Antitumor and anti-metastatic effects of photolon-mediated Sono-Photodynamic therapy on mice bearing Lewis lung Carcinoma

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Abstract

The aim of present study is to investigate the antitumor and anti-metastatic effects of sono-photodynamic therapy (SPDT) with photo sensitizer (PS) photolon on C57Black mice bearing Lewis lung carcinoma (LLC).

Materials and methods

Experiments were performed on 64 female mice C57Bl/6 with LLC. The tumors were sonicated (SDT) with prior intraperitoneally administration of photolon (5 mg/kg b.w). Sonication was performed in continuous mode with 1.5 W/cm² power density at 1.1 and 3 MHz frequencies for 5 min, 3 h after photolon administration using «Phyaction U»; Gymna Uniphy). Photo irradiation (PDT) of tumors was carried out immediately after sonication with semiconductor laser («UPL PDT», Lemt, λ=660±5 nm) at exposure dose of 50 J/cm² with output of 0.25 W for 3 min. The evaluation criteria of antitumor efficacy were tumor volume, tumor weight and a absolute tumor growth factor (K). About metastatic disease severity was assessed by the frequency of metastasis, the average number of metastases, the average weight of lung metastases and metastasis inhibition index (MII). Differences between groups were determined using Student’s t test, with p values of < 0.05 deemed significant.

Results

We have found that the greatest antitumor and anti metastatic effect has ultrasound (1.1 MHz, 1.5 W/cm²), and laser irradiation (50 J/cm²) in combination with photolon. Under combined treatment the following results were achieved: K coefficient was 8.88±2.93 and was significantly lower than in controls (78.25±18.57; p=0.002) and 1.1 MHz SDT group (31.31±9.03; p=0.029); the number of lung metastases decreased 1.9 times in comparison with photoirradiation (p=0.0008), 1.67 times - from SDT groups (p=0.03) and 2.6 times - to the control (p<0.0001). MII in SDT 1.1 and 3 MHz; PDT; SDT 1.1 MHz + PDT and SDT 3 MHz + PDT was 35.54; 42.82; 26.88; 61.51 and 41.46; respectively.

Conclusion

These results indicated that photolon-mediated SPDT significantly inhibited tumor growth and metastasis in mice LLC tumor model compared with SDT and PDT alone.

Keywords

Sono-photodynamic therapy; Photolon; Lewis lung carcinoma; Antitumor and anti-metastatic effects.

Abbreviations used

SPDT — sono-photodynamic therapy; PS — photo sensitizer; LLC — Lewis lung carcinoma; SDT — sono-dynamic therapy; PDT — photodynamic therapy; K — tumor growth factor; MII — metastasis inhibition index; US — ultrasound.

Introduction

Sono-photodynamic therapy (SPDT) is a new trend in modern experimental and clinical oncology [1]. SPDT based on the significant increase of the cytotoxicity of drugs combined with ultrasound (US) and photo irradiation of the tumor tissue. According to numerous studies of sono-photochemical reactions include a direct interaction of excited molecules with the help of ultrasonic radiation, the PS on the substrate and forming transient radicals that react with oxygen [2,3].

Interaction initiates a complex cascade of free radicals, such as singlet oxygen (1O2), hydroxyl radical (·OH), hydrogen peroxide (H2O2) and superoxide anion radical (·O2−), causing the development of oxidative stress syndrome. As a result, SPDT

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effectively induced tumor-cell apoptosis and necrosis. The two possible mechanisms might be: a) promoting mitochondria to release Cyto-C and activate Caspase-3, then to initiate apoptosis; b) the destroying of micro vessels, inhibition of angiogenesis and the induction of ischemia and anoxia of tumor cells, resulting in ischemic necrosis [4,11].

A number of experimental studies in vitro and in vivo confirmed the effectiveness of SPDT of malignant tumors with chlorine6, synoporphyrin sodium and hematoporphyrin monomethyl ether [4, 12, 13, 14, 15, 16].

At the moment not enough studied is the question about the features of metastasis of malignant tumors undergoing combined ultrasound and laser irradiations. In the available literature we have found few publications devoted to its study. Wang P. et al. concluded that the combined use of PS, US and laser radiation results in significant inhibition of tumor growth and metastatic processes in experimental tumors. This fact makes SPDT potential and effective option in testing the method in a clinical setting [17].

