J Cancer Res Clin Oncol (1994) 120:657–661

Cancer Research
Clinical Oncology
© Springer-Verlag 1994

Photodynamic therapy of experimental colonic tumours with 5-aminolevulinic-acid-induced endogenous porphyrins

K. Orth[1], K. König[2], F. Genze[1], A. Rück[2]

[1] Department of General Surgery University of Ulm, Germany
[2] Institute for Laser Technology in Medicine, Ulm, Germany

Received: 15 September 1993/Accepted: 25 May 1994

Abstract. 5-Aminolevulinic acid (5-ALA) is a precursor in the biosynthesis of haem. External application of 5-ALA leads to the formation of protoporphyrin IX, the last intermediate product before haem, which is an effective sensitiser. The 5-ALA-induced endogenous photosensitisation of tumour cells has been exploited for photodynamic therapy (PDT). Experimental human G-3 colonic tumours were transplanted into nude mice, and ten mice were treated by PDT. Ten animals served as controls. We measured a fluorescence intensity of the tumour that was about eight times higher than in the surrounding tissue; a good correlation between the fluorescence intensity and the photodynamic effect was found. Tumour growth was inhibited significantly after PDT, two tumours being destroyed completely after the second PDT treatment. In addition, on-line fluorescence detection during PDT showed a change in the intensity and the fluorescence spectrum of protoporphyrin IX caused by photobleaching and the formation of photoproducts.

Key words: Photodynamic therapy – Fluorescence – 5-ALA – Experimental colonic tumour

Introduction

Photodynamic therapy (PDT) is a new treatment modality that involves the systemic or topical administration of a photosensitiser followed by subsequent exposure to light delivered from lasers or appropriately filtered lamp sources. This therapy results in damage to tumour cells (Kelly et al. 1975) and destruction of the vasculature of the tumour (Henderson et al. 1985). If the sensitisers accumulate more in malignant than in normal tissue, selective tumour therapy is possible (Zalar et al. 1977; Barr et al. 1991). A strong absorption in the red or infrared region is advantageous because there is a deep tissue penetration of light in that frequency region.

In our research efforts in this area we used 5-aminolevulinic acid (5-ALA), a precursor and the first committed step in the biosynthesis of haem (Fig. 1). In the presence of excess 5-ALA the natural regulatory feedback system is disturbed, allowing accumulation of endogenous protoporphyrin IX, which is an effective sensitiser (Malik and Djaldetti 1979; Sima et al. 1981; Kennedy et al. 1992; Charlsworth et al. 1993). Divaris et al. (1990) showed that, after intraperitoneal administration of 5-ALA to mice, protoporphyrin IX accumulated in the skin, the urothelium of the bladder and the endometrium of the uterus in sufficient amounts. This group has also demonstrated that the underlying layers in these organs exhibited a relatively small concentration of sensitisers, which could enable selective treatment of cancers in the endothelium and urothelium without causing perforation of the uterus or bladder. Barr et al. (1988, 1991) could destroy small experimental colon cancers without producing any damage to adjacent normal colon tissue exposed to similar doses of light. Bedwell et al. (1992) and Loh et al. (1993) showed necrosis of normal mucosa and tumour in the rat colon sparing normal muscle after PDT, when 5-ALA was given intravenously or orally.

The purpose of our study was to investigate fluorescence modification during PDT and the tumour response after PDT in colonic-tumour-bearing nude mice.

Materials and methods

Chemicals. In this study we used 5-ALA at 7 mg/mouse (Sigma, Germany). Topical application was carried out with the solvent phosphate-buffered saline (PBS) dimethylsulphoxide 1 : 1. Intravenous and oral application was performed with 5-ALA dissolved in PBS and neutralized.

