In vivo investigation of photosensitizers pharmacokinetics in mouse models of cancer by transilluminative fluorescence imaging

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1. Introduction

In recent years numerous photosensitive dyes have been developed for fluorescence diagnostics and photodynamic therapy of cancer. Efficiency of photodynamic therapy is known to be dependent on the photosensitizer concentration, tumor-to-normal tissue ratio, the interval between photosensitizer administration and irradiation [1]. For investigation of the tumor selectivity and pharmacokinetics such methods as fluorescence spectroscopy, spectrophotometry, confocal microscopy are widely used in animals studies. In many cases these techniques deal with ex vivo tissue samples which can affect photochemical characteristics of photosensitiser. Moreover, they lack the capability to observe tumor in their entirety. This is particularly important as tumor have been shown to be spatially and temporally heterogeneous. Current advances in molecular imaging and chemistry of photosensitive dyes offer new opportunities for application of optical technologies in experimental oncology [2-6].

The purpose of the study was to determine the feasibility of transilluminative fluorescence imaging for *in vivo* investigation of photosensitizers pharmacokinetics in mice.

2. Materials and Methods

Mice and Tumors. Experiments were performed on 10 male BALB/c mice bearing solid Erlich carcinoma, 25 female CBA mice bearing cervical carcinoma, 25 female BDF-1 mice bearing Lewis lung carcinoma. Tumors were transplantated in the subscapular region subcuteniously and used 14-20 days after inoculation (1-1.2 cm diameter).

Photosensitizers. Three dyes were studied: Photosens, Alasens and Photoditazine because their tumor selectivity and pharmacodynamics properties have been completely described by now.

Photosens is sulfo-substituted aluminium phthalocyanine $AlPcS_2$ (Niopic, Russia). The mice were injected with Photosens (1 mg/kg) per delivery route i.v. (in the lateral tail vein). In aqueous solution Photosens shows maximum absorption at 675 nm.

Alasens (Niopic, Russia) is a preparation on the basis of 5-aminolaevulinic acid. Although ALA is itself non-fluorescent, it induces the accumulation of endogenous protoporphyrin IX (PpIX) in tumor. PpIX exhibits fluorescence with maxima 635 nm and 700 nm. Alasens was administered per os to mice at 400 mg/kg.

Photoditazine is N-methyl glucosamine chlorine e6 salt (Veta-Grand, Russia). It has a powerful absorption band with a maximum at 662 nm. Mice were injected with 10 mg/kg Photoditazine i.v.

In vivo fluorescence imaging was performed with a fluorescence imaging setup (Russia). In this setup synchronous scanning of the object in the transilluminative configuration is provided by a single pair of a source and a detector set [6]. As a source of excitation light we employed a semiconductor laser at 635 nm. As a detector of fluorescent light we used a high-sensitivity cooled photomultiplier tube Hamamatsu H7422-20. Emission signal was filtered using 685 to 735 nm band-pass filter. For scanning procedure an animal was placed vertically in a glass container and slightly compressed to 1.2 cm. Image acquisition time per animal was 3–5 minutes. The mice were imaged *in vivo* at 10 min, 0.5 h, 1 h, 2 h, 3h, 4 h, 6 h, 8 h, 24 h, 48 h, 72 h and 96 h following the photosensitizer administration. The image obtained before injection was used as a control.

Fluorescence analisis. After imaging at the time points of 3 h, 24 h and 96 h animals were euthanized, and the tumors and normal organs and tissues were removed. Fluorescence was measured *ex vivo* using a spectrometer (QE65000, Ocean Optics Inc., USA). The tissue samples were excited with 635-nm light, and emission was collected between 660 nm and 760 nm. The presence of the photosensitizer in the tumor was also confirmed using an inverted laser scanning confocal fluorescence

microscope Axiovert 200M LSM 510 META (Carl Zeiss, Germany). For images obtaining we used excitation at 633 nm and signal collection in 650-710 nm range.

3. Results

Our results show that fluorescence imaging of photosensitizers is feasible in transplantable mouse tumors. Due to the selective accumulation of fluorescence dyes the tumors are clearly visible on the images. Results of serial imaging in the same animal demonstrate that transilluminative imaging technique is able to depict photosensitizer accumulation in tumor and washout over time (in individual animals). As an example, the *in vivo* images of Photoditazine in cervical carcinoma of CBA mouse are presented (Fig. 1). The average fluorescence signal in tumor varied from 57 a.u. in control image obtained before injection to 612 a.u. in 5 h after i.v. administration. At the time period from 5 to 8 h we observed maximum selectivity of Photoditazine. In 8 h the level of signal decreased to 501 a.u., in 24 h – to 236 a.u. By 96 h intensity of signal reduced to 70 a.u. which meant that selectivity is nearly lost.

