Experimental Research

Oedema formation in experimental photo-irradiation therapy of brain tumours using 5-ALA

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Summary

Background. Five-aminolevulinic acid (5-ALA) induces the specific accumulation of photosensitising porphyrins in malignant gliomas and has been explored for photo-irradiation therapy of these tumours. However, information is unavailable on whether and to what extent this treatment modality may induce the formation of brain oedema, and how potential oedema might be treated.

Methods. Rats were implanted with C6 gliomas. Eight days later magnetic resonance images (MRI) were obtained. On day 9 rats received 100 mg 5-ALA/kg b.w. and were craniotomized for photo-irradiation of tumours 6 hours later (100 J/cm², 635 nm argon-dye laser). Part of the animals was treated with daily dexamethasone injections (0.3 mg/kg), beginning 6 hours before phototherapy. 72 hours later, brains were removed and dissected according to tumour dimensions on pre-therapy MRI into ‘‘tumour’’, ‘‘brain around tumour’’ (BAT), residual cortex and basal ganglia, for measurements of water contents. Measurements were also performed in untreated animals with tumours, with or without steroid treatment and in control animals. An additional group of animals lacking tumours, with or without steroid treatment, underwent 5-ALA-phototherapy to determine effects on normal brain.

Results. C6 gliomas induced brain oedema, which responded to steroid treatment. 5-ALA-phototherapy resulted in additional oedema, which responded partly to steroids. 5-ALA-phototherapy of normal brain increased water content moderately in irradiated cortex. This oedema was also partly counteracted by steroids.

Conclusions. Photo-irradiation therapy with 5-ALA induces oedema which is partly counteracted by steroid therapy. The possibility of steroid resistant oedema formation should be considered when planning human trials with this treatment modality.

Keywords: 5-Aminolevulinic acid; photodynamic therapy; malignant glioma; porphyrins; oedema.

Introduction

The prognosis of patients suffering from malignant gliomas remains poor with inevitable tumour recurrence only months after initial treatment. Available treatment is essentially palliative with the aim of prolonging high quality survival as long as possible. Following surgery and radiotherapy, tumour generally recurs from the margins of the former resection cavity [2, 1, 9] suggesting that patients might benefit from more aggressive local therapies which selectively target residual tumour cells. In this respect, photodynamic therapy (PDT), a form of photo-irradiation therapy, has been under investigation. PDT is a cancer treatment based on the apparently selective accumulation of photosensitising drugs in malignant tissues. When activated by light of an appropriate wavelength, the photosensitizer exerts tumourtoxic properties. PDT has proved useful in the therapy of a number of different neoplastic lesions [8] and has also been utilised for local adjuvant therapy of malignant gliomas [16, 24, 26, 28, 29, 40]. Hematoporphyrin derivative (HpD), and its purified versions, porfimer sodium (Photofrin®) and dihematoporphyrin ether (Photofrin II®) have been used for photo-irradiation therapy in the brain. However, these sensitisers have the disadvantage of causing prolonged skin sensitization [19, 29] and damage to normal brain tissue has been reported from preclinical studies [4, 7, 40, 15].

Recently, we have investigated a novel sensitiser, 5-aminolevulinic acid (5-ALA), for photo-irradiation therapy of gliomas [25]. 5-ALA is a naturally occurring metabolite in the heme biosynthesis pathway. Excess exogenous 5-ALA leads to accumulation of highly photosensitising and highly fluorescent heme precursor
porphyrins, such as protoporphyrin IX (PpIX), in a number of malignant tissues [17, 21, 27], including malignant gliomas [35–37], where accumulation is highly specific. In contrast to hematoporphyrin derivatives, side-effects of 5-ALA are mild [10, 20, 30, 37]. Photoirradiation therapy using 5-ALA induced porphyrins has been found to selectively damage experimental brain tumours with negligible effects on normal or adjacent, oedematous brain [25]. Thus, 5-ALA appears to fulfill a number of pre-requisites for an ideal photosensitizer.

However, PDT using hematoporphyrin derivatives has been reported to induce significant brain oedema [15, 19], a complication that should also be considered for photo-irradiation therapy with 5-ALA. To our knowledge it has not yet been determined whether brain oedema is a potential complication of 5-ALA phototherapy. Prior to the clinical application of 5-ALA for phototherapy, therefore, the extent and therapeutic accessibility of brain oedema should be examined more closely. For this reason, the present study investigates the development of brain oedema subsequent to photoradiation therapy of experimental brain tumours using 5-ALA and tests the efficacy of steroid treatment for counteracting oedema formation.

**Material and methods**

**General**

All experiments were conducted in accordance with Bavarian State animal protection laws and were approved by the Bavarian State Government. Procedures were performed using male Wistar rats weighing 240–260 g (Charles River, Sulzfeld, Germany).

