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The role of photodynamic therapy in posterior fossa brain tumors

A preclinical study in a canine glioma model

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✓ Photodynamic therapy was studied in dogs with and without posterior fossa glioblastomas. This mode of therapy consisted of intravenous administration of Photofrin-II at doses ranging from 0.75 to 4 mg/kg 24 hours prior to laser light irradiation in the posterior fossa. Tissue levels of Photofrin-II were four times greater in the tumor than in the surrounding normal brain. Irradiation was performed using 1 hour of 500 mW laser light at a wavelength of 630 nm delivered through a fiberoptic catheter directly into the tumor bed via a burr hole. All animals receiving a high dose (4 or 2 mg/kg) of Photofrin-II developed serious brain-stem neurotoxicity resulting in death or significant residual neurological deficits. A lower dose (0.75 mg/kg) of Photofrin-II produced tumor kill without significant permanent brain-stem toxicity in either the control animals or the animals with cerebellar brain tumors receiving photodynamic therapy.

KEY WORDS • **brain neoplasm** • **posterior fossa** • **photodynamic therapy** • **dog**

PHOTODYNAMIC therapy (PDT) is a relatively new cancer treatment modality involving administration of a localized photosensitive dye which, upon activation with light, destroys tumor cells.[4,6–9,16,29] Currently, the most common regimen for treating brain tumors consists of an intravenous administration of Photofrin-II 18 to 72 hours prior to localized photoirradiation using laser light at a 630-nm wavelength.[22,23,26,27] The laser light can then be delivered to the sensitized tumor cells by directly inserting a fiberoptic diffuser into the tumor mass.[13] Alternatively, the tumor can be resected and the resulting cavity irradiated using a fluid-filled balloon through which the light diffuses.[23,41]

Uptake and biodistribution of dihematoporphyrin (DHE) and hematoporphyrin derivative (HPD) have been investigated in several studies using a variety of normal tissues and tumor models.[3,5,10,11,14,15,17,21,24,25,34,40] For these studies, HPD/DHE was labeled with a radionucleotide. Tritium-labeled (^{3}H) HPD, carbon (^{14}C)-labeled HPD, indium (^{111}In)-labeled HPD, and copper (^{64}Cu)-labeled HPD have been successfully synthesized for the purpose of studying preferential uptake/retention at different postinjection time intervals for a range of doses of the radiolabeled HPD/DHE. Doses ranged from 0.5 to 20 mg/kg. Overall, these studies indicated that there is preferential uptake and retention of HPD/DHE in tumor cells as compared to normal cells.

Doses of Photofrin-II and laser light used in PDT have varied significantly between clinical study groups.[22,23,26,27] In general, the Photofrin-II dose ranged from 0.75 to 5 mg/kg and the light dose varied between 150 and 2000 J. Additionally, treatment duration (light exposure) varied between 20 and 60 minutes at a laser power output of 300 to 2000 mW.

A number of clinical studies have been published updating the use of PDT in human brain tumors.[1,18–20,22–24,26–28,32–36] Overall, complications of PDT are within acceptable limits and many malignant gliomas showed a response to PDT that translated into prolonged survival.[23] However, most of these studies included only adults with supratentorial brain tumors. There are currently few published cases involving the use of PDT to treat tumors in the posterior fossa or brain tumors in children.[32]

The treatment of pediatric brain tumors requires special considerations.[2,12,13,31] In children, 60% to 70% of brain tumors are located in the posterior fossa. These infratentorial neoplasms include astrocytomas, medulloblastomas, ependymomas, and brain-stem gliomas. The close proximity of these malignant and benign childhood brain tumors to the brain stem raises special safety concerns for the use of PDT in the posterior fossa. We believe that the clinical trials in adults and animal studies did not sufficiently address this issue. In our preclinical animal study, we specifically investigated the safety and efficacy of PDT used in the posterior fossa. Our canine glioma model enables us to grow malignant tumors in the cerebellum adjacent to the brain stem.[37-39] Therefore, our model closely resembles a pediatric brain tumor situation and allows us to evaluate the use of PDT for patients with posterior fossa neoplasms.

Materials and Methods

Tumor Cell Culture

Canine glioma clones were grown in monolayer tissue culture in P100 tissue culture dishes at 37°C in a humid 5% to 10% CO_2 atmosphere, as previously described.[37-39] Cultures were harvested by gently dislodging the cells from their substratum with a Pasteur pipette, and cell suspensions of approximately 10 million cells/0.5 cc were inoculated into each dog.

