Review Article

Photoradiation therapy and its potential in the management of neurological tumors

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Photoradiation therapy is a form of local treatment that depends on the selective retention of a photosensitizer, such as hematoporphyrin derivative (HpD), by the tumor followed by treatment with light of an appropriate wavelength to activate the sensitizer in the tumor. The selective uptake of HpD by cerebral tumors has been demonstrated both in laboratory animal model studies and in clinical studies, and selective destruction of intracerebral tumors has been demonstrated in animal glioma models. The biological basis for photoradiation therapy and, in particular, the mechanisms for the selective uptake of the sensitizer into the tumor and the destruction of tumor with photoradiation therapy are discussed. Current evidence suggests that singlet oxygen is the major intermediary leading to cell damage, although other radicals such as hydrogen peroxide and hydroxyl radicals may be involved. Other studies suggest that the initial damage is to the blood vessels, and the tumor subsequently undergoes ischemic changes.

Sixty-four patients treated with photoradiation therapy have been reported in the literature. The initial clinical studies were disappointing in their therapeutic effect but these studies often included treatment of recurrent gliomas and low doses of light were used. Technical advances, particularly in laser technology, have enabled more effective photoradiation therapy and the clinical trials are reviewed.

KEY WORDS • brain neoplasm • hematoporphyrin derivative • glioma • laser • photoradiation therapy

At present, there is no satisfactory treatment for malignant cerebral glioma. The best available treatment, using surgery, radiation therapy, and systemic chemotherapy, results in a median survival time of less than 1 year. Most treatment failures occur because of local recurrence of the tumor, indicating that a more aggressive local therapy to the tumor could be beneficial. Recently it has been suggested that photoradiation therapy (PRT) could be used for the therapy of gliomas.

This mode of therapy depends on the selective retention of a photosensitizer such as hematoporphyrin derivative (HpD) by malignant tissue, followed by treatment with laser light of appropriate wavelength to activate the sensitizer. The photodynamic properties of porphyrins were known to German scientists around the turn of the century. In 1900, Raab reported the effects of acri- dine dye-sensitized Paramecium to visible light. In 1903, eosin was used to treat patients with skin tum- ors, and Auler and Banzer suggested in 1942 that hematoporphyrin could be used in cancer therapy due to its ability to localize in tumor tissue. In 1961, Lipson, et al. reported tumor fluorescence with HpD, a mixture of porphyrins prepared by acetylating hematoporphyrin with an acetic acid-sulfuric mixture followed by
hydrolysis under basic or nearly neutral conditions. This technique was first used in 1966 by Lipson, et al., to treat a patient with a fungating breast tumor. In 1972, Diamond, et al., examined the effect of hematoporphyrin activated by white light on glioma cells in culture and on a transplanted glioma tumor in rats. They demonstrated both the death of cells in culture and considerable destruction of tumor in the rats. In 1975, Dougherty and colleagues reported that systemic HpD, activated by red light (600 to 700 nm) from a xenon arc lamp, could cause complete eradication of a transplanted mouse mammary tumor without excessive damage to the surrounding skin that was included in the light field; neither the application of light nor HpD alone resulted in any gross effects on tumor or skin. A clinical trial was commenced by this group in 1976. Hematoporphyrin derivative has now been used for the detection of tumors and PRT for the treatment of several different tumor types, including tumors of the esophagus, bladder, skin, and lung, and has been demonstrated to be useful for the control of local disease. In 1980, Perria, et al., reported the first attempts at the photodynamic treatment of human gliomas.

For a photosensitizer to be widely used in patients, it should be nontoxic in clinically useful doses, be selectively taken up and/or retained in malignant tissue, be activated by penetrating light (> 600 nm), and be relatively photochemically efficient. Hematoporphyrin derivative and its purified active component di-hematoporphyrin ether (DHE) meet these criteria. Other photosensitizers have not yet been tested clinically. The laboratory and clinical studies that have been undertaken to establish the role of PRT in the treatment of cerebral tumors will be discussed. The optimal characteristics of sensitizers for systemic tumors may not be the same as those for cerebral tumors, where drugs that do not cross the blood-brain barrier but can enter into gliomas may be the best agents. We will discuss sensitization of both systemic and cerebral tumors.

