Aluminium phthalocyanine mediated photodynamic therapy in experimental malignant glioma

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The efficacy of aluminium tetrasulfonated phthalocyanine (AlSPc) as a photosensitizer for photodynamic therapy (PDT) was investigated in the C6 glioma model in Wistar rats. The depth and extent of tumour necrosis was dependent on both the dosage of intravenously (IV) administered AlSPc, and the dose and time post-sensitization of 675 nm light administration. There was no effect on tumour or normal brain if the sensitizer dose was less than 0.5 mg/kg. Selective necrosis of tumour was evident 6 hours post-sensitization at an AlSPc dose up to 1 mg/kg and light doses up to 200 J/cm². Necrosis occurred in normal brain at higher doses of light and sensitizer. There was no photosensitizer effect in animals treated 24 hours post AlSPc administration. The ability of AlSPc to act as a selective photosensitizer causing photonecrosis provides a basis for the generation of modified AlSPc species as future agents in the PDT of brain tumours, especially as these sensitizers absorb light at a higher wavelength than those that are currently available.

Keywords: Brain tumour, Glioma, Photodynamic therapy, Aluminium tetrasulfonated phthalocyanine

Introduction

Cerebral tumours are responsible for approximately 2% of all cancer deaths and the high grade cerebral glioma, glioblastoma multiforme (GBM), is the most common cerebral tumour in adults. Conventional therapies for the treatment of malignant gliomas have only limited success. The best available protocol, using surgery, radiation therapy and systemic chemotherapy result in a median survival time of less than one year for GBM.1,2,3 Most treatment failures occur because of local recurrence of the tumour indicating that a more aggressive local therapy would be desirable for the treatment of gliomas.4,5,6,7

Photodynamic therapy (PDT) is a treatment modality that involves the selective uptake of a photosensitizer by the tumour followed by irradiation of the tumour with light that will activate the sensitizer and cause selective tumour death. PDT utilising the sensitizers haematoporphyrin derivative (HpD) or its more enriched commercial form, Photofrin, has been used in a number of trials in the treatment of patients with brain tumours,4,5,6,7,8 with over 120 patients being treated in the Royal Melbourne Hospital study.4,5

Despite showing clinical efficacy,4,5 HpD and Photofrin possess many characteristics which make them less than ideal photosensitizers. In the synthesis of HpD and Photofrin, a hydrolysis mixture of acetic and sulphuric acid is used to treat haematoporphyrin, resulting in a large number of oligomeric, monomeric ether and ester components.9 Numerous attempts have been made to determine the 'active' compound in HpD, and several candidates have been nominated.8,10,11,12 The composition of HpD may also vary from batch to batch ultimately altering its biological efficacy.13 Additional disadvantages are that HpD absorbs poorly in the region of light (600-700 nm) desirable for optimal tissue penetration and that patients remain photosensitive for several weeks due to retention of appreciable amounts of dye in the skin.14 These constraints have encouraged the search for alternative photosensitizers for clinical use.

One potential group of compounds are the phthalocyanines, which have been suggested as possible candidates for replacing HpD in PDT protocols.15 They are characterised by strong light absorption in the 670-700 nm range and are easily synthesised in a pure form. In this study, the efficacy of AlSPc as a potential photosensitizer in the PDT treatment of the C6 glioma in the Wistar rat has been investigated and the appropriate dose of sensitizer and light dosimetry which caused destruction of tumour with sparing of normal brain was established.
Materials and methods

Chemicals

AISPc was purchased in powder form in 1g quantities (Porphyrin Products, Utah, USA). The molecular structure of the tetralsulfonated compound is shown in Figure 1, although high performance liquid chromatography of this proprietary AISPc showed it to be a mixture consisting of the mono-, di-, tri- and tetrasulfonated forms (unpublished results). On receipt, it was stored desiccated and shielded from light at -20°C until subsequent use. Immediately prior to use, it was suspended in isotonic saline and as a precaution was stored in the dark at 4°C.

Cells

The C6 glioma cell line was obtained from the American Type Culture Collection, and the cells grown at 37°C in a humidified atmosphere containing 5% CO2 in RPMI 1640 medium supplemented with 5% newborn calf serum (Commonwealth Serum Laboratories, Parkville, Australia).

Animals and tumour implantation

All in vivo animal procedures were performed in accordance with requirements established by the Royal Melbourne Hospital Institutional Animal Ethics Committee.