Brain Tumor Inoculation

Adult mongrel dogs, each weighing approximately 20 kg, were anesthetized with either general barbiturates or halothane/nitrous oxide inhalation, and placed on ventilatory assistance with endotracheal intubation. Craniotomies were performed using a posterior cerebellar approach under sterile conditions, and canine glioma cell suspensions were injected via a No. 27 needle through burr holes to a depth of approximately 1 cm into either the cerebellar vermis or the left cerebellar hemisphere adjacent to the brain stem under direct vision. All tumor-bearing and normal control dogs were maintained throughout the study on oral cyclosporine (100 mg twice a day), azathioprine (50 mg twice a day), and prednisone (20 mg twice a day) to prevent rejection of the canine glioma allografts and to provide proper controls. Wounds were closed in appropriate layers after the application of bone wax, and the animals were observed daily for neurological symptoms.

Magnetic Resonance Imaging

Magnetic resonance (MR) images were obtained in all dogs at about 2 weeks after tumor implantation using a 1.5-tesla unit with standard T_1- and T_2-weighted and gadolinium-enhanced T_1-weighted sequences. Tumor location was confirmed and tumor volumetric measurements were made using continuous MR image slices.[37-39] All animals were placed under general barbiturate anesthesia and received endotracheal intubation throughout the MR imaging procedures.

Additional MR images were obtained at 1-week intervals following PDT in order to assess the effects of the therapy on the cerebellar tumor and surrounding brain, particularly the brain stem.

Photofrin Uptake Studies

Tumor and nontumor brain tissue samples were homogenized in a phosphoric acid solution (pH 3.2) to determine Photofrin levels.[30] The homogenate was extracted with chloroform and methanol. Water and protein layers were discarded while the chloroform layer was evaporated to dryness with N_2 at 45°C. The Photofrin residue was resuspended in 1.0 N HCl by sonication and transferred to a tube containing tetrahydrofuran. The solution was mixed and heated to 100°C for 30 minutes. The acid hydrolysate was allowed to return to room temperature and was then neutralized with 10 N NaOH, 1 M KH_2PO_4, and 100 mM cetyltrimethylammonium bromide (pH 7.0). Photofrin fluorescence measurements were obtained at an excitation wavelength of 410 nm and an emission wavelength of 638 nm. Results are expressed as ng Photofrin-II/mg brain tissue as compared to the standard curve of known Photofrin-II concentrations prepared using known spiking doses of Photofrin-II added to the tissue sample being tested.

Drug and Light Dosimetry

Photodynamic therapy was administered to dogs with and without tumors at 2 weeks after tumor inoculation. All animals received Photofrin-II* 24 hours prior to laser light exposure. Photofrin-II was dissolved in 5% dextrose and administered intravenously. The dogs without tumors were given 4, 2, or 0.75 mg/kg of Photofrin-II prior to surgery. Laser power at the tip of the fiber ranged from 300 to 800 mW. Treatment time varied between 20 and 60 minutes, thus the total light dose ranged accordingly between 960 and 1800 J. Once it became clear that the dogs receiving 4 or 2 mg/kg of Photofrin-II developed severe, usually fatal brain-stem neurological deficits, thereafter all dogs received the lower dose of 0.75 mg/kg (Table 1).

The dogs with brain tumors received the safer Photofrin-II dose of 0.75 mg/kg. The total light dose was always 1800 J. Treatment time ranged from 60 to 120 minutes and the laser power at the tip of the fiber from 250 to 500 mW. Control dogs received either no PDT, laser light treatment only, or Photofrin-II administration only (Table 1).

Light Delivery System

The laser light source was the Aurora/M laser system which produces light at a wavelength of 630 nm, verified periodically with a spectroscope.† The light from the laser was delivered to the target tissue via an interstitial spherical diffusion fiber.‡

* Photofrin-II supplied by Quadra Logic Technologies, Inc., Vancouver, British Columbia, Canada.

† Laser system and spectroscope manufactured by Cooper Lasersonics, Inc., Santa Clara, California.

‡ Diffusion fiber manufactured by Laser Therapeutics, Inc., Buellton, California.

