Journal of Applied Spectroscopy, Vol. 68, No. 3, 2001

FLUORESCENCE OF THE POLYMETHINE DYE TIKS AND DIAGNOSTICS OF CANCER

E. S. Voropai,[a] M. P. Samtsov,[b*] V. N. Chalov,[c] and É. A. Zhavrid[c]

UDC 535.37

It is shown that the fluorescence of the polymethine dye TIKS, whose absorption and fluorescence bands are located in the spectral region of transmission of biological tissues, can be recorded from a depth of up to 1.5 cm of an animal's body. The intensity of the fluorescence recorded from the surface of the animal's body in intravenous injection of the dye (1–2 mg/kg) is in direct proportion to its concentration in tumor nodes and muscles. In rapidly growing tumors, a high (up to 3.6) degree of contrast of the content of the dye is attained in tumor tissues as compared to the surrounding normal tissues. Over the course of 7 days after the injection, the dye is practically completely removed from both the tumor and normal muscular tissues. From the change in the fluorescence intensity in scanning the surface one can determine the regions of localization of tumor nodes against the background of the surrounding normal tissues and the presence of regions with a nonuniform distribution of the dye.

Keywords: *polymethine dye, fluorescence, localization, degree of contrast, tumor and normal tissues.*

For rapid identification of the region of localization of malignant tumors, one can use fluorescent compounds with properties differing markedly in tumor tissues and in normal ones [1–6]. Polymethine dyes (PDs) whose absorption and fluorescence bands are located in the spectral region of transmission of biological tissues seem promising for such investigations. The important characteristics which enable one to assess the possibility of using such a dye are: distance from the surface from which the fluorescence of the dye is recorded; relation between the useful signal and the luminescence of other stained compounds contained in the organs; influence of the saturation of the tissues in question with blood vessels on investigation results. It is also necessary to determine how much the spectral-luminescence properties of these luminophors differ in tumor and normal tissues. The present work seeks to solve the problems formulated above in relation to a glucose-modified tricarbocyanine indolenine dye (TIKS).

Objects and Procedures of Investigation. TIKS — a tricarbocyanine indolenine dye with 4-chlorine-substituted heptamethine conjugate chain and nitrogen-grafted glucose — was developed and synthesized for the first time at the Spectroscopy Laboratory of the A. N. Sevchenko Scientific-Research Institute of Applied Physical Problems. This compound belongs to the class of polymethine dyes that are promising as sensitizers for phototherapy [7–12].

*To whom correspondence should be addressed.

[a]Belarusian State University, Minsk, Belarus; [b]A. N. Sevchenko Institute of Applied Physical Problems, 7 Kurchatov Str., Minsk, 220064, Belarus; e-mail: samtsov@pfp.bsu.unibel.by; [c]N. N. Aleksandrov Scientific-Research Institute of Oncology and Medicinal Radiology, Minsk, Belarus. Translated from Zhurnal Prikladnoi Spektroskopii, Vol. 68, No. 3, pp. 359–362, May–June, 2001. Original article submitted January 30, 2001.

0021-9037/01/6803-0468$25.00

The investigations were carried out on non-thoroughbred white rats with tumors of the following strains (the tumors were reinoculated in the hip/thigh area): sarcoma 45, (S-45), sarcoma M1 (SM-1), alveolar hepatocarcinoma PC-1, and Worker's carcinosarcoma (W-256). Groups of 6–8 animals with regularly shaped tumor nodes were selected. The preparation was injected intravenously at a rate of 1–2 mg per kilogram of body mass.

The fluorescence of the dye *in vivo* was measured using a modified spectrometer based on a double monochromator of the Spex Company (USA) or an Lésa-6 spectrometric complex (developed by the Institute of General Physics of the Russian Academy of Sciences, Moscow). In the first case, the fluorescence was excited by radiation of a krypton laser (λ = 647.1 or 676.4 nm; the power is controlled within 0.01–0.20 W); an RCA 31034 A photomultiplier operating in the regime of photon counting was used as the radiation detector. In the Lésa-6 complex, a He–Ne laser (power at the outlet from the light guide is 15 mW) was used as the source of exciting radiation. In both experimental setups, supply of the exciting radiation to the object in question and recording of the fluorescence were carried out using Y-shaped light guides. The fluorescence was recorded directly through the skin. The light guide was applied to the portion in question at 5 to 6 points, and the values obtained were averaged. To take account of the level of scattered light and the intrinsic fluorescence of biological tissues, we performed check measurements in animals who did not receive injections of the preparations. In the course of the experiment, the animals who received the injection of a photosensitizer were kept under conditions which excluded the action of direct solar light on them. The absorption spectra of the dyes in solutions and specimens of the tissues were recorded by a PV 1251A spectrophotometer, while the fluorescence spectra were recorded by a Fluorolog spectrofluorimeter of the Spex Company.