**Photoradiation Therapy**

**Hematoporphyrin Derivative**

Hematoporphyrin derivative (Fig. 1) has been the sensitizer used for the last 10 years for clinical PRT, although it is not entirely satisfactory as a photosensitizer. A major problem is that HpD persists in the skin for up to 1 month, so patients need to stay out of sunlight for this period or longer to avoid developing sunburn. Hematoporphyrin derivative absorbs light maximally at 390 nm, but this wavelength of light penetrates tissues poorly; however, HpD has a minor absorption peak around 630 nm which penetrates tissues better. Hematoporphyrin derivative is a complex mixture of porphyrins and minor uncontrollable variation in its components may result during its synthesis. There has been extensive investigation of HpD to identify which components sensitize cells to kill, which components localize in tumors, and whether tumor-localizing components can be distinguished from those that persist in the skin and lead to skin photosensitivity. In vitro comparison of high-pressure liquid chromatography (HPLC)-separated HpD has demonstrated that the least polar components of HpD play the major role in tumor sensitization. The tumor-localizing components are postulated to be dimers and candidate molecules include DHE (Fig. 2) and dihematoporphyrin ester. Dougherty, et al., and Kessel and Cheng suggested that HpD was composed of ether-linked dimers or oligomers. More recently, Kessel, et al., have presented evidence that ester-linked porphyrins are present in HpD and that these are the tumor-localizing components.
Photonics. A major problem has been the difficulty in the purification of the different porphyrins in HpD. Recently, Scourides, et al.,\textsuperscript{115} took an alternative approach: they synthesized oligomers of DHE and showed that these had very similar properties to HpD. However, it was not determined whether dihematoporphyrin ester oligomers also had these properties. Using gel chromatography, Dougherty, et al., have produced Photofrin II, which it was claimed is a better sensitizer than HpD and is associated with less skin photosensitivity. Although Photofrin II is “enriched” with a higher concentration of DHE, it is also a poorly characterized mixture of porphyrins (PAScourides, personal communication, 1987).

Currently, HpD and Photofrin II remain the major sensitizers in clinical trials. However, other sensitizers are under examination. The desirable properties of a sensitizer include uniform distribution of sensitizer throughout the tumor, rapid excretion from the body, a well-characterized molecule, and better absorption of long wavelength light which penetrates tissues to a greater depth than short wavelength light. Porphyrin C occurs naturally as the iron porphyrin, which is the chromophore in cytochrome c and fulfills some of these criteria. We have recently developed a preparative reverse-phase HPLC method for porphyrin C purification\textsuperscript{116} and a synthetic method.\textsuperscript{115}

Porphyrin C can be synthesized in pure form and it selectively sensitizes tumors to kill \textit{in vivo} and \textit{in vitro}. Porphyrin C is rapidly excreted from the body, which results in less photosensitization of the skin and makes it a potentially useful sensitizer for tumor detection and therapy. Porphyrin C has been shown to selectively localize in the C6 intracerebral tumor model at 90 minutes after administration with a ratio of at least 100:1 compared to blood and to cause selective tumor kill (AH Kaye, unpublished data, 1987). The tumor:brain ratio was 1000:1. The cellular localization of the fluorescence has not yet been determined.

\textbf{Basis for Selective Localization of HpD in Tumors}

Studies attempting to elucidate the basis for the tumor specificity of HpD have not been conclusive. When studying the tumor specificity of photosensitizers, it is necessary to distinguish differences in the amount taken up by tumors from that retained by tumors, and to establish whether the photosensitizer is localized in the cells or in the stroma of the tumor. It is important to determine exactly what is being retained in the tumor, and this is difficult because porphyrins are usually measured by the level of fluorescence emission which can vary depending on whether the porphyrins are aggregated and on the buffer used for the preparation. There is some evidence that the sensitizing material is not particularly fluorescent.\textsuperscript{72} An alternative to fluorescent techniques for detecting porphyrins is offered by radiolabeled methods. Because HpD is a complex mixture of porphyrins, studies with radiolabeled HpD such as tritium (\textsuperscript{3}H)-labeled material are not entirely convincing because the \textsuperscript{3}H may not be equal in all components.\textsuperscript{22} Synthesizing metalloporphyrins from porphyrins by the insertion of iron or gallium into the porphyrin mix is also not satisfactory because the properties of the metalloporphyrin differ from original porphyrin. Despite these technical difficulties, studies of HpD uptake and retention using radiolabeled HpD have been undertaken. Gomer and Dougherty\textsuperscript{50} reported similar tissue uptake and retention with HpD labeled with carbon-14 and \textsuperscript{3}H; they also noted that tumor retained more HpD than skin or muscle. Gomer, et al.\textsuperscript{51} studied the uptake of \textsuperscript{3}H-HpD into human retinoblastoma heterotransplanted intraocularly into nude mice and showed increased uptake and retention in the eyes containing the tumor.