Tumours and animals. Human G-3 colonic tumours (adenocarcinoma) were transplanted subcutaneously into 24 female nude mice (NMRI *nu/nu*; body weight about 25 g). When the tumour had reached 1.5 cm in diameter (after about 4 weeks) PDT treatment was started. 5-ALA was administered intravenously in ten mice. After an incubation period of 3 h the tumour was irradiated with the 633-nm wavelength of an argon-laser-pumped dye laser (100 mW/cm^2, 150 J/cm^2). As controls we used three tumour-bearing mice that had received i.v. 5-ALA but no irradiation, as

Abbreviations: PDT, photodynamic therapy; 5-ALA, 5-aminolevulinic acid

Correspondence to: K. Orth, Steinhövelstrasse 9, Chirurgische Klinik I, D-89075 Ulm, Germany

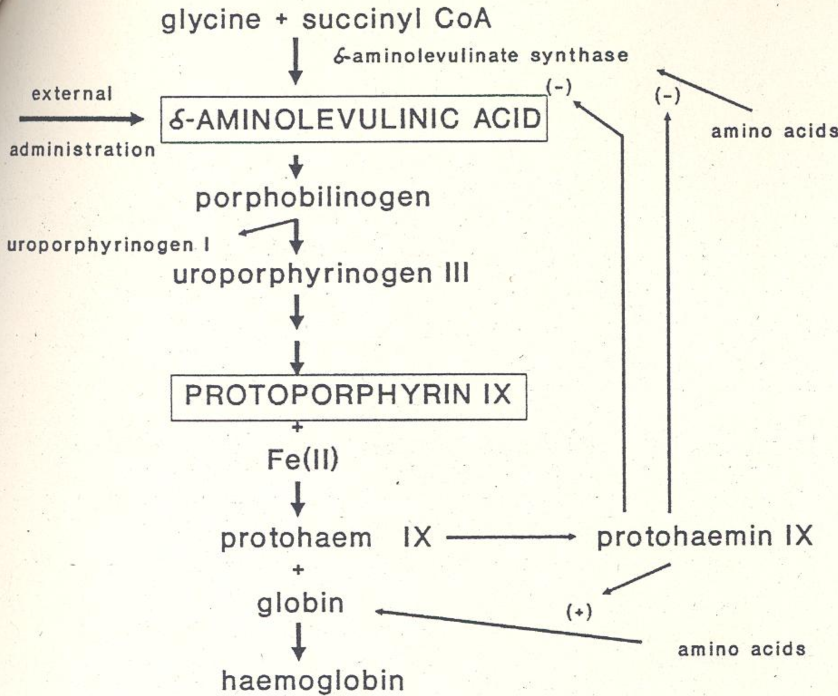

Fig. 1. Biosynthesis of fluorescent protoporphyrin IX (McGilvery and Goldstein 1979) using external 5-aminolevulinic acid administration

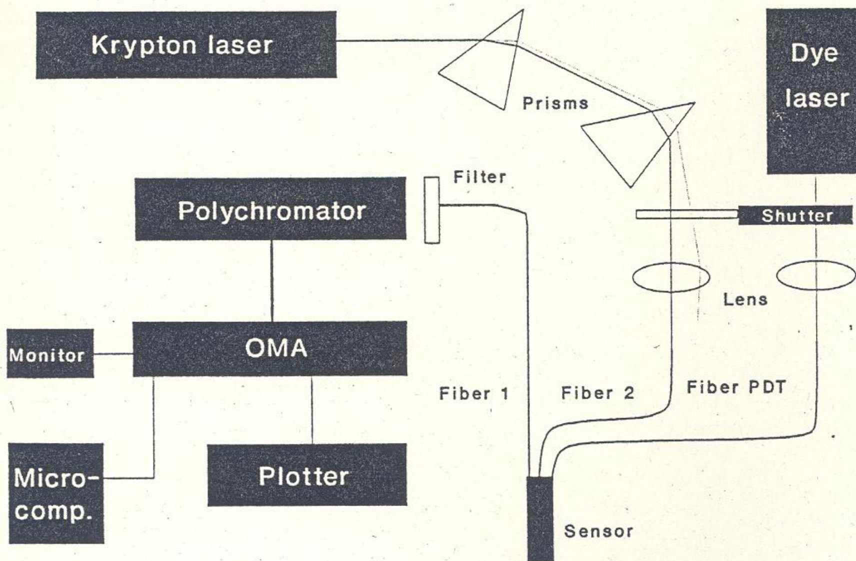

Fig. 2. Experimental equipment for in vivo fluorescence measurements during photodynamic therapy (PDT). *Fibre 1*, fibre for fluorescence measurements; *fibre 2*, fibre for excitation of fluorescence (407 nm); fibre PDT, fibre for PDT treatment (633 nm); *OMA*, optical multichannel analyser

