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Possible advantages of aluminum-chloro-tetrasulfonated phthalocyanine over hematoporphyrin derivative as a photosensitizer in photodynamic therapy

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Summary. The potency of aluminum-chloro-tetrasulfonated phthalocyanine (AlS4Pc) as a photosensitizer in photodynamic therapy was evaluated in in vitro and in vivo studies. Compared with hematoporphyrin derivative (HpD), the following advantages of AlS4Pc were revealed: (1) AlS4Pc was less toxic than HpD in vitro without light irradiation. (2) AlS4Pc showed more photodynamic-dependent cytotoxicity and anti-tumor effect in the red area of the spectrum (>660 nm) at which tissue penetration is high. (3) AlS4Pc appeared to be removed more rapidly from normal tissues such as muscle and skin. (4) AlS4Pc showed less photodynamic-dependent cytotoxicity in vitro and milder cutaneous phototoxicity in vivo with UVA irradiation. On the basis of these observations, AlS4Pc shows considerable promise as a photosensitizer for PDT.

Key words: Hematoporphyrin derivative – Photodynamic therapy – Phthalocyanines – Skin photosensitivity

Photodynamic therapy (PDT) for cancer is a relatively new technique for the selective destruction of malignant tumors. Selectivity of effect is a consequence of both the preferential accumulation of the dye in tumors and the accuracy of light delivery. At present the photosensitizer of choice in PDT is hematoporphyrin derivative (HpD), which shows preferential tumor-localizing ability in a variety of human tumors while being retained to some extent in normal tissues as well.

Using PDT we have treated patients with superficial bladder cancer, especially carcinoma in situ of the bladder [8, 10]. Skin photosensitivity and a reduction in bladder capacity were experienced as a consequence of retention of HpD in the skin and normal portion of the bladder. These adverse reactions have, in part, restricted the development of PDT and underlie the search for alternative photosensitive compounds for clinical use.

Phthalocyanines (AlSPc), which can be regarded as azaporphyrins, appear to be more suitable photosensitizers because of their strong absorption above 650 nm (where the light penetration of tissue is good), their photochemical and thermal stability in solution, and their relatively well defined chemical properties. Moreover, AlSPc have been reported to be capable of photoinactivating cells in tissue culture [2], to exhibit good tumor-localizing capacity and to reduce the tumor burden of various murine tumors with light irradiation [3].

Recently, aluminum-chloro-tetrasulfonated phthalocyanine (AlS4Pc), the most hydrophilic derivative of AlSPc, was shown to have the greatest retention in solid tumor [5]. Our interest, therefore, has been attracted to AlS4Pc as one of a second generation of photosensitizers for PDT of bladder cancer. In the present study, the relative potencies of AlS4Pc and HpD as photosensitizing agents was evaluated in vitro and in vivo.

Materials and methods

Photosensitizers

Hematoporphyrin derivative (HpD: Photofrin I) was obtained from Oncology Research and Development, New York, N.Y. Aluminum-chloro-tetrasulfonated phthalocyanine (AlS4Pc) was provided by Sumitomo Pharmaceuticals, Osaka, Japan.

Light source

A gold vapor laser (GVL), model AU-10, was developed by Oxford Lasers, Oxford, UK. The instrument delivered pulsed monochromatic light at 627.8 nm. The pulse width at base was 80 ns and the pulse repetition rate was 10 kHz. Argon-dye laser (ADL; Spectra Physics, Mountain View, Calif.) combined with rhodamine B or 4-dicyanomethylene-2-methyl-6-p-dimethylaminostyryl-4H-pyran was used to obtain a wavelength of 630 nm or 670 nm, respectively. The light beam was coupled to a 600-μm quartz fiber. Visible light

