereas in control it was 14 %.

The increase in the laser radiation power density up to 0.44 W/cm^2 (gr. 2) yielded positive results in most animals. At day 3, the tumor was not palpable in 100 % of mice, whereas at day 7, some animals showed continued tumor growth. However, in comparison with normal tumor-bearing mice, tumor growth was considerably lower during the whole period of observation (statistical difference in different time periods of investigation was $p < 0.003 - p < 0.015$). At day 7, complete tumor regression was achieved in 86 % of mice, at day 14 – in 43%, and at day 21 – only in 29 % of animals. At day 3, ITG was 100 % comparing to control. But at day 21, it decreased up to 74 %.

The increase in the dose of the photosensitizer (gr. 3) led to the statistically significant positive results: over 14 days, C was considerably lower than in control ($p < 0.010 - 0.030$). However, at day 10, some mice showed continued tumor growth, but it was lower than in control. At day 10, the percentage of complete tumor regression was 84 %, at day 14 – 84 % and at day 21 – 70%. (Figure 3). At this time, ITG made 80 % versus control.

In group 4, the power density increased up to 0.44 W/cm^2 . The energy density (150 J/cm^2) and the dose of boronated chlorin (10 mg/kg)

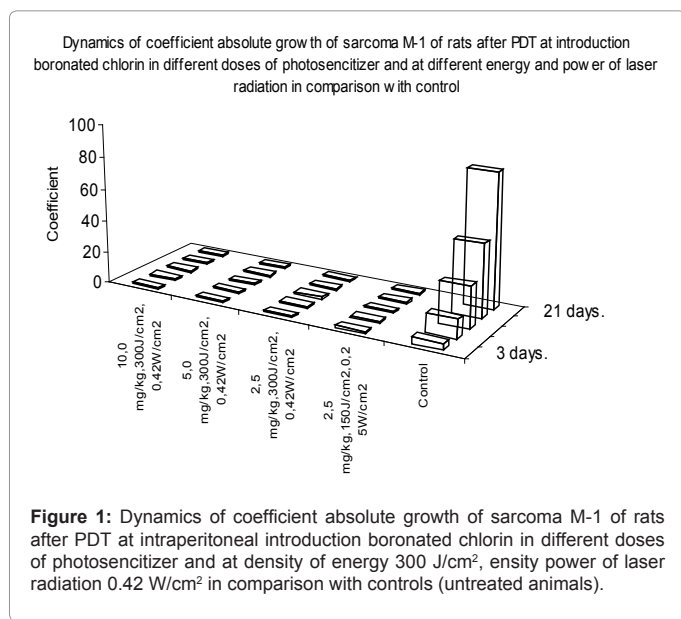


Figure 1: Dynamics of coefficient absolute growth of sarcoma M-1 of rats after PDT at intraperitoneal introduction boronated chlorin in different doses of photosensitizer and at density of energy 300 J/cm^2 , ensity power of laser radiation 0.42 W/cm^2 in comparison with controls (untreated animals).

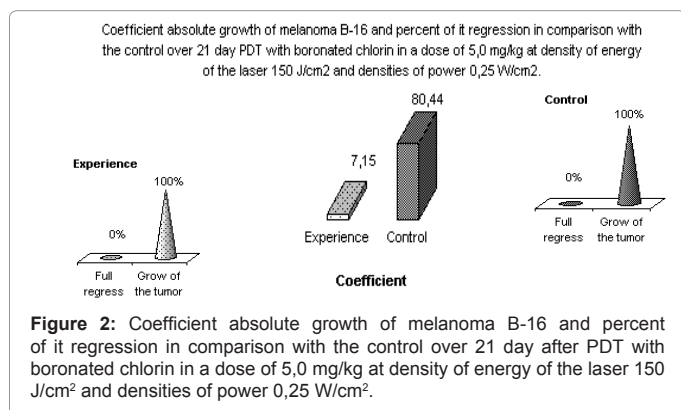


Figure 2: Coefficient absolute growth of melanoma B-16 and percent of it regression in comparison with the control over 21 day after PDT with boronated chlorin in a dose of $5,0 \text{ mg/kg}$ at density of energy of the laser 150 J/cm^2 and densities of power $0,25 \text{ W/cm}^2$.

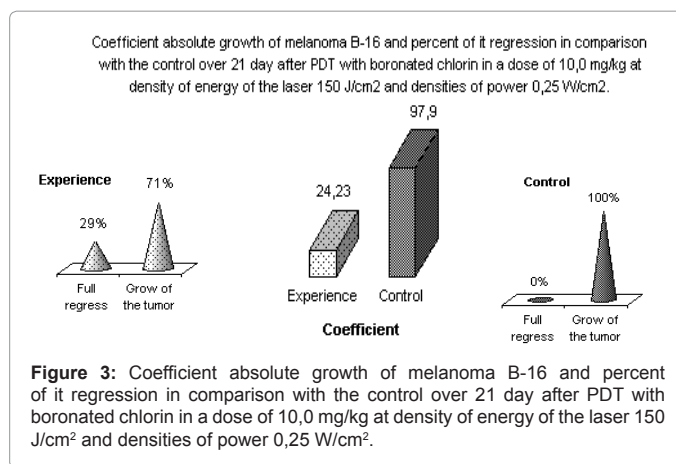


Figure 3: Coefficient absolute growth of melanoma B-16 and percent of it regression in comparison with the control over 21 day after PDT with boronated chlorin in a dose of $10,0 \text{ mg/kg}$ at density of energy of the laser 150 J/cm^2 and densities of power $0,25 \text{ W/cm}^2$.

