Mechanisms of tumor necrosis in photodynamic therapy with a chlorine photosensitizer: experimental studies

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ABSTRACT

A photodynamic therapy experiment on 118 inbred white mice with transplanted Ehrlich's tumor (mouse mammary gland adenocarcinoma) is performed to reveal mechanisms of necrosis formation. In 7-10 days the tumor of 1-1.5 cm diameter is formed under skin at the injection point, and PDT procedure is applied. There were used a chlorine type photosensitizer Radachlorine\textsuperscript{TM} and 662 nm wavelength diode laser. The drug is injected by intravenously at the dose of 40 mg/kg; the irradiation is executed in 2-2.5 hours at the surface dose of about 200 J/cm\textsuperscript{2}. Each of the mice had a photochemical reaction in form of destructive changes at the irradiation region with subsequent development of dry coagulation necrosis. After rejection of the necrosis there occurred epithelization of defect tissues in a tumor place. Histological investigations were conducted in different follow-up periods, in 5 and 30 min, 1, 3, 6, and 12 hours, 1, 3, 7 and 28 days after irradiation. They included optical microscopy, immune marker analysis, morphometry with measurements of volume density of epithelium, tumor stroma and necroses, vascular bed. The investigations showed that an important role in damaging mechanisms of photodynamic action belongs to hypoxic injuries of tumor mediated by micro vascular disorders and blood circulatory disturbances. The injuries are formed in a few stages: microcirculation angiospasm causing vessel paresis, irreversible stases in capillaries, diapedetic hemorrhages, thromboses, and thrombovasculitis. It is marked mucoid swelling and fibrinoid necrosis of vascular tissue. Progressive vasculitises result in total vessel obliteration and tumor necrosis.

Keywords: Laser, photodynamic therapy, histological investigations, transplanted tumor, Ehrlich's carcinoma, necrosis

1. INTRODUCTION

Photodynamic therapy (PDT) of malignant tumors with a new chlorine type photosensitizer “Radachlorine”\textsuperscript{TM} is known about 10 years. The method was investigated in experiment and demonstrated high efficiency, good chemical stability, and PDT activity. Pre-clinical and first-phase clinical trials demonstrated significant advantages of this photosensitizer: high excretion rate (94% in the first day after injection) very low dark toxicity, high contrast of tumor accumulation, high photodynamic activity and practical absence of side effects of this substance during experimental tumor treatment\textsuperscript{2-4}. The main effect of PDT is photochemical damage and destruction of tumor cells, development of aseptic necrosis of tumor and subsequent rejection of necrotic tissues\textsuperscript{5,6}. However, the mechanisms of antineoplastic PDT activity are not completely studied yet\textsuperscript{7}. The aim of this research was to reveal the mechanism of formation of tumor necrosis after PDT with Radachlorine.

2. MATERIAL AND METHODS

The experiment was carried out in 111 white inbred mice. Tumours were reproduced by subcutaneous inoculation of Ehrlich tumor (breast adenocarcinoma of mice). On 7-10 day a tumor 1-1.5 cm in size was formed in the place of injection of Ehrlich tumour cell suspension. Then PDT was performed in the basic group of animals. Photosensitizer Radachlorine was introduced (intraperitoneal injection; 40 mg/kg; 0.35 % solution), and 2-2.5 hours later the tumor was irradiated with laser (662 nm wavelength, total dose 200 J/cm\textsuperscript{2}).

The animals were euthanized in 5 and 30 minutes, 1, 3, 6, 12 and 24 hours, 3, 7 and 28 days after PDT, and histological analysis was carried out to reveal some mechanisms of tumor necrosis formation. The researches included the light
microscopy, immunohistochemistry, morphometry with studying of volume density of epithelium, stroma and necrotic tissue, and also changes of its vascularity.

Mice after sensitizer introduction without laser irradiation (control group I) and after irradiation without sensitizer introduction (control group II) served as a control. Intact animals with transplanted tumor served as methodical control (control group III).