The concentration of the dye in the blood and the specimens of animal tissue was determined by extracting them with a 2% solution of triton X-100 and recording their fluorescence using the Fluorolog spectrofluorimeter. The optical density of the specimens of the extracts was necessarily controlled; the linear correspondence between the fluorescence signal and the concentration of the dyes was ensured by dilution of the specimens when needed.

Results and Their Discussion. Figure 1 presents the absorption and fluorescence spectra of the dye in ethanol, the serum of calves' blood, and biological tissues. Since the fluorescence spectra of the dye in the specimens of muscular, cutaneous, and tumor tissues coincided, we gave one spectrum normalized to the maximum. The fluorescence quantum yield of the dye is 0.29 in ethanol and 0.30 in the blood serum. The maximum of the longwave absorption band of the dye in ethyl alcohol is located at λ = 725 nm, and the molar extinction coefficient is equal to $3.0 \cdot 10^5$ M^{-1} cm^{-1}. The absorption spectrum of the dye in the blood serum retains, in general terms, its shape in the organic solvent. At the same time, a bathochromic shift of 15 nm occurs (λ_{max} = 740 nm) and the halfwidth increases by ~10 nm. The fluorescence spectrum of the dye in biological tissues is also shifted to the longwave region by more than 10 nm as compared to the spectra in ethanol, and the halfwidth increases by 20 nm. The fluorescence spectra of the dye in the blood serum and the tissue specimens which are similar in position and shape show that it is difficult to determine the type of tissue in which the dye is contained *in vivo* from the analysis of spectral parameters.

In injecting the rats with different amounts of the dye, the intensity of its fluorescence *in vivo* for the hip/thigh muscles and sarcoma M1 and PC-1 tumors increased in proportion to the injected dose. The data on the change in the fluorescence intensity of the dye in the muscular tissues and tumor nodes of PC-1 with a twofold increase in the dose of the preparation are given in Table 1. There was a linear dependence of these parameters throughout the time of the experiment (24 h). A correspondence between the fluorescence intensity *in vivo* and the concentration of the dye in the tumor and muscular tissues and the skin was also observed in the case of its extraction from these tissues. The obtained data together indicate that the recorded signal of fluorescence of the dye in tissues *in vivo* is in proportion to its concentration indeed.

When animals receive an intravenous injection of dyes, a certain part of them is in the blood. In order to elucidate the influence of the saturation of tumor and muscular tissues with blood on measurement

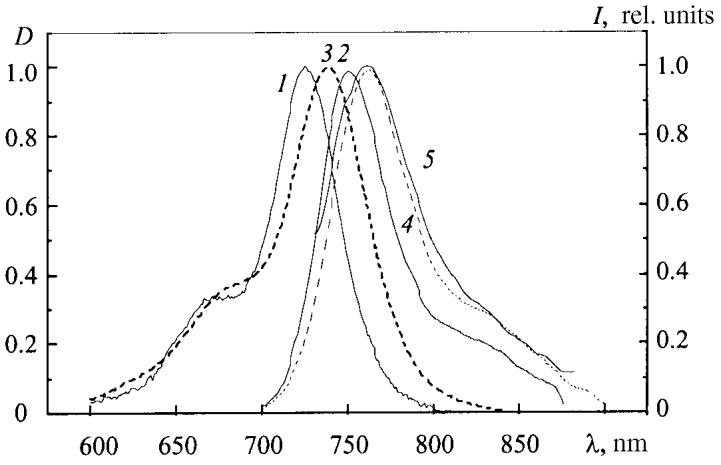

Fig. 1. Absorption (1, 3) and fluorescence (2, 4, 5) spectra of the dye TIKS in ethanol (1, 2), blood serum (3, 4), and muscular tissue (5).

TABLE 1. Ratio (I_2/I_1) of the Fluorescence Intensity of the Dye in Injecting the Animals with It*

Time, h	0.25	0.5	0.75	1.0	1.5	2.0	2.5	3.0	24.0
PC-1									
I_2/I_1	2.0	2.1	1.9	2.1	2.0	2.1	1.9	2.0	2.1
Muscle									
I_2/I_1	2.1	1.9	1.8	1.8	2.2	1.9	2.1	1.8	1.9

*A concentration of 1 mg/kg corresponds to I_1 and a concentration of 2 mg/kg corresponds to I_2.

results, we compared the fluorescence intensities of the dyes in these tissues just before removing the blood from the tissues of animal organs and immediately after it. It was established that in 1 h or more after injection of the dye, removal of the blood leads to a decrease of ~10% in the signal from the tissue surface. Furthermore, we recorded the kinetics of change in the content of TIKS in the blood by extraction by a 2% solution of triton X-100. A nearly exponential decrease in the concentration of the dye in the blood as a function of the time after injection was observed. In 1 h after the injection the content of the dye in the blood decreases by nearly an order of magnitude (see Fig. 2).