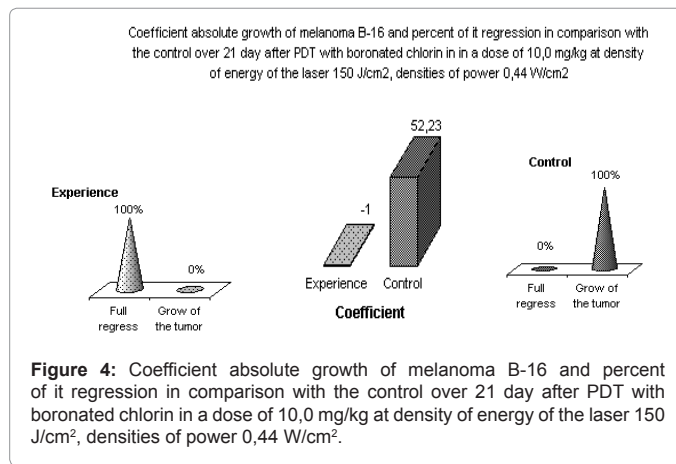


Figure 4: Coefficient absolute growth of melanoma B-16 and percent of it regression in comparison with the control over 21 day after PDT with boronated chlorin in a dose of $10,0 \text{ mg/kg}$ at density of energy of the laser 150 J/cm^2 , densities of power $0,44 \text{ W/cm}^2$.

kg.) were equal to group 3. The results of this study indicated that the efficiency of PDT increased substantially. Over 21 days, complete tumor regression was noted in 100 % of mice (Figure 4). ITG was also 100 % comparing to control.

Histologically, mice without palpable tumors showed an extensive postirradiation necrosis of the skin and hypodermic tissues (hypodermic connective tissue, muscles, fat), growth of the fibrous tissue at site of damaged structures, their infiltration by segmental leucocytes and macrophageal elements. At site of irradiated tumor, a mini focus of necrotic tumor tissue infiltrated both by segmental lymphocytes and lymphocytes and surrounded by the connective tissue capsule was found. Edema of the connective and muscular tissue, foci of fibrous growth. Thrombosis of great vessels. No tumor residues or separate tumor cells were detected.

PDT performed with energy density of up to 300 J/cm^2 , boronated chlorin administered at a dose of 5.0 mg/kg and power density of 0.25 W/cm^2 (gr. 5) yielded a good therapeutic effect resulting in complete tumor regression for 21 days.

In group 6, a day after PDT with boronated chlorin at a dose of 5 mg/kg with laser radiation energy density of 300 J/cm^2 and power density of 0.42 W/cm^2 , not only a strong damage of the tissue of the extremities but also the amputation of the extremities in the most cases occurred.

No lung metastases were found in experimental groups. At the

same time, in control animals they were detected in 28.6 % - 43.3 % of cases.

Discussion

Taking into account all these findings, it is obvious that the effectiveness of PDT with boronated chlorin as a photosensitizer depended on laser energy density and power density, dose of the photosensitizer and tumor type. Thus, PDT of rat M-1 sarcoma yielded good therapeutic results at all three doses of the photosensitizer, which manifested in 100 % tumor regression persisting for 21 days. In our study, optimal conditions of PDT were dose 2.5 mg/kg and laser irradiation 150 J/cm² and 0.25 W/cm². However, it does not mean that other parameters of PDT should be excluded. If necessary, they can be used, but this question needs to be scrutinized.

As for using boronated chlorin for PDT of B-16 melanoma in mice, the conditions of its performance appeared to be more limited. There was a discrepancy between the results in different groups. The optimal parameters were as follows: boronated chlorin at a dose of 10.0 mg/kg of animal's body weight, energy density of 150 J/cm² and power density of 0.44 W/cm². This regimen enabled the most marked and stable complete tumor regression. The use of other parameters produced continued tumor growth at 10 – 14 days after PDT. However, the coefficient of absolute tumor growth was considerably lower than in control (untreated animals).

It is important to keep in mind that during PDT with boronated chlorin no lung metastases were seen over the whole period of observation even in those animals which showed continued tumor growth, whereas in different control groups melanoma metastases were detected at day 21 in 28.6 % - 43.3 % of untreated mice. Over this period of time, none of animals died of progressive disease. In control groups, the death of animals occurred in 14 % of cases.

A comparison between the results of our studies with use of boronated chlorin as a photosensitizer for PDT of B-16 melanoma and those of our previous studies with use of photolon, the derivative of chlorin e6 [1], suggested that PDT with chlorin e6 at doses of 9.0 and 12.0 mg/kg of animal's body weight and laser radiation power densities of 0.38 W/cm² and 0.25 W/cm² caused a considerable decrease of the coefficient of absolute tumor growth. PDT with chlorin e6 at a dose of 12.0 mg/kg, laser radiation energy density of 150 J/cm² and power density of 0.25 W/cm² resulted in complete melanoma regression only in two of eight animals within 21 days of observation. Such an effect occurred in one of 12 animals after the administration of chlorin e6 at a dose of 9.0 mg/kg, energy density of 150 J/cm² and power density of 0.38 W/cm².

Together, these findings support the idea that boronated chlorin is more preferable than chlorin E6 for PDT. Further research is required to bring some new insights into this problem.

Conclusion

1. Boronated chlorin is an effective photosensitizer for PDT of rat M-1 sarcoma. At its doses from 2.5 up to 10.0 mg/kg of animal's body weight, laser energy density of 150 and 300 J/cm² and power density of 0.25 and 0.42 W/cm² complete tumor regression was achieved.
2. PDT of B-16 melanoma with boronated chlorin was the most

effective when boronated chlorin was administered at a dose of 10.0 mg/kg of animal's body weight under laser radiation conditions of 150 J/cm² and 0.42 W/cm².

3. PDT of B-16 melanoma with boronated chlorin prevents arising of lung metastases in mice for 21 days.

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