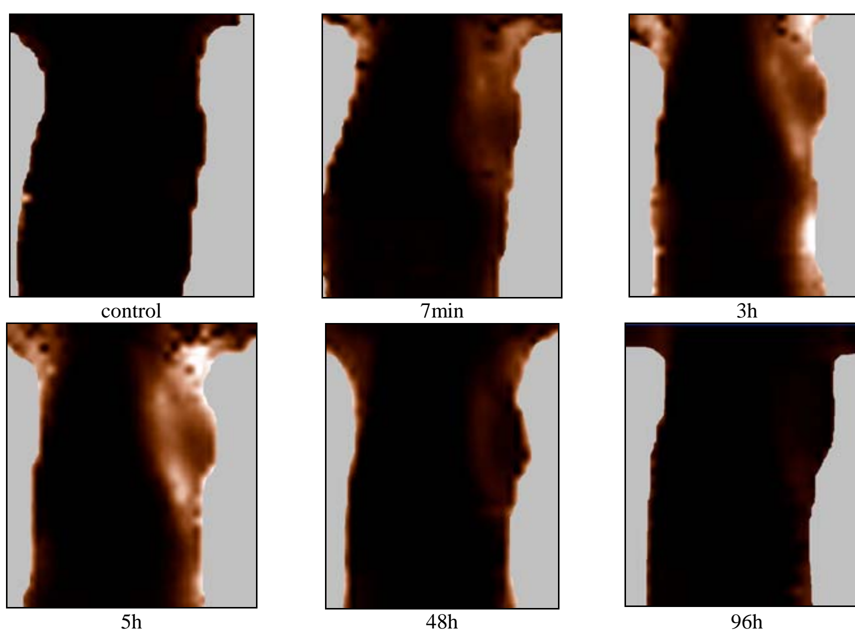


Fig. 1. *In vivo* fluorescence images of tumor-bearing mouse before (control) and after administration of Photoditazine. Images obtained in a transillumination configuration of source and detector of fluorescence imaging setup.

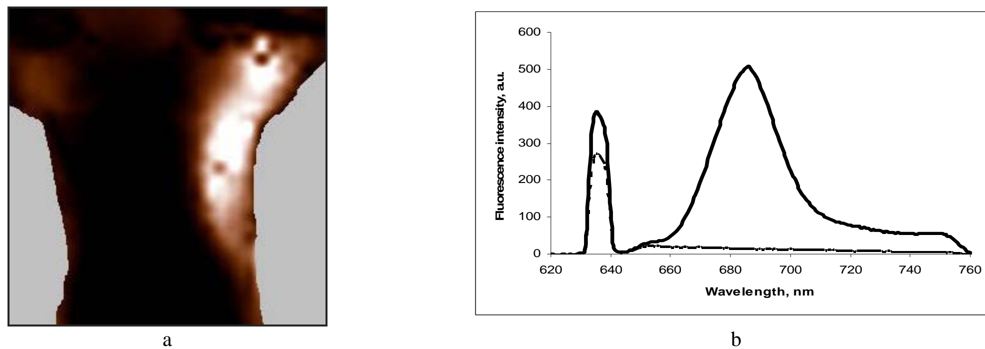
By using *in vivo* imaging, we determined that Photosens was located within tumor already in 10 minutes after i.v. injection. Tumor accumulation of Photosens became maximal by 3-7 h. At this time point the signal intensity reached to 1150 a.u. In 24 h the signal decreased to 870 a.u. Unlike the Photoditazine and Photosens, fluorescence of PpIX in tumor after external Alasens administration was visualized since the time point of 1 h (105 a.u.). Then the level of signal was gradually increased and reached maximum in 6 h (502 a.u.).

It is necessary to notice that Photoditazine and Alasens were used at the therapeutic doses converted from human to mice. As regards Photosens, it was taken at the dose two times less as therapeutic. Therefore, the sensitivity of imaging setup is sufficient for effective visualization of fluorescence dyes in animal study.