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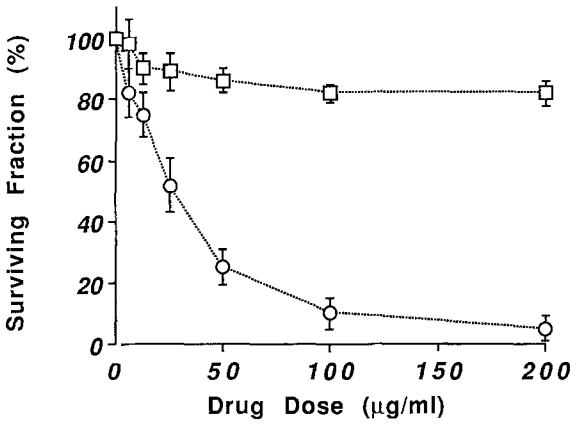


Fig. 1. Cytotoxicity of aluminum-chloro-tetrasulfonated phthalocyanine (AlS4Pc, □) and hematoporphyrin derivative (HpD, ○) on KK-47 cells. The surviving fraction was determined by MTT assay after 72 h of continuous exposure. The mean of four separate experiments and SD are shown

and UVA were delivered from a slide projector lamp (Kodak Ektagraphic, 375–750 nm with a peak of 615 ± 10 nm) and an ultraviolet (UV) lamp (Dermaray, model M-DMR-1, mounting Toshiba FL20S, BLB, 300–430 nm; Eisai, Tokyo), respectively.

In vitro PDT

Eight thousand cells of KK-47 human bladder cancer cell line [13] in a well of a 96-well microplate were incubated with each photosensitizer at different concentrations for 24 h. After washing with phosphate-buffered saline (PBS), the cells were treated with graded doses up to 8 J/cm^2 of UVA, or red light at >600 nm and >660 nm. Immediately after the light exposure, an MTT assay [9] was performed to determine the surviving fractions.

Kinetics of dyes

A suspension of KK-47 cells containing 6×10^6 cells in 0.2 ml culture medium was injected subcutaneously into the back of nude mice. When tumors were 8–12 mm in diameter, the mice were given a single intraperitoneal injection of AlS4Pc or HpD in 0.1 ml PBS. At various times after dye administration animals were killed and tissue samples were resected and stored at −20°C until used for dye extraction. Extraction of AlS4Pc was performed according to the method of Chan et al. [3], that is, thawed tissues were digested with 0.1 M NaOH for 4 h in a 50°C water bath with occasional shaking. The dye concentration of the extracts was calculated from the fluorescence obtained by measurement in a spectrofluorimeter (Hitachi, Tokyo, Japan) using 630 nm excitation and 673 nm emission. Extraction of HpD was carried out as described by Evensen et al. [6]. In brief, thawed tissues were homogenized in 5 ml 0.15 M acetic acid. After adding 15 ml ethylacetate : acetic acid (3 : 1), the suspension was stored at −18°C for 1 h and then thawed and filtered through a glass filter. To the filtrate was added 25 ml of a saturated solution of sodium acetate. The organic phase was extracted with an aqueous solution of 1.5 M HCl. Solid sodium acetate was added to pH 3–3.5, and the porphyrins extracted with ether, which was evaporated under reduced pressure. After the samples had been dissolved in methanol, the concentration of the extracts was calculated from the fluorescence obtained by measurement in the spectrofluorimeter using 400 nm excitation and 630 nm emission.

In vivo PDT

Nude mice bearing KK-47 cells and C3H/He mice bearing spontaneous squamous cell carcinoma [15], transplanted at a diameter of 5–7 mm to the depilated back, were used for studies of photodynamic tumor destruction. Each photosensitizer was administered to the mice intraperitoneally in 0.2 ml physiological saline (the three doses used were 0.1, 1.0, 10.0 mg/kg body weight). KK-47 cell tumors were irradiated 48 h later by ADL at 150 mW/cm^2, 1503/cm^2 as the total light dose, and squamous cell carcinomas were irradiated 24 h later by GVL at 200 mW/cm^2, 200 J/cm^2 as the total light dose, since the peak concentrations of the two drugs in transplanted tumor were reported to occur between 24 and 48 h [3, 7]. The tumor volume following irradiation was estimated using a slide caliper by applying the following formula: Tumor volume $=\pi/6 \times a \times b \times c$, where a is the length, b the width and c the thickness of the tumor. Complete tumor remission (CR) was defined as no palpable tumor at 60 days after treatment.

