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Rapid communication

Photochemotherapy of animal tumors with the photosensitizer Methylene Blue using a krypton laser

K. König [1], V. Bockhorn [2], W. Dietel [1], and H. Schubert [3]

[1] Department of Physics, [2] Clinic of Urology, [3] Centre for Experiments on Laboratory Animals, Friedrich-Schiller University, Jena, German Democratic Republic

Summary. The cytotoxic effect of the photosensitizer Methylene Blue activated with long wavelength laser radiation has been investigated using mice with solid Ehrlichcarcinomas. A krypton laser was used as a light source emitting laser radiation at 647 nm and 676 nm. The red spectral range is important for photochemotherapy because it is easily transmitted through tissue. A significant tumor reduction, including complete tumor destruction, has been achieved.

Key words: Photochemotherapy – Photosensitizer – Methylene Blue – Laser

Introduction

The development of laser and fiber techniques has provided new possibilities for tumor therapy. On the one hand high-power lasers act as an "optical scalpel" by thermal and ablative effects, on the other hand low-power lasers are better used for photochemotherapy. In this case, laser radiation activates special dyes (photosensitizers) which can cause cytotoxic reactions. If the dye accumulates in malignant tissues more than in normal ones, selective therapy is then possible. Photosensitizers of greater importance are dyes with a strong absorption in the red or the infrared region (range of high tissue penetration). In this way it is also possible to treat more deeply lying tumors. The most widely used photosensitizer with tumor-localizing properties is a mixture of different porphyrins and their aggregates called hematoporphyrin, HpD (Kessel 1984). Selective destruction of tumors via photochemotherapy with HpD and lasers has been demonstrated by several clinical groups (Dougherty et al. 1978; Hayata et al. 1982).

Offprint requests to: K. König, Friedrich-Schiller University, Jena, Department of Physics, Max-Wien-Platz 1, DDR-Jena 6900, GDR

HpD has some disadvantages, however. It poorly absorbs red light and consists of an active component which has not yet been synthesized in a pure form. Furthermore, HpD stays in the body over a long period of time and causes skin photosensitivity. Therefore the search for alternative photosensitizers is of great interest (Doiron and Gomer 1983).

This paper deals with investigations on the photochemical action of Methylene Blue, which strongly absorbs longer wavelength red light. Its cytotoxicity in connection with light was verified in 1966 (Berg and Jungstand 1966). The cytotoxic effect of Methylene Blue was noticed in animal tumors following intratumoral application and excitation with a high-pressure xenonlamp. Because a correlation between the staining of tissue with Methylene Blue and the histological grading was found (Fukui et al. 1983) the question arises, in what respect does Methylene Blue act as a photosensitizer in photochemotherapy by radiation with red light.

The aim of these investigations was to examine the cytotoxic effects of Methylene Blue activated with long wavelenght laser radiation. The solid Ehrlichcarcinoma has been used as an animal tumor model in these experiments.

Materials and methods

Chemicals. The Methylene Blue used in this investigation was a component of the drug Coloxyd® produced by SPOFA United Pharmaceutic Plants Prague. The drug was diluted with isotonic NaCl solution and injected by intratumoral (into and around the tumor mass) administration (0.25 ml). In order to get a better dye diffusion, dimethyl sulfoxide was added.

Animals and tumor model. The tumors were solid s. c. Ehrlichcarcinomas in male ICRmice, induced by injection of 0.2 ml Ehrlichascites cells (5×10^6 cells/ml). The treatment followed 4 days after administration of the tumor cell suspension. Tumors were macroscopically noticed at this time on the shaved skin of the mice and were in the early stage of expansion.

