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Photodynamic lasertherapy using aminolaevulinic acid and desferrioxamine

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Key words: ALA – Tumor – PDT – Desferrioxamine

Summary: The formation, accumulation and photodynamic activity of endogeneous protoporphyrin IX in xenotransplanted tumors induced by exogenous 5-aminolaevulinic acid (ALA) and administration of desferrioxamine was studied. The chelating agent desferrioxamine possesses a high affinity for iron. The additional topical administration of that iron complexing compound leads to an accelerated accumulation and an increased concentration of protoporphyrin in tumor tissue determined by in-vivo fluorescence measurement. This results in a significantly enhanced efficiency of the photodynamic tumor therapy.

Photodynamische Lasertherapie mittels Aminolävulinsäure und Desferrioxamin

Schlüsselwörter: ALA – Tumor – PDT – Desferrioxamin

Zusammenfassung: Die durch exogene 5-Aminolävulinsäure und Gabe von Desferrioxamin induzierte Formation und Akkumulation, sowie die photodynamische Aktivität von endogenem Protoporphyrin IX in xenotransplantierten Tumoren wurde untersucht. Das Chelat Desferrioxamin zeichnet sich durch eine hohe Eisenaffinität aus. In-vivo-Fluoreszensmessungen zeigen, daß die zusätzliche topische Applikation dieses Eisenkomplexbildners eine beschleunigte Anreicherung und eine erhöhte Konzentration von Protoporphyrin im Tumorgewebe bewirkt. Die Effizienz der Photodynamischen Tumortherapie kann dadurch signifikant erhöht werden.

The external administration of 5-aminolaevulinic acid (ALA) leads to a high-efficient biosynthesis of the fluorescent photosensitizer protoporphyrin IX in the mitochondria of different cells [1,2]. This results, first, in an ALA-induced autofluorescence in the red spectral region offering the possibility of a fluorescence diagnosis and, second, in a photosensitivity of tumor tissue which can be used for photodynamic therapy. Kennedy et al. [1, 2] were the first who treated patients with basal cell carcinoma after topical ALA administration and light irradiation.

Figure 1 indicates the schematic pathway of heme synthesis. Catalytic reactions lead over the formation of the precursor ALA to the synthesis of the intermediate substance protoporphyrin IX followed by the process of iron insertion forming heme. The concentration of heme causes a negative feedback to the activity of the enzyme ALA synthase and therefore to a limitation of the ALA concentration. The external administration of ALA bypasses the heme regulation and therefore leads to an increased synthesis of protoporphyrin and heme.

The additional administration of desferrioxamine, a chelating agent with high affinity for iron, leads to a reduced rate of iron insertion in protoporphyrin molecules. This should cause a fast increase of protoporphyrin concentration. In addition, more protoporphyrin should be synthesized by an enhanced ALA formation as an answer to the reduced heme concentration due to the feedback regulation.

Ortel and Hönigsmann [3] demonstrated the enhanced photosensitization of ALA-incubated mouse keratinocytes by desferrioxamine and the decreased supression of proliferation by supplementation with iron ions. No in-vivo studies on an ALA- and desferrioxamine-induced PDT were found in the literature.

The aim of this in-vivo investigation was to study the time-dependent formation of protoporphyrin and the ef-