3. MORPHO-FUNCTIONAL CHANGES IN TUMOR TISSUE DURING PHOTODYNAMIC THERAPY

3.1 Intact tumor group (control III)

**Macroscopic examination:** The intact intradermal tumor was up to 1.5 cm in size, soft, elastic, light pink, heterogeneous with whitish and dark red sites up to 0.2 cm in size. In 3 mice of intact group the tumor extended through muscular tissue, invading vertebra bodies.

**Light Microscopy.** The tumor was formed by atypical tissue in derma without involving of epidermis, and there was a narrow sheet of derma, free from tumor infiltration, between epidermis and tumor node. Tumor parenchyma was formed by 2 types of structures: rare tubular and numerous solid cellular layers (Fig. 1 a).

![Figure 1. Intact Ehrlich tumor: a – Solitary tubular structures among layers of atypical cells: “enclosed” muscle bundles, tumor necrosis fields. Hematoxylin and eosin staining x 100; b - polymorphic alcianophilic tumor cells, the advanced vascular network. PAS – alcian blue staining x 200; c – fine bundles of fuchsinophilic fibers. Van Gieson's picrofuchsin staining×100; d - loose reticular matrix of tumor stroma. Gomori's silver impregnation.×200.](image)

The volume density of parenchyma was 34.6±2.4 %. Tumor epithelial cell were polymorphic, with a ring of alcianophil cytoplasm (Fig. 1 b). Nuclei of cells were large, hyperchromatic, the majority with several nucleoli and numerous figures of pathological mitoses. All animals had secondary necrotic changes of tumor tissue. The average volume density of tumor necrosis was 8.3±2.4 %. The stromal component of tumor was 44.3±2.7 %; it consisted of rare bundles of mature fuchsinophil collagen fibers and loose reticular matrix (Fig. 1 c, d). Microcirculatory network was advanced enough, its volume density was 10.2±1.0 %. Irregular capillary filling and focal hemorrhages were registered on border of the tumor with the top layers of the skin. Average diameter of tumor vessels was 15.7±1.1 µm. In many fields of view the smooth muscle bundles were enclosed into invasive carcinoma and islands of fatty tissue were found out; their volume density was 2.6±0.8 % (Table. 3.1).

3.2 Basic group

Morphological research revealed circulatory disturbances in the basic group of tumor bearing animals 5 minutes after PDT session. In all fields of view capillary and arteriole spasm and extensive zones of tumor tissue with the devastated vessels were seen (Fig. 2 a). There were no significant differences in the volume ratio of epithelial and stromal components, and tumor necrosis after PDT in comparison with intact group (Table. 3.1).
Table 1. Morphometric changes of tumor structural components in intact and basic groups

<table>
<thead>
<tr>
<th>Values</th>
<th>Average diameter of vessels, µm</th>
<th>Volume density, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epithelium</td>
<td>Stroma</td>
</tr>
<tr>
<td>Groups (M±m) and time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact tumor</td>
<td>15.7±1.1</td>
<td>34.6±2.4</td>
</tr>
<tr>
<td>Basic group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 minutes</td>
<td>12.5±0.8*</td>
<td>35.4±2.3</td>
</tr>
<tr>
<td>30 minutes</td>
<td>9.5±0.7*</td>
<td>36±2.5</td>
</tr>
<tr>
<td>1 hour</td>
<td>11.8±0.6*</td>
<td>30.1±1.8</td>
</tr>
<tr>
<td>3 hours</td>
<td>22.4±2.0*</td>
<td>19.7±1.8*</td>
</tr>
<tr>
<td>6 hours</td>
<td>20.5±1.3</td>
<td>9.2±1.4*</td>
</tr>
<tr>
<td>12 hours</td>
<td>18.9±1.4</td>
<td>9.7±1.3</td>
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<tr>
<td>1- day</td>
<td>18.1±1.3</td>
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<tr>
<td>3-days</td>
<td>19.3±1.9</td>
<td>6.7±1.5</td>
</tr>
<tr>
<td>7-days</td>
<td>19.5±2.9</td>
<td>5.2±1.6</td>
</tr>
<tr>
<td>28 days</td>
<td>9.2±1.3*</td>
<td>2.8± 0.8</td>
</tr>
</tbody>
</table>

Note: * - Differences are statistically significant comparing with the previous time of observation (p <0.05).