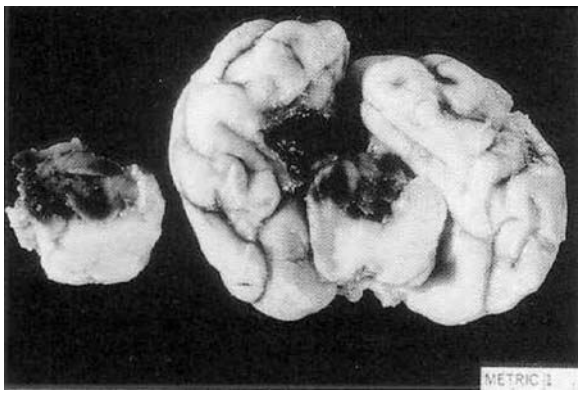

FIG. 1. Gross pathology demonstrating brain-stem hemorrhage and necrosis in a dog following photodynamic therapy with the high dose (4 mg/kg) of Photofrin-II.

TABLE 1

Summary of treatment protocols used

No. of Dogs	Photofrin Dose (mg/kg)	Laser Light Dose (J)	Results
normal dogs/no tumor			
4	4	1800	brain-stem hemorrhage, necrosis
2	2	1800	brain-stem hemorrhage, necrosis
2	0.75	1800	brain stem intact
1	0	1800	brain stem intact
tumor-bearing dogs			
3	4	0	Photofrin levels 4:1 tumor:brain; died of progressive tumor growth
2	0	1800	died of progressive tumor growth
1	0.75	0	barbiturate anesthesia death before laser light treatment*
3	0.75	1800	barbiturate anesthesia deaths before posttreatment MRI*
5	0.75	1800	tumor necrosis; brain stem intact

* Anesthesia technique changed from barbiturate injection to gas inhalation. MRI = magnetic resonance imaging.

Laser Power Output Monitoring

Photodynamic therapy was monitored in all cases with light dosimetry consisting of: 1) initial determination of laser output at the tip of the spherical diffusion fiberoptic catheter by a photometer; 2) monitoring of the intracerebellar light output from this spherical diffusion tip by another adjacent fiberoptic catheter in the brain and connected to a photometer throughout surgery; and 3) continued monitoring of the light emanating from the laser by a fiberoptic catheter connected to yet another photometer from which readings were taken continuously throughout PDT. The photoirradiation by laser was stopped if the laser output fluctuated by more than 10%, and the laser was recalibrated before proceeding with further photoirradiation in the cerebellum/cerebellar tumor.

Photodynamic Therapy Procedure

Prior to surgery, the laser was calibrated to the appropriate wavelength and power levels as described above. All dogs received a general barbiturate or gas inhalation anesthesia with endotracheal intubation and ventilatory assistance. The dogs were placed, under sterile conditions, in a stereotactic head frame that allowed access to the posterior skull. A craniotomy was performed that exposed either the cerebellar vermis or the left cerebellar hemisphere. The interstitial spherical diffusion fiber was then inserted at a depth of 1 cm into the cerebellar or the tumor tissue. For the dogs with tumors, the previous craniotomy site from the brain tumor inoculation was usually enlarged in order to directly visualize the tumor. Direct visualization and preoperative gadolinium-enhanced T_1-weighted MR images were used to assure the proper placement of the fiberoptic diffuser in the tumor. After PDT, bone wax was applied to the craniotomy site and the wound was closed.

Pathological Study

All animals were sacrificed by intravenous barbiturate overdose when significant neurological deficits developed, producing discomfort and/or difficulty feeding, or at 1-month posttreatment. The dogs' brains were fixed in formalin for 1 to 2 weeks and then sliced along the coronal plane for gross inspection. Measurements were made of the needle/fiberoptic catheter tracks as well as tumors and areas of hemorrhage or necrosis. Sections were then cut for histopathological study and stained with hematoxylin and eosin.

Statistical Analysis

This study involved a series of experiments in which the toxicity and efficacy of PDT for brain tumors were evaluated in the cerebellum and adjacent brain stem. The primary outcomes of interest were toxicity and tumor volume reduction. Tumor volume measurements were based on MR imaging volumetrics, comparing pre-and posttherapy measurements. Posttherapy tumor and volumetric measurements were analyzed as the percentage reduction from pretherapy measurements. The one-sample t-test was used to determine the significance of the tumor reductions. Concurrent control groups were included in each experiment when possible.