Skin photosensitivity

The back skin of C3H/He mice was irradiated with UVA or visible light at 5 mW/cm^2, 10 J/cm^2 as the total light dose, 24 h after intraperitoneal injection of each drug. The thickness of the skin was measured using a dial thickness gauge at various times after irradiation. The results were expressed as the relative increase in dorsal skin fold thickness over that of the pre-irradiation controls.

Results

Although no cytotoxicity was observed when KK-47 cells were exposed to up to 200 μg/ml of AlS4Pc or HpD for 24 h, cytotoxicity of HpD became significant after continuous exposure for 72 h as shown in Fig. 1.

The photodynamic-dependent cytotoxicity of HpD was significantly higher than that of AlSPc with UVA irradiation (Fig. 2A). With red light irradiation at >600 nm, there was no difference between HpD and AlS4Pc, while red light at >660 nm produced a greater cytotoxicity on the cells treated with AlS4Pc compared with those treated with HpD (Fig. 2B). The photodynamic effect was dependent on the light dose (data not shown) as well as the drug dose (Fig. 2).

The highest concentration of AlS4Pc (in tumors as well as in skin and muscle) was obtained at 3 h after injection, while the peak HpD concentration in these tissues was observed at 24 h after injection (Fig. 3). However, concentrations of AlS4Pc in the tumor as well as the skin were higher than those of HpD at all phases in this nude mouse model. Relative uptake of AlS4Pc in the tumor compared with muscle was in general higher than that of HpD. The highest tumor/muscle ratio in AlS4Pc was found at 96 h after injection (Fig. 4A). The skin/muscle ratio decreased with time for AlS4Pc, but tended to increase for HpD, suggesting that HpD was preferentially retained in the skin compared with AlS4Pc. This was also supported by the changes in the tumor/skin ratios (Fig. 4B, C).

Figure 5 shows the growth curves of transplanted tumors in nude mice after PDT. Equivalent tumor regression was observed after ADL irradiation at 630 nm with 10 mg/kg of AlS4Pc and with 10 mg/kg of HpD. Moreover, much greater regression was produced by

