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ALUMINUM SULFONATED PHTHALOCYANINE DISTRIBUTION IN RODENT TUMORS OF THE COLON, BRAIN AND PANCREAS

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Abstract—The most widely discussed aspect of photodynamic therapy (PDT) is the preferential uptake and retention of sensitisers by malignant tissues. The sensitiser usually used is hematoporphyrin derivative (HPD) but this compound is not an ideal photosensitiser for this purpose and we have therefore studied an aluminum sulfonated phthalocyanine (AlSPc) as a possible alternative. Here we have studied the uptake and retention of this compound in a rat colon cancer, a hamster pancreatic cancer and a mouse glioma, using an alkali extraction technique to estimate tissue AlSPc and comparing the results with those from the corresponding normal tissues. All of the tumors studied reached accumulation peaks at 24–48 h after intravenous administration of AlSPc compared with peaks at 1–3 h in the normal tissues. The tumors outside the central nervous system (CNS) reached peak tumor : normal tissue ratios of 2–3 : 1 and the tumor within the CNS, the malignant glioma, reached a far higher ratio of 28 : 1. These ratios are similar to those reported by others using HPD.

INTRODUCTION

Photodynamic therapy has attracted considerable interest because it offers the possibility of a selective treatment for cancer. The theory is that there is preferential uptake and retention of intravenously administered sensitisers in malignant tissue in relation to the surrounding normal tissues. This sets up a therapeutic ratio so that when activated with light of an appropriate wavelength, the malignant tissue would be destroyed selectively or at least to a greater extent than the surrounding normal tissues.

There have been numerous studies reported on the tumor uptake of HPD.† Most of these studies report tumor : normal ratios of 2–5 : 1 in tumors outside the central nervous system (Agrez, 1983; Gomer and Dougherty, 1979), but they often fail to compare tumor with the tissue of origin.

As has widely been discussed HPD is a far from ideal photosensitiser. It has a variety of problems including being an unpredictable mixture of incompletely defined porphyrins, low absorption in the red region of the spectrum, where light penetration of tissue is good, and poor selective uptake by tumors. One of the most promising alternative groups of agents for photodynamic therapy is the phthalocyanines. We and others have been studying these agents as potential photosensitisers. It has been shown that aluminum sulfonated phthalocyanine (AlSPc) will cause photosensitised cell death *in vitro* (Ben-Hur and Rosenthal, 1986; Chan *et al.*, 1986).

We have shown that AlSPc will effectively sensitise normal liver tissue to a greater extent than HPD (Bown *et al.*, 1986) and also a malignant fibrosarcoma (Tralau *et al.*, 1987). Phthalocyanines have therefore been shown to have many of the properties required of an ideal photosensitiser but there is one property which although essential for a photosensitiser, has been little studied. This is the uptake and retention of the sensitiser in tumor tissues and their associated normal tissues.

Frigerio (1962) was the first to show selective uptake of a phthalocyanine by implanted brain tumors in mice, he reported a tumor : normal ratio 50 : 1. Rousseau *et al.* (1985) used a technetium (^{99m}Tc) and gallium (^{67}Ga) radiolabelled tetrasulphophthalocyanine in a mammary adenocarcinoma of Fischer 344/CRBL rats. Tc-TSPC achieved 3.8 : 1 tumor : muscle ratio at 24 h and Ga-TSPC achieved a ratio of 15 : 1 tumor : muscle at 6 h. Straight and Spikes (see Spikes, 1986) found selective retention of zinc sulfonated phthalocyanine in S180 tumors of Swiss Webster mice but no tumor : normal tissue ratio was stated. There are few studies of tumor photosensitiser content *vs* the normal organ of origin of the tumor.

In this paper we report preliminary studies on the uptake of aluminum sulfonated phthalocyanine (AlSPc) by a range of rodent tumors of the colon, pancreas and brain, compared with uptake in the corresponding normal tissues.

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†*Abbreviations*: AlSPc, aluminum sulfonated phthalocyanine; CNS, central nervous system; HPD, hematoporphyrin derivative; PDT, photodynamic therapy.

