PDT OF TUMOR-BEARING MICE USING LIPOSOME DELIVERED TEXAPHYRINS

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SUMMARY

The krypton ion laser (752.5 nm) induced photodynamic effects in bladder tumor-bearing nude mice after i.v. application of waterinsoluble cadmium texaphyrins were investigated. Liposomes using sojalecithin as phospholipid act as carriers. Cd-texaphyrin synthesized by Sessler, possesses strong absorption transitions at 765 nm and a high quantum effiency of singlet oxygen production. The phototreatment 2 hours after i.v. injection of the photosensitizer led to significant tumor destruction, whereas the treatment 24 hours after administration shows no effect. This corresponds well to studies on the accumulation behaviour of the carriers in tumor, skin and inner organs using fluorophore-labeled liposomes.

INTRODUCTION

Photosensitizers for the photodynamic therapy (PDT) should possess strong absorption transitions mainly in the "optical window" of biological tissue, that means in the red or NIR spectral region. The lack of intrinsic absorbers there leads to optical penetration depths of 2 to 10 mm and avoid thermal damage. In addition, the phototoxic side effect of skin damage in the case of photosensitizer accumulation in the skin by sun radiation is low because of relative small light intensity in this spectral region. And, a high efficiency rate of singlet oxygen production is given for a triplet energy of around 1 eV. This supposition is given by an absorption in the long-wavelength red and in the NIR spectral region assuming small energy gap between triplet and singlet state. For the practical handling and safety of a phototherapy it is more convenient to avoid nonvisible NIR radiation. Thus, the ideal photosensitizer should absorb mainly in the far red region.

The derivatives of texaphyrin synthesized by Sessler /1/ possess a high absorbance in the far red spectral region around 720 nm - 780 nm. The excellent photophysical and photochemical properties of this tripyrroledimethine-derived expanded porphyrin-like system are listed in Table 1.

Table 1
Properties of Cd-texaphyrin

ε_A = 40 000 $M^{-1}cm^{-1}$	(765 nm, ethanol)	/1/
ϕ_T = 0.90	(CH_3CN)	/2/
τ_T = 0.0225 msec	(CH_3CN	/2/
η_F = 0.001	(CH_3CN)	/2/
$\phi_{\Delta O_2}$ = 0.7	methanol	/3/

However, only few in-vitro studies exist on the photodynamic activity of the texaphyrins /4,5/. It was the aim to study in-vivo the cytototoxic effect of liposomal Cd-texaphyrin using tumor-bearing nude mice.

MATERIALS AND METHODS

Apparatus. A krypton ion laser at 752.5 nm (200 mW/cm^2) was used for photodynamic treatment, the radiation at 406.7 nm served as excitation source for fluorescence measurements. The fiberoptic fluorescence detector system is described in /6/. The distribution of liposomes into tumor cells was studied using a video-enhanced fluorescence microscope (Zeiss, Axiophot) combined with a SIT-camera and dual image processor.

Chemicals. Cd-texaphyrin was kindly given by J.L. Sessler, Austin. Its concentration was 100 mg per ml liposomes (phospholipid: sojalecithin, ratio molar 70:1, diameter: 100 nm /7/). Liposomes were resuspended in PBS (pH=7.4). Small unilamellar vesicles were formed by sonication. Tetramethylhematoporphyrin (TMHP) was used as fluorophore.

Animals. Female nude mice had two transplanted subcutaneous G3 bladder tumors. One tumor was irradiated, the other one served as control. The tumor volume was determined by measuring length, height and width (assumed shape of the tumor: semiellipsoid).

RESULTS AND DISCUSSION

Fluorescence measurement were carried out to get information on the accumulation of liposomes into tumor tissue. As Cd-texaphyrin emits in the infrared spectral region with very low fluorescence quantum yield, liposomes were labeled with the fluorophore TMHP. The strongest emission was found into the liver as seen from Fig. 1 according to a preferential uptake by the reticoloendothelial system. The fluoresecence of tumor tissue achieved the highest values between 1 and 4 hours after i.v. injection. However, the fluorescence signal was small compared to the emission of the liver.

The distribution of liposomes (fluorescent spots) inside a single tumor cell (Fig. 2) showed an accumulation in the cytoplasm, but not in the nucleus.