well as three mice that had undergone tumour irradiation but no 5-ALA application. Four mice did not receive 5-ALA and were not irradiated with light. Under general anaesthesia (intramuscular Ketanest), the skin covering the tumour was carefully removed for treatment. Two weeks after the first PDT we performed a second PDT with 5-ALA. The tumour volume was calculated every week by three-dimensional measuring. In addition to i.v. injection of 5-ALA, we applied the sensitiser topically (n=2) and orally (n=2). The topical application was done by fixing a 5-ALA-containing cotton swab to the skin above the tumour for 3 h. 5-ALA was mixed into the mouse food for oral application.

Experimental equipment. The equipment of our experimental apparatus is demonstrated in Fig. 2. An argon-laser-pumped dye laser emitting at 633 nm was chosen as the light source for PDT. The 407-nm line of a krypton laser was used for excitation of the fluorescence. The background radiation of the krypton laser, consisting of spontaneous radiation and luminescence from the Brewster windows, was separated using two quartz prisms. The 407-nm radiation was coupled into the 0.3-mm central fibre of a fibre bundle. Eight peripheral fibres were used for detection, transmitting the fluorescence radiation into a polychromator and an optical multichannel analyser (König et al. 1993; Schneckenburger et al. 1992). The registration of a single spectrum took 20 ms, but was integrated over 1 s. During fluorescence measurement, a shutter stopped PDT radiation for 1 s. Power densities on the surface of the tumour were 100 mW/cm^2 for PDT (633 nm) and 5 mW/cm^2 for fluorescence detection (407 nm).

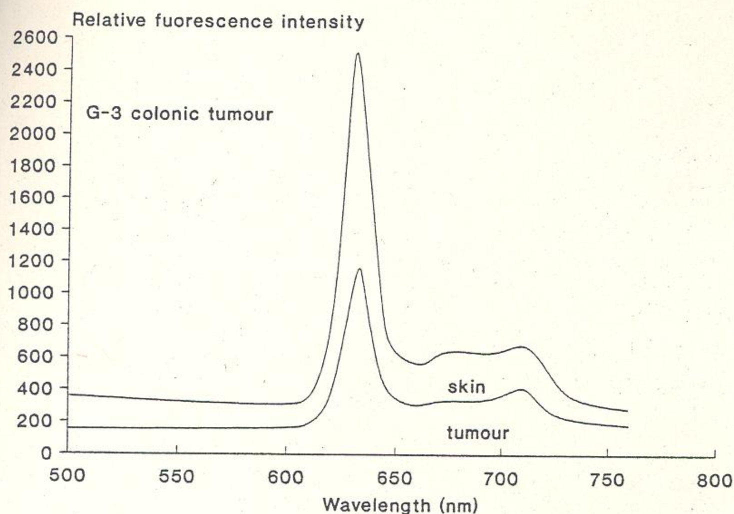

Fig. 3. Fluorescence of tumour and skin after intravenous aminolevulinic acid (5-ALA) injection (7 mg/mouse)