MATERIALS AND METHODS

Photosensitiser

Aluminum sulfonated phthalocyanine (AlSPc) was obtained from Ciba-Geigy and used as received. Although this compound was pure AlSPc the molecule had a variable number of sulfonic acid groups which McCubbin (1985) estimated to be an average of three sulfonates per molecule. This compound was administered in 0.9% saline and although it was photochemically and chemically stable, as a precaution it was stored in the dark.

Animals and tumor models

Three tumor models were studied, the first two being chemically induced autochthonous models and the other being a transplantable model. The induction of these models is outlined separately.

1. *Colon cancer model.* Animals used were male Wistar weanling rats of approx. 100 g at time of induction. The animals were given 1,2-dimethylhydrazine dihydrochloride (Aldrich Chemical Co., Gillingham, Dorset). This was prepared by the method of Filipe (1975). Each rat received five sub-cutaneous injections of DMH (40 mg kg^{-1} body wt $week^{-1}$) in the left flank. The rats were housed in temperature controlled quarters in subgroups of five in suspended cages with open wire mesh floors to prevent coprophagia. They were weighed weekly and inspected for signs of illness. Animals were colonoscoped using a bronchoscope (Olympus BF1TR), in order that they should be used only when useful tumors were present which usually occurred at weeks 25–30. The phthalocyanine was administered (5 mg kg^{-1}) *via* a tail vein injection and animals were sacrificed by cervical dislocation at a range of times from a few minutes to 1 week after administration. At *post mortem* the whole distal colon was removed and the tumors were excised together with a piece of adjacent 'normal' colon. Samples were frozen separately (−20°C) until they were assayed for AlSPc content. Blood samples were taken from normal rats over a range of times (0.1–72 h), these samples were placed in heparinised blood tubes and centrifuged. Plasma was removed and assayed for AlSPc content using the technique described previously (Bown *et al.*, 1986).

From 20 animals there were 25 normal tissue and 25 tumor tissue extractions.

2. *Pancreatic cancer model.* The animals used were female Syrian hamsters of approx. 150 g. The carcinogen used was 2,2′-dioxodi-*n*-propyl nitrosamine (BOP; Ash Stevens Inc., Detroit, USA). This was prepared by the method of Pour (1980). Animals were given weekly subcutaneous injections of 10 mg kg^{-1} for 10 weeks and were used at 18–25 weeks. Animals were anesthetised with ether, and an exploratory laporotomy was performed to see if pancreatic tumors were present. Animals with tumors were given an intravenous injection of AlSPc (5 mg kg^{-1}) into the inferior vena cava and allowed to recover from the anaesthetic. They were sacrificed at a range of times from a few minutes to 3 weeks after administration. At *post mortem* the pancreas was excised and obvious tumors were dissected free of surrounding 'normal' tissues, both normal and tumor samples were frozen separately at −20°C until being assayed for AlSPc content.

From 21 animals there were 21 normal tissue extractions and 9 tumor tissue extractions.

3. *Brain tumor model.* The animals used were white mice (VM) and the murine glioma was induced using cells which were cultured from a spontaneous murine astrocytoma (glioma). The cells were implanted intracranially and took approx. 7 days to grow to a usable size. Animals were anesthetised using intraperitoneal injections of Valium (Diazepam) and Hypnorm (fentanyl and fluanisone), injected with AlSPc intravenously (5 mg kg^{-1}) into the tail vein and sacrificed at a range of times after administration, from a few minutes to a week. Animals were killed by cervical dislocation and the brains were removed. It proved impossible to dissect tumor from normal tissues, due to the small size and infiltration by the tumor, and so we assayed brain containing tumor and normal brain for AlSPc content. Specimens were kept frozen (−20°C) until they were assayed. Blood samples were taken at a range of times (1–72 h after sensitisation) and assayed for AlSPc content as described before (Bown *et al.*, 1986). From 40 animals there were 40 normal tissue (left hemisphere) and 40 tumor extractions (right hemisphere).

