Experimental Research
Photodynamic Effects in Perifocal, Oedematous Brain Tissue

C. Goetz1, A. Hasan2, W. Stummer1, A. Heimann3, and O. Kempski3

1Department of Neurosurgery, Ludwig-Maximilians-University of Munich, Germany
2Department of Neurosurgery, Klinikum Kassel, Germany
3Institute for Neurosurgical Pathophysiology, Johannes-Gutenberg-University of Mainz, Germany

Summary

Background. Photodynamic therapy (PDT) has been under discussion as additional treatment option for malignant gliomas. However, damage not only to tumour tissue but also to normal brain has been demonstrated. The mechanisms of this unwanted side effect have not yet been clearly identified. Spreading of photosensitiser with oedema after disruption of the blood-brain-barrier and potential sensitisation of normal tissue has been found previously. The present study investigates the time- and dose-dependency of normal tissue damage to photodynamic therapy using Photofrin II after disruption of the blood-brain-barrier.

Methods. Male wistar rats anaesthetised with chloral hydrate were subjected to focal, cerebral cold lesions. Simultaneously, Photofrin II® (PFII) was injected (2.5 or 5 mg/kg b.w.). Laser irradiation (630 nm) was performed after 4 h, 12 h and 24 h with varying light doses. Control groups were subjected to focal cold lesion alone, cold lesion with laser irradiation, PFII followed by laser irradiation, or laser irradiation alone (n = 6 all groups). 24 h later, brains were removed for assessment of necrosis in coronal sections.

Findings. Light dose had a significant impact on the extent of necrosis. Compared to control animals (lesion only: 0.84 ± 0.2 mm²; lesion and irradiation alone: 0.7 ± 0.3 mm²), the area of necrosis was increased to 2.8 ± 0.5 (50 J/cm²), 3.5 ± 1.3 (100 J/cm²) and 4.3 ± 0.7 mm² (200 J/cm², 5 mg/kg b.w.; p < 0.01). This effect was time-dependent. Maximal necrosis (6.3 ± 1.6 mm²) was observed when brains were irradiated 12 h after PFII injection, with less necrosis occurring at 24 h (2.8 ± 0.4 mm², p < 0.01). Reducing sensitiser dose to 2.5 mg/kg b.w. resulted in a reduction of necrosis (2.09 ± 0.2 mm², p < 0.05).

Interpretations. Damage to oedematous tissue after photodynamic therapy using i.v. PFII and laser light at 630 nm depends on laser dose, sensitiser dose and the time point of laser irradiation. The time point of PDT should be considered to prevent unwanted tissue reactions. In the clinical setting however, defined damage to peritumoural tissue may be advantageous. This should be achievable by optimised timing and dosage of photodynamic therapy.

Keywords: Photodynamic therapy; porphyrins; oedema; cold lesion.

Introduction

Despite ongoing research the prognosis of patients suffering malignant gliomas remains poor, with a median survival time of one year for glioblastoma and 3 years for anaplastic astrocytoma [7]. Following surgery, tumour recurs locally in more than 80% of cases [3, 13, 46]. Therefore, augmental therapies targeting the peritumoural region might serve to prolong survival in these patients. In this respect, photodynamic therapy (PDT) may provide a local treatment modality. This method utilises photosensitising compounds, which apparently accumulate in malignant tumour tissue. When exposed to light of an appropriate wavelength and energy, tumours are selectively damaged. Although the concept has been under investigation for many years now [4, 5, 10, 14, 17, 28, 32, 42, 48] a number of questions are still unanswered.

Of special interest is the question of damage to normal brain tissue. Blood-brain barrier breakdown within malignant tumours leads to exudation of oedema fluid into normal brain. Oedema fluid propagates through white matter not only by diffusion but by convection [27, 38, 45]. In an earlier study we were able to demonstrate the wash-out of photosensitiser by oedema fluid generated in a cerebral lesion. Photosensitiser was detectable at considerable distances from the lesion border [42]. Thus, normal tissue may be sensitised and damaged if irradiated with light. A certain degree of damage to peritumoural tissue may be acceptable or

---

This work was supported by a grant (No. 0706903A5) from the former Bundesministerium für Forschung und Technologie (BMFT).
even desirable in order to reduce the risk of tumour recurrence due to solitary residual tumour cells in this region. However, to make PDT a useful tool in the hands of a neurosurgeon, the time course of sensitisation and the mechanisms of damage to peritumoural tissue must be well investigated. We therefore studied the time- and dose-dependency of PDT delivered to oedematous brain tissue.

