In vivo autofluorescence investigations on animal tumors

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The in vivo fluorescence behavior of unmarked tissue (autofluorescence) of tumor-bearing mice was investigated. An argon-ion laser was used as the excitation source emitting radiation at 351 nm and 364 nn. Fluorescence bands in the red spectral range were recorded. The spectral distribution of the fluorescence of the solid Ehrlich carcinoma was different from that of the neighboring tissue.

Key words: Autofluorescence, laser, solid Ehrlich carcinoma.

The in vivo fluorescence diagnosis of cancer is based on the use of special fluorescent dyes and their property of tumor-selective retention. Because of this property tumors exhibit a greater amount of fluorescence than their neighboring tissues after the application of these tumor markers and light excitation. Hematoporphyrin derivative (HpD) a mixture of different porphyrins, is the most frequently used tumor marker [3].

The measured in vivo fluorescence signals of the applied marker are superimposed by background radiation resulting from the tissue fluorescence. This tissue fluorescence exhibits an unspecific autofluorescence with continuously increasing intensity from the red towards the blue-green wavelength range. In addition to this unspecific fluorescence, tissue-specific autofluorescence with a characteristic structure can occur. However, only a few investigations of this unmarked naturally occurring fluorescence have been carried out. Lasers with an appropriate emission wavelength and sensitive detector systems are necessary because of the small quantum yield of autofluorescence.

MONTAN et al. [4] recorded autofluorescence spectra of animal tumors with a peak of 480 nm using an excitation wavelength of 337 nm. YANMING et al. [5] measured fluorescence bands of about 632 nm and 700—720 nm on human tumors excited with UV radiation at 364 nm. The position and structure of these bands are in accordance with those of some porphyrins. Red peaks of about 634 nm (rat tumor TCT-4904) and 600 nm (mouse bladder tumor MBT-2), and a common fluorescence band of about 520 nm were found by ALFANO et al. [1]. The excitation source was an argon ion laser that emitted radiation at 488 nm. A high-power density was used. In this paper we report on the measurement of autofluorescence of different mouse

tissues excited by the UV lines of an argon ion laser including the fluorescence behavior of tumor tissue in comparison with the neighboring normal tissue.

Materials and methods

Animals and tumors. White male ICR mice were used. During measurements the animal was anesthesized (Rompun 5%), Ursotamin (10%), NaCl — 1 : 3 : 6). The tumor (d = 5 mm in diameter) was a solid s.c. growing Ehrlich carcinoma, induced by injection of 0.2 ml Ehrlich ascites cells (5 × 10^6 cells/ml). The skin above the tumor and the neighboring area was removed carefully.

Laser equipment. An argon ion laser (ILA 120, Carl Zeiss, Jena, equipped with UV mirrors) emitting up to 100 mW at 351 and 364 nm was used. The laser radiation was chopped and coupled to a 400 μm step index quartz fiber. A special device was constructed [2] in order to avoid falsifications of the fluorescence signals caused by background laser radiation (spontaneous emission from the Ar-discharge tube). The fluorescence radiation was coupled to a fibre bundle and transmitted to a prism monochromator. The detection of the weak tumor fluorescence was performed by the use of a red-sensitive photomultiplier and a lock-in device (Fig. 1). The examined tissues were excited with 150 mW/cm² at 364 nm (the irradiated area was 0.1 cm²).

Results

The autofluorescence of the tumors was investigated in the spectral region of 600—720 nm. Figure 2 shows the in vivo spectra of the tumorous and the neighboring tissues. Skin (epidermis and subcutis) in the tumor surroundings exhibited a fluorescence band with a maximum of about 670 nm, whilst peritoneum (with muscle tissue) showed a weak fluorescence in the red spectral range only.