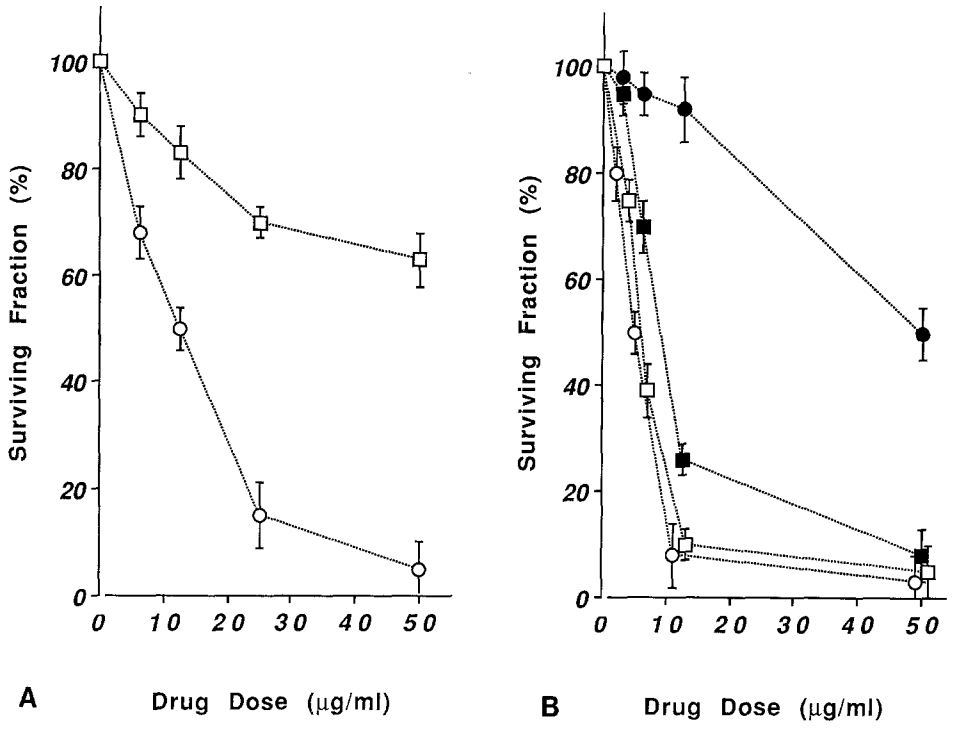
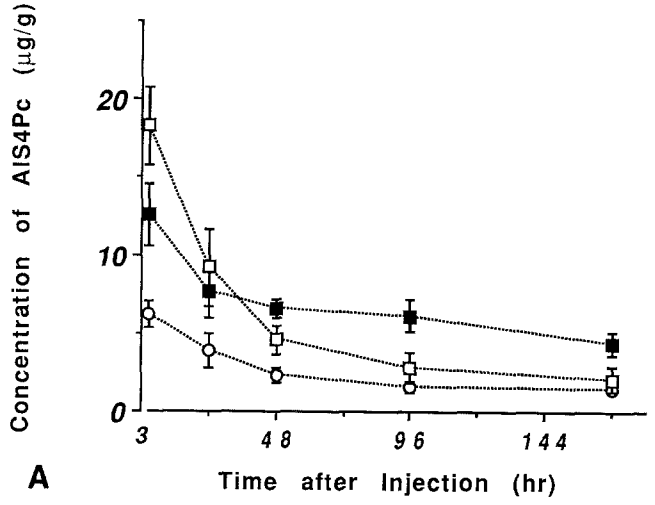
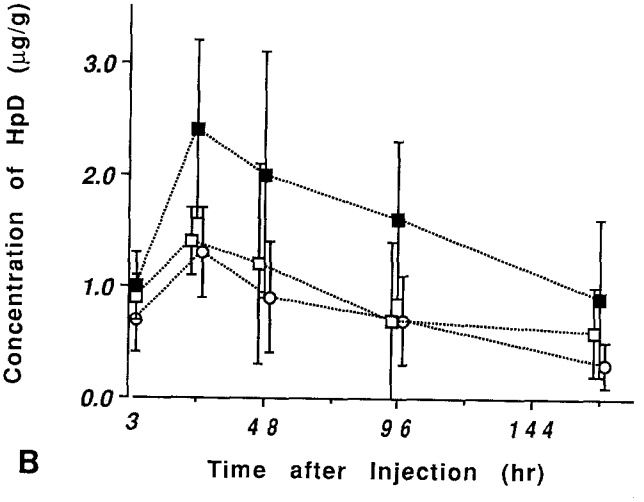


Fig. 2. A Photodynamic-dependent cytotoxicity of AlS4Pc (□) and HpD (○) on KK-47 cells with UVA irradiation at 2 J/cm^2. B Photodynamic-dependent cytotoxicity of AlS4Pc (□) and HpD (○) with red light at >600 nm at 8 J/cm^2, and of AlS4Pc (■) and HpD (●) with red light at >660 nm at 8 J/cm^2. The mean of four separate experiments and SD are shown





irradiation at 670 nm with 10 mg/kg of AlS4Pc compared with the above conditions. There was no significant rise in temperature in the treated tumor.