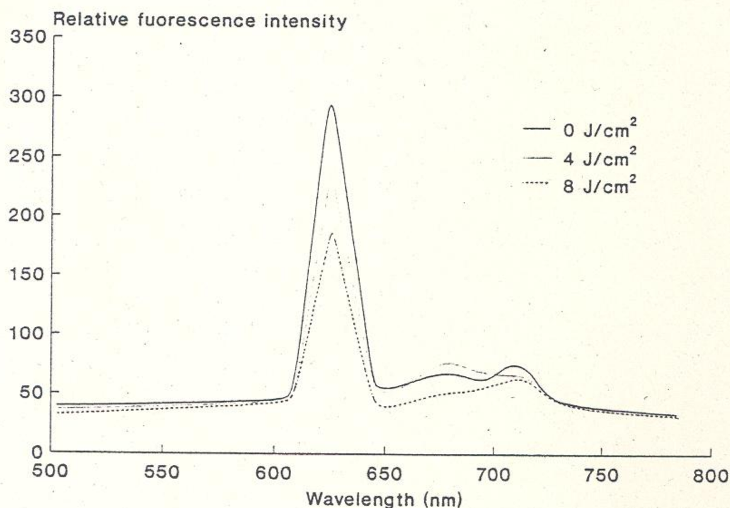

Fig. 4. Fluorescence modification during PDT treatment with 5-ALA

Results

A strong fluorescence with bands around 635 nm and 710 nm in the tumour after intravenous and oral application of 5-ALA was found. The relative fluorescence distribution after 5-ALA feeding was the same as after i.v. application. However, the total intensity was lower than after i.v. application. No protoporphyrin IX fluorescence was found in tumours that had received 5-ALA topically on the skin with intact stratum corneum. All tissues followed the same fluorescence profile. However, we found the fluorescence intensity in tumours to be about eight times higher than in the surrounding muscle tissue, but lower than in the skin (Fig. 3).

We also found modifications of the fluorescence spectrum during PDT (Fig. 4). The fluorescence intensity around 670 nm increased to a maximum and then slowly decreased. In contrast, the fluorescence intensities at 635 nm and 710 nm decreased as a result of photobleaching. At the end of PDT, after a total radiant exposure of 150 J/cm^2, no fluorescence in the red spectral region could be measured any more.

The tumour volume in mice was calculated every week (Fig. 5). We found no difference in tumour growth within the three groups of control mice. The proportion of the median tumour volume after the first week and the first PDT was 1 : 2.2 in favour of the PDT-treated animals. After the third week and the second PDT the proportion was 1 : 6.9. In two mice the transplanted tumour was destroyed completely after the second PDT. In the other animals tumour growth could be inhibited by superficial tumour necrosis but not stopped. After 5 weeks, the mean tumour volume proportion was 1 : 8.8.

Discussion

A new method of tumour treatment with PDT has been studied using 5-ALA. We found that the i.v. injection of 5-ALA together with the oral application of 5-ALA leads to sufficient protoporphyrin IX to cause tumour reduction when ex-

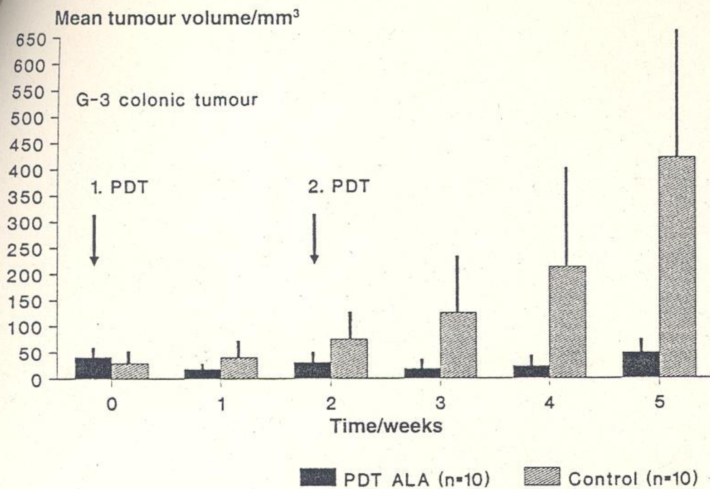

Fig. 5. Photodynamic tumour therapy with 5-ALA, 633 nm, 100 mW/cm^2, 150 J/cm^2, 3 h after intravenous 5-ALA injection