There is clearly selective retention of HpD as detected by fluorescence in human tumors such as those of the bronchus\textsuperscript{9} and bladder,\textsuperscript{11,12} and there is a good correlation between fluorescent intensity and biopsy-proven cancer. However, the selectivity of uptake and retention of HpD by tumor tissue \textit{in vivo} does not seem to be reproduced by malignant cells \textit{in vitro}.

Moan, et al.\textsuperscript{92,93} examined HpD uptake by fibroblast cells and found that the ability to produce tumors did not correlate with the uptake and retention of HpD. Henderson, et al.\textsuperscript{57} reported no differences in uptake of HpD between normal and malignant cells, although Andreoni, et al.\textsuperscript{9} did find more uptake by malignant cells. In a study undertaken in our laboratory,\textsuperscript{22} the factors determining uptake of HpD were examined in detail by flow cytometry. The major factors were the size of the cell, the pH, and the serum concentration of the mixture. It was found that large cells took up more HpD measured by fluorescence, and that low pH and low serum concentration also increased uptake. It is not clear how the \textit{in vitro} findings relate to retention \textit{in vivo}. Studies of tumor fluorescence have not conclusively indicated the exact location of the porphyrins within the tumor, which may be extracellular or intracellular, and in the normal cells or in the malignant cells of the tumor. Variation in fluorescence has also been noted in different portions of a tumor, with the more vascular sections usually having the higher fluorescence. When administered to patients,\textsuperscript{94} HpD was
thought to be bound to albumin; several studies of the binding of fractions of HpD to albumin have been reported.\textsuperscript{46} Recently, it has been stated that HpD binds to serum lipoproteins and that high porphyrin concentrations develop in tissue with high levels of lipoprotein receptors such as the liver.\textsuperscript{94,109} How the binding of porphyrins to albumin or lipoproteins leads to tumor retention is not clear.

**Mechanisms of Sensitization of Cells by Porphyrins**

There are two types of reactions possible when a sensitizer is excited by light. These are Type I reactions which involve direct interaction of the electronically excited dye with a cellular target followed by reaction of transients formed with oxygen, and Type II reactions in which singlet oxygen is first formed by reaction of oxygen with the dye in its excited triplet state. Current evidence suggests that singlet oxygen is the major intermediary leading to cell damage.\textsuperscript{18,71,102,129,131} Oxygen appears to be essential for tumor damage, and under anoxic conditions the photosensitizing effect of HpD is very low.\textsuperscript{79,90} The possibility of the optical detection of singlet oxygen production during in vivo phototherapy was recently reported.\textsuperscript{101} This is particularly important as it may lead to a device for the detection of singlet oxygen during therapy which would be a function of the light dose delivered and sensitizer concentration within the tumor, and so could improve the dosimetry of PRT. Hematoporphyrin derivative and Photofrin II are aggregated in aqueous solution and it appears that there is an inverse relationship between the degree of aggregation and the production of singlet oxygen.\textsuperscript{9,105,119}

Other radicals that may be involved include hydrogen peroxide,\textsuperscript{27} hydroxyl radicals,\textsuperscript{36,54} and superoxide;\textsuperscript{48} however, these appear to be less important than singlet oxygen. In cells, the porphyrins appear to be mainly located within membranes including the outer membrane, mitochondria,\textsuperscript{8,92} and lysosomes. The initial damage after phototherapy appears to occur to these structures.\textsuperscript{15,16,49,53} There is little material in the nucleus. Whether the cells damaged first are the tumor cells or the endothelial cells is not clear. The studies of Henderson, et al.,\textsuperscript{57} suggest that blood vessels are damaged initially and the tumor subsequently undergoes ischemic changes.