Efficacy of PDT in vivo was further investigated in the C3H/He mouse model with GVL irradiation, which produced a considerable rise in intratumoral temperature up to 45°C. The hyperthermic effect produced considerable tumor reduction in the group treated with GVL alone. Significant tumor regression was demonstrated ($P<0.05$, Kruskal-Wallis' nonparametric ANOVA followed by Hollander-Wolfe's multicomparison test) in the three groups treated by GVL with HpD 10 mg/kg or with AlS4Pc 1 mg/kg and 10 mg/kg, as shown in Fig. 6. Concerning the CR rate, only the group teated by GVL with AlS4Pc 10 mg/kg showed a statistically significant CR rate compared with the control group ($P<0.05$, Fisher's exact probability test), although a higher number of CRs was obtained by increasing the injected doses of AlS4Pc or HpD.

Results of the skin photosensitivity test were expressed as relative increases in the thickness of the mouse back skin following PDT (Fig. 7). With UVA irradiation, a stronger skin reaction was observed in the HpD-injected group than the AlS4Pc-injected group, whereas the degree of skin reaction induced by the two drugs was equivalent with the visible light.

Fig. 3A, B. Kinetics of AlS4Pc (A) and HpD (B) uptake and retention in subcutaneous growing tumors (■), adjacent skin (□) and muscle (○). The mean concentration from five mice and SD are shown

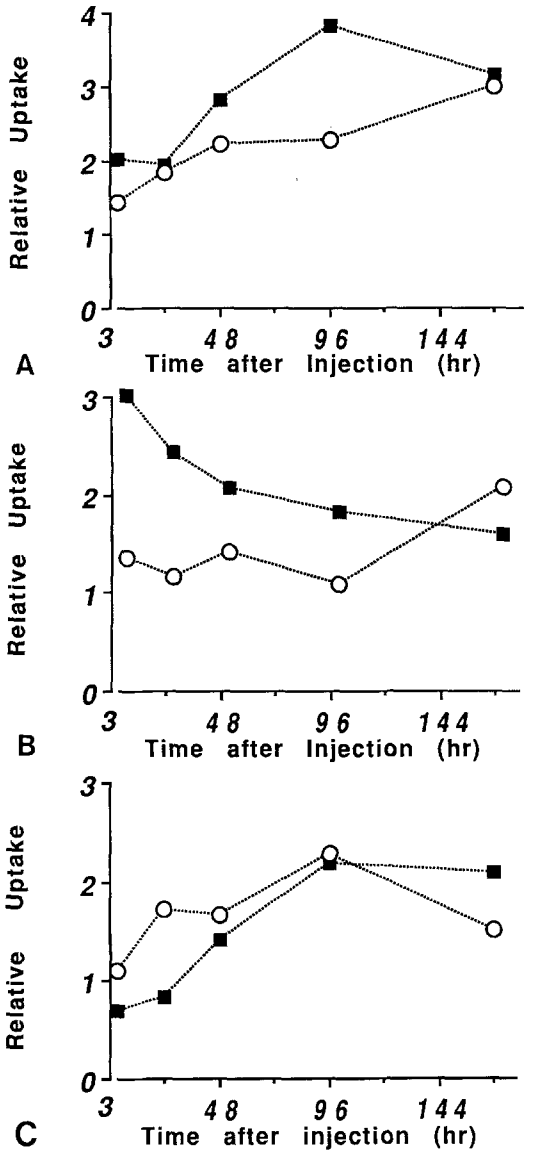
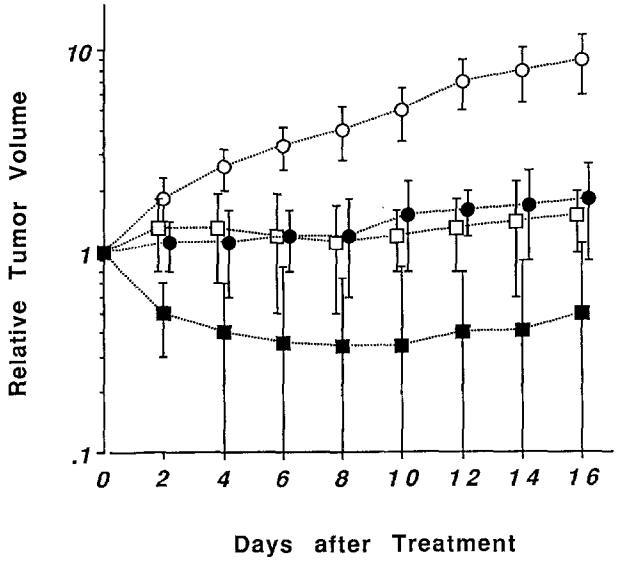


