A Method for Improving the Efficiency of Therapy for Melanoma

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A method for photodynamic therapy was developed in experiments on the model of melanoma B16. The photosensitizer is injected by two doses, while laser exposure is carried out during coincidence of its maximum accumulation phases in tumor vessels and cells. This method increased the percentage of animals with complete regression of the tumor, decreased the coefficient of absolute tumor increase in animals with progressive tumor growth, increased survival of mice, and significantly decreased melanoma metastasizing to the lungs in comparison with untreated animals and even with the standard photodynamic therapy.

Key Words: *melanoma; photodynamic therapy; laser*

Melanoma is a malignant disease difficult to treat because of its rapid metastasizing to distant organs. Local removal of the tumor does not guarantee the arrest of the tumor process [1-3]. Therefore, the search for therapeutic methods directed against the primary focus and preventing metastasizing is a pressing problem. Tumor cells are transported to organs distant from the primary focus mainly through the vascular (blood, lymph) system. Hence, inhibition of tumor cell migration from the primary focus can presumably limit or at least reduce the metastatic process. We think that photodynamic therapy (PDT) is a modern method best of all suitable for this purpose; after appropriate improvements, this method will modulate not only the primary focus, but will also minimize migration of tumor cells.

The curve reflecting accumulation of the photosensitizer (PS) in the tumor after its intravenous injection has two peaks. One during the vascular phase, when the greatest quantity of the agent is in tumor vessels, and the second when accumulation is maximum in tumor cells; PS is virtually absent from the vessels during this period or its level is the minimum. Hence, the photodynamic effect of laser exposure manifests mainly at the level of tumor cells. Traditional PDT is usually carried out during the phase of maximum accumulation of the agent in tumor cells. We think that PDT during coincidence of the two phases of maximum accumulation of the agent in the tumor will not only destroy the tumor cells, but will also block the routes of their elimination.

We developed an experimental method for improving the efficiency of melanoma treatment by PDT.

MATERIALS AND METHODS

Experiments were carried out on 60 F_1(CBA×C57Bl/6) hybrids (20 g). Melanoma B16 served as the tumor model. The strain was obtained from the Bank of Tumor Strains, Laboratory of Combined Therapy of Tumors, N. N. Blokhin Cancer Research Center. Tumor cell suspension was transplanted subcutaneously into the hip. The animals were taken into the experiment on days 4-5, when visible diameter of the tumor reached 3-5 mm. The fur on the hip was removed. The animals were irradiated under thiopental narcosis (1.25% solution, 0.05 ml intraperitoneally). Tumor diameter was measured before injection of the agent (V_0) and on

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days 3, 7, 10, 14, and 21 (V_t) after PDT. Experiments were carried out with photolon as the PS in a total dose of 9 mg/kg. A Lameda device (λ=662 nm) served as radiation source. Irradiation was performed through a quartz monofiber lightguide with a lens at the tip. The diameter of the light spot was 1 cm, radiation energy density 150 J/cm^2, power density 0.12 W/cm^2, duration of exposure 21 min.

The animals were divided into 3 groups. Group 1 animals (experiment; n=20) were intraperitoneally injected with $^2/_3$ of total photolon dose (6 mg/kg; 0.5 ml) and after 1.5 h intravenously with the remaining $^1/_3$ dose (3 mg/kg; 0. 25 ml). The tumor was exposed to laser directly after repeated injection of PS. Due to this order of PS injections, the phases of the maximum accumulation of the agent in tumor vessels and cells coincided. Group 2 mice (control 1; n=20) received a single intraperitoneal injection of photolon in a dose of 9 mg/kg (0.75 ml). Laser exposure was carried out after 1.5 h (traditional PDT). Group 3 (control 2; n=20) consisted of untreated mice with tumors. The conditions of irradiation were the same in all groups.

The efficiency of PDT was evaluated by the following parameters.

Time course of tumor growth. Tumor volume was calculated by the formula:

$$V = \frac{1}{6}\pi \times d_1 \times d_2 \times d_3 \quad (\mathbf{1}),$$

where d_1, d_2, d_3 are three perpendicular diameters of the tumor and V is its volume (cm^3).

Coefficient of absolute increment of the tumor (K) was calculated by the formula:

$$K = \frac{V_t - V_0}{V_0} \quad (\mathbf{2}),$$

where V_0 is tumor volume before exposure and V_t tumor volume during a certain period of observation.

Percentage of complete tumor regression (CR) and growth inhibition (TGI) in comparison with control 2 were evaluated. The absence of visible and palpated tumor was considered as complete regression (K= -1.00). All tumors developing during the observation at the edge of or beyond the exposure area were considered as continuing growth (but not relapses), because there was no tumor in the exposed area, as a rule.

Tumor growth inhibition was calculated by the formula:

$$\mathrm{TGI} = \frac{V_c - V_e}{V_c} \times 100\% \quad (\mathbf{3}),$$

where V_c is the mean volume of tumor in the control group and V_e mean volume of tumor in experimental group.

Number of animals dead during the period of observation (21 days).

Incidence of tumor metastases in the lungs.

In order to detect metastases, the animals were sacrificed by chloroform overdosage after the end of the observation period. The lungs were removed, washed from blood in water, the lobes were separated, and the metastases were counted under an MBS-1 microscope (×12). The proportion of number of animals with metastases to the total number of animals in the group, expressed in percent, was taken for the incidence of metastases.

The data were statistically processed using Statistica 5.0 software. Descriptive statistics and Kruskal—Wallis dispersion method were used for comparison of 3 groups. Paired comparison was carried out using Mann—Whitney U test. The results were considered significant at p<0.05.