Assay of AlSPc content of tissues

Preparation of a standard curve. One problem of fluorescent assays of this kind is that tissue components may interfere with the expression of fluorescence from the AlSPc under study. Components will cause scattering of the exciting light and probably quenching of AlSPc fluorescence. In order to allow for these effects we prepared standard curves of AlSPc in the extracting medium (NaOH) and also in supernatants of the tissues of non-sensitised animals. The supernatants were prepared using the methods given below. The standards were then prepared using these supernatants instead of the NaOH. The fluorescence of both standard curves was then read on a spectrofluorimeter (Perkin–Elmer LS-5 Luminescence Spectrofluorimeter). The exciting wavelength was 610 nm, slit width was 2.5 nm and the emission wavelength was 675 nm (the wavelength of maximum absorption by AlSPc) and slit width was 5.0 nm. The emission was filtered with a Schott long pass RG 645 filter in order to filter out scattered light at wavelengths below 645 nm (50% transmission at 645 nm).

Tissue content of AlSPc. The tissue samples were thawed and then approx. 0.5 g of tissue was weighed and placed in clean glass test tubes; 1.5 mℓ of 0.1 *M* NaOH was added and the samples were thoroughly mixed using a laboratory mixer. Samples were incubated in a water bath (Grant Instruments Ltd., Cambridge) at 50°C for 4 h, during which they were mixed twice. The solution was then placed in micro-centrifuge tubes and spun at top speed in a Hawksley micro-haematocrit centrifuge for 10 min. The supernatant was removed and the fluorescence was read as above. AlSPc concentrations were read from the tissue corrected standard curve, corrected for sample weight and expressed as μg AlSPc g^{-1} tissue. The results of this extraction technique are reproducible and although it is difficult to estimate precisely what percentage of the total AlSPc is extracted, it has been estimated to be about 70% for liver tissue (Bown *et al.*, 1986) and this is similar in all tissues studied.

RESULTS

The results of this study are summarised in Table 1 together with published data for HPD for comparison.

Normal rat colon reaches a peak of AlSPc accumulation at 1–3 h after administration, after which time the concentration decreases to an undetectable level approx. 1 week after administration. The tumors reach a peak at 48 h and at this time the tumor : adjacent normal colon ratio reached its maximum value of 2.8 : 1 (Fig. 1). Plasma levels peak at 0.1 h and decline rapidly being undetectable by 48 h (n.b., These results are quantitatively differ-

Table 1. Summary of the results

Tumor model	AlSPc Max. ratio tumor : normal	AlSPc Max. ratio tumor : normal	HPD Time (h)	HPD Time to reach max. ratio (h)
Colon	48	2.0 : 1	72	2.0 : 1 (Agrez, 1983)
Pancreas	24–48	3.0 : 1	3	2.2 : 1 (Schroder, 1986)
Brain	24	28.0 : 1	4	26.0 : 1 (Wharen, 1983)
Fibrosarcoma (Tralau *et al.*, 1987)	48	2.7 : 1	—	—

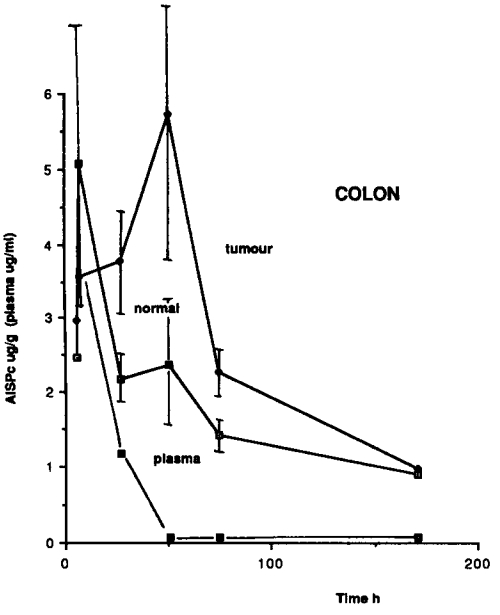

Figure 1. The concentration of AlSPc extracted from normal colon and the adjacent tumor tissue and plasma with time in the DMH colonic cancer model (mean ± SD, No. per point = 3 or 4).