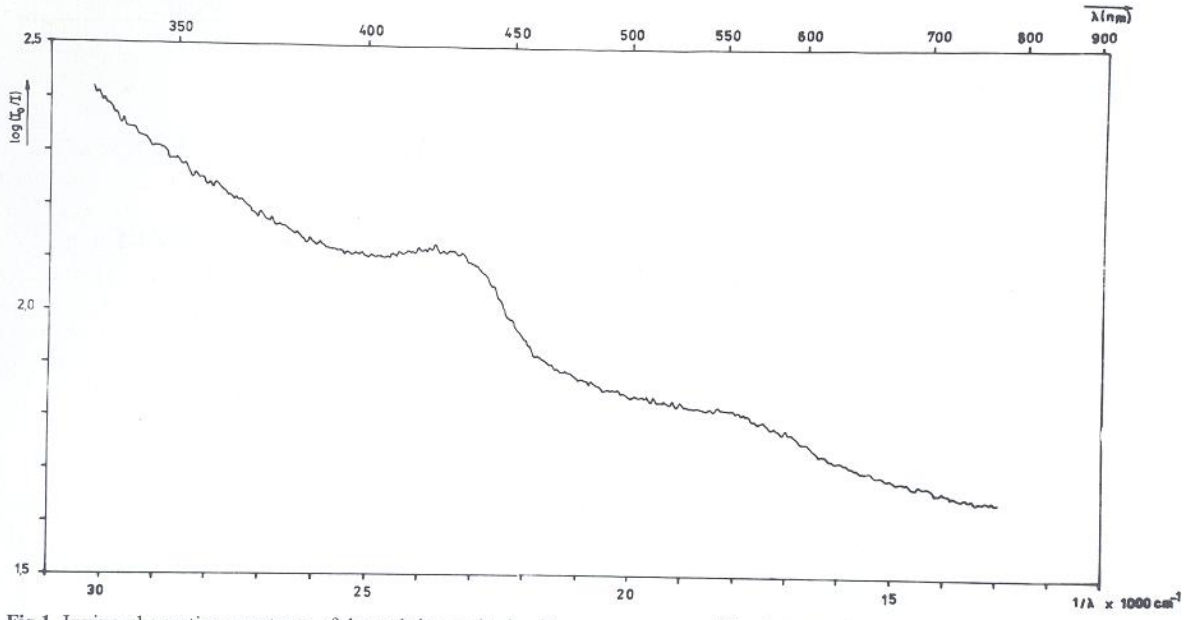

Fig. 1. Invivo absorption spectrum of dermal tissue obtained by measurement of the 0.1-mm thick ear of a white mouse

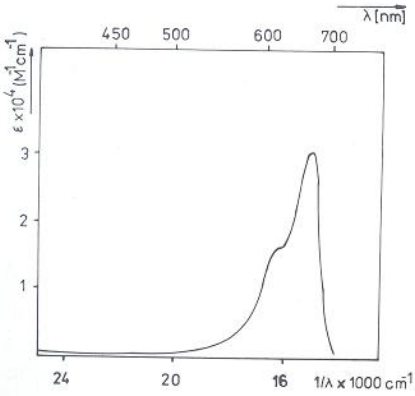

Fig. 2. Absorption spectrum of Methylene Blue (concentration $c = 3 \times 10^{-5}\ M^{-1}$; diluted with phosphate-buffered saline, pH 7.2)

The mice were fed ad libitum with standard pellets R 13 (VEB Versuchstierproduktion Schönwalde, GDR) and had permanent access to water. They were housed in plastic cages, temperature 22° to 25 °C. During treatment the mice were anesthetized (Rampoon®, Ursotamin®, NaCl).

Laser. The light source was selected taking into consideration the absorption spectrum of the dye and the tissue transmission. Figure 1 shows the absorption spectrum of dermal tissue. Because of the absorption of hemoglobin and pigments, there is only a small penetration of short-wave visible light into blood-containing tissue. In order to treat largely and deeply lying (millimeter range) malignant tissues, penetration of the light is a necessity. Therefore, the longer wavelength red and near infrared region is especially suitable for light penetration into the tumor tissue.

Methylene Blue shows a strong absorption band in the red spectral range (Fig. 2). A krypton laser was chosen as a light source emitting radiation at 647 nm and 676 nm. The ratio of the intensities of the lines was 4:1. The laser beam was coupled to a 200 µm step-index single optical fiber. The fieber tip was fixed so that the laser light illuminated the tumor completely. The beam had an intensity of 100 to 200 mW and a diameter of approximately 1 cm.

Treatment. At 30 min after intratumoral injection of Methylene Blue the tumors were irradiated for 10 to 30 min at intervals of 24 h. The animals were divided into different groups kept under the same conditions. The first group consisted of animals without drug administration and without laser irradiation, the second group received intratumoral drug therapy without irradiation, and the third group received both drug administration and laser treatment (see Table 2). With laser irradiation alone ($P = 200$ mW, irradiation time: 30 min, $\lambda = 647$ nm, 676 nm) no detectable influence on the growth of the tumors was observed.