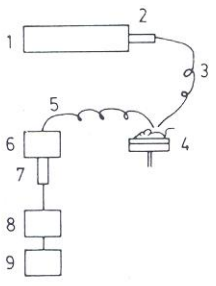

Fig. 1. Arrangement of the fluorescence detection equipment. A mirror on the top of the fibre 5 reflects the scattered excitation radiation. 1 — Laser, 2 — adapter with modulator, 3 — fiber (excitation), 4 — sample, 5 — fiber (fluorescence), 6 — monochromator, 7 — photomultiplier, 8 — lock-in, 9 — recorder.

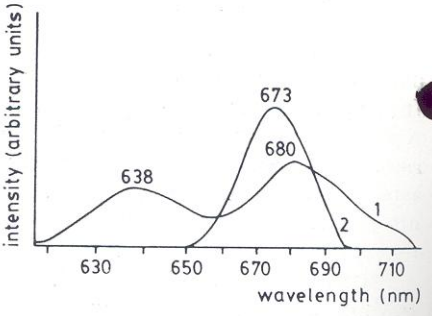

Fig. 2. In vivo fluorescence spectrum of the solid Ehrlich carcinoma and the neighboring tissue (without the unspecific autofluorescence). 1 — Tumor, 2 — skin.

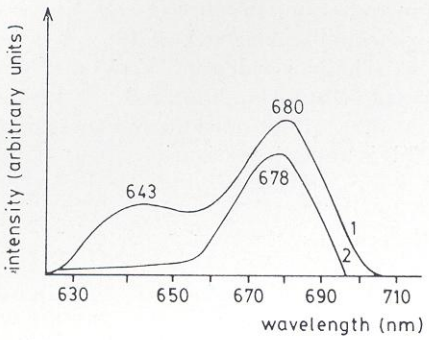

Fig. 3. In vivo fluorescence spectrum of external tissue (without the unspecific autofluorescence). 1 — Ear, 2 — tail.

The fluorescence of the examined EC-tumors consisted of two bands with maxima of 638 and 680 nm, respectively. The latter had a greater intensity. It was possible to distinguish between the tumor and the surrounding tissue with an accuracy of about 3 mm by means of the spectra.

It is interesting that other external tissues (such as the ear and the tail) exhibited a spectrum similar to that of the tumor (Fig. 3). The same characteristic spectral distribution of the fluorescence was found by measurements on other mice. However, the fluorescence behavior inside the tumor area varied because of the structure of the tumor (e.g., content of blood vessels).

After killing the animals the inner organs (liver, lung, spleen) were examined. No characteristic red fluorescence was found.

Discussion

Without any administration of fluorescent drugs, the tissue of the solid Ehrlich carcinoma showed a structured fluorescence in the red spectral range between 600 and 710 nm after UV excitation. The fluorescence spectrum is characterized by two main emission bands of about 638 and 680 nm. The fluorescence of hematoporphyrins has maxima of nearly the same wavelength but with reverse intensity distribution. It may be possible that the porphyrins of the body caused the obtained signals. A similar suggestion can be found in the study of YAUMING et al. [5]. Two characteristic red fluorescence bands were observed by investigations on human tumors [5] after excitation also at 364 nm (the position of the maxima differs about by 10—20 nm). In the neighboring tissue a red fluorescence was also found, nevertheless it was possible to identify the tumor by its spectral fluorescence characteristics.

It is interesting that all external dermis tissues showed a strong fluorescence in the red spectral range as opposed to the examined inner organs (measurements performed some minutes after killing).

ALFANO et al. [1] reported on a tumor-specific peak of nearly 520 nm of different rat and mouse tumors excited with 488 nm. The red bands measured in our experiments were not observed there. The use of different excitation sources and tumors might explain this inconsistent behavior. In the case of porphyrin fluorescence detection the excitation of the tumor surface with UV or violet radiation is more convenient than the use of longwave visible light because of the higher absorption of 350—420

nm radiation. Because of this, an undesired high-power radiation by the use of the excitation wavelength of 488 nm is necessary for detection.

The power density of 150 mW/cm^2 of UV used here is not low. It is known that porphyrins form photoproducts during irradiation. Because of this it cannot be ruled out that the obtained fluorescence spectra are influenced by fluorescent photoproducts. This is under investigation.

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