**Methods**

Male Wistar rats (body weight approx. 300 g) were anaesthetised with chloral hydrate (i.p., 3.6%, 1.3 ml/100 g b.w.). After cannulation of the right external jugular vein the animals heads were fixed in a stereotactic holder prior to performing a left parietal craniotomy (3 x 6 mm) leaving the dura intact. During drilling the area was permanently irrigated with saline solution to prevent heating of the underlying structures. A cold lesion according to KLATZO [27] was applied through the intact dura to the underlying cortex, using a copper stamp (diameter 1 mm) cooled to –68°C. At the same time Photofrin II (PFII, Fa Photomedica Inc., Raritan, New York) in 0.9% saline was injected at different doses (see table). Oedematous brain was irradiated 4 h after induction of trauma with laser light at a wave length of 630 nm (Argon-Rhodamin-Dye-Laser, Coherent, Deaborg, Germany). The laser was adjusted to 100 mW/cm². Different laser-energy doses were achieved by longer irradiation times instead of higher energy to minimise thermal side effects. Irradiation was applied through a metal frame (0.8 x 5 mm) to protect identical irradiation fields.

Animals were sacrificed 24 h after laser irradiation by intracardiac perfusion with 2% paraformaldehyde. The brains were removed and placed in paraformaldehyde-solution for at least 24 h. Serial coronal sections (5 µm) were stained (Nissl-stain).

Coronal sections were digitised and the area of necrosis was quantified planimetrically using an electronic image analysis processing system (Kontron Electronics, Erding, Germany). An specially designed image analysis program [42] calculated the extent of necrosis after the necrotic area was marked as region of interest. For a single animal the medium value of 3 sections with the maximum extent of necrosis was used for comparison.

**Experimental Groups**

The extent of necrosis under different conditions of PDT was determined in 6 experimental groups and 4 control groups.

- To test for the influence of laser light dose 6 animals in each group were irradiated with 50 J/cm², 100 J/cm² or 200 J/cm² after induction of cold lesion and administration of 5 mg PFII.

Table 1. *Experimental Groups*

<table>
<thead>
<tr>
<th>Group</th>
<th>PFII-dose (mg/kg b.w.)</th>
<th>Laser-dose (J/cm²)</th>
<th>Irradiation-time after trauma (h)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>200</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>100</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>50</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>200</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>200</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>2.5</td>
<td>200</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

Fig. 1. Necrotic area in different control groups

- The influence of the PFII-dose was evaluated in further 2 groups receiving 2.5 or 5 mg PFII/kg b.w.. These animals were irradiated with 200 J/cm² 4 h after induction of trauma with simultaneous administration of PFII.
- The dependency on time of irradiation on the induction of necrosis was tested in another 2 groups irradiated 12 or 24 h after trauma and simultaneous administration of PFII.
- Additional 4 groups were used as control groups. The first group was irradiated with 200 J/cm² without having received PFII and without trauma. In the second group only trauma was induced, without further treatment. The third group received a combination of trauma and irradiation (200 J/cm²) and the fourth group was irradiated 4 h after application of 5 mg PFII/kg b.w..

**Results**

**Controls**

Irradiation of normal tissue with laser light alone (200 J/cm²) provoked no visible damage in the histological sections. After induction of the cold lesion without application of PFII or laser light, the necrotic area measured 0.84 ± 0.18 mm². Irradiation of oedematous perifocal brain with 200 J/cm² laser light resulted in a necrotic area of 0.65 ± 0.30 mm². There was no significant difference between both groups. However animals receiving PFII and irradiation showed an extensive lesion measuring 3.11 ± 0.51 mm² (Fig. 1).

**Light Dose**

Increasing energy doses (50, 100 and 200 J/cm²) after induction of the cold lesion and simultaneous administration of PFII (5 mg/kg b.w.) resulted in a dose-dependent increase of the necrotic area. Compared to the corresponding control group (5 mg PFII/kg b.w. and 200 J/cm²), the necrotic area was sig-
significantly increased when the additional cold lesion was applied (Fig. 2).