**Interaction Between Phototherapy and Other Forms of Treatment**

**Hyperthermia.** Hyperthermia can occur during photoradiation, and a synergistic tumoricidal effect has been shown using the SMT-F mouse mammary tumor.\textsuperscript{126} It is possible that some complications associated with phototherapy are due to direct kill by hyperthermia.

**Radiotherapy.** An early study suggested that HpD may be a radiosensitizer,\textsuperscript{86} although more recent studies do not support this. Wharen, et al.,\textsuperscript{132} studied the in vitro interaction between x-irradiation and HpD in 9L rat glioma cells and a clone using a dye exclusion assay to assess survival. At high intracellular HpD concentrations and large x-irradiation fraction sizes there appeared to be a potentiation of radiosensitivity. The relevance of this is not clear and there seems to be no added toxicity between radiotherapy and phototherapy (AH Kaye, unpublished data, 1987).

**Chemotherapy.** While it is generally thought that there are no interactions between chemotherapy and phototherapy, this question has not been exhaustively investigated. We have studied in vitro phototherapy in combination with chemotherapy.\textsuperscript{21} A major finding was that there appeared to be a physical interaction between anthracyclines and HpD, whereby each appeared to interfere with the uptake of the other by cells.

**Delivery of Light for Phototherapy**

Therapy of tumors is usually undertaken with red light (630 nm). The depth of tumor kill is a function of the depth that the light penetrates and the concentration of sensitizer in the tumor. A useful concept is the attenuation depth,\textsuperscript{122,123} this is the depth at which the intensity of light drops to e\textsuperscript{-1} (37%) of the starting value. This depth varies for different tissues but appears to be 2 to 4 mm in vivo. Currently, the effective depth of tumor necrosis appears to be three times the attenuation depth.\textsuperscript{44}

Most clinical studies are currently being undertaken with argon ion lasers that pump a dye laser to produce 3 to 4 W of power at 630 nm. The light is delivered to the tumor bed with quartz optical fibers. These lasers produce continuous wavelength light and can have problems of alignment and require extensive cooling. Recently, several manufacturers\textsuperscript{*} have developed a gold metal vapor laser\textsuperscript{93,95} which produces pulsed light at 628 nm with an average power of 5 to 6 W. The beam produced by the gold metal vapor laser is wider than the beam of an argon-pumped dye laser, but improvements in its coupling should allow 80% of the light to be delivered to the tumor bed. There are no problems of alignment associated with this instrument and it is fully portable. There has been recent interest in a new type of laser called an excimer-dye laser.\textsuperscript{†} This is not currently a practical instrument for delivering light for phototherapy but may be useful for tumor detection.\textsuperscript{59}

A comparison between the effectiveness of pulsed and continuous wavelength light\textsuperscript{37} showed no difference in the extent of tumor kill. We have recently described our experience with the gold metal vapor laser in 33 patients with esophageal, rectal, and cerebral tumors and malignant ascites.\textsuperscript{74} There did not appear to be any additional tumor kill or toxicity than might be expected from similar doses and dose rates from a continuous wavelength laser, but this requires further study.

\* Including Quentron, Adelaide, South Australia.

\† Excimer-dye laser manufactured by Lambda Physik, Göttingen, West Germany.
Laboratory Investigation of Photoradiation Therapy

Photoradiation therapy has been used to kill glioma cells in vitro and in vivo. Although a positive correlation between the intensity of light and tumor cell killing efficiency has been demonstrated in cell lines, including the 9L glioma cell line, Wharen, et al., showed no difference in the cell killing efficiency using the U87MG glioblastoma cell line and densities of red light from 10 to 100 mW/sq cm.

Highly selective uptake of HpD into cerebral tumor animal models has been demonstrated. The basis for the selective localization to tumor areas and its exclusion from the normal brain is probably related to a relative lack of blood-brain barrier within the tumor. The evidence for this includes the fact that uptake of HpD into normal brain tissue occurs only into areas known to be outside the blood-brain barrier or when the blood-brain barrier is disrupted by mannitol.