30 minutes after PDT dystrophic and necrobiotic changes in tumor cells and epidermis developed along with vascular disturbance. The edema and swelling of tumor cells and increasing of volume density of epithelium up to 36.0±2.5 % were found out. The necrosis ratio increases up to 10.0±2.8 % (p> 0.05). The alternations of spastic strictures and paresis on the adjacent sites of the arteriole. Hematoxylin and eosin staining. x200; c – paretic capillary ectasia. . Biotin-streptavidine-peroxidase method for CD34 endothelial cells, hematoxylin additional staining x400; d - vessel plethora , blood stasis . PAS – alcian blue staining x 200.

1 hour after PDT angioparesis and hemorheological disturbances prevailed in tumor tissue: arteriole and capillary pareses, blood stasis with agglutination of erythrocytes in the lumen of vessels were seen (Fig. 2 c,d). The volume density of vascular bed and average diameter of vessels increased up to 6.9±0.8 % (p=0.09) and 11.8±0.6 µm (p=0.01) respectively in comparison with the previous term. The area of the tumor necrotic detritus enlarged up to 13.6±3.2 %, the
volume fraction of tumor epithelium decreased down to 30.1±1.8 % (p> 0.05). The epidermal layer of the skin above the tumor was necrotized too.

3 hours after session microvessel thrombosis and focal hemorrhages were found out in the tumor tissue (Fig. 3 a). The areas of not restored blood flow with collapsed capillaries (Fig. 3 b) were also found. The volume density of necrosis in parenchyma was 20.7±2.1 % (p=0.000 comparing with the intact group, and 5, 30 minutes groups; p=0.01 in comparison with the 1-st hour). The augmentation of average diameter of vessels and the volume of vascular bed and the decrease of epithelium volume density were statistically significant in comparison with those after 30 minutes, 1 hour (Table 1). Perivascular edema and hemorrhagic infiltrates caused by vascular disturbance were seen in stroma.

![Figure 3. Morphological changes in Ehrlich's tumour 3-24 hours after photodynamic therapy: a – necroses, hemorrhagic infiltration of tumor tissue. Hematoxylin and eosin staining x 100; b - necrosis of epidermis, devastated vessels. Hematoxylin and eosin staining x 100; c – plasma suffusion of arteriole walls. Hematoxylin and eosin staining x 100; d - weak leukocyte infiltration on periphery of extensive necroses. Hematoxylin and eosin staining ×200.](image)

6 hours after PDT, alterative and exudative changes in stroma and vessels of the tumor prevailed. Plasma suffusion of arteriole walls and swelled endothelium of vessel were seen (Fig. 3 c). There were the mucoid swelling signs in stroma with light pink PAS - positive staining and accumulation of acidic glycosaminoglycans. Gomori’s silver impregnation revealed swelled coarse reticular network. Tumor necrosis areas increased essentially up to 27.1±4.0 % (p=0.007; comparing with the 1-st hour), volume density of epithelium was 9.2±1.4 % (p=0.000). The volume fraction of stroma was 54.3±3.0 % (p=0.04; comparing with 5 minute group).

In 12 hours the tumor was represented by the fields of necrotic detritus (volume density 28.4±4.5 %), surrounding the layers of cancerous cells (9.7±1.3 %). The feeble round cell inflammatory infiltration was registered on the periphery of acidophilic necrotic masses in contrast to the previous terms of experiment and the intact group (Fig. 3 d). It testified to the necrosis caused by tumor damage, i.e. PDT action. Average diameter of vessels was 18.9±1.4 µm; their volume density decreased down to 5.2±0.6 % (p=0.02).