Intracranial implantation of C6 glioma in Wistar rats

Adult Wistar rats weighing between 200 and 400 g, obtained from the joint animal facility of the Ludwig Institute for Cancer Research (Melbourne Tumour Biology Branch) and Department of Surgery, Royal Melbourne Hospital, were used for C6 glioma intracranial implantation. The rats were anaesthetised using methoxyflurane inhalation followed by administration of chloral hydrate (0.1 ml/10g body weight in a solution containing 3.6 g/100 ml water (wt/vol)) by intraperitoneal injection. The method developed by Kaye et al16 was used to establish intracranial gliomas. In brief, the head was shaved, a 1.5 cm midline scalp incision was made, and a burr hole was placed just in front of the coronal suture, 3 mm to the left of the midline. The C6 glioma cells were suspended in a solution of 2X RPMI 1640 containing 1% Sea plaque agarose (FMC Corp, Rockland, Maine) at a density of 106 cells per 25 μl. This volume was injected using a Hamilton Microlitre syringe with a 27 gauge disposable needle covered by a plastic sleeve to a depth of 2 mm from the outer table of the bone. The needle was withdrawn 30 seconds after injection of the suspension, and the hole was sealed with bone wax. The wound was then closed with clips. The operation was performed using an operating microscope.

Photodynamic therapy

PDT was administered to animals on the 10th day following tumour cell implantation. The animals were sensitized with AISPc via intravenous (IV) tail vein injection either 6 hours or 24 hours prior to the PDT procedure as described.

Normal animals

A 2 cm midline incision was made to expose the skull and allow the pericranium to be reflected. A craniotomy of 4-5 mm long and 3-4 mm wide was performed with a high speed dental drill (Faro Pty Ltd, Italy). The exact dimensions of the craniotomy were recorded to be used later in calculating the laser light dose. The anterior margin of the craniotomy was placed 1 mm in front of the coronal suture and medially extended to within 1 mm of the midline.

Tumour bearing animals

An incision was made as before, but incorporating the previous burr hole after reflection of the pericranium. A craniotomy was performed as in the normal rats over the C6 tumour injection site to ensure the exposure was over the surface of the tumour.

PDT of rats

PDT was administered 6 or 24 hours after the IV administration of AISPc. Light of 675 nm was generated using an Argon—ion pumped Kiton red dye laser producing a broad band (615—695 nm) output (Spectra-Physics, Model 164-05 Argon Ion Laser, Model 375 dye laser, Mountain View, California). A birefringent grating was used to tune the output to 675 ± 2 nm and the wavelength output verified using a slit monochrometer. A 600 μm diameter flat cut quartz fibre was used to deliver the light. The light output was measured by placing the tip of the fibre in a photoelectric cell (Spectra Physics Model 404, Spectra Physics, Bayswater, Australia) and calculating the light dose relative to the craniotomy area. The fibre tip was held at a distance of 3 to 4 mm over the craniotomy so that red light evenly covered the exposed tissue.
Laboratory investigations

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The laser power at the fibre tip varied from 50 to 500 mW and the dose of light that was delivered varied from 0 to 800 J/cm². The surface of the brain was kept cool by irrigation with isotonic saline at room temperature, which has been shown in previous studies to prevent hyperthermia of the irradiation surface and underlying tissue. On completion of the irradiation, a single layer of Surgicel (Johnson and Johnson, North Ryde, Australia) was used to cover the craniotomy site, and the incision closed with wound clips.

Quantification of photodynamic effect

The animals were culled 5 days after laser treatment. The brains were removed, fixed in 10% formaldehyde, sectioned through the area of irradiation, and stained with haematoxylin and eosin. The extent of normal brain or tumour necrosis was measured on serial sections using a graticule micrometer (Leitz, Wetzler, West Germany) in a Nikon Diaphot microscope.

Results

Effect of PDT and AlSPc on normal brain

The effect of light dosage on normal brain in animals sensitized with AlSPc, but without implanted tumour, was studied to determine the dosimetry limits of selective kill. The PDT effect on normal brain was studied following AlSPc doses of either 0, 0.5, 1 or 5 mg/kg, and light doses up to 800 J/cm². Necrosis of the normal brain was not evident following light doses up to 800 J/cm², both in animals sensitized at a dose of 0.5 mg/kg and non-sensitized animals. Following administration of AlSPc at a dose of 1 mg/kg, normal brain necrosis was not evident up to a light dose of 200 J/cm², but was apparent at higher light doses (Fig. 2). At a dose of 5 mg/kg and a light dose greater than 50 J/cm², necrosis of normal brain was observed, with the depth and extent of this necrosis dependent on the light dose administered.