Tumor Models for Studying Photoradiation Therapy

An accurate and easily reproducible animal tumor model is critical for the study of PRT. The tumor model must resemble as closely as possible the histological features of the human glioma if the laboratory results are to be extrapolated to the clinical situation. Although many tumor systems have been developed for studying the treatment of glioma, none of these is completely satisfactory for the study of spontaneous human glioma tumors. Chemical and viral induction in adult animals, transplacental chemical induction, and transplantation will induce tumors of different histological types which to a greater or lesser extent simulate human tumors. The 9L gliosarcoma cell line has been used frequently in the investigation of PRT; however, we have employed the C6 glioma cell line, as its histological pattern resembles more closely the human glioma and the 9L cell line is a very malignant tumor and resistant to therapy. The C6 glioma rat model is an appropriate size for studying the effect of PRT. Uptake studies are more economically performed using a mouse model due to the number of animals required to obtain statistically significant data. There have been difficulties in developing an accurate mouse glioma model. Although the nude mouse model has been established for the study of glioma, it has disadvantages: as an immunosuppressed animal, it is not entirely representative of the clinical situation and there are difficulties in housing and handling these mice. An ependymoblastoma tumor has been used as an implantable intracerebral tumor model in normal mice, but this is a rare tumor in humans. We have found that the C6 rat glioma can be grown as a xenograft in both neonatal and adult mouse brains. This model and the monoclonal antibody to C6 have proved useful for the study of phototherapy as both the size and the position of the tumor can be judged accurately, and the monoclonal antibody clearly defines the tumor extension into adjacent brain.

Photosensitizer Uptake

Wise and Taxdal first demonstrated by fluorescence that hematoporphyrin was selectively localized in cerebral lesions and was excluded from the normal brain. In the C6 model, HpD has been shown to be highly selectively localized to the tumor areas, with slight fluorescence in the brain adjacent to the tumor and no significant fluorescence in the normal brain. These findings correlate well with the measurements of blood-brain barrier permeability in an autochthonous rat glioma model which showed increased permeability of the blood-brain barrier within the tumor, with partial preservation of the barrier in the brain adjacent to the tumor. In this region, the edematous brain may contain tumor cells that are within a relatively intact blood-brain barrier.

Boggan, et al., showed a patchy uptake of HpD into the 9L gliosarcoma rat model: only 33% to 44% of the tumor area was fluorescent and maximum fluorescence did not occur until 24 hours after the administration of HpD. In the C6 glioma model, maximum uniform fluorescence occurred throughout the tumor by 6 hours, and the fluorescence began to decline at 24 hours. These differences may well reflect differences between the tumor models. Wharen, et al., using tritiated HpD, showed a twofold higher ratio of HpD in tumor:brain at 4 hours compared to 24 hours after injection in ethyl nitrosourea-induced rat central nervous system tumors. However, maximum uptake of HpD has been reported at 24 hours in extracerebral transplanted tumors. This difference in the time to maximum fluorescence further suggests that the basis for tumor selectivity of HpD in gliomas and systemic tumors is different because, in extracranial tumors, selective retention rather than selective uptake seems to be important.

In the C6 glioma model there was no significant fluorescence in normal brain. Wharen, et al., using tritiated HpD, similarly showed that the amount of photosensitizer in normal brain was extremely small in both animals and humans. Boggan, et al., used digital video fluorescence microscopy to demonstrate fluorescence of the pial gray matter margin. Although Rounds, et al., reported phototoxicity in normal mouse brain treated with HpD and exposed to light and green wavelength, suggesting that the normal brain contained HpD, high doses of HpD were used, and heat energy from the irradiation may have caused some of the damage.