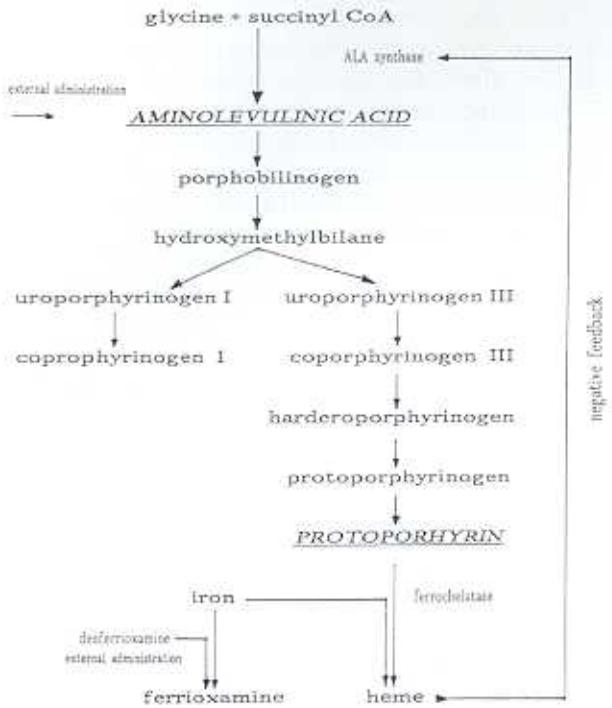

Fig. 1 Schematic pathway of the biosynthesis of heme and the alternative way in case of administration of the iron-complexing agent desferrioxamine

ficiency of a photodynamic tumor therapy after administration of ALA and desferrioxamine.

Materials and methods

Apparatus

Protoporphyrin fluorescence was excited by the 407 nm line of krypton ion laser (5 mW/cm^2). Protophyrin monomers absorb mainly in this spectral region (Soret band). The fluorescence radiation was detected using a fiber-optic fluorescence sensor, polychromator and optical multichannel analyzer, as described elsewhere [4]. Photodynamic therapy was carried out using a tuneable dye laser with the active medium DCM. The radiation was coupled into a single quartz fibre. The tumor was irradiated by means of an optical system consisting of a lens and diaphragm. The irradiance was 100 mW/cm^2 (630 nm) and varied by less than 20 % over the tumor surface. The irradiated area was slightly greater than the tumor region. Three phototreatments were carried out; the first with a radiant exposure of 200 J/cm^2, the second 2 weeks later (100 J/cm^2) and the third 1 week after the second.

Animals

50 days old female nude mice (NMRI nu-nu, body weight about 25 g) bearing two s. c. transplanted bladder tumors (G2) were used. One tumor was irradiated, the other one served as a control. The skin covering the tumor was carefully removed for PDT and fluorescence measurements. ALA was given intraveneously (vene of tail, 7 mg/mouse) 3 hours before each irradiation. Desferrioxamine was applied intratumoral at the same time (0.75 mg/mouse). The tumor volume was calculated with V = 0.5 x l x b x h based on the measured length l, height h and width b and the assumed shape of a semiellipsoid.

Chemicals

ALA (Sigma) was dissolved in NaOH and PBS (final pH = 6.8). The agent desferroxamine mesylate ($C_{25}H_{48}N_6O_8\,CH_4O_3S$, molecular weight: 656,8 g, dissolved in NaCl, Sigma) was used. 1 mol desferrioxamine binds 1 mol iron ions forming the stable watersoluble iron-complex ferrioxamine.

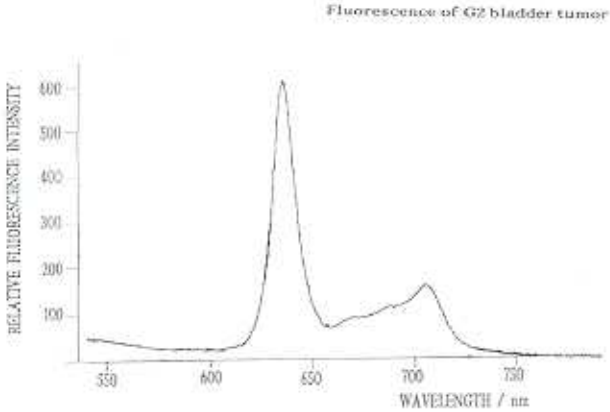

Fig. 2 In-vivo fluorescence spectrum of a tumor incubated with ALA and desferrioxamine

Results

Figure 2 shows the in-vivo fluorescence spectrum of one tumor 2 hours after administration of ALA and desferrioxamine. The spectrum with main peaks at 635 nm and around 710 nm is typical for protoporphyrin IX in lipophilic environment. Tumor tissue without any incubation showed no fluorescence in the red spectral region. In contrast, the skin of the control animals emits in the long-wavelength red spectral region with a broad peak around 670 nm. Figure 3 demonstrates the time-dependent average fluorescence signal of 4 tumors at 635 nm. A faster fluorescence rise and higher intensities were found in the case of ALA and simultaneous desferrioxamine administration compared with an injection of ALA alone. No significant differences in the fluorescence intensities were measured in the skin three centimeters away from the tumor region, see Figure 4.