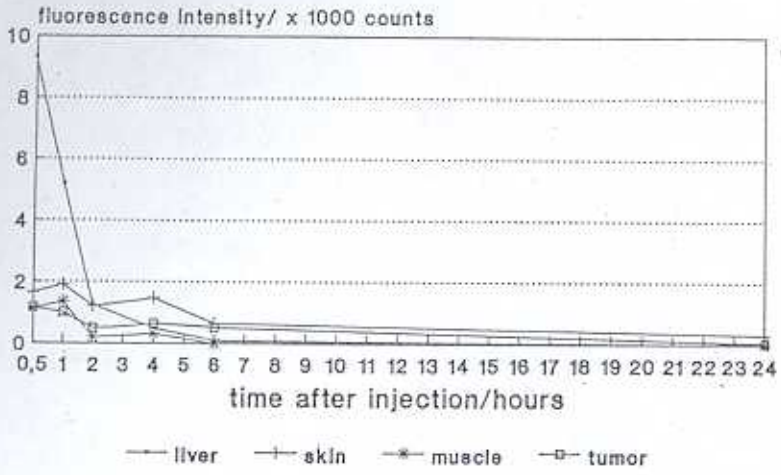

Fig. 1. Relative fluorescence intensities in dependence of the time after injection of the liposomal fluorophores

This rapid accumulation in tumor tissue correlated with the obtained cytotoxic effect. A treatment 24 hours after administration of the photosensitizer showed no tumor destruction, whereas the therapy 2 hours after i.v injection resulted in a significant tumor reduction, see Fig. 3. However, no mouse showed complete tumor destruction. The survival of tumor cells led to a renewed tumor growth 3 to 4 weeks after the photodynamic therapy. A possible explanation for the low efficiency of the therapy seems to be the poor accumulation of liposomal Cd-texaphyrin in tumor tissue.

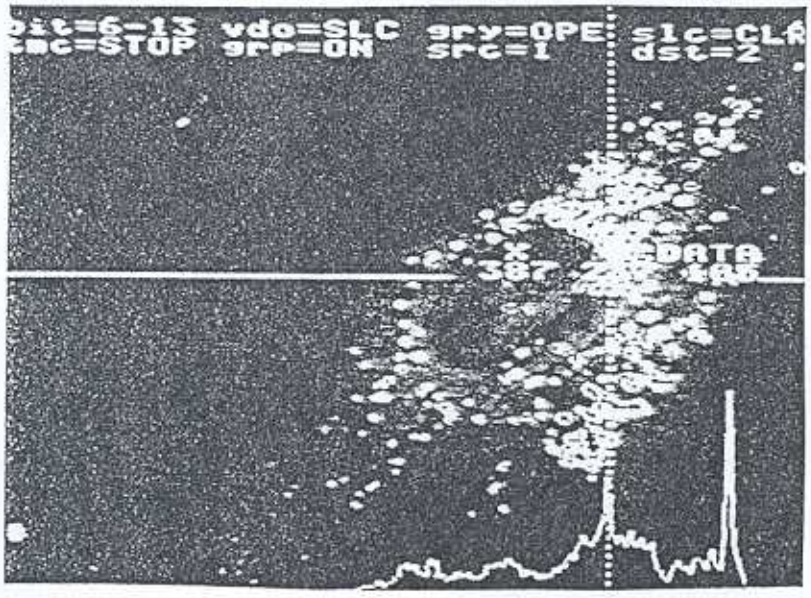

Fig. 2. Fluorophore distribution inside a G3 bladder tumor cell

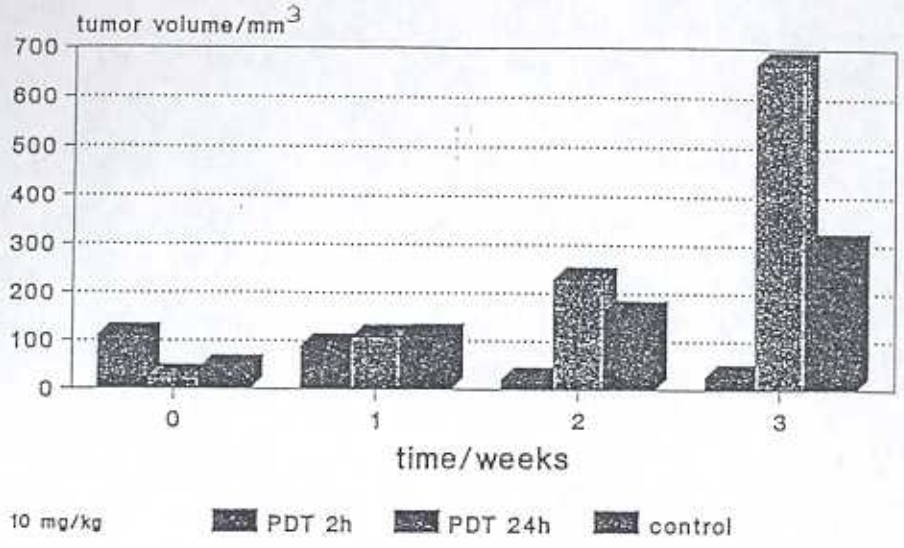

Fig. 3. Tumor volumes (average value of 10 tumors) and standard deviation in dependence of the time after treatment.

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