Results

Tissue Photofrin Levels

Three dogs with brain tumors were injected with 4 mg/kg Photofrin-II in order to determine Photofrin-II uptake. All dogs showed increased Photofrin levels in tumor tissue compared to normal brain tissue; the selective retention of Photofrin-II was 4.1 ± 0.77 (mean $\pm$ standard deviation) times greater in tumor than in surrounding normal brain. Published data on injected Photofrin doses ranging from 0.5 to 20 mg/kg agree with this ratio, with higher uptake in tumor than in normal brain tissue.[40]

Photodynamic Therapy of Normal Brains

Initially, the dogs without tumors were treated with

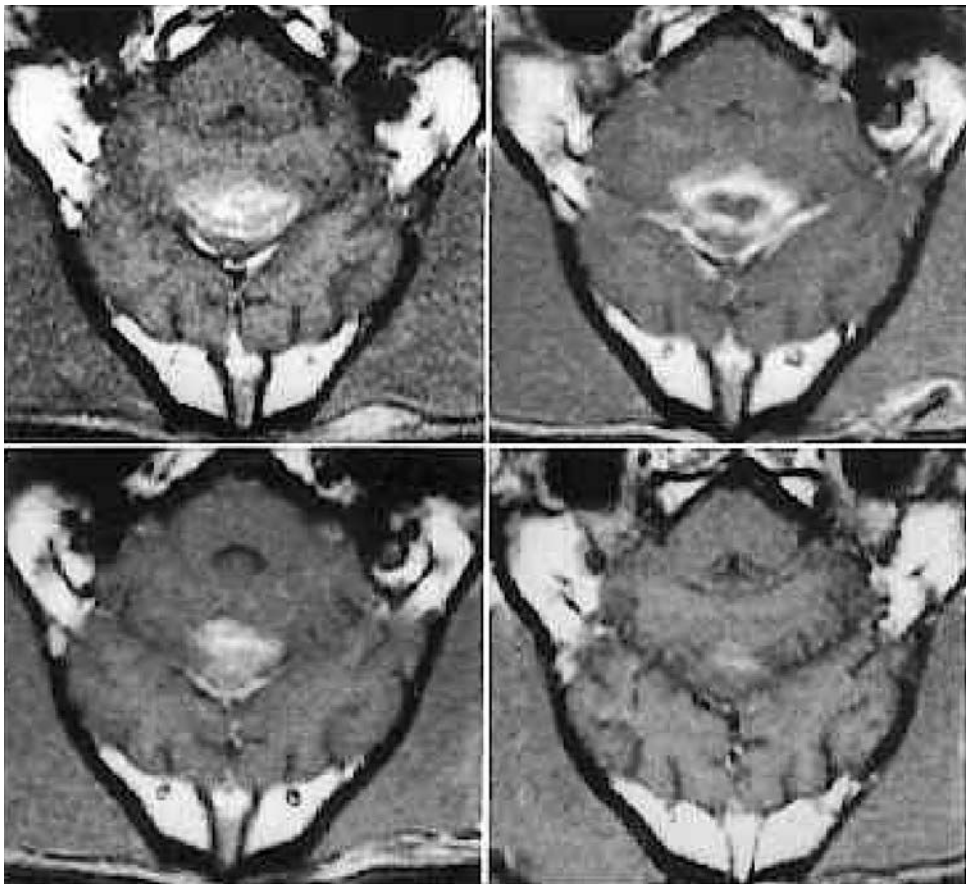

FIG. 2. Magnetic resonance images of a dog with a posterior fossa tumor obtained just prior to photodynamic therapy (0.75 mg/kg of Photofrin-II) (PDT, *upper left*), at 1 week after PDT *(upper right)*, at 2 weeks after PDT *(lower left)*, and at 3 weeks after PDT *(lower right)*. Note the significant shrinkage of the tumor by 2 and 3 weeks posttherapy.

PDT in the cerebellar vermis or left cerebellar hemisphere after receiving 4 mg/kg of Photofrin-II 24 hours before photoirradiation. All of the surviving dogs developed severe brain-stem neurological deficits consisting of ataxia, feeding difficulties (dysphagia), and respiratory instability; in the remaining dogs, immediate postoperative death occurred from respiratory insufficiency, later determined to be the result of brain-stem necrosis/hemorrhage (Table 1 and Fig. 1). Brain-stem necrosis/hemorrhage and similar neurological deficits also occurred in dogs receiving 2 mg/kg Photofrin-II 24 hours before photoirradiation (Table 1).