1 day after the session the histological picture was characterized by increase of dystrophic processes in stroma and vessels of the tumor. The accumulation of PAS - positive material in the thickened arteriole walls, segmental fibrinoid necrosis, perivascular infiltrates with the cells of lymphohistiocyte origin took place. The average diameter of vessels was 18.1±1.3 µm. The capillary network was disturbed, collapsed. The volume density of the vascular bed was 4.3±0.6 % at the average. Collagen fibers of stroma lost their fuchsinophilia, and were stained orange with picrofuchsin. The volume density of swelled stroma was 60.4±3.2 %.

In 3 days the epithelium in tumor structure was found out only in particular fields of view, its volume density was 6.7±1.5 %. The stroma with dystrophic changes, i.e. partial edema (64.8±2.9 %), and denuclearized necrotic detritus (24.6±4.8 %) prevailed. The signs of formation of granulation tissue, i.e. proliferation of fibroblast located in bundles on the border with necrosis were observed outside from necrotic masses. The arteriole walls were infiltrated with lymphoid cells, the signs of vasculitis were registered (Fig. 4 a).
On the 7th day of experiment the proliferation and marginal growth of the basal layer of epithelium under the crust was found out, the mature granulation tissue rested below (Fig. 4 b). The volume of non-resorbed necrotic masses came to 11.8±0.6 %. The volume density of necrosis was significantly decreased in comparison with the previous terms, i.e. 3, 6, 12, 24 hours after PDT. The groups of tumor cells (volume density 5.2±0.6 %) were found out in 2 mice among granulations. The vessels 19.5±2.9 µm in diameter were situated among the tumor cells, a part of them demonstrated the productive vasculitis and lumen stenosis. In others the organization of blood clot masses and thrombovasculitis development were seen (fig. 3.4). Capillaries in the tumor islands were revealed with immunohistochemical staining; the average volume of the vascular bed in parenchyma was 1.1±0.3 %.

By the 28th day the restoration of continuous layer of epidermis in 1 animal was seen; all layers of derma contained the mature fibrous connective tissue. Tumor cells were revealed in 1 mouse, they were situated among the bundles of collagen fibers; their volume density was 2.8±0.8 % (Fig. 4 d). Capillaries in the tumor tissue were revealed by immunohistochemical staining (volume density 0.9±0.2 %); their average diameter was 9.2±1.3 µm.

Thus, according to our morphological research, the photodynamic therapy resulted in vascular, destructive and regenerative processes in the experimental tumor. In the early stage of the experiment the consequent vascular disturbances, i.e. angiospastic and angioparetic changes of microvessels, blood stasis development, local thrombogenesis were revealed. Since 3 hours after treatment hemorheological disturbances resulted in increasing ischemia, dystrophy and necrosis of the tumor; the maximal development of the process was achieved by 6-12 hours. In the later terms (3, 7 day) the mucoid swelling, fibrinoid necrosis of vessel walls, perivascular infiltrates, thromb organization and development of productive vasculitis and thrombovasculitis which resulted in obliteration of vessel lumen were observed. Thus complete regress of the tumor has been achieved in 2 animals; but in one case the tumor cells were found out on the 28 day of experiment.

### 3.3 Control groups I and II

In the control groups I - II the tumor invaded the deep layers of derma, the epithelial layer of skin above the carcinoma was preserved, tumor necrosis without demarcation inflammatory reaction was seen in all terms of the experiment (Fig. 5 a).
Statistically significant differences in volume density of epitheliocytes, stroma and vascular bed, necrosis and average diameter of vessels were not revealed in 5, 30 minutes, 1, 3, 6, 12 hours after the treatment. On the 3 and 7 day of experiment the insignificant increase of volume density of tumor necrosis and decrease of average diameter of vessels were recorded, probably caused by tumor progressive growth, i.e. neoangiogenesis, rate of proliferation and destruction of tumor cells.