Fig. 4A–C. Relative uptake of AlS4Pc (■) and HpD (○), expressed as tumor/muscle (A), skin/muscle (B) and tumor/skin (C) ratio



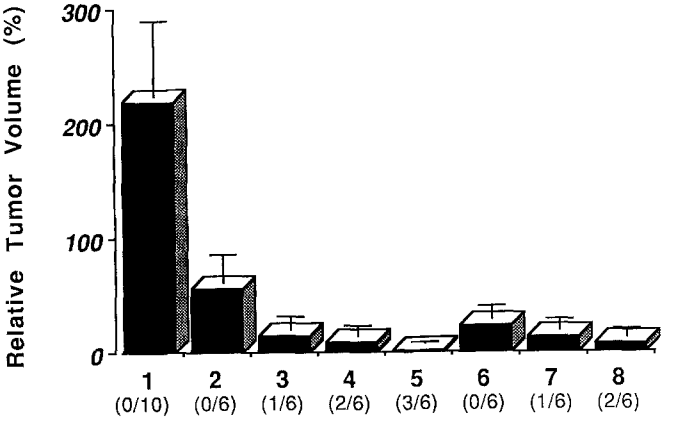


Fig. 6. Effect of PDT on autologous tumor in C3H/He mice: *1*, control; *2*, GVL only; *3*, AlS4Pc (0.1 mg/kg) and GVL; *4*, AlS4Pc (1 mg/kg) and GVL; *5*, AlS4Pc (10 mg/kg) and GVL; *6*, HpD (0.1 mg/kg) and GVL; *7*, HpD (1 mg/kg) and GVL; *8*, HpD (10 mg/kg) and GVL. Relative tumor volume (%) = tumor volume on day 30 after treatment/tumor volume on day 0 × 100. Figures in *parentheses* indicate CR rate. The mean response from six mice and SD are shown

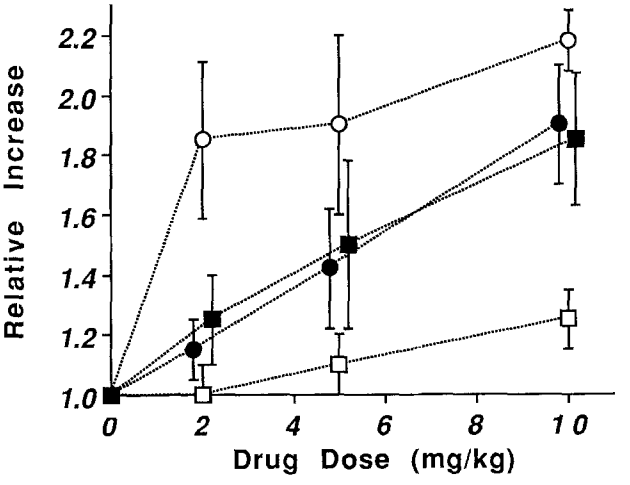


Fig. 7. Changes in back skin thickness in C3H/He mice as an estimate of photodynamic-induced skin photosensitivity: AlS4Pc (10 mg/kg) with UVA (□) or visible light (■); HpD (10 mg/kg) with UVA (○) or visible light (●). The mean response from five mice and SD are shown

Discussion

The present study reveals some advantages of AlS4Pc as a photosensitizer for PDT as compared with HpD.