RESULTS

No local (at the site of exposure) reaction was detected directly after PDT in groups 1 and 2. In group 1, sharp edema of the entire paw was recorded on day 3 after the start of therapy. Palpation showed that muscle tissue was compact and there was a crust at the site of irradiation. The tumor was undetectable; in other words, it completely regressed in 100% animals. The TGI in comparison with untreated animals (group 3) was 100%.

Paw edema developed also in group 2, but muscle tissue was rather pliant, with a crust at the site of laser exposure. Complete regression of the tumor was observed in 40% animals. A small induration above the site of exposure in 8 mice was considered as continuing tumor growth, which was later confirmed. TGI in comparison with untreated animals (group 3) for this term was 58%.

In group 3, the tumor was palpated in 100% mice. Comparative analysis of the coefficient of absolute tumor increment showed statistically significant differences not only between groups 1 and 3 (p<0.000), but also between groups 1 and 2 (p<0.04; Fig. 1).

On day 7, a tumor appeared above the site of irradiation in one mouse in group 1. Complete regression was observed in 95% mice. TGI by this period was 99%. In group 2, during the same period tumor growth continued in 50% animals, at 80% TGI. The significance of differences between these groups was p<0.03.

On day 10, the previously detected tumor in group 1 increased in size, but no new tumors emerged. The CR and TGI values were the same as on day 7 (95 and 99%, respectively). In group 2 the tumor growth continued during this period in 60% mice, TGI being

76%. Statistical difference between groups 1 and 2 by the coefficient of absolute tumor increment remained significant ($p<0.004$).

On day 14, the percentage of animals with complete regression of the tumor decreased in group 1 (60%), but still surpassed that in group 2. The TGI in comparison with group 3 was 94%. Though the coefficient of absolute tumor increment in this group somewhat increased, it remained significantly lower ($p<0.001$) than in group 2. In group 2, the percentage of CR by this period dereased to 10%, while TGI was 64% compared to group 3.

By day 21, the number of animals with CR decreased significantly in group 1 in comparison with the previous periods, but still 2.5-fold surpassed that in group 2 and was 25%. The coefficient of absolute tumor increment was also significantly (4-fold) lower. The significance of differences between groups 1 and 2 was $p<0.001$. By this time, no mortality was observed in group 1, while in group 2 5% animals died. In group 3, the mortality was 21%. It is noteworthy that in none cases in this group no complete regression of the tumor was detected; moreover, progressive growth was observed in all cases. The coefficient of absolute tumor increment was significantly higher not only in comparison with group 1 ($p<0.000$ for all periods), but even in comparison with group 2 ($p<0.03$ on day 21 and $p<0.000$ during the rest periods).

Despite continuing tumor growth in 75% animals of group 1 by the end of the study, metastases in the lungs were detected in only 2 mice. In group 2, tumor growth continued in 90% animals and metastases were detected in 12 mice. In untreated animals (group 3), metastases were detected in 76.9% animals. The total number of metastases in group 1 was significantly lower in comparison with untreated animals ($p<0.000$) and with animals subjected to PDT ($p<0.001$).

Hence, the studies showed that under the same conditions of laser exposure, the method of PDT with photolon injected so that the phases of maximum PS accumulation in tumor (B16 melanoma) cells and vessels coincide is more effective than standard method, when the drug is injected in a single dose and the exposure is delayed until the period of its maximum accumulation in the tumor. The positive effect of this PDT method manifests in higher incidence of the tumor CR and in cases with its continuing growth by inhibition of its growth in comparison with untreated animals and with animals receiving traditional PDT; the effect manifests also in the absence of animal mortality during the entire period of observation and (the most important) in a statistically significant reduction of the incidence of metastases in the lungs in comparison with control groups 1 and 2.

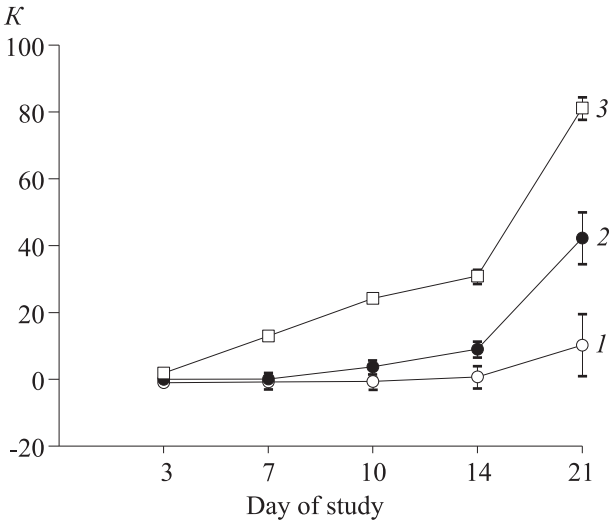

Fig. 1. Dynamics of coefficient of absolute increment of B16 melanoma in experimental and control mice. *1*) group 1 (experiment); *2*) group 2 (control 1); *3*) group 3 (control 2).

As for continuing tumor growth, only visible part of the tumor is exposed to laser with slight involvement of the adjacent tissues. It is quite possible that some cells, which were transported into regions distant from the exposure zone with the blood or lymph flow or via interfascial spaces during inoculation of the tumor emulsion, later start to grow. However, this hypothesis is to be specially verified and this factor is principally unimportant, because the conditions of tumor transplantation were the same in all cases. All marginal (in comparison with the irradiation site) tumors were taken into consideration and regarded as continuing tumor growth.

Hence, the method of PDT for melanoma with consideration for two phases of maximum accumulation of PS in the tumor is more effective in comparison with the traditional PDT for local treatment of the tumor and for reducing its metastases in the lungs.

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