**Time**

When laser light was applied at different time points (4, 12 and 24 h) after the cold lesion and PFII, a small but not significant increase of necrotic area was found in the 12-hour-group as compared with the 4-hour-group. However, in the 24-hour-group a significant decrease of the necrotic area was measured in comparison with the 4-hour-group ($p < 0.002$). The necrotic area was more pronounced in the treatment group than in the corresponding control group (cold lesion alone, $p < 0.02$, Fig. 3).

**Sensitiser**

Application of different doses of sensitiser (0, 2.5 and 5 mg/kg b.w. PFII) resulted in a linear increase of the necrotic area which was doubled in the 5 mg-group compared to the 2.5 mg-group ($p < 0.001$, $n = 18$, Fig. 4).

**Discussion**

Gliomas are the most malignant of brain tumours. 1 year after diagnosis more than 50% of patients are dead even if all possible therapeutic options had been tried [7, 9]. Tumour removal followed by conventional radiotherapy and, especially in younger patients, adjuvant chemotherapy, is still state of the art [30]. More recently introduced treatment such as the local application of chemotherapeutic agents into the tumour cavity, have resulted in no relevant improvement of survival times [6, 43]. Glioblastomas recur in almost 100% of cases and usually tumour recurrence is observed in the direct vicinity of the primary tumour location [1, 3, 13, 31]. Recurrence results from minimal tumour remnants or from single tumour cells infiltrating surrounding, possibly functionally intact tissue through which they progress with oedema. Optimal adjuvant tumour therapies should therefore selectively target these residual tumour cells.

PDT may be a promising tool in the treatment of malignant brain tumours [24, 32, 34, 36]. The sensitiser is supposed to accumulate selectively within the tumour. Irradiation with laser light of an appropriate wave length leads to necrosis of sensitised cells [16, 18, 22]. Theoretically, the extent of necrosis should depend on the location of the sensitiser in the irradiated tissue and on the penetration depth of laser light. Penetration depth of laser light depends on its wave length and on the properties of the penetrated tissue. Penetration depth is defined as the range of penetration at which the light intensity decreases to 37% of the initial value.
and is greater in tumour tissue than in oedematous tissue, where it is greater than in normal tissue [33, 44, 47]. At a wave length of 630 nm, photodynamic effects have been observed up to a depth of 5–8 mm.

Ever since the first description of PDT at the beginning of the century [37] many different photosensitising agents have been investigated. Most work has focused on hematoporphyrins. The frequently applied hematoporphyrins consist of a mixture of different porphyrins, which makes it difficult to investigate interactions of this substance with surrounding tissue [11, 12, 25, 26]. The ratio of sensitiser accumulation in tumour to normal tissue is of utmost importance. The better the ratio the higher the expected selectivity of tumour destruction without damage to normal tissue. In a 9L-gliosarcoma model in rats treated with PFII, the ratio tumour: normal tissue was 13:1 [8]. The same group found that the ratio was significantly higher for PFII encased in liposomes than for PFII dissolved in dextrose [20]. Studies with benzoporphyrine derivative showed a tumour to normal tissue ratio of 12:1 for intracerebral tumours with maximal tumour uptake between 3 and 5 h [40]. In a Phase-I study with boronated porphyrins a dose dependent tumour concentration and tumour: plasma ratio was found after application of different doses of sensitizer [39]. PFII – a hydrophilic photosensitiser – is mainly bound to albumin and globulin; in blood it is partly transported as pseudomicelles [41]. In experimental as well as in human brain tumours, photosensitiser was detected in mitochondria [21]. Photosensitiser has also been found within endothelial cells and, as we have demonstrated in a previous paper, photosensitiser has also been found in oedematous perifocal tissue, travelling with “bulk flow” [42].

Tumour toxic PDT effects in brain tumours have been observed in experimental as well as in clinical studies [5, 22, 23, 24, 28, 32, 34]. In some cases however, PDT resulted in the development of oedema even in normal brain tissue. Other findings were haematomas and haemorrhage. In recent publications, severe vascular damage to both tumour and surrounding tissue was reported, involving the swelling of endothelial cells, the formation of thrombosis and coagulation necrosis [14, 48]. Even tissue damage without any detectable sensitiser accumulation in tumour cells was reported [15].

