

TUMOR NECROBIOSIS STUDY IN PHOTODYNAMIC THERAPY WITH A CHLORINE TYPE PHOTSENSITIZER ON TRANSPLANTED EHRLICH'S ADENOCARCINOMA IN MICE

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Photodynamic therapy (PDT) of malignant tumors with a new chlorine type photosensitizer "Radachlorine"¹ 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 treatment²⁻⁴. 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^{5,6}. However, the mechanisms of antineoplastic PDT activity are not completely studied yet⁷. The aim of this research was to reveal the mechanism of formation of tumor necrosis after PDT with Radachlorine.

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; 40mg/kg; 0.35 % solution), and 2-2.5 hours later the tumor was irradiated with laser (662nm wavelength, total dose 200 J/cm²).

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).

An 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. The tumors were formed by atypical tissue in derma without involving of epidermis. Tumor parenchyma was formed by 2 types of structures: rare tubular and numerous solid cellular layers. In the control groups I - II the tumors also have usual structure with deep invasion in derma. 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.

In the basic group in all cases PDT induced photochemical reactions resulted in destructive changes in tumors with formation of dry coagulation necrosis in the end. We have defined four periods in the process of PDT tumor destruction.

The first period, named as *functional microcirculation disturbances* one, 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. 1a).



Figure 1. 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. x200

The second - *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. 2b).

In the third, *irreversible changes*, 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. Tumor necrobiosis realized in significant increase of tumor necrosis zone on the background of decrease of volume density of epithelium. By the 12th hour the tumor was represented by fields of necrotic detritus (Fig. 3b). 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. 3a).

In the fourth period, named as *necrosis sloughing and cicatrix formation* one, 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. 4).

So, the hemodynamic disturbances play the main role in the development of tumor damage after PDT. The changes are phasic, and may be divided into four periods: functional microcirculatory disturbances, transition, irreversible changes and tumor necrosis, tumor necrosis sloughing and cicatrix formation. One of the main mechanisms of tumor necrosis development after PDT is the hypoxic damage of tumor, mediated by disturbances of microcirculatory bed.

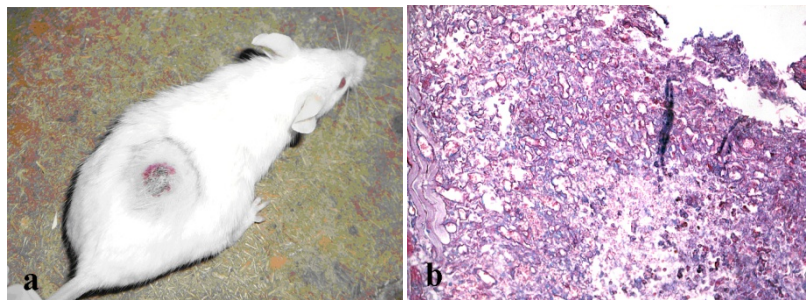


Figure 2. Vascular disturbances in Ehrlich's tumor after photodynamic therapy, 1 hour after PDT session: a - general appearance of tumor after PDT; b - vessel plethora, blood stasis. PAS – alcian blue staining x 200

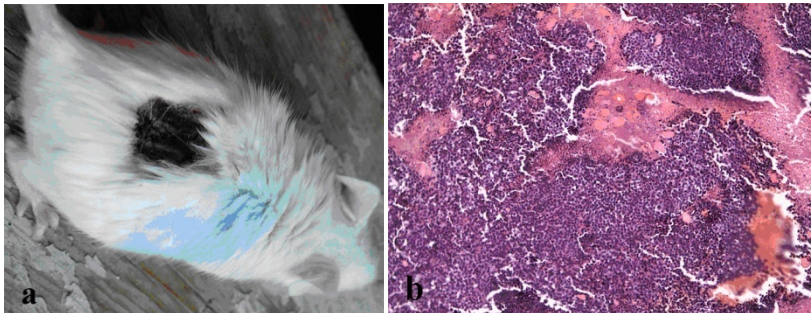


Figure 3. Necrosis of Ehrlich tumor, 12 hours after PDT. a – general view; b - histological picture: necroses of confluent character, hemorrhagic infiltration of tumor tissue. Hematoxylin and eosin staining x 100

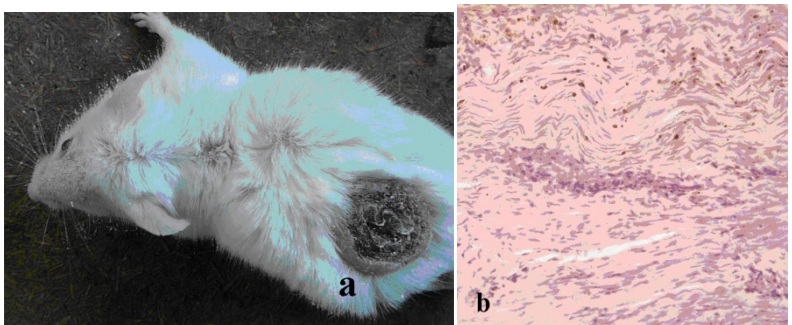


Figure 4. Formation of cicatrix tissue after necrosis sloughing. a – the beginning of cicatrix formation; b - the fibrous connective tissue in all layers of derma. Hematoxylin and eosin staining x 200

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