The *in vivo* experiments involved three tumor models of different histological origin. We revealed that mouse cervical carcinoma is more appropriate for transilluminative fluorescence imaging because of absence of large necrotic regions, mainly spherical-shaped nodules and slow growth rate. However, no considerable differences in selectivity and pharmacokinetics of photosensitizers concerning one or another model have been found out.

Our results match with information reported in literature on pharmacokinetics of the photosensitizers [7-8], as well as with correlative spectroscopic and microscopic imaging data.

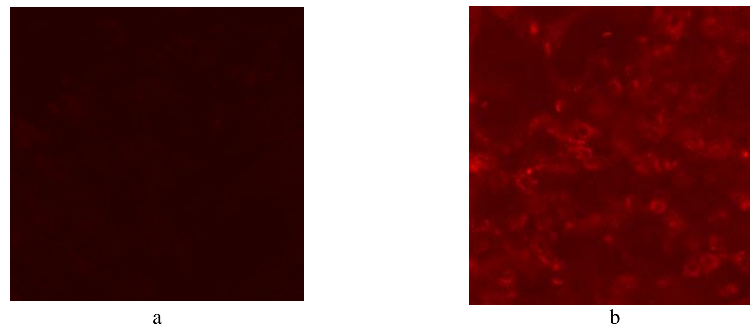
Fig. 2 shows the fluorescence spectrum of mouse cervical carcinoma tissue in 3 h after i.v. administration of Photosens when there was a maximum of photosensitizer selectivity. The spectrum has a peak at 685 nm and is identical to that of Photosens solution.



a b

Fig. 2. Maximum Photosens selectivity at 3 h after i.v. injection. a – in vivo fluorescence image obtained by transilluminative imaging setup; b – fluorescence spectrums of the same tumor (solid line) and tumor without photosensitizer (dashed line).

The fluorescence microscopic images of the same tumor, corresponding to Fig 2a, are shown in Fig. 3. The images illustrate the accumulation of Photosens in neoplastic tissue after 3 h.



a b

Fig. 3. *Ex vivo* confocal fluorescence image of the cervical carcinoma: a – no photosensitizer, b - at 3 h after i.v. administration of Photosens.

4. Conclusions

The results demonstrate the possibility of *in vivo* investigation of fluorescent dyes by means of transilluminative imaging technique. Described method may prove useful for evaluation of tumor-selectivity and pharmacokinetics of photosensitizers in animal studies. In principle, the fluorescence imaging setup used in this work allows to realize 3D-reconstruction of fluorophore distribution [6]. However, as the photosensitizer is located both in the tumor and in the normal surrounding tissue, it makes great difficulties for reconstruction in depth. Nevertheless, we suppose that an accurate and robust 3D imaging of the photosensitive dyes can be achieved after certain modification of reconstruction algorithms.

5. References

[1] B.C. Wilson, M.S. Patterson, “The physics, biophysics and technology of photodynamic therapy,” Phys. Med. Biol. **53** R61–R109 (2008).

[2] V. Ntziachristos, J. Ripoll, L. V. Wang, R. Wesslender, “Looking and listening to light: the evolution of whole-body photonic imaging,” Nat. Biotechnol. **23**, 313–320 (2005).

[3] R. M. Hoffman, M. Yang, “Whole-body imaging with fluorescent proteins,” Nat. Protocol. **1**, 3, 1429–38 (2006).

[4] Andersson-Engels S. Johnsson J., Svanberg K., Svanberg *S.* “In vivo fluorescence imaging for tissue diagnostics,” Phys. Med. Biol. **42** 815-824 (1997).

[5] X. Montet, J.-L. Figueiredo, H. Alencar, et al. “Tomographic fluorescence imaging of tumor vascular volume in mice,” Radiology**, 242**, 3 (2007).

[6] I.V. Turchin, V.A. Kamensky, V.I. Plehanov, et al. “Fluorescence diffuse tomography for detection of red fluorescent protein expressed tumors in small animals,” J. Biomed. Opt., **13**, 041310-10 (2008).

[7] C. Perotti, A. Casas, H. Fukuda, P. Sacca, A. Batlle “ALA and ALA hexyl ester induction of porphyrins after their systemic administration to tumour bearing mice,” British Journal of Cancer, **87**, 790 – 795 (2002).

[8] Kazachkina N.I., Zharkova N.N., Fomina G.I. et al. “Pharmocokinetical study of Al- and Zn-sulphonated phthalocyanines,” Proc. SPIE, **2924**, 233 (1996).

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