Investigation of the dynamics of accumulation of TIKS showed that for rapidly growing S-45, SM-1, and W-256 tumors (for three of the four investigated strains) we observe a higher intensity of its fluorescence and accordingly a higher concentration than in normal tissues. The content of the dye in both the tumor and normal tissues is maximum during the first 5 h after the injection; then a smooth decay begins and in 7 days just trace amounts are present. The relation between the fluorescence intensity of the dye in a tumor and hip/thigh muscles depends on the time after its injection (Fig. 3). For sarcoma M1, the ratio I_t/I_m is equal to 1.04 in 1 h after the injection of the dye; then it increases to 1.69 (in 1 day) and 2.17 (2 days). Subsequently the degree of contrast of the content of the dye decreases smoothly. The content of TIKS in Worker's carcinosarcoma is 1.2–3.6 times higher throughout the observation than in the tissues of rat's hip/thigh. For sarcoma 45, the concentration of the dye in the tumor during the first day after the injection is more than 1.5–2 times higher than the content of the preparation in the normal hip/thigh of rats. And it is just in tumor PC-1 that the content of TIKS is ~20% lower than in the normal hip/thigh, except for the point of 1 day after the injection when the content of the preparation in the normal and tumor tissues is nearly the same. Conse-

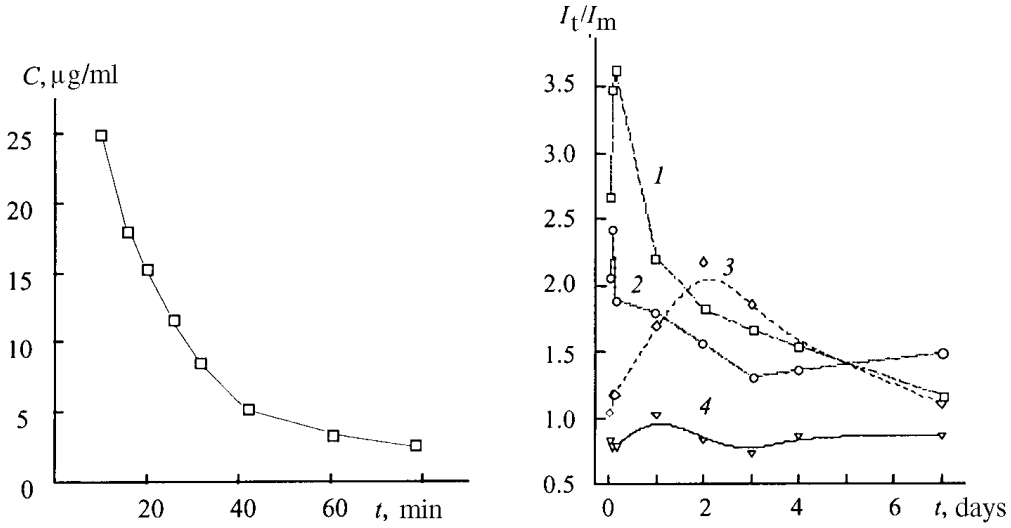

Fig. 2. Change in the concentration of the dye TIKS in animals' blood as a function of the time after its injection (the first measurement corresponds to 10 min).

Fig. 3. Ratio of the fluorescence intensity of the dye TIKS in tumor nodes [1) W-256, 2) S-45, 3) SM-1, and 4) PC-1] and muscles of rat's hip/thigh as a function of the time after its injection.

quently, time intervals after the injection of the dye exist when the boundaries of rapidly growing tumors can be determined by recording the fluorescence intensity of TIKS.

In order to determine the distance from the surface of the animals' body from which the fluorescence of the dyes is recorded, we analyzed the influence of the thickness of the tissue specimens on the fluorescence signal under concrete experimental conditions. Animals with rather large tumors were selected for investigation. In 1 h after injecting the animals intravenously with the dye, we identified tumor nodes together with a segment of the skin above the node. We applied a light guide to the tumor on the source side of the skin and recorded the fluorescence intensity as the specimen thickness was successively decreased. In recording in the region 800 nm using the spectrometer based on the double monochromator of the Spex Company and excitation of radiation with λ = 676.4 nm (the power at the exit from the light guide was 30 mW), we recorded a change in the fluorescence intensity with a decrease of 1 cm in the tumor thickness. For a thickness of the tumor tissue of 1.0, 1.5, 1.8, 2.2, and 2.5 cm, the fluorescence intensity was 1.0, 2.4, 3.0, 3.0, and 3.0 rel. units, respectively, i.e., for a larger thickness the fluorescence signal was invariant. In recording of the fluorescence by the Lésa-6 spectrometer, we observed a change in the fluorescence intensity with a decrease of less than 0.5 cm in the tumor thickness. Hence, when the appropriate equipment is used, one can record the dye molecules not only in the skin and layers of tumor tissue directly adjacent to it but at a considerable depth (to 1.5 cm) as well.