The method of measuring porphyrin uptake is a major limitation in the qualitative and quantitative estimation of the uptake of HpD and Photofrin II. As previously discussed, there are problems in using radiolabeled porphyrins. The major problem with fluorescence methods is that it is an indirect technique for measuring uptake; at high concentrations of porphyrin...
light through the tumor and, depending upon the position of the tumor within the normal brain, through the surrounding normal brain. Svaasand and Ellingsen\textsuperscript{123} found that the penetration depth of light in malignant gliomas was usually twice the penetration depth in the surrounding normal brain. They showed that the penetration depth in the red part of the light spectrum in glioma was between that in adult brain and in neonatal brain and was approximately 2.5 mm, defined as the depth at which light is $e^{-1}$ of the starting value. The highest penetration depth was in the most malignant gliomas. It is evident that the extent of tumor destruction reported in the C6 model\textsuperscript{66} is two to three times greater than the reported penetration depth of red light, and the additional tumor destruction may result from vascular damage. Experimental studies that support the vascular damage hypothesis include those of Bugelski, et al.,\textsuperscript{29} who showed evidence of vascular endothelial damage 15 minutes after PRT; after HpD injection, fluorescence was shown to be distributed maximally in blood vessels surrounding the tumor, and vascular walls have been seen to independently fluoresce. In the C6 rat model, no tumor necrosis was detectable in the first 6 hours after irradiation,\textsuperscript{66} which supports the suggestion of Henderson, et al.,\textsuperscript{52} that necrosis follows ischemia due to small-vessel occlusion.

### Optimization of Phototherapy in a Laboratory Model

The rat C6 glioma model has been used to investigate the delivery of PRT for glioma and to study the effect of PRT on the normal brain to determine the most appropriate dose of light to obtain maximum kill with minimum damage to normal tissue.\textsuperscript{66} Selective tumor kill of a cerebral glioma with sparing of the normal brain can be achieved with PRT.\textsuperscript{66} This selective tumor destruction in the C6 glioma model\textsuperscript{66} occurred at HpD doses of less than 20 mg/kg and light doses of less than 200 joule/sq cm. With HpD doses of 20 mg/kg body weight and red light, 200 joule/sq cm, from an argon dye laser, the mean depth of tumor kill was 4.5 mm and in 20% of animals the depth of tumor destruction was greater than 6 mm. Increasing the dose of HpD from 20 mg/kg body weight to 40 or 60 mg/kg body weight did not increase the depth of destruction but it did increase both the likelihood of developing edema and necrosis in normal brain and the extent of that necrosis. There was a significantly greater depth of tumor kill if higher red light doses (400 or 600 joule/sq cm) were used in animals that had been pretreated with HpD at doses of either 20 or 40 mg/kg body weight; in such cases the mean depth of tumor destruction was 5.8 mm and in five of 20 animals tumor destruction was greater than 7 mm in depth with a maximum depth of 1 cm. However, increasing the dose of red light above 200 joule/sq cm significantly increased the likelihood and extent of necrosis in normal brain. Despite the relationship of depth of tumor destruction to dose of red light, a dose rate effect with PRT was not evident.\textsuperscript{66}

We have been able to demonstrate prolonged survival times in rats with C6 intracerebral tumors treated with red light at 200 joule/sq cm and HpD at 20 mg/kg body weight 1 week after inoculation with $10^6$ C6 cells (AH Kaye, unpublished data, 1987). Boggan, et al.,\textsuperscript{19} have also shown prolonged survival times in rats with the 9L intracerebral tumors when treated with similar doses of red light and HpD.

Provided sufficient HpD is present within the tumor to provide a photochemical reaction, the depth of tumor necrosis is largely related to the penetration of red light through the tumor and, depending upon the position of the tumor within the normal brain, through the surrounding normal brain. Svaasand and Ellingsen\textsuperscript{123} found that the penetration depth of light in malignant gliomas was usually twice the penetration depth in the surrounding normal brain. They showed that the penetration depth in the red part of the light spectrum in glioma was between that in adult brain and in neonatal brain and was approximately 2.5 mm, defined as the depth at which light is $e^{-1}$ of the starting value. The highest penetration depth was in the most malignant gliomas. It is evident that the extent of tumor destruction reported in the C6 model\textsuperscript{66} is two to three times greater than the reported penetration depth of red light, and the additional tumor destruction may result from vascular damage. Experimental studies that support the vascular damage hypothesis include those of Bugelski, et al.,\textsuperscript{29} who showed evidence of vascular endothelial damage 15 minutes after PRT; after HpD injection, fluorescence was shown to be distributed maximally in blood vessels surrounding the tumor, and vascular walls have been seen to independently fluoresce. In the C6 rat model, no tumor necrosis was detectable in the first 6 hours after irradiation,\textsuperscript{66} which supports the suggestion of Henderson, et al.,\textsuperscript{52} that necrosis follows ischemia due to small-vessel occlusion.
that in two series PRT has improved the survival times occurring independently of a hyperthermia the effective depth of kill is 4 to 7 mm, that tumor kill of fight is critical in achieving selective tumor kill, that selective tumor kill, that the dose of HpD and the dose photodynamic cell kill or to protect normal tissue.