Subsequently, all dogs were given the lower dose of Photofrin-II (0.75 mg/kg) and developed milder or no brain-stem deficits (Table 1). Brain histopathology demonstrated small cystic areas of photodynamic tissue destruction at the fiberoptic catheter tip in the cerebellum, completely sparing the brain stem. The dog receiving photoirradiation only, without Photofrin-II administration, showed no histopathological changes other than the existence of a fiber track (Table 1).

Photodynamic Therapy of Brain Tumor

All dogs with cerebellar brain tumors documented by MR images during the week prior to treatment underwent PDT. Four dogs died before completing therapy/postoperative MR imaging due to the additive respiratory depression produced by barbiturate anesthesia in animals with posterior fossa tumors; such deaths were subsequently prevented by using gas inhalation anesthesia. All dogs receiving PDT at the Photofrin-II dose of 0.75 mg/kg tolerated the procedure well and demonstrated no damage to the brain stem. Magnetic resonance images of the brain tumors demonstrated large areas of central necrosis the week following PDT that were not present prior to PDT (Fig. 2). All pretreatment brain tumors were homogeneously gadolinium-enhancing masses on MR images. Subsequent MR images demonstrated significant ($p < 0.05$) mean shrinkage of residual ring-enhancing brain tumor tissue of 42% ± 8% and 81% ± 1% by 2 and 3 weeks posttherapy, respectively, without regrowth throughout the 1-month period of post-PDT observation and

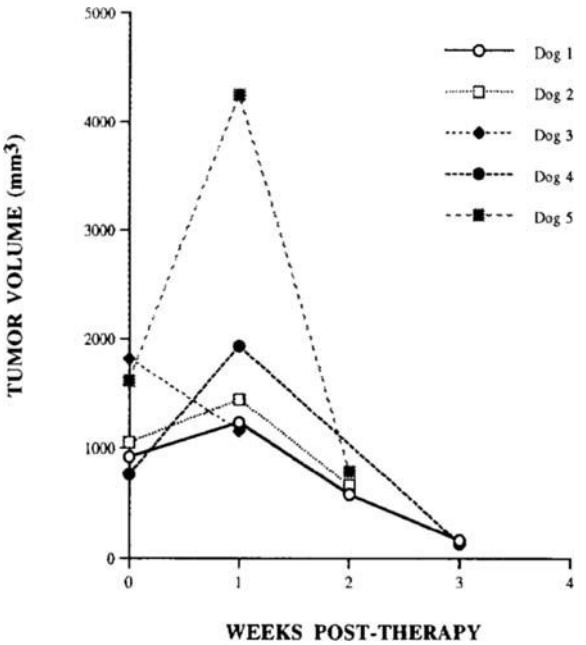

FIG. 3. Graph showing brain tumor volumes in five dogs, calculated from magnetic resonance images obtained just prior to photodynamic therapy (PDT, Week 0) and at 1, 2, and 3 weeks after PDT. The tumor volume in each slice was computed using the formula for the volume of an ellipsoid: $\frac{4}{3} \cdot \pi \cdot a \cdot b \cdot c$, where a = length/2, b = width/2, and c = depth/2. The total volume of each brain tumor was the sum of the volumes of tumor in the slices from each brain.

weekly neuroimaging (Figs. 2 and 3). Minimal clinical neurological deficits occurred in these animals, and in many cases the neurological deficits that were caused by the brain tumors actually improved following PDT. All areas of canine glioma tissue and of hemorrhage/necrosis in tumor tissue and normal brain were confirmed by histopathology (Figs. 4 and 5).

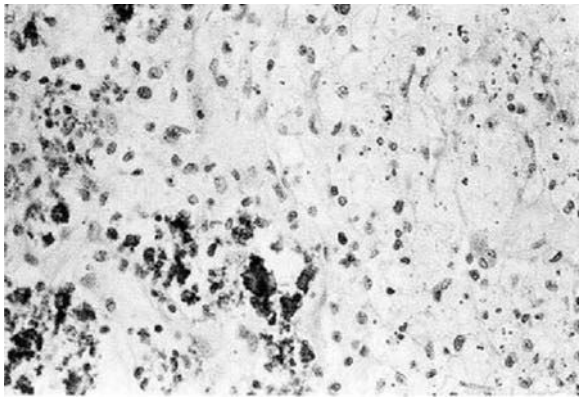

FIG. 4. Photomicrograph of tumor tissue treated with photodynamic therapy showing that the tumor is replaced by macrophages, mononuclear inflammatory cells, and calcification. H & E, × 21.