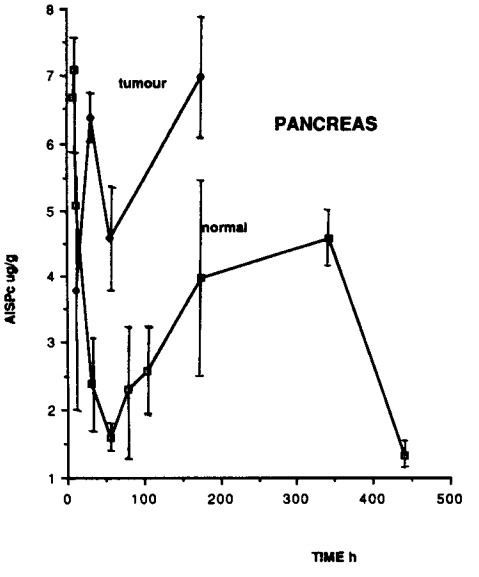

Figure 2. The concentration of AlSPc extracted from normal pancreas and the adjacent tumor tissue with time in the BOP pancreatic cancer model (mean ± SD; No. per point (normal) = 4 or 5, (tumor) = 2 or 3).

ent to those reported in Bown *et al.*, 1986, the method of collection of samples and assay of AlSPc content being improved in the results reported here.)

Normal pancreas reaches an initial accumulation peak immediately after administration, levels decline rapidly to 48 h and then rise again at 1 week, but in the normal pancreas tissue levels had declined again by 3 weeks. At 24–48 h the maximum tumor : normal pancreas ratio of 3 : 1 is seen (Fig. 2). These results are only preliminary and more samples are required.

Normal brain tissue reaches a peak of accumulation of AlSPc immediately after administration after which levels decline until it is undetectable at 10 weeks although there does appear to be a small second peak at 1 week. The brain containing glioma had a very different accumulation pattern to the normal brain. This reached considerably higher levels of AlSPc, reaching a peak at 24 h after administration. At 24 h the brain containing glioma : normal brain ratio reached its maximum of 28 : 1 (Fig. 3). Plasma levels were higher than the tissue levels at all times studied, they peaked at less than 1 h and declined rapidly. It is interesting to note that tumor levels of AlSPc in the brain are at no time greater than the plasma levels. The absolute levels of AlSPc present in the brain and brain tumor are far lower than in colon or pancreas.

DISCUSSION

For selectivity of PDT to be clinically relevant there must be selectivity between the tumor and the

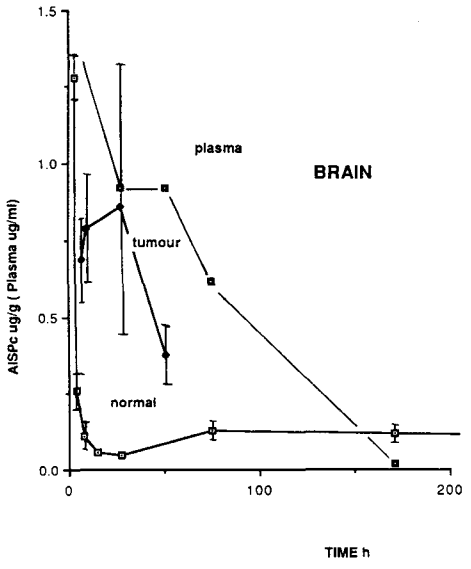

Figure 3. The concentration of AlSPc extracted from normal brain and tumor containing brain (opposite hemisphere) and plasma with time in the mouse glioma model (mean ± SD; No. per point = minimum 5).

normal tissue in which that tumor arose or into which that tumor has spread. It is surprising how rarely such comparisons are made. For example, many early studies compared uptake in a transplantable mammary carcinoma with muscle instead of comparing it with normal mammary tissue. The aim in this study was to compare selectivity of uptake of HPD as reported by others and our own results for AlSPc between three rodent tumors and the individual host tissues.

It has been reported that HPD is selectively taken up and retained for longer periods in tumor tissues than normal tissues. Although many studies have shown tumors fluorescing selectively under an ultraviolet light source after the administration of porphyrins (Benson, 1985; Gregorie, 1968), there have been few quantitative studies of tissue concentration of porphyrins which support the concept of selective retention. Gomer and Dougherty (1979) studied the distribution of tritium and ^{14}C-labelled HPD in a spontaneous mammary carcinoma and a methylcholanthrene induced mammary carcinoma of mice. They reported tumor : muscle ratios of approx. 4 : 1 at 24 h but showed no prolonged retention of HPD. Agrez (1983) studied the uptake of HPD in the DMH colon cancer model used here; he showed selective uptake in malignant tissues but it was not selective enough for detection purposes and no prolonged retention was seen, although studies only extended to 72 h after administration of HPD.