Thermal Effect

The thermal effect resulting from PRT has been investigated, and a hyperthermic response has been shown to occur in the irradiated tumor. Following irradiation with either 800 or 1200 mW, a temperature rise occurred which reached a maximum within 1 minute of up to 5° and 7°C, respectively. This temperature rise was avoided by irrigation with normal saline at room temperature. There was no significant difference in the temperature rise in either normal or tumor-bearing animals, and prior administration of HpD did not affect the temperature response. There is some controversy concerning the role hyperthermia plays in the tumoricidal effect with PRT. Although some authors consider a hyperthermic response to be a significant component, tumor destruction has clearly been shown to occur when there is no significant heating of the tissue. Other studies have suggested that a decrease in the temperature may also enhance the photodynamic cytotoxic effect. It is possible that temperature manipulation, such as hyperthermia or hypothermia, could be used in association with PRT to enhance the photodynamic cell kill or to protect normal tissue.

The laboratory findings show that PRT will produce minimal cerebral edema similar to that noted in nontumor-bearing cerebral tissue. It is difficult to be certain of the effect of PRT on cerebral edema in deep intracranial tumors, as the brain adjacent to the C6 tumor is usually edematous. In this situation, a problem arises in determining the extent of cerebral edema due to the intracerebral tumor and that caused by the PRT. However, in the study investigating the ability of PRT to produce selective tumor kill there was no significant cerebral edema in the cerebral cortex superficial to deep tumor in 40% of animals treated with HpD at 20 mg/kg and red light at 200 joule/sq cm, implying that these doses of PRT produce minimal cerebral edema similar to that noted in nontumor-bearing cerebral tissue.

Photodynamic Therapy in Clinical Neurosurgery

Perria, et al., reported the first attempts at photodynamic treatment of human gliomas. Since 1978, 64 patients with cerebral tumors treated with PRT have been reported (Table 1). The light has been administered by either a xenon arc lamp, a helium neon laser, an argon dye laser, or a gold metal vapor laser. The initial clinical studies of PRT have been disappointing in their therapeutic effect; however, in these studies, recurrent gliomas were often treated and doses of light 10 to 100 times lower than are used in systemic tumors were applied. This was done partly because of the fear of side effects of PRT in high doses (especially when combined with x-irradiation therapy) and also due to lack of availability of powerful light-producing sources.

Technical problems associated with the administration of PRT in the treatment of neurosurgical tumors include the sensitizer to be used and the dose and time of its administration, the type of irradiating system and dose to be given, the possible effects of preoperative steroid administration, and the possible postoperative and postphototherapy complications including cerebral edema, skin sensitization, and interaction with x-irradiation therapy.

Sensitizers

The two sensitizers that have been used clinically are HpD and Photofrin II. The two sensitizers that have been used clinically are HpD and Photofrin II. Photofrin II is a somewhat enriched form of HpD, and its possible benefits might be that a lower dose could be used to diminish the possible sensitizer-associated side effects. The sensitizer has been administered intravenously, although direct intratumor injection has been advocated. Intratumor administration would seem to be inappropriate as the basic concept of phototherapy is its "selectivity" for tumor rather than for normal brain. Any adjuvant therapy for cerebral gliomas should be directed toward an improved tumoricidal effect on the spreading edge of the tumor, rather than the central mass which can usually be easily resected by standard surgical techniques. An intratumor injection would certainly provide very high doses of...
sensitizer within the tumor mass but is unlikely to provide a selectivity for the tumor cells spreading out into the normal and often vital area of brain.