Control Canine Studies

The dogs without tumors receiving laser light treatment only demonstrated no apparent deficits. The animals with tumors receiving laser light treatment only or Photofrin-II administration only showed progressive neurological deterioration. These animals died within 2 to 3 weeks after tumor inoculation due to the rapidly growing tumor.

Discussion

The use of PDT for posterior fossa brain tumors may provide an adjuvant form of treatment to our already existing armamentarium of surgical resection, chemotherapy, and external beam radiation therapy. While anecdotal success has been reported in the treatment of supratentorial brain tumors in adults, there has been no well-controlled clinical study of PDT for brain tumors in any group to date. Nonetheless, this technique has appeared promising with many dramatic partial

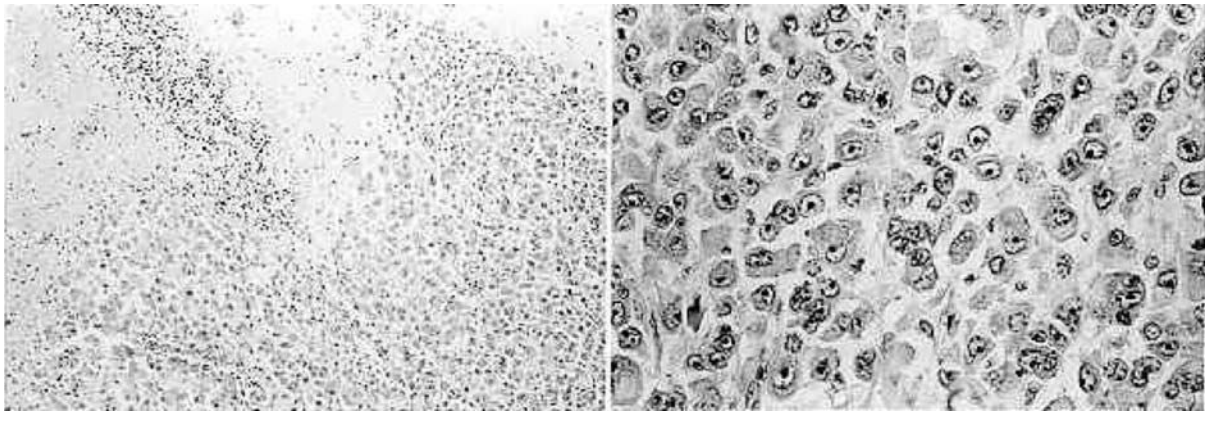

FIG. 5. Photomicrograph of untreated tumor tissue showing infiltration into the cerebellum *(left)* and large pleomorphic, occasionally multinucleated tumor cells containing large nuclei, prominent nucleoli, and abundant cytoplasm *(right)*. H & E, × 20 *(left)* and × 200 *(right)*.

and complete responses reported in adult glioblastoma patients.[22,23,26,27,29] Most patients treated thus far have had supratentorial brain tumors, many of which were partially resected prior to PDT. In these cases, PDT was applied through intracavitary balloon laser photoirradiation or the placement of multiple interstitial fiberoptic catheters to photoirradiate large areas of tumor bulk.

With posterior fossa tumors located in close proximity to the brain stem, different issues of safety and therapeutic efficacy are raised.[2,12,13,31] It is our experience with the canine glioma model studied here that cerebellar brain tumors adjacent to the brain stem become clinically significant at sizes much smaller than those that are often treated by PDT in adult supratentorial locations. Therefore, the intratumoral laser light application by spherical diffusion catheter photoirradiation seems more appropriate than surgically creating large cavities and irradiating through these balloon or multiple fiber techniques.

Our study indicates that under proper conditions PDT can be performed in the posterior fossa of dogs. A Photofrin-II dose of 0.75 mg/kg administered 24 hours prior to photoirradiation with a total light dose of 1800 J appears to fulfill this condition. At this dose, we were able to eliminate brain tumors without causing fatal or permanent brain-stem toxicity. We believe that PDT may be a safe and effective treatment modality for tumors in the posterior fossa sufficient to be considered for trials. Conventional treatment with external beam radiation has often failed in such patients. Therefore, our laboratory is currently assessing the toxicity of posterior fossa PDT following external beam radiation.

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