The purpose of this study is to investigate the antitumor and anti-metastatic effects of SPDT with photolon on a Lewis lung carcinoma model in C57Black mice.

Materials and methods

Experimental Animals

Sixty four C57Black female mice 20-25 g. obtained from the vivarium of the N.N. Alexandrov National Cancer Center of Belarus (Minsk, Republic of Belarus) were used. The animals received a standard diet and had permanent access to water. All manipulations were carried out according to the international scientific ethic standards of the quality of planning and carrying out animal investigations, according to «Methodic instructions for carrying out preclinical investigations of pharmacokinetics of pharmacologic substances and drugs» presented in the «Good Laboratory Practice TKP 125-2008» (Health Ministry of Republic Belarus, Minsk, 2008). Before treatment, all animals were anesthetized by intramuscular administration of a 0.2 ml 0.025% solution of droperidol. Removal of animals from the experiment was carried out using conventional methods of euthanasia. All effects were carried out for 10-12 days after tumor inoculation.

Tumor model

The model of tumor growth is epidermoid lung carcinoma Lewis (LLC), received from the bank of tumor strains of the Oncological Research Centre of the Russian Academy of Medical Sciences. LLC tumor tissue were transplanted under the skin of animals left groin in a volume of 0.3 ml of cell suspension in 10% Hank's solution («Biolot», Russia).

PS

Chlorine 6 conjugated with polyvinyl pyrro-lidone (Photolon® produced by Scientific Pharmaceutical Center of RUE «Belmedpreparaty», Minsk, Republic of Belarus) was injected intra peritoneally at a dose of 5 mg/kg.

SDT

Tumor insonation procedure was performed 3 h after photolon administration using («Phyaction U»; Gymna Uniphy, Germany) with an emitter of 1.5 cm²; 1,1 and 3 MHz ultrasound frequency in a continuous mode with 1.5 W/cm² intensity for 5 min employing stable techniques.

PDT

Photo irradiation of tumors was carried out immediately after sonication using diode laser with 661 nm wavelength (IMAF-AXICON, Minsk, Republic of Belarus) at doses of 50 J/cm² with 0.14 W/cm² fluence rate. The output was 0.25 W, the light spot diameter 1.5 cm, irradiation for 5 min.

Study groups are presented in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of animals per group, n</th>
<th>Ultrasound (parameters)</th>
<th>Photoirradiation (parameters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact control</td>
<td>14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SDT_1</td>
<td>10</td>
<td>1,1 MHz; 1,5 W/cm²; 5 min.</td>
<td>-</td>
</tr>
<tr>
<td>SDT_2</td>
<td>10</td>
<td>3 MHz; 1,5 W/cm²; 5 min.</td>
<td>-</td>
</tr>
<tr>
<td>PDT</td>
<td>10</td>
<td>-</td>
<td>50 J/cm²</td>
</tr>
<tr>
<td>SPDT_1</td>
<td>10</td>
<td>1,1 MHz; 1,5 W/cm²; 5 min.</td>
<td>50 J/cm²</td>
</tr>
<tr>
<td>SPDT_2</td>
<td>10</td>
<td>3 MHz; 1,5 W/cm²; 5 min.</td>
<td>50 J/cm²</td>
</tr>
</tbody>
</table>
Antitumor efficacy

The volume of tumors registered throughout the experiment. The animals were decapitated on 24th day after tumor transplantation. Evaluation of anti-tumor efficacy was performed on the effects of indicators characterizing the dynamics of the tumor volume (V), tumor weight (M) and the coefficient of absolute growth of the tumor (K).

Tumor volume was calculated by the formula: \[ V = \frac{4}{3} \pi d^3 \times d \times d \]

Where \( d_{1,2,3} \) - three mutually perpendicular tumor diameter; \( \pi / 6 = 0.52 \) - constant; \( V \) - Tumor volume, cm\(^3\).

Absolute tumor growth factor (K) was calculated by the formula:

\[ K = \frac{V_t - V_o}{V_o} \]

Where \( V_o \) – initial tumor volume (before photonol administration and treatment procedures); \( V_t \) – tumor volume for a certain period of observation.

The dynamics of tumor growth was recorded at 10, 12, 14, 17, 19, 21 and 24 days after transplantation.