posed to red light. However, we found the fluorescence intensity higher after intravenous administration for the same initial 5-ALA concentration. In contrast, topical application of 5-ALA was not possible with intact stratum corneum. Our findings are consistent with those of Loh et al. (1993) who found similar fluorescence kinetics also by giving 5-ALA orally. However, a higher oral dose of ALA was required to achieve the same level of photosensitivity as an intravenous administration in normal mucosa in rats. As reported in the same paper, sufficient fluorescence levels were achieved in human colonic adenocarcinoma with an oral dose of 30 mg/kg 5-ALA, which is much less than the efficient dose in small animals. It appears that the oral administration of 5-ALA is an important and easy aspect of PDT treatment of colonic tumours.

PDT treatment, performed with intravenous application of 5-ALA, led to a significant reduction in macroscopic tumour volume or tumour destruction. The tumour reduction is caused by partial tumour necrosis, induced by cytotoxic reactions and possibly indicated by the formation of photoproducts. However, the depth of tumour necrosis is dependent on the concentration of the synthesized protoporphyrin IX and on the tissue penetration of the light. We found a different tumour response even when the PDT treatment was the same. Probably the behaviour of tumour regrowth is different in different animals. Bedwell et al. (1992) have shown that a rat colon tumour has a higher protoporphyrin IX synthetic capacity compared with normal colonic mucosa and less in the muscle wall. This correlates with an observed necrosis in the tumour after PDT treatment, while the normal muscle region is spared. A similar effect is described for the treatment of basal cell carcinomas with a complete response rate of 90% (Kennedy et al. 1990). Only a small amount of fluorescence could be found under a confocal laser-scan microscope in muscle tissue of mice bearing mammary carcinoma and treated with 5-ALA, which again explains the selective necrosis in tumour tissue after PDT treatment (Peng et al. 1992). These results again correlate with findings of Bedwell et al. (1992) who found a fluorescence difference of 1 : 6 between tumour and normal tissue in the rat colon after i.v. application of 5-ALA. The fluorescence intensity in our experimental colonic tumours was about eight times as high as in the surrounding muscle tissue. This concentration difference raises the possibility of selective necrosis in the tumour during PDT.

The high fluorescence intensity in the carcinoma also allows tumour detection, using in vivo fluorescence measurements. The change in fluorescence during PDT can be explained by the photoreaction of the sensitiser. This process results finally in photobleaching (König et al. 1993) and could be used for in vivo measurements of the light dosage. The formation of a new fluorescence band (around 670 nm), which we observed at the beginning of irradiation, is possibly due to the formation of fluorescent photoproducts or less photobleachable aggregates (Inhoffen et al. 1969; Seidlitz et al. 1992; Valat et al. 1988). König et al. (1990, 1993) found similar modifications of fluorescence during light exposure when using haematoporphyrin derivative or 5-ALA-induced protoporphyrin IX. In line with this, the fluorescence decay kinetics can be related to the fluorescent photoproducts (Schneckenburger et al. 1992; Yamashita et al. 1984). It is important to mention that the photochemical process of producing new photoproducts requires oxygen (König et al. 1990). This is an indication that singlet oxygen is involved. Obviously this is the main cytotoxic agent responsible for the photodynamic process.

Further studies will show whether there is a correlation between tumour fluorescence modifications during PDT and the cytotoxic effect. Perhaps fluorescence detection during PDT may give direct information about the biological efficiency and reaction mechanism of the tumour treatment.

References

Barr H, Tralau CJ, MacRobert AJ, Morrison I, Phillips D, Bown SG (1988) Fluorescence photometric techniques for determination of microscopic tissue distribution of phthalocyanine photosensitizers for photodynamic therapy. Lasers Med Sci 3:81–86

Barr H, Chatlani P, Tralau CJ, MacRobert AJ, Boulos PB, Bown SG (1991) Local eradication of rat colon cancer with photodynamic therapy: correlation of distribution of photosensitiser with biological effects in normal and tumour tissue. Gut 32:517–523