Each of the tumors studied here had a different distribution of AlSPc to the associated normal tissue. The tumor tissue in general reached an accumulation peak at a later time than the associated normal tissue. From our own studies most normal tissues reach early accumulation peaks, within the first 4 h (unpublished data).

All of the tumors studied, including a fibrosarcoma studied by us earlier (Tralau *et al.*, 1987), showed AlSPc accumulation peaks at 24–48 h after administration by which time the levels of AlSPc in associated normal tissues were declining. This later peak for tumor than normal tissues indicates the presence of a possible therapeutic window for photodynamic therapy. Detailed fibrosarcoma data are not included here as there is no convenient normal tissue with which to compare the tumor data.

It is of interest to consider whether the tissue concentration of AlSPc in tumor *vs* normal could be improved by giving multiple injections of AlSPc at different times, but an analysis of the accumulation curves shows that the fall off in AlSPc concentration in tumors after the peak at 24–48 h is too rapid to achieve any improvement in the ratio with multiple injections.

From these results it appears that for tumors outside the central nervous system the largest tumor : normal tissue ratio obtainable with AlSPc is 2–3 : 1, but in the tumor within the CNS the ratios obtained are far higher (28 : 1). The extractions were done on brain containing tumor so the actual ratio brain tumors : normal brain may be far higher than the 28 : 1 measured. Preliminary results using a more precise detection method suggest ratios of up to 60 : 1 in this model (unpublished). It is interesting that the plasma levels in the mouse were at all times greater than the levels of AlSPc in the normal brain or glioma; this could be due to the hydrophillic nature of AlSPc causing it to be poorly taken up by the hydrophobic brain tissue. This suggests that much of the preferential retention by this tumor is due to disruption of the blood brain barrier, although other factors may be involved. Also if the tumor was treated by PDT at a time when there were large amounts of AlSPc in the plasma we would expect extensive damage to areas of normal brain exposed to light as was observed with HPD by Berenbaum *et al.* (1986). These results suggest that although AlSPc is a potentially better agent than HPD for the PDT of tumors on other grounds (Bown *et al.*, 1986), it is not taken up more selectively by tumor tissue. Selectivity is in fact very similar between the two. Agrez *et al.* (1983) showed a tumor : normal tissue ratio of 2 : 1 at 72 h after administration of HPD in the DMH colon model; Schroder (1986) used the same pancreatic model and found a tumor : normal pancreas ratio of 2.2 : 1 at 3 h and Wharen *et al.* (1983) showed a tumor: normal brain ratio of 26.0 : 1 at 4 h in an ENU induced glioma of mice. Although various methods of detection of sensitiser have been used, there is a very good correlation between HPD and AlSPc tumor : normal tissue ratios in similar models. The time at which these maximum ratios are observed

varies but this may be due to the distribution not being studied over a long enough period (Table 1). These results also suggest that accumulation of HPD and AlSPc in tumor tissue is unlikely to be a process specific to any particular chemical and probably involves some type of non-specific property of tumors such as poor lymphatic drainage (Bugelski *et al.*, 1981). Perhaps PDT will have its best application for brain tumors where uptake is more selective, unless other sensitisers with better selective uptake are found. Alternatively other methods of increasing the selectivity of PDT could be used, such as directing the light source solely at the tumor or showing that there is a different biological response in normal and tumor tissue.

The phthalocyanine used here is a mixture of molecules with differing numbers of sulfonic acid groups. Species with more negative groups, i.e. $-SO_3^-$, will be more water soluble than species with less negative groups, which will be more lipid soluble. The different species present in the mixture used here would be expected to have differing tissue distributions and thus have different degrees of uptake by malignant tissues and possibly different photosensitising abilities. We are currently studying the effects of the pure species but it is unlikely that the selectivity of tumor uptake will be significantly improved.