Anti-metastatic effect

To assess the intensity of the process of metastasis the following criteria were used:

1. The frequency of metastasis - the percentage of animals with metastases in relation to the total number of animals in a group;
2. Average number of metastases per animal in the group;
3. The average weight of lung metastases LLC;
4. The degree of lung metastases LLC:
   - 0 degree – no metastases;
   - 1 degree – less than 10; diameter not exceeding 1 mm;
   - 2 degree – from 10 to 30 metastases;
   - 3 degree – more than 30 metastases with different size;
   - 4 degree – less than 100 metastatic nodes without formation of conglomerates;
   - 5 degree – more than 100 metastatic nodes with formation of conglomerates.

The metastasis inhibition index (MII) was calculated by the formula:

\[ MII = \left( \frac{A_k}{A} \right) \times \left( \frac{B_k}{B} \right) \times 100\% \]

Where \( A_k \) and \( A \) – frequency of metastasis to the lung in mice in the control and experimental groups;
\( B_k \) and \( B \) – average number of lung metastases per animal in control and experimental groups.

The number of lung metastases was counted after fixation of Carnoy's fluid (exposure time is not more than 20 min.) using a binocular microscope (magnification × 9).

Statistical analysis

The values obtained were processed using standard statistical methods of Origin Stat 7.0 software. The statistic processing of obtained results was conducted with the help of Student’s t-criteria. The significance level was determined as 0.05.

Results

The study of antitumor and anti-metastatic effectiveness of SPDT was performed on 64 C57Black female mice with LLC, obtained from the vivarium N.N. Alexandrov National Cancer Center of Belarus. Laboratory animals were divided into 5 groups of 10 animals in each group. In the control group there were 14 tumor-bearing mice receiving no treatment.

Data on antitumor efficacy of SDT, PDT and SPDT are presented in Table 2.

Table 2. – Antitumor efficacy of sono-photodynamic therapy with photolon on C57BLack female mice with Lewis lung carcinoma

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean tumor volume, cm(^3)</th>
<th>( p )</th>
<th>Tumor weight, g.</th>
<th>( p )</th>
<th>K level</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact control</td>
<td>1.52±0.35</td>
<td>–</td>
<td>4.52±0.98</td>
<td>–</td>
<td>78.25±18.57</td>
<td>–</td>
</tr>
<tr>
<td>SDTØ 1 MHz</td>
<td>1.11±0.18*</td>
<td>( p&gt;0.05 )</td>
<td>3.76±0.69</td>
<td>( p&gt;0.05 )</td>
<td>31.31±9.03*</td>
<td>( p=0.035 )</td>
</tr>
<tr>
<td>SDTØ 3 MHz</td>
<td>1.22±0.19*</td>
<td>( p&gt;0.05 )</td>
<td>4.19±0.71</td>
<td>( p&gt;0.05 )</td>
<td>43.26±11.61</td>
<td>( p=0.05 )</td>
</tr>
<tr>
<td>PDT° 50 J/cm(^2)</td>
<td>0.46±0.12*</td>
<td>( p=0.01 )</td>
<td>3.13±0.65</td>
<td>( p&gt;0.05 )</td>
<td>19.73±10.36*</td>
<td>( p=0.013 )</td>
</tr>
<tr>
<td>SDT 1 MHz + PDT 50 J/cm(^2)</td>
<td>0.32±0.14*</td>
<td>( p=0.014 )</td>
<td>2.01±0.55*</td>
<td>( p=0.033 )</td>
<td>8.88±2.93*</td>
<td>( p=0.002 )</td>
</tr>
<tr>
<td>SDT 3 MHz + PDT 50 J/cm(^2)</td>
<td>0.51±0.13*</td>
<td>( p=0.005 )</td>
<td>3.32±0.81</td>
<td>( p&lt;0.05 )</td>
<td>13.28±4.11*</td>
<td>( p=0.003 )</td>
</tr>
</tbody>
</table>

*\( p < 0.05 \) vs. intact control group; Ø SDT – sonodynamic therapy; ° PDT – photodynamic therapy.
LLC growth dynamics in laboratory animal control and main groups is shown in Figure 1. In the main subgroups (SDT 1.1 MHz; SDT 3 MHz; PDT 50 J/cm²) marked lower growth rates of tumor volume compared to control.