Therefore the question arises whether PDT is a safe treatment option for brain tumours and, if not, how may it be modified. Of great interest is the question whether there is an optimum time-point and an optimum dosage for sensitiser or laser light to make PDT both effective concerning its tumour toxic effects and safe regarding undesirable damage to normal brain tissue. In an earlier study using a focal cold lesion model our group was able to demonstrate the spreading of PFII with oedema into surrounding tissue to a distance of 5 mm after 5 h [42]. This implies that malignant tumour cells which are usually found in peritumoural tissue, may be reached by PDT. It is also possible that laser irradiation of sensitised normal tissue may lead to undesired necrosis in functionally important regions. The present study was undertaken to determine the time-dependency of necrosis in perifocal tissue after PDT. Furthermore we investigated the influence of sensitiser dose and laserlight dose on the extent of the necrotic area.

We used the same well-established model of vasogenic brain oedema used in our earlier study [42]. Vasogenic brain oedema in this model is very similar to peritumoural oedema with regard to electrolyte- and protein-content. The properties of perifocal oedema following a focal cold lesion have been investigated in various studies [2, 38, 45]. Furthermore, the lesion with surrounding perifocal oedema is located on the cortical surface and is ideally localised for irradiation. Animal tumour models have the disadvantage that tumour growth and oedema production are very irregular. Since the presumed effects of sensitisation and irradiation are in part dependent on oedema flow, reproducible oedema production is very important. Induction of a focal cold lesion results in very reproducible oedema with regard to volume and distribution.

The energy of light produced by the argon Rhodamin Dye laser is exactly measurable, i.e. a defined dose of laser light can be applied to a certain region. The applied doses may be modified easily by increasing or decreasing the application time. The emitted light with a wave length of 630 nm closely matches the absorption maximum of PFII.

Our results show a clear dependency between applied light- or sensitiser dose and the volume of the resulting necrotic area. These findings are in accordance with other studies, showing a sensitiser dose dependent increase of oedema after irradiation of normal rat brain [19] as well as a light-dose-dependend risk of side effects [29]. According to Berenbaum et al., a significant part of the effects of PDT is due to accumulation of the sensitiser within the endothelial cells. Irradiation of the sensitised cells leads to vascular damage and
thus tumour necrosis. Swelling of endothelial cells results in reduced tumour blood flow and reduced tumour nutrition as well as in increased interstitial pressure within the tumour. After 4 h, only minimal doses of sensitiser are found in tumour wash-out, but the amount of sensitiser localised within the endothelial cells remains remarkably stable [35]. This phenomenon explaines in part the increased necrotic area found after sensitisation and irradiation of normal tissue. Damage to normal tissue, however, is normally an undesirable side effect of PDT. Consequently, these phenomena have to be investigated further and to be considered in a potential clinical set-up of PDT for brain tumours, in order to avoid unwanted side-effects. A combination of PDT with modern methods for tumour localisation, such as neuronavigation or intraoperative tumour-detection, may be used for generating defined areas of necrosis in peritumoural tissue. It is conceivable that following macroscopic tumour resection in non-functional brain, well-defined regions of tissue necrosis to tissue surrounding the tumour might be intentionally induced in order to destroy residual tumour cells in these areas, decreasing the probability of tumour regrowth.

In earlier studies we were able to demonstrate extravasation of PFII within the focal lesion and its propagation with the oedema bulk-flow through subcortical white matter. Furthermore, time-dependent accumulation of porphyrin fluorescence in oedema fluid was observed, with maximum fluorescence after 4 h. Conversely, the present study demonstrated maximal necrosis after 12 h as opposed to 4 h. Thus, it has to be assumed that PFII-fluorescence in oedema does not strictly correlate to the photodynamic effect. Other mechanisms such as vascular damage caused by accumulation of the photosensitising compound within the endothelial cells or localisation of the sensitiser within astrocytic cells might be responsible for this “delayed” damage [12, 15, 48].