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REFERENCES

Agrez, M. V., R. E. Wharen, R. E. Anderson, E. R. Laws and D. M. Ilstrup (1983) Hematoporphyrin derivative, quantitative uptake in DMH induced murine colo-rectal carcinoma. *J. Surg. Oncol.* **24**, 173–176.

Ben-Hur, E. and I. Rosenthal (1986) Photosensitisation of Chinese Hamster cells by water soluble phthalocyanines. *Photochem. Photobiol.* **43**, 615–619.

Benson, R. C., G. M. Farrow and J. H. Kinsey (1985) Hematoporphyrin photosensitisation and the argon laser. In *Lasers in Urological Surgery* (Edited by J. A. Smith), Chicago Yearbook. Medical Publications, Chicago.

Berenbaum, M. C., G. W. Hall and A. D. Hoyes (1986) Cerebral photosensitisation by haematoporphyrin derivative. Evidence for an endothelial site of action. *Br. J. Cancer* **53**, 81–89.

Bown, S. G., C. J. Tralau, P. D. Coleridge-Smith, D. Akdemir and T. J. Wieman (1986) Photodynamic therapy with porphyrin sensitisation: quantitative studies in normal rat liver. *Br. J. Cancer* **54**, 43–52.

Bugelski, P. J., C. W. Porter and T. J. Dougherty (1981) Autoradiographic distribution of HPD in normal and tumour tissue of the mouse. *Cancer Res.* **41**, 4606.

Chan, W. S., R. Svensen, D. Philips and I. R. Hart (1986) Cell uptake, distribution and response to aluminium chloro phthalocyanine, a potential anti-tumour photosensitiser. *Br. J. Cancer* **53**, 255–264.

Frigerio, N. A. (1962) Metal phthalocyanines. U.S. Patent No. 3,027,391 (Patented 27 March 1962).

Filipe, M. I. (1975) Mucous secretion in rat colonic mucosa during carcinogenesis induced by dimethylhydrazine. A morphological and histochemical study. *Br. J. Cancer* **32**, 60.

Gregorie, H. B., E. O. Horger, J. L. Ward, J. F. Green, T. Richards, H. C. Robertson and T. B. Stevenson (1968) Haematoporphyrin derivative fluorescence in malignant neoplasms. *Ann. Surg.* **167**, 820.

Gomer, C. J. and T. J. Dougherty (1979) Determination of ^{3}H and ^{14}C hematoporphyrin derivative distribution in malignant and normal tissue. *Cancer Res.* **39**, 146.

Lipson, R. L. and E. J. Baldes (1960) The photodynamic properties of a particular hematoporphyrin derivative. *Arch. Derm.* **82**, 508.

McCubbin, I. (1985) Photochemistry of some water soluble phthalocyanines. PhD thesis, University of London.

Pour, P. M. and R. B. Wilson (1980) Experimental tumours of the pancreas. In *Tumours of the Pancreas* (Edited by A. R. Moossa), p. 37. Williams and Wilkins, Baltimore, MD.

Rousseau, J., M. Ali, G. Lamoureux, E. Lebel and J. E. Van Lier (1985) Synthesis, tissue distribution and tumour uptake of ^{99m}Tc and ^{67}Ga-tetrasulphophthalocyanine. *Int. J. Appl. Radiat. Isotopes* **36**, 709.

Schroder, T., I.-W. Chen, M. Sperling, R. H. Bell, K. A. Bracklett and S. N. Joffe (1986) Haematoporphyrin uptake and photodynamic therapy in pancreatic carcinoma. *Proc. Eur. Laser Association*, Amsterdam, November 1986, No. 63.

Spikes, J. D. (1986) Phthalocyanines as photosensitisers in biological systems and for the photodynamic therapy of tumours. *Photochem. Photobiol.* **43**, 691.

Tralau, C. J., A. J. MacRobert, P. D. Coleridge-Smith, H. Barr and S. G. Bown (1987) Photodynamic therapy with phthalocyanine sensitisation: quantitative studies in a transplantable fibrosarcoma of rats. *Br. J. Cancer* **55**, 389–395.

Wharen, R. E., B. A. S. Anderson and E. R. Laws (1983) Quantitation of hematoporphyrin derivative in human gliomas, experimental central nervous system tumours and normal tissues. *Neurosurgery* **12**, 446.