We found that the average volume of tumors in laboratory animals treated with PDT and SP 1.1 MHz and SPDT 3 MHz was statistically significantly lower when compared to the control group (p<0.05). This parameter in mice treated with SDT 1.1 MHz and SDT 3 MHz with an intensity of 1.5 W/cm² was 26.97 and 19.74% and less than the control, but produced the results are not statistically significant (p>0.05).

We found a statistically significant difference between the groups SDT (1.1 MHz) and SDT (1.1 MHz) + PDT 50 J/cm² (p=0.0026) and SDT (3 MHz) and SDT (3 MHz + PDT 50 J/cm²) (p=0.006) for comparing the average tumor volume in main groups.

We noted that at day 24 of the experiment, only mice subjected to combined US (1.1 MHz; 1.5 W/cm²; 5 min.) and photo irradiation (50 J/cm²) after photolon administration this figure was significantly less compared with laboratory animals of the control group (p=0.033) (Figure 2).

Absolute tumor growth coefficients (K) in the main groups in laboratory animals compared to the control are shown in Table 2.
As evidenced by the data presented, statistically significant differences were obtained in all groups (p<0.05), except for laboratory animals, treated by SDT with a pulse intensity of 3 MHz (p>0.05). K in groups of combined treatment with US intensities of 1.1 MHz and 3 MHz with photoirradiation (50 J/cm²) was 8.88±2.93 (p=0.002) and 13.28±4.11 (p=0.003). We found a statistically significant difference between the groups SDT (1.1 MHz) with SDT (1.1 MHz) + PDT 50 J/cm² (p=0.029), and SDT (3 MHz) with SDT (3 MHz) + PDT 50 J/cm² (p=0.025).

Based on these results, we can conclude that the greatest antitumor efficacy has a treatment regimen that includes a preliminary photonon administration, ultrasonic treatment at a frequency of 1.1 MHz pulses; intensity 1.5 W/cm² and subsequent photoirradiation with light exposure dose of 50 J/cm².

Data on anti-metastatic efficacy of SDT, PDT and SPDT are presented in Table 3.

According to Table 3, the average number of lung metastases after SDT with frequencies of 1.1 MHz and 3 MHz, and photo irradiation 50 J/cm² decreased to 1.55 (p=0.019), 1.75 (p=0.007) and 1.37 times (p=0.038) for compared with laboratory animals in the control group, respectively.

It is worth noting that in the combination therapy group also observed a statistically significant decrease in the test indicator (1.1 MHz US + photo irradiation 50 J/cm² - 2.6 times (p<0.0001); 3 MHz US + photo irradiation 50 J/cm² - 1.71 times (p=0.003). However, when comparing paired experimental groups we noticed the following tendency: the combination of ultrasound pulses with a frequency of 1.1 MHz and photo irradiation (50 J/cm²) marked decrease in the number of lung metastases 1.9 fold compared to photoirradiation only (p=0.0008) and 1.67 times - with ultrasound (p=0.03). When used in the proposed scheme ultrasound treatment at a frequency of 3 MHz statistically significant difference was not. We noted a slight decrease in the test indicator compared to photoirradiation (1.25 times; p>0.05) and an increase of 1.02 times compared with ultrasound (p>0.05).

Table 3. – Anti-metastatic efficacy of sono-photodynamic therapy with photolon on C57Black female mice with Lewis lung carcinoma

<table>
<thead>
<tr>
<th>Groups</th>
<th>Average number of metastases per group</th>
<th>p</th>
<th>Lungs weight, g.</th>
<th>p</th>
<th>Frequency of metastasis, %</th>
<th>MII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact control</td>
<td>43.9±4.3</td>
<td>–</td>
<td>0.856±0.09</td>
<td>–</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>SDTØ 1 MHz</td>
<td>28.3±4.4*</td>
<td>p=0.019</td>
<td>0.568±0.05</td>
<td>p=0.01</td>
<td>100</td>
<td>35.54</td>
</tr>
<tr>
<td>SDTØ 3 MHz</td>
<td>25.1±4.6*</td>
<td>p=0.007</td>
<td>0.544±0.04</td>
<td>p=0.004</td>
<td>100</td>
<td>42.82</td>
</tr>
<tr>
<td>PDT° 50 J/cm²</td>
<td>32.1±3.2*</td>
<td>p=0.038</td>
<td>0.575±0.04</td>
<td>p=0.009</td>
<td>100</td>
<td>26.88</td>
</tr>
<tr>
<td>SDT 1 MHz + PDT 50 J/cm²</td>
<td>16.9±2.1*</td>
<td>p=0.001</td>
<td>0.495±0.02</td>
<td>p=0.001</td>
<td>100</td>
<td>61.51</td>
</tr>
<tr>
<td>SDT 3 MHz + PDT 50 J/cm²</td>
<td>25.7±3.3*</td>
<td>p=0.003</td>
<td>0.751±0.08</td>
<td>p&gt;0.05</td>
<td>100</td>
<td>41.46</td>
</tr>
</tbody>
</table>