For certain tumor nodes of sarcoma M1 we observed considerable changes (of 2–3 times) in the fluorescence intensity of the dye in scanning over their surface. With the aim of elucidating the reasons for this, we investigated the dye distributions directly in the volume of tumor tissue. We prepared sections of the tumors and performed scanning over their plane. We investigated tumor nodes of PC-1 and sarcoma M1 (continuous and having necroses of the tissues, i.e., spontaneous segments atrophied intravitally). We emphasize that the sarcoma M1 strain with a tumor size larger than 1.5 cm is characterized by the appearance of spontaneous necroses in tumor nodes, while in the case of strain PC-1 tumor nodes grow without necroses,

as a rule. It was established that in tumors without spontaneous necroses the content of the dye at the surface of a tumor and inside it differed by no more than 7% for both strains. In scanning over the surface of such homogeneous tumors, the change in the fluorescence intensity was also no higher than 7%. In the region of spontaneous necrosis, the dye-fluorescence intensity was 3–5 times lower than in the remaining region. For tumor nodes in which the presence of spontaneous necrosis was subsequently revealed, the fluorescence intensity for different points differed by a factor of 2–3 in scanning over the tumor surface. It follows that under the conditions *in vivo* the fluorescence signal is affected to a considerable extent by the presence of inhomogeneities in the tissues of tumor nodes.

Thus, the dye TIKS, having the absorption of light and fluorescence in the region of transmission of biological tissues, can be recorded from a depth of up to 1.5 cm from the surface of an animal's body. The intensity of fluorescence recorded from the surface of the animal's body in injection of the dye in the range 1–2 mg/kg is in direct proportion to its concentration in tumor nodes and muscles. In rapidly growing tumors, a high concentration and degree of contrast of the content of the dye is attained as compared to the surrounding normal tissues. Over the cource of 7 days after the injection, the dye TIKS is removed practically completely from both the tumor and normal muscular tissues. The observed features of accumulation of the dye in rapidly growing tumors make it possible to determine the regions of localization of tumor nodes against the background of the surrounding normal tissues.

This work was carried out with support from the Belarusian Republic Basic Research Foundation, project No. F99-174

REFERENCES

1. J. C. Kennedy, S. L. Markus, and R. H. Pottier, *J. Clin. Laser Med. Surg*., **14**, 289–292 (1996).
2. M. M. Kriegmair, R. Baumgartner, R. Knuechel, et al., *Urology*, **44**, 836–839 (1994).
3. M. Kriegmair, R. Baumgartner, R. Knuechel, et al., *Urology*, **155**, 105–109 (1996).
4. K. Licha, B. Riefke, V. Ntziachristos, et al., *Photochem. Photobiol*., **72**, 392–398 (2000).
5. A. Becker, B. Riefke, B. Ebert, et al., *Photochem. Photobiol*., **72**, 234–241 (2000).
6. J. S. Reynolds, T. L. Troy, R. H. Mayer, et al., *Photochem. Photobiol*., **70**, 87–94 (1999).
7. Z. Diwu and W. Lown, *J. Pharmac. Ther*., **63**, 1–35 (1994).
8. C. S. Lipshutz, D. J. Castro, R. E. Saxton, et al., *Laryngoscope*, **104**, 996–1002 (1994).
9. D. A. Bellnier, W. R. Potter, A. Lurine, et al., *Photochem. Photobiol*., **62**, 896–905 (1995).
10. E. S. Voropai, M. P. Samtsov, A. P. Lugovskii, E. N. Aleksandrova, É. A. Zhavrid, and I. N. Zhuravkin, *Vestn. Bel. Gos. Univ*., Ser. 1, No. 3, 23–27 (1996).
11. E. S. Voropai (Voropay), M. P. Samtsov, A. P. Lugovskii (Lugovskiy), E. A. Zhavrid, E. N. Aleksandrova, I. N. Zhuravkin, U. P. Istomin, and S. I. Shastak, *Exp. Oncol*., **19**, 56–60 (1997).
12. S. I. Schastak, V. Enzmann, A. Jngel, E. A. Zhavrid, E. N. Alexandrova, E. S. Voropai, M. P. Samtsov, A. P. Lugovskiy, P. Wiedermann, *Laser Medizin*, **13**, 50–54 (1997).