Bedwell J, MacRobert AJ, Phillips D, Bown SG (1992) Fluorescence distribution and photodynamic effect of ALA-induced PP IX in the DMH rat colonic tumour model. Br J Cancer 65:818–824

Charlsworth P, Truscott TG (1993) The use of 5-aminolevulinic acid (ALA) in photodynamic therapy (PDT). J Photochem Photobiol [B] 18:99–100

Divaris DXG, Kennedy JC, Pottier RH (1990) Phototoxic damage to sebaceous glands and hair follicles of mice after systemic administration of 5-aminolevulinic acid correlates with localized protoporphyrin IX fluorescence. Am J Pathol 136:891–897

Henderson BW, Waldow SM, Mang TS, Potter WR, Malone B, Doughtery TJ (1985) Tumor destruction and kinetics of tumor cell death in two experimental mouse tumors following PDT. Cancer Res 45:572–576

Inhoffen HH, Brockmann H, Bliesener KM (1969) Photoprotoporpyrine und ihre Umwandlung in Spirographis sowie Isospirographis-Porphirin. Liebigs Ann Chem 730:173–185

Kelly JF, Snell ME, Berembaum MC (1975) Photodynamic destruction of human bladder carcinoma. Br J Cancer 31:237–244

Kennedy JC, Pottier RH (1992) Endogenous protoporphyrin IX, a clinical useful photosensitizer for photodynamic therapy. J Photochem Photobiol [B] 14:275–292

Kennedy JC, Pottier RH, Pross DC (1990) Photodynamic therapy with endogenous protoporphyrin IX: basic principles and present clinical experience. J Photoechem Photobiol [B] 6:143–148

König K, Wabnitz H, Dietel W (1990) Variation in the fluorescence decay properties of hematoporphyrin derivative during its conversion into photoproducts. J Photochem Photobiol [B] 8:103–111

König K, Schneckenburger H, Rück A, Steiner R (1993) In vivo photoproducts formation during PDT with ALA-induced endogenous porphyrins. J Photochem Photobiol [B] 18:287–290

Loh CS, MacRobert AJ, Bedwell J, Regula J, Krasner N, Bown SG (1993) Oral versus intravenous administration of 5-amino-levulinic acid for photodynamic therapy. Br J Cancer 68:41–51

Malik Z, Djaldetti M (1979) 5-Aminolevulinic acid stimulation of porphyrin and hemoglobin synthesis by uninduced friend erythroleukaemic cells. Cell Differ 8:223–233

McGilvery RW, Goldstein G (eds) (1979) Biochemistry, functional approach. Saunders, Philadelphia London Toronto, pp 632–635

Peng Q, Moan J, Warloe T, Nesland JM, Rimington C (1992) Distribution and photosensitizing efficiency or porphyrins induced by application of exogenous 5-aminolevulinic acid in mice bearing mammary carcinoma. Int J Cancer 52:433–443

Schneckenburger H, Strauss W, Rück A, Seidlitz H, Wessels JM (1992) Microscopic fluorescence spectroscopy and diagnosis. Opt Eng 31:995–999

Seidlitz HK, Stettmaier K, Wessels JM, Schneckenburger H (1992) Intracellular fluorescence polarization, picosecond kinetics and light-induced reactions of photosensitizing porphyrins. Opt Eng 31:1482–1486

Sima AAF, Kennedy JC, Blakeslee D, Robertson DM (1981) Experimental porphyric neuropathy: a preliminary report. Can J Neurol Sci 8:105–113

Valat P, Reinhart GD, Jameson DM (1988) Application of time-resolved fluorometry to the resolution of porphyrine-product mixtures. Photochem Photobiol [B] 47:787–790

Yamashita M, Jomura M, Kobayashi S, Sato T (1984) Picosecond time resolved fluorescence spectroscopy of HPD. IEEE J Quantum Electron 20:1363–1369

Zalar GL, Poh-Fitzpatrick M, Krohn DL, Jacobs R, Harber LC (1977) Induction of drug photosensitisation in man after parenteral exposure to hematoporphyrin. Arch Dermatol 113:1392–1397