The intravenous dose of HpD has varied from 2.5 to 5 mg/kg, and Photofrin II has been administered at 2.5 mg/kg. Although HpD has been administered up to 3 days prior to irradiation, the Royal Melbourne Hospital-Ludwig Institute of Cancer Research (RMH-LICR) series \(^{58}\) of 28 patients with malignant cerebral tumors received HpD, 5 mg/kg, intravenously 24 hours before surgery, as the laboratory data indicate this to be the optimal time.\(^{67,131}\)

Tumor Resection

The type and extent of tumor excision prior to the administration of the phototherapy depends on the position and size of the tumor. Laws, \textit{et al.},\(^{58}\) reported the use of a fiberoptic probe inserted into the tumor or cyst cavity without excision of the tumor. In view of the limited depth of irradiating light penetration, it is apparent that the most favorable tumors for phototherapy are those in which a radical debulking operation can be performed so as to leave as little residual tumor as possible. In the RMH-LICR series,\(^{68}\) a radical debulking procedure was performed on all 28 patients with the aid of the Cavitron ultrasonic surgical aspirator (CUSA).\(^{13}\) In nine of the patients, the tumor was situated so that a polar lobectomy could be performed; however, a complete resection of the tumor was performed in only three patients. With the appropriate instrumentation it would be possible to perform stereotaxic phototherapy on small deep tumors that could not be resected.

Irradiating Systems

The light irradiating systems have included incandescent lamp systems such as a xenon arc lamp\(^{131}\) and laser systems such as the helium neon laser,\(^{103,104}\) argon dye laser,\(^{58,78,88,96}\) or gold metal vapor laser.\(^{68,88,95}\) The most efficient method of delivering red light is from a laser system. The laser light sources have the advantage that a single known wavelength of light is being used, the light output can be accurately measured, the light can be administered selectively to parts of the tumor cavity, deep cavities can be effectively irradiated, and high doses of light can be used. The argon rhodamine pumped-dye laser produces continuous light; however, the coupling is inefficient so that only a relatively small dose of light can be produced from a high-energy laser. The other major disadvantage of the argon rhodamine pumped-dye laser is that it is relatively immobile and requires an area dedicated to its use. The gold metal vapor laser is portable, produces a much higher light intensity at 627.8 nm, and has the theoretical advantage of providing a pulsed light rather than a continuous beam. Consequently, it produces very high peaks of light at approximately 10,000 pulses/sec. These pulses of power may improve sensitizer activation and increase the depth of penetration, although this has not yet been proven experimentally.

Laser light in the RMH-LICR series\(^{68}\) was generated by an argon rhodamine pumped-dye laser\(^{5}\) in nine patients and a gold metal vapor laser\(^{11}\) in 19. The light was delivered through a flat cut quartz fiber (600 \(\mu\) diameter in 17 patients and 1 mm diameter in 11 patients) which was placed in a photoelectric cell to calculate the dose. The laser power was in the range of 0.7 to 2.2 W at the fiber tip. The surface area to be irradiated was carefully calculated, the fiber was attached to a Yasargil brain-retractor arm,\(^{1}^{*}\) and the tumor bed was irradiated for between 43 and 94 minutes (median 60 minutes). To ensure even distribution of the hot spot from the fiber tip, the fiber was moved at regular intervals to completely and evenly cover the tumor bed surface. If tumor resection resulted in a cavity to be irradiated, 0.5% Intralipid\(^{†}\) was used as a diffusing agent as suggested by Dr. Laws (E Laws Jr, personal communication, 1985). The temperature on the surface of the brain was monitored using a thermal diffusion cerebral blood flow monitor, and the brain deep to the irradiated surface was monitored with a trigeminal neuralgia electrode lesion generator inserted to a depth of 2 mm.\(^{‡}\) The temperature was kept below 37°C by irrigation with either normal saline or 0.5% Intralipid. Following PRT the dura was closed, the brain flap replaced, and the craniotomy wound was closed. The total time for surgery including the PRT varied from 3\(\frac{1}{2}\) hours to 5\(\frac{1}{2}\) hours.

The argon rhodamine pumped-dye laser was used in the early part of the RMH-LICR series. Because the power that could be obtained from this instrument was less than 1.5 W at the fiber tip, the maximum dose of light to the tumor bed was 