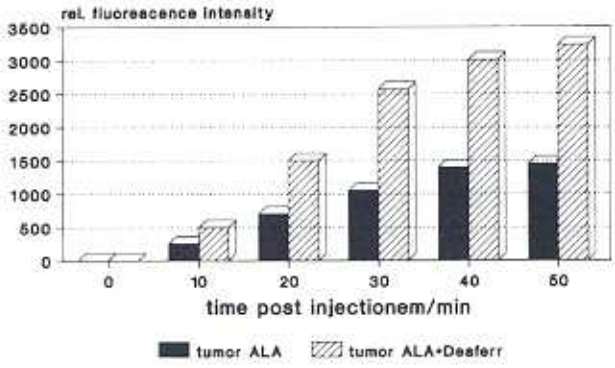

Fig. 3 In-vivo fluorescence at 635 nm vs. time after drug administration. Average value of 4 tumors

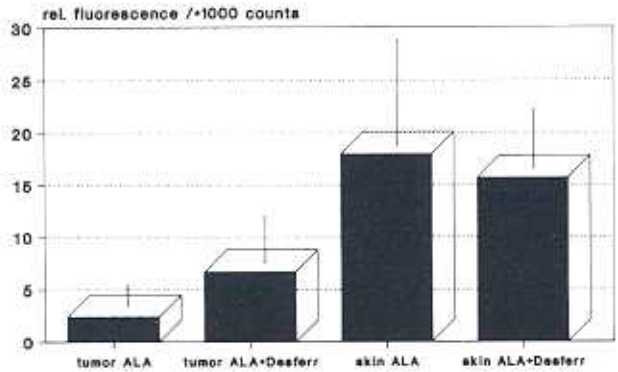

Fig. 4 Fluorescence of tumor and skin. The additional intratumoral administration of desferrioxamine results in a higher intensity of protopophyrin fluorescence

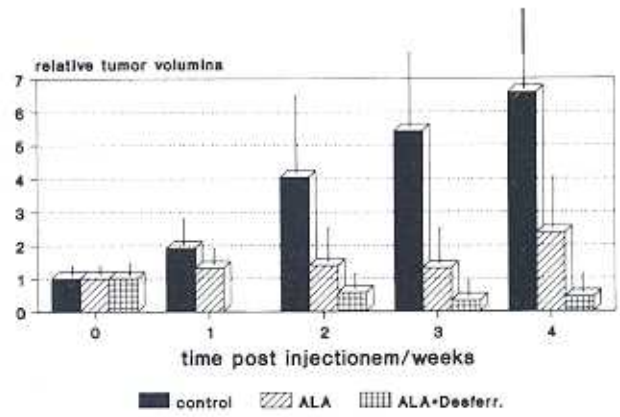

Fig. 5 Relative tumor volumes (average volume from 10 mice and standard deviation) in dependence of the time after first treatment (first after 0 weeks, second after 2 weeks, third after 3 weeks)

These findings correspond well with the results of the photodynamic treatment. The average tumor volumes vs. time after the first treatment are shown in Figure 5 indicating a strong cytotoxic effect. It is obvious, that the irradiated tumors with an additional administration of desferrioxamine showed the highest volume reduction. However, no mouse showed a complete tumor remission.

Discussion

The additional topical administration of the iron-complexing agent desferrioxamine results in a faster accumulation and in higher concentrations of protoporphyrin IX in ALA-incubated tissue. The region of high protoporphyrin IX concentration seems to be limited to the diffusion-controlled area of accumulated desferrioxamine. The local increase of endogeneous protoporphyrin in tumor tissue results in an enhanced efficiency of the photodynamic treatment. Desferrioxamine is a pharmaceutic agent (e.g. the main component of the drug Desferal, CIBA-GEIGY GmbH) used for the treatment of cases associated with excessive iron concentrations in tissue such as heamochromatosis, haemosiderosis, thalassaemia or acute iron poisoning [5]. Therefore, a photodynamic therapy of tumors, skin- and other deseases using the precursor of the porphyrin synthesis, ALA, in combination with the drug desferrioxamine may become a highly efficient therapeutic method.

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