First, less cytotoxicity of AlSPc than HpD in vitro was demonstrated by the MTT assay, which measures the activity of mitochondria in the cells. This is in agreement with the results reported by Chan et al. [2], who evaluated the cytotoxicity of both drugs by counting the number of

Fig. 5. Effect of PDT on transplantable tumor in nude mice: control (○); HpD (10 mg/kg) and light at 630 nm (●); AlS4Pc (10 mg/kg) and light at 630 nm (□); AlS4Pc (10 mg/kg) and light at 670 nm (■). The mean response from eight mice and SD are shown

nonadherent cells in culture after 3 days of exposure. AlSPc was less toxic despite the fact that it was taken up by cells in relatively greater amounts.

Second, AlS4Pc showed more photodynamic-dependent cytotoxicity and a greater antitumor effect in the red area of the spectrum (>660 nm) where tissue penetration is high. Although no significant differences in tumor reduction were demonstrated between the groups treated with AlS4Pc and HpD with light irradiation at 630 nm from ADL or GVL, the antitumor efficacy of AlS4Pc became greater when a longer wavelength (670 nm) of light was employed. This is also in agreement with the results from the in vitro study with red light at >660 nm. In addition to the absorption spectra of AlS4Pc, the deep penetration of the red light might account for the more efficient tumor destruction.

Third, AlS4Pc appeared to be removed more rapidly from normal tissues such as muscle and skin, since tumor/muscle ratios of AlS4Pc were higher than those for HpD, and the skin/muscle ratio of AlS4Pc decreased with time while that of HpD tended to increase. Moreover, higher absolute amounts of AlS4Pc than HpD were taken up by the tumors when equivalent doses were injected. Thus, AlS4Pc appeared to accumulate and be retained more efficiently in the tumors.

The retention patterns of AlS4Pc and HpD were different. The concentration of AlS4Pc decreased with time in tumors as well as in skin and muscle, while the peak concentration was seen 24 h after injection of HpD. AlSPc and its derivatives (AlS1Pc, AlS2Pc, AlS3Pc, AlS4Pc) were reported to have a peak concentration in transplantable tumors at 24 h after intravenous administration [5]. This discrepancy can be explained by the different route of drug administration: intravenous vs intraperitoneal. An immunohistochemical study, however, revealed different clearance patterns of AlSPc derivatives, that is, hydrophobic members such as AlS1Pc and AlS2Pc accumulated up to 48 h after intraperitoneal injection, while hydrophilic members such as AlS3Pc and AlS4Pc decreased with time [11], consistent with our results.

Fourth, a reduction in skin photosensitivity in PDT might be achievable by substituting AlS4Pc for HpD, since AlS4Pc showed less photodynamic-dependent cytotoxicity in vitro and milder cutaneous phototoxicity in vivo with UVA irradiation (which is responsible for photodynamic-dependent skin reactions [16]). Although Tralau et al. [14] reported greater cutaneous photocytotoxicity of dihematoporphyrin than AlS4Pc to simulated solar radiation in mice, we found no differences in skin edema between the groups treated with AlS4Pc and HpD when exposed to visible light. This discrepancy may be attributed to the fact that the visible light used had a peak power at 615 ± 10 nm, shifting to a longer-wavelength region as compared with sunlight, while Tralau et al. used light between 300 to 600 nm with peaks at 450–500 nm.

Considering these advantages of AlS4Pc over HpD, AlS4Pc can be regarded as one of the most promising photosensitizers for PDT. However, prior to clinical application of AlS4Pc in patients with bladder cancer, the kinetics of AlSPc need to be clarified not only the cancerous portion but also in the normal portion of the human bladder. According to animal investigations by Chan et al. [4], the anatomical site of tumor growth had a marked effect on AlSPc accumulation in the tumor in comparison with adjacent normal tissue, i.e. tumors located at subcutaneous, intrapulmonary, intrathoracic or intrarenal sites took up greater amounts of AlSPc than normal tissue, while the reverse obtained in the liver or spleen. Although Chan et al. did not investigate the bladder, there is a distribution study of AlSPc in a rat bladder cancer in which AlSPc was shown to localize diffusely in the rat bladder tumor after intravenous administration, and to be superficially retained in the tumor but also in normal urothelium after intravesical administration [1]. Furthermore, the kinetics of individual derivatives of AlSPc may vary, since different degrees of sulfonation of AlSPc were shown to be related to different penetration patterns in normal rat bladder wall [12] or to different intratumoral localization [11].