Seven groups of animals were studied (Table 1). Four groups of animals were implanted with C6 gliomas for measuring either oedema derived from tumour (n = 9), for determining the effects of steroid treatment on tumour oedema (n = 10), for determining the effects of tumour phototherapy using 5-ALA (5-ALA-PT) on oedema accumulation (n = 13), or for determining the influence of steroid treatment on oedema evolution after 5-ALA-PT (n = 13). Two additional groups of animals without tumours were studied for elucidating the effect of 5-ALA-PT on normal cortex (n = 6) and whether ensuing oedema might be counteracted by steroid treatment (n = 6). A final group of animals was studied (n = 6) in order to determine the water contents of normal brain tissue without foregoing treatments.

Spontaneously breathing rats were anesthetized with isoflurane (1–2%) in O2 during tumour implantation, photooirradiation treatment and drug administration. For magnetic resonance imaging studies (MRI) they were placed under chloralhydrate anaesthesia (3.6%, 1.2 ml per 100 g body weight), delivered by intraperitoneal injection. Animals were maintained on temperature controlled, feed-back heating pads at 37 °C. For tumour implantation and photo-irradiation procedures, their heads were immobilized in a stereotactic head holder. Craniotomies were performed using a high-speed dental drill utilising copious irrigation to avoid thermal damage. Care was taken to leave the dura mater unharmed.

Five-aminolevulinic acid (5-ALA; Medac GmbH, Wedel, Germany) was obtained as hydrochloride. For intravenous administration, 5-ALA was dissolved in phosphate buffered saline (PBS) at a concentration of 30 mg/ml immediately before use and adjusted to a pH of 6.5. Injections of 5-ALA were performed by direct puncture of the exposed left femoral vein.

An argon-pumped rhodamine dye laser (argon laser: Coherent INNOVA Sabre R DBW 15 dye laser: Coherent 599; Coherent, Darmstadt, Germany) was used for photo-illumination at 635 nm red light. The power density was adjusted to 100 mW/cm² and an energy density of 100 J/cm² was applied. Prior to and after photo-illumination the power-density was checked using a powermeter (Labmaster, Coherent, Darmstadt, Germany). For monitoring of power density during photo-irradiation, a beamsplitter deflected a constant portion of laser light onto a photodiode. Light density was manually readjusted in case of deviations of more than 5%.

**Phototherapy of experimental brain tumours**

For the present experiments, the C6 model of malignant glioma was used as previously described, with minor modifications [36]. C6 glioma cells were grown in monolayer tissue cultures in Dulbecco’s Minimal Essential Medium (SeromedBiochrom KG, Berlin, Germany) supplemented with 10% fetal calf serum and sodium-pyruvate (1 mM) at 37 °C in a 5% CO₂ atmosphere. Penicillin G (300 IU/ml) and streptomycin (300 μg/ml) were added to the medium in order to prevent bacterial infection. Cultures between passage 72 and 88 were harvested by trypsinization and cells were suspended in saline at a concentration of 2 × 10⁶ cells/μl. Five μl (10⁶ cells) were stereotactically implanted via a small burr hole 2 mm lateral and 2 mm caudal to the bregma, i.e. the region designated for later craniotomy, using a 10 μl syringe (Microliter syringes, type N-10C, Microsyringe, Oberkochen, Germany). The operation was performed under sterile conditions in a laminar flow cabinet. After implantation of the cell suspension, the skin incision and the parietal burr hole were closed with sutures.

**Table 1. Experimental groups**

<table>
<thead>
<tr>
<th>Tumour bearing animals</th>
<th>n</th>
<th>purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour only</td>
<td>9</td>
<td>measure oedema induced by tumour alone</td>
</tr>
<tr>
<td>Tumour + steroids</td>
<td>10</td>
<td>determine effects of steroids on tumour oedema</td>
</tr>
<tr>
<td>Tumour + 5-ALA-PT</td>
<td>13</td>
<td>determine changes in water content induced by 5-ALA-PT in tumour and brain</td>
</tr>
<tr>
<td>Tumour + 5-ALA-PT + steroids</td>
<td>13</td>
<td>determine effects of steroids on 5-ALA-PT induced oedema in tumour and brain</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-tumour controls</th>
<th>n</th>
<th>purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal brain</td>
<td>6</td>
<td>measure water contents of normal brain without tumour or 5-ALA-PT</td>
</tr>
<tr>
<td>Normal brain + 5-ALA-PT</td>
<td>6</td>
<td>determine oedema in normal brain in response to 5-ALA-PT</td>
</tr>
<tr>
<td>Normal brain + 5-ALA-PT + steroids</td>
<td>6</td>
<td>determine effects of steroids on 5-ALA-PT induced oedema in normal brain</td>
</tr>
</tbody>
</table>

**Tumour** Prior implantation of C6 glioma cells; **Steroids** Steroid treatment; **5-ALA-PT** phototherapy after administration of 5-ALA.