Effect of PDT and AlSPc on intracerebral tumours

When C6 tumour cells were implanted 2 mm deep from the outer bone margin, intracerebral tumours grew up to the surface of the brain at 10 days post inoculation. This allowed for the surface of the tumour to be irradiated with red laser light after the craniotomy was performed. The depths of PDT mediated tumour kill 6 hours post administration of doses of AlSPc from 0-5 mg/kg, and of 675 nm light from 0-800 J/cm² at 6 hours, are shown in Figure 3.

The major finding was that following administration of AlSPc at a dose of 5 mg/kg and 100 J/cm² of 675 nm light, there was variable animal death, with 5 out of 9 animals surviving the 5 day post laser irradiation period. Animals that were not able to move or feed were sacrificed. Mso, sensitized animals treated at a light dose of 900 J/cm² were sacrificed within 24 hours of treatment as they were suffering from neurological symptoms, which were not apparent in non-sensitized control animals administered the same light dose. Wide spread cerebral oedema was evident in these animals. Based on these results, animals administered 5 mg/kg AlSPc were not treated at light doses higher than 100 J/cm². There was no tumour necrosis in animals which received laser treatment alone up to light doses of 800 J/cm², or laser treatment following sensitization at an AlSPc dose of 0.5 mg/kg.
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Following a sensitizer dose of 1 mg/kg, and light doses up to 200 J/cm², selective kill of the C6 rat glioma with sparing of normal brain was evident (Fig. 4). The depth of necrosis was similar in rats treated with light doses between 200 and 800 J/cm². At the highest selective laser dose of 200 J/cm², the mean depth of tumour necrosis was 3.4 mm, with 4.9 mm being the deepest depth of necrosis, and in 2 out of 5 animals the depth of necrosis was greater than 3.5 mm.

Delayed effect of PDT and AlSPc on normal brain and intracerebral tumours

The depth and extent of necrosis following AlSPc mediated PDT of normal brain and C6 tumour was also investigated at 24 hours post sensitization. AlSPc was administered at either 0.5 mg/kg or 1.0 mg/kg and 675 nm light at doses up to 800 J/cm². No evidence of normal brain necrosis in sensitized animals was evident even at the highest light dose of 800 J/cm² (Fig. 5a). In contrast, there was minimal tumour necrosis at an AlSPc dose of 1.0 mg/kg and 800 J/cm² of light (Fig. 5b) to the same dose regime 6 hours post AlSPc administration (Fig. 6) which resulted in a mean depth of necrosis of 3.5 mm.

Discussion

Most cerebral gliomas recur locally after the present conventional therapies of surgery, radiotherapy and chemotherapy indicating a failure to control the local tumour.1,2,3 PDT is a targeted adjuvant therapy which has been shown to aid local tumour control4,5 and has been the subject of several clinical trials,6,8 some of which have demonstrated it to be clinically effective.4,5