References

1. Bachor R, Flotte T, Scholz M, Dretler S, Hasan T (1992) Comparison of intravenous and intravesical administration of chloro-aluminum sulfonated phthalocyanine for photodynamic treatment in a rat bladder cancer model. J Urol 147:1404–1410
2. Chan WS, Svensen R, Phillips D, Hart IR (1986) Cell uptake, distribution and response to aluminum chloro-sulfonated phthalocyanine, a potential anti-tumor photosensitizer. Br J Cancer 53:255–263
3. Chan WS, Marshall J, Lam G, Hart IR (1988) Tissue uptake, distribution, and potency of the photoactivatable dye chloroaluminum sulfonated phthalocyanine in mice bearing transplantable tumors. Cancer Res 48:3040–3044
4. Chan WS, Marshall J, Hart IR (1989) Effect of tumour location on selective uptake and retention of phthalocyanines. Cancer Lett 44:73–77
5. Chan WS, Marshall J, Svensen R, Bedwell J, Hart IR (1990) Effect of sulfonation on the cell and tissue distribution of the photosensitizer aluminum phthalocyanine. Cancer Res 50:4533–4538
6. Evensen J, Sommer S, Moan J, Christensen T (1984) Tumor-localizing and photosensitizing properties of the main components of hematoporphyrin derivative. Cancer Res 44:482–486
7. Gomer CJ, Dougherty TJ (1979) Determination of [^{3}H] and [^{14}C] hematoporphyrin derivative distribution in malignant and normal tissue. Cancer Res 39:146–151
8. Hisazumi H, Miyoshi N, Naito K, Misaki T (1984) Whole bladder wall photoradiation therapy for carcinoma in situ of the bladder: a preliminary report. J Urol 131:884–887
9. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63
10. Naito K, Hisazumi H, Uchibayshi T, Amano T, Hirata A, Komatsu K, Ishida T, Miyoshi N (1991) Integral laser photodynamic treatment of refractory multifocal bladder tumors. J Urol 146:1541–1545
11. Peng Q, Moan J, Farrants G, Danielsen HE, Rimington C (1991) Localization of potent photosensitizer in human tumor LOX by means of laser scanning microscopy. Cancer Lett 58:17–27
12. Pope A, MacRobert A, Phillips D, Bown SG (1991) The detection of phthalocyanine fluorescence in normal rat bladder wall using sensitive digital microscopy. Br J Cancer 64:875–879
13. Taya T, Kobayashi T, Tsukahara K, Uchibayashi T, Naito K, Hisazumi H, Kuroda K (1977) In vitro culture of malignant

tumor tissues from the human urinary tract. Jpn J Urol 68: 1003–1010
14. Tralau C, Young A, Walker N, Vernon DI, MacRobert AJ, Brown SB, Bown SG (1989) Mouse skin photosensitivity with hematoporphyrin ether (DHE) and aluminum sulphonated phthalocyanine (AlSPc): a comparative study. Photochem Photobiol 49: 305–312
15. Urano M, Nesumi N, Ando K, Koike S, Ohmura N (1976) Repair of potentially lethal radiation damage in acute and chronically hypoxic tumor cells in vivo. Radiology 118: 447–451
16. Zalar G, Poh-Fitzpatrick M, Krohn D, Jacobs R, Harber LC (1977) Induction of drug photosensitization in man after parenteral exposure to hematoporphyrin. Arch Dermatol 113: 1392–1397

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