* p < 0.05 vs. intact control group; ° PDT – photodynamic therapy;

Data on weight of lung, affected by metastatic foci, shown in Figure 3.

Characteristics of the degree of metastatic lung lesions: the absence of lesions 4 and 5 degrees and a minimum number of laboratory animals with 3-th degree of the defeat in the combination-therapy group.
Discussion

In recent years the study of anti-tumor efficacy issues of SPDT with different PS is becoming increasingly important. These experimental data further indicate the prospects of the use of this method as an alternative to both traditional and effective options integrated circuits in the treatment of cancer [12, 13, 14, 15, 16]. However, the question of the anti-metastatic effect of this method is poorly understood. According to M. Trendowsky, using sonication in vitro on K562/A02 and human hepatocellular HepG2/ADM carcinoma circulating tumor blood cell lines resistant to chemotherapy in the presence of photosensitizing agents significantly reduces their number, reduces the viability without causing toxic effects on normal cells blood [17].

Wang P. et al. (Key Laboratory of Medicinal Resources and Natural Pharmaceutical Chemistry, China) investigated the antitumor and anti-metastatic effects of SPDT with PS chlorine6 (Ce6) in experiments in vitro and in vivo on tumor cell lines of malignant human breast (MDA-MB-231, MCF-7 and 4T1).

Research groups in vitro: control, Ce6 + US (SDT), Ce6 + photo irradiation (PDT), Ce6 + US + photo irradiation (SPDT) and Ce6 + photo irradiation + US (PSDT). Scheme in vitro: photo irradiation was performed on a semiconductor laser in the exposure dose of 1.2 J/cm² (λ=650 nm); sonication - an ultrasonic apparatus with the radiant coil of 35 mm with a pulse frequency of 1 MHz, the intensity of 0.36 W/cm² for 60 seconds. Irradiation of cell cultures was made after 4 hours of incubation in culture medium Ce6 at 1 μg/ml. Antitumor efficacy was evaluated on the basis of the number of viable tumor cells.

A similar distribution groups in the experiment performed on linear mice Balb/c. Photo irradiation was performed on subcutaneously transplanted tumors through 2 hours following intravenous administration Ce6 at a dose 10 mg/kg with exposure dose 120 J/cm² (λ=650 nm) and next US parameters: 1.9 MHz; 1.6 W/cm²; 3 min. Antitumor efficacy of treatment was evaluated on the basis of data on the dynamics of the increase in the mass of tumor and histological examination.

On three cell lines (MDA-MB-231, MCF-7 and 4T1) the authors observed a statistically significant decrease in the number of viable tumor cells in groups on SDT and SPDT and 52.17%; 55%; 44.34% and 55.71%; 48.8%; 53.62%, respectively, compared with groups of PDT (p<0.01; p<0.01; p<0.01) and PDT (p<0.01; p<0.01; p<0.05).

On day 22 of the experiment inhibition of tumor growth in the groups of PDT, SDT, PSDT and SPDT was 24.22%; 25.87%; 47.48% and 52.2%, respectively. Combined exposure led to a decrease in tumor mass compared with controls (p<0.01), SDT and PDT groups (p<0.05).

The average number of metastases in the control, PDT, SDT, PSDT and PSPTD was 63.43; 39.14; 38.43; 24.43 and 16.43; respectively (p<0.05) [18].

In our study, we have shown antitumor and anti-metastatic efficacy of the combination of ultrasonic and laser irradiation on pre-sensitized tumor tissue LLC with PS photolon. The effectiveness of the proposed treatment regimen characterized by a marked inhibition of tumor growth and metastasis by inhibiting processes on experimental tumors. The findings give hope that SPDT with photolon can find clinical application in the treatment of a number of nosological forms of malignant tumors.

References