We have previously shown that AlSPc administered via the IV route into CBA mice bearing the intracranial C6 glioma was selectively retained by the tumour, with peak uptake at 6 hours post-sensitization.12 The data reported here indicate that selective kill of a cerebral glioma with sparing of the normal brain can be achieved with AlSPc mediated PDT. Selective tumour destruction occurred at an AlSPc dose of 1 mg/kg and at light doses less than 200 J/cm² administered 6 hours post-sensitization. Increasing the light dose from 200 J/cm² to 800 J/cm² (in 200 J/cm² increments) did not significantly increase the depth of tumour necrosis but did result in damage to the normal brain. The depths of tumour kill reported here are similar to those previously reported by Kaye and Morstyn using HpD in doses between 5 to 20 mg/kg.16 The timing of the PDT procedure was found to be critical since tumour bearing and non-tumour bearing rats injected with 0.5 mg/kg or 1.0 mg/kg AlSPc, and treated with 0 J/cm² to 800 J/cm² 24 hours post AlSPc administration did not show any evidence of normal brain or tumour necrosis, a result in accord with the pharmacokinetic data.12 These results are in contrast to a report using the VM/Dk murine glioma model, where mice were treated with AlSPc mediated PDT18 and non-tumour bearing animals treated at 48 hours post-sensitization with 0.5 mg/kg or 5.0 mg/kg. These workers found that PDT administered 48 hours
following a dose of 0.5 mg/kg resulted in all animals exposed to 200 J/cm\(^2\) light dying within 24 hours of light exposure. Our results show that if PDT was administered 6 hours post AISPc dosage, the time of maximal sensitizer uptake,\(^{17}\) no deaths occurred immediately postoperatively or in the 5 day postoperative period at light and sensitizer doses of 50 J/cm\(^2\) and 5 mg/kg AISPc respectively. However, using a dose regime of 5 mg/kg AISPc and 100 J/cm\(^2\), there was variable death of the animals, suggesting the threshold light dose is between 50 and 100 J/cm\(^2\). Animals treated at 24 hours with either 0.5 mg/kg or 1.0 mg/kg showed minimal evidence of either normal brain or tumour necrosis. The differences in the results reported here and those previously published,\(^{18}\) may reflect either variations in the tumour models or differences in the composition of the sensitizer preparation. Variations in tumour models have been recognised, with conflicting reports regardingHpD peak uptake times in different brain tumour models.\(^{19,20}\) Boggan et al\(^{20}\) observed a patchy uptake of HpD into the 9L gliosarcomat rat model with only 33-44% of the tumour area being fluorescent, and the maximal fluorescence not apparent until 24 hours after the administration of HpD. After IV administration of HpD into mice bearing the C6 glioma, patchy uptake was evident at 4 hours, and maximal and uniform fluorescence was present throughout the tumour at 6 hours, with a decline in fluorescence beginning at 24 hours. Kaye et al\(^{16}\) have also shown that the C6 glioma cell line in vitro is more sensitive to kill by light after exposure to HpD than V79 fibroblast cells. Differences in cellular uptake of the amount of HpD or its various components by cells of different origin may also explain the discrepancies in uptake in different in vivo models. Variations between the C6 and 9L models may also be critical since Boggan et al\(^{21}\) have suggested that in the 9L model heterogeneity among tumour capillaries, some of which were highly permeable and some of which maintained characteristics of the normal blood-brain barrier, caused the patchy uptake. In addition, it has also been observed that protein bound drugs and high molecular weight aggregates such as HpD diffuse only minimal distances from permeable capillaries.\(^{22}\) A similar situation may apply to the phthalocyanines as we have shown that they can associate with serum proteins, particularly lipoproteins, which may affect the degree of tumour uptake. Variations in the degrees of sulfonation in a phthalocyanine mixture, such as that used in this PDT study, will affect the hydrophobic properties of the compound with hydrophilicity increasing with increasing sulfonation. We have shown that the pharmacokinetics of uptake and distribution in the glioma cells in vitro and the C6 model in vivo, are dependent on the degree of sulfonation.\(^{17}\) This variation in time to peak uptake may explain the variations between the results reported here, and those of Sandeman et al.\(^{18}\) Thus further PDT studies utilising the purified sulfonated forms of AISPc (mono-, di-, tri- and tetra-) are needed to determine both the optimal timing of PDT, and the degree of sulfonation required for maximal tumour kill. Few studies addressing these issues have been reported, although Brasser et al\(^{23,24}\) have compared the photodynamic activity of various phthalocyanines with that of HpD in treating a subcutaneously implanted EMT-6 mammary tumour in mice. The aluminium and gallium chelated complexes of the phthalocyanines were as tumouricidal as HpD, while disulfonated phthalocyanine has been shown to elicit superior tumouricidal activity to an AISPc mixture, various sulfonated gallium phthalocyanine complexes and HpD.\(^{23,24}\)

The disulfonated aluminium phthalocyanine has also been shown to be superior to HpD as a PDT sensitizer in mice bearing a MS-2 fibrosarcoma or B16 melanoma.\(^{25}\) With light penetration being significantly reduced in the pigmented melanoma, it is possible that localisation of the disulfonate form to a critical subcellular target may have resulted in greater tumouricidal effects.

Whilst hyperthermia has been regarded by some investigators as a major component of PDT,\(^{26,27,28}\) this study and previous studies using HpD have shown that hyperthermia does not occur if the site is irrigated with isotonic saline, and that PDT's efficacy is independent of hyperthermia. The critical tumour targets of HpD sensitized PDT are yet to be definitively described, although evidence from some studies suggests that since the observed depth of tumour damage\(^{16}\) is beyond the proposed depth of penetration of light,\(^{29,30}\) it is probable that vascular damage with subsequent deeper tissue infarction may be a major basis for some of the tumour destruction.\(^{16,31}\)

As AISPc is comprised of a mixture of the various sulfonate species (J Bommer, Porphyrin Products, personal communication, Stylli et al, unpublished observations), it is possible that the amount of the more active disulfonate component is proportionately low. Thus a description of the tumour distribution of the various sulfonate species comprising AISPc is needed to determine the critical targets for this sensitizer.

The results presented in this study show that AISPc possesses characteristics that may allow it to be considered for Phase I clinical trials of PDT. AISPc shows selective tumour uptake, and mediates selective photonecrosis of implanted glioma. Further studies examining the efficacy of the separate sulfonated forms of AISPc warrant additional evaluation and are currently under investigation.

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