3.4 Comparative analysis of the basic group and the control groups I and II

To reveal the possible differences in tumor morphological changes we carried out the comparative analysis of morphometric parameters in the control groups I-III and the basic group.

In the basic group the volume density of vascular bed significantly reduced 5 minutes after PDT in comparison with control group II (Fig. 6.). Statistically significant tendency of vessel volume fraction decreasing continued for about 7 day in the basic group comparing with control groups I and II.

![Volume density of vascular bed, %](image)

**Figure 6. Changes of volume density of vascular bed in the control groups and the basic group.**

Average vascular diameter in the tumor of basic group was significantly smaller, than in the animals of the control groups 5, 30 minutes and 1 hour after the treatment.

As evident from the figure 7, statistically significant differences between the groups related to augmentation of vessel diameter in the tumor after PDT were revealed in 3, 6, 12, 24 hours and 3 days. On the 7th day there was no significant difference in the average diameter of vessels between the control and basic groups that was probably caused by tumor angiogenesis.
The main differences in volume density of tumor epithelium were found out in 3 hours (Fig. 8). At this time the appreciable decrease of volume fraction of epitheliocytes in the basic group was registered; and in the control groups the volume of epithelium retained at the former level. In 6 hours after treatment the volume density of epithelium in the basic group decreased 4 times in comparison with control groups I and II ($p=0.000$), i.e. the significant decrease of tumor tissue parenchyma volume was observed in the mice of the basic group in contrast to the control groups.

The volumes of tumor necroses demonstrated the similar dependence. So, by the 3d hour the necrosis fraction grew almost twice up to $20.7\pm2.1\%$ in the basic group ($p=0.000$) against $12.1\pm3.7\%$ in control group I and $12.0\pm3.3\%$ in control group II. The significant augmentation of volume density of necrosis was revealed in the basic group in comparison with the control groups ($p=0.005$) 6, 12 hours and 1 day after treatment. On the 3-7 day the volume of
necrosis in the basic group significantly exceeded those in control groups; on the 7th day the necrosis volume was 11.8±2.6 % in the basic group, 13.6±4.1 % in the group I and 10.7±3.9 % in the group II (Fig. 9).

The volume density of stroma in tumors of the basic group exceeded similar parameters in control group II in 1 hour ($p=0.006$); in 3 hours statistically significant differences were revealed between the basic group and the both control groups. The tendency of increasing of stroma volume after PDT in comparison with control was obvious at all terms of experiment (Fig. 10).

This tendency was caused by increase of dystrophic processes in stroma: plasmmorrhagia, perivascular and intercellular edema, mucoid swelling of collagen fibers on the early stage of experiment (1, 3, 6, 12 hours, 1 day). On 3 day there was granulation tissue growth into necrotic masses in the tumors exposed to PDT. Such effect was not observed in the mice.
of the control groups. By the 7-th day extensive fields of granulation were formed in all animals of the basic group, and by the 28-th day this tissue matured into tender fibrous connective tissue replacing the dermal defect.

4. DISCUSSION

The received results of clinical and morphological researches allowed us to create a chronological picture of photodynamic damage of tumor. We have defined four periods in the process of tumor destruction after PDT: the period of functional microcirculation disturbances, the transition period, the period of irreversible changes and necrosis formation, the period of tumor necrosis sloughing and cicatrix formation.

4.1 The period of functional microcirculation disturbances

The first period of functional microcirculation disturbances lasts about 1 hour from the PDT treatment. The initial response to the photochemical damage of the tumor is the microcirculatory bed vasospasm. Thus, already at the end of the PDT session the surface of the tumor, as a rule, became pale (ischemic), that testifies for circulation disturbance. The skin above the tumor became "glassy"-whitish with precise contours and slight hyperemia crown on the border with the healthy tissue. The tumor retains such appearance within one hour from irradiation (Fig. 11a).