According to other authors (Berg and Jungstand 1966) and our own investigations, an appropriate time for beginning the treatment should be after the first macroscopical appearance of the tumor and before the necrotic stage. The mice were killed 10 days after treatment and the weights of the tumors determined.

Results

At first the lethal rate 10 days after treatment was determined, dependent on the applied dose of Methylene Blue and laser irradiation (see Table 1). The radiation power used was 100 mW (80 mW at 647 nm and 20 mW at 676 nm), irradiation time was 10 min, 4 irradiations being applied in a period of 24 h. The drug was injected s.c. 30 min before irradiation of animals which had tumors. Response typical of a photosensitizer was observed.

In the following investigations, a drug dose of 25 mg/kg body weight was used. The sensitizer was now used in the treatment of solid Ehrlichcarcinoma. Table 2 shows the tumor masses after 4 laser irradiations ($P = 150$ mW, $t = 30$ min in a period of 24 h).

Table 1. Lethal rate dependent on the drug dose administered and irradiation

Injected dose of Methylene blue	Lethal rate (%) after drug administration without irradiation $n=10$	Lethal rate (%) after drug administration and irradiation $n=10$
250 mg/kg BW	0	90
125 mg/kg BW	0	50
25 mg/kg BW	0	0

BW: body weight (around 20 g), n: number of animals

Table 2. Tumor masses after laser irradiation

Group	MB	IR	n	Tumor mass/mg	Average tumor mass/mg	σ/mg
1	–	–	6	930; 690; 900; 840; 1140; 1040;	923	156
2	×	–	6	560; 420; 490; 400; 600; 290;	460	114
3	×	×	8	170; 200; 120; 100: 150; 200; 0; 0;	118	80

MB: Methylene Blue administration, σ: standard deviation, IR: irradiation with krypton laser, n: number of animals, tumor mass 0: not detectable by preparation (in groups 2 and 3 one animal showed tumor infiltration into the peritoneum. Because of no clear limitation with respect to the normal surrounding tissue these animals were not included in the table)

There were two drug administrations only, one before the first irradiation, the other before the third one. Methylene Blue therapy without laser treatment reduced the tumor mass (group-2) and this reduction depended on the drug dose and the number of administrations. The results obtained are in agreement with investigations of other authors (Berg and Jungstand 1966). Photochemotherapy using a krypton laser (with laser radiation of 647 nm and 676 nm) increased these effects significantly (group-3). In the present investigation, a tumor reduction of about 90% was achieved. As a side effect of the irradiation, edema, erythema, and partial inflammation were observed for a time of 1 to 2 weeks. The animals without exposure (group-2) showed no visible side effects.

The tumors were examined histologically and the treated animals (group-3) showed necroses and cell destruction. Temperature measurements by means of thermocouples inside the tumor mass indicated a temperature increase smaller than 4 °C during the irradiation period. Thus thermally induced necroses should be excluded.

Discussion

The cytotoxic effect of the photosensitizer Methylene Blue in connection with the radiation of a kryptonion laser was shown in mice with s.c. Ehrlichcarcinoma. The tumor model was chosen in accordance with former experiments with Methylene Blue (Berg and Jungstand 1966). Because of high tissue penetration in the long wavelength spectral range, photosensitizers with absorption in the red and infrared range are of importance in photochemotherapy. (Methylene Blue has a particularly strong absorption in the red range). A significant tumor reduction, and in some cases complete tumor destruction, were achieved using red laser radiaton (krypton laser with 647 nm and 676 nm). A cytotoxic effect caused by administration of the photosensitizer alone without any irradiation has been noted by other authors (Berg and Jungstand 1966). The photochemotherapy with Methylene Blue activated with long wavelength laser light can be of clinical importance mainly for the treatment of bladder tumors. Fukui et al. reported on the instillation of Methylene Blue in the bladder of 129 patients with bladder carcinoma and found a correlation between the extent of staining and the histological grading (Fukui et al. 1983). Selective photochemotherapy is then possible. In addition, the instillation of photosensitizers into the bladder can considerably reduce side effects due to sensitivity of the skin to light. Furthermore, laser light can be directed into the bladder via light fibers and endoscopes. We have initiated treatment with the photosensitizer Methylene Blue on patients with bladder tumors, the results of which will be published in the future.

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