Conclusions

For clinical use of PDT the following conclusion can be drawn:

- As the concentration of sensitiser in the perifocal oedema was shown to be time-dependent, the time-point of laser irradiation might influence the extent of necrosis. Since the time point of maximum necrosis lagged behind the time point of maximum oedema fluorescence, other mechanisms than extravasation of photosensitiser alone seem to determine the photodynamic effect.
- In light of the results of the present study, PDT should be applied within a short time interval after i.v.-application of the photosensitiser to selectively destroy tumour tissue.
- If a defined lesion to the surrounding tissue is tolerated or even desired with the purpose of eliminating microscopic tumour remnants after resection of a brain tumour, PDT should be applied more than 12 h after administration of the photosensitiser.
- Three components of PDT, i.e. laser dose, sensitiser dose and time point of irradiation influence the extent of necrosis. It therefore seems possible to “tailor” PDT according to the localisation and operability of a distinct tumour.
- To optimise PDT, a sensitiser would be desirable which allows tumour detection during surgery and which is characterised by a better tumour/brain tissue-ratio compared to PFII, thus reducing the risk of damage to the surrounding normal tissue.

References


37. RAAB O (1900) Ueber die Wirkung fluoreszierender Sto¨re auf Infusorien. Z Biol 39: 524


Comments

Photodynamic therapy (PDT) emerges as a promising additional treatment option for malignant gliomas. PDT utilizes photosensitizing compounds like Photofrin II that accumulate in malignant brain tumors and subsequent exposure to laser light should destroy the tumor tissue selectively. However damage not only to the tumor but also to normal brain has been demonstrated where the edematous border zone between tumor and normal brain tissue is vulnerable. The authors performed a thorough laboratory investigation to study the time- and dose-dependency of normal tissue damage to photodynamic therapy in the setting of a broken blood-brain barrier. As the results show there is damage to non-tumoral edematous brain tissue. The extent depends on laser-light dose, sensitizer (Photofrin II) dose as well on the timepoint of laser irradiation. Taking these results into account PDT can be used as a more precise neurosurgical tool. Furthermore C. Goetz suggests to utilize optimal timing and dosage of PDT as a local treatment modality to damage peritumoral tissue in a controlled fashion which could be advantageous in the clinical setting of glioma treatment with respect to local tumor recurrence.

V. Seifert

Photodynamic therapy (PDT) is a form of adjuvant therapy aimed to improve local control in patients with brain gliomas. However, the experience with this technique is still limited and the more appropriate selection of parameters such as the sensitizer and light doses, or the time interval between sensitizer injection and light application for decreasing the risk of damage to normal tissue adjacent to the tumor, remains to be determined. The present study was designed to investigate the time- and dose-dependency of normal tissue damage with PDT in the rat. After using combinations of sensitizer and light doses and different intervals for light application, the authors conclude that tissue damage is dependent on the dose of sensitizer, the dose of light and the timing of application. It seems clear that PDT should be used later that 12 hours after administration of the sensitizer in order to diminish the damage of peritumoral brain tissue containing tumor remnants.

As the authors state, optimal adjuvant therapies in patients with brain glioma should target residual tumor cells, and certain degrees of damage to peritumoral tissue may be desirable in order to eliminate nests of tumor cell distant from the core of the lesion, thus reducing the risk of tumor recurrence.

However, we still do not know the more appropriate doses of both sensitizers and lights to be used in the clinical setting. Since the sensitizer injected before surgery is taken up not only by the tumor but also by the tumor-invaded, yet functional brain tissue neighbouring the tumor, PDT could result in an unacceptable damage to normal brain tissue. In one study porphyrin levels into the brain tissue adjacent to the tumor were found to be as high or even higher than those into the tumor itself [1].

In respect with this problem it is worth mentioning that in a previous study the authors observed that sensitizer extravasation was maximal after 4 hours of injection, whereas maximal tissue necrosis in the present experiments was observed after 12 hours. Thus, there must be other mechanisms influencing the photodynamic effect apart of the concentration of the sensitizer. Further studies are needed to establish the dynamics of sensitizers into the peritumor oedematous brain tissue and the most appropriate method for the administration of light (wavelengths, dosimetry).


Ramiro D. Lobato

Correspondence: Dr. med. Claudia Goetz, Department of Neurosurgery, Marchioninistr. 15, 81477 München, Germany.