![Figure 11](image-url)

Figure 11. Early vascular disturbances in Ehrlich's tumor after photodynamic therapy (5 – 30 min): a - general appearance: tumor became pale after PDT, initial manifestations of cyanosis; b- light microscopy: alternating of spasm and paresis in the adjacent sites of the arteriole. Hematoxylin and eosin staining. х200.

In the first 5 minutes after PDT only the microcirculation vasospasm and paretic dilatation of vessels in some areas were significant (Fig. 11 b). By 30 minute the initial manifestations of thrombosis were observed as a result of blood flow deceleration and blood stasis, along with functional microcirculation disturbances (spasm and paresis of vessels). We have not got reliable data about direct damage of tumor cells up to 30 minutes of our observation. There were no statistically significant changes of any parameters in the control group III (intact tumor) and control group I (Radachlorine without laser irradiation). In the control group II (laser irradiation without photosensitizer) tissues of irradiated area were slightly hyperemic during a short-term period after the irradiation (0.5 - 3 hours). Morphological research also demonstrated statistically significant increase of average diameter and volume density of skin and tumor vessels and their plethora that corresponded to clinical hype remia in the early terms, since 5 minutes. These phenomena can be regarded as thermal effect of laser radiation on the tissue, which on the one hand impacted the microcirculatory bed of the tumor causing hyperemia, and on the other hand promoted focal destructions of superficial layers of epidermis expressed as focal hemorrhages and edema. This alterations were temporary and reversible (till 6 hours), and we considered them as the result of thermal damage (fist-degree burn). When the laser power was decreased from 0.5 – 1.0 W to 200 mW with the same irradiation doze 200 J/cm², the vascular changes were not expressed that testifies for the thermal character of changes.

4.2 The transition period

The following transition period (1-3 hours after PDT session) was characterized by aggravation of the processes in tumor vessels, gradually getting irreversible. At this time microcirculatory vasospasm, decrease of the average diameter of vessels and volume density of vascular bed were still observed. However, alternating of spastic strictures and paresis in the adjacent sites of vessels with formation and aggravation of thrombosis were observed here and there (Fig. 12 b).
Paretic vasodilatation increased, and 1 hour after PDT angioparetic and hemorheologic disturbances were revealed: i.e. arteriole and capillary paresis and blood stasis with erythrocytes agglutination in the lumen of vessels. Clinical observations demonstrated that paleness was replaced with cyanosis (Fig 12 a), that testified for the progress of tissue ischemia. The volume density of vascular bed and average diameter of vessels increased in comparison with the previous term. In the first period the spastic stricture of arterioles resulted in decrease of blood filling and angiospastic ischemia; in the second period the spastic strictures were replaced with paretic vasodilatation of microcirculatory bed. Vessel paresis resulted in increase of blood filling in tumor, but venous drainage did not change, that caused postischemic venous hyperemia. It explains the edema and cyanosis of tumor. The transition period is the beginning of irreversible processes induced by incompetence of tumor vascular system. Such deep and irreversible vascular changes with clot formation resulted in the progressive ischemic necrosis of the tumor after PDT.

In the control group II (laser irradiation without Radachlorine) vascular changes and changes of other tumor components were statistically significant during the transitional period, however the changes were reversible and not so rough. Clinically there were no manifestations of deep necrosis; the irradiated skin was darkly red, with formation of limited perifocal edema by the 3-rd hour. In other control groups no visible and significant changes of tumor components were revealed.

4.3 The period of irreversible changes

In the third period the progressive changes in tumor vascular system were also evident in the basic group; however tumor necrobiosis was of the main interest. Thus, the given period should be considered as a result of two processes: vascular and necrobiotic. During this period, thromboses were formed and developed, focal hemorrhages increased and extensive areas of not restored blood flow were observed in the vascular system of the tumor. By the 6th hour the plasmatic suffusion of vessel walls was seen and the arteriole lumens were constricted down to complete absence due to swelled endothelium. Later, 12 and 24 hours after PDT, the expressed dystrophic changes of vessels, perivascular edema and infiltration were observed, and the tumor vascular network was completely disorganization of by the end of the first day.

The significant increase of tumor necrosis zone on the background of decrease of volume density of epithelium took place during all periods of observation, beginning with transition (1 hour after PDT). In three hours the perivascular edema and hemorrhagic infiltration were observed, they progressed with manifestation of mucoid swelling by the 6th hour. By the 12th hour the tumor was represented by fields of necrotic detritus (Fig. 13b) with feeble inflammatory infiltration of surrounding tissues on periphery. This infiltration demarcated the necrotizing tumor. All this effects became more expressed by the end of the first - second day after PDT.

Clinically the first signs of necrosis, i.e. dark sites on tumor surface, were seen by the 3-6th hour after PDT; and by the 12-24th hour the entire tumor was completely black in color with even distinct borders with surrounding tissue on periphery (Fig. 13a).
There were no statistically significant changes in the control groups III (intact tumor) and I (Radachlorine without laser) as before. In the control group II (laser without sensitizer) clinical and morphological changes coursed as a first-degree burn with the tendency to recovery. By the end of the 1st day, the skin got its usual color; the irradiated surface was dry and flaking.

4.4 The period of necrosis sloughing and cicatrix formation.

In the basic group the dry tumor crust was clearly demarcated from the healthy skin and then sloughed; granulation tissue occurred on border with necrotized tumor (6 day) and the edge epithelization initiated (Fig. 14).

In all three control groups the expressed, clinically significant signs of active tumor growth were observed during this period.

Thus, our results of clinical and morphological comparison, have allowed us to specify some mechanisms of damaging action of PDT, i.e. necrosis formation, and to offer classification of tumor necrobiosis stages. We divided this process into four periods. Such division allows us to understand the cause-effect relation between photodynamic action and tumor destruction (Table 2).

The results of the research allowed us to consider irreversible microcirculatory bed damage to be one of the reasons of tumor destruction after PDT, however it does not exclude other possible mechanisms.
Table 2. Formation of tumor tissue necrosis after photodynamic therapy.

<table>
<thead>
<tr>
<th>Period of tumor necrobiosis</th>
<th>Term</th>
<th>Clinical picture</th>
<th>Morphological picture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional microcirculation disturbances</td>
<td>5 – 60 min.</td>
<td>Tumor blanching</td>
<td>Vasospasm, vessel paresis, beginning of clot formation</td>
</tr>
<tr>
<td>Transition period</td>
<td>1 – 3 hours</td>
<td>Paleness is changed with cyanosis, tissue swelling begins to develop in tumor</td>
<td>Arteriole and capillary paresis, blood stasis with agglutination of erythrocytes in the vessel lumen, thrombosis increasing</td>
</tr>
<tr>
<td>Irreversible changes and necrosis formation</td>
<td>6 hours – 7 -8 days</td>
<td>Dark sites in tumor, the tumor became black, the necrotic crust is formed with clear demarcation from healthy skin</td>
<td>Microcirculatory disturbances and endothelium damage lead to vascular network disorganisation and total tumor necrosis.</td>
</tr>
<tr>
<td>Necrosis sloughing and cicatrix formation</td>
<td>2 – 5 weeks</td>
<td>Tumor necrosis sloughing and tender cicatrix formation</td>
<td>Development of granulation tissue on border with necrotized tumor, sloughing, initiation of boundary epithelisation.</td>
</tr>
</tbody>
</table>

5. CONCLUSION

The hemodynamic disturbances play the main role in the development of tumor damage after PDT. The changes are phasic, and may be divided into:
- the period of functional microcirculatory disturbances;
- the transition period;
- the period of irreversible changes and tumor necrosis formation;
- the period of tumor necrosis sloughing and cicatrix formation.

One of the mechanisms of tumor necrosis development after PDT is the hypoxic damage of tumor, mediated by disturbances of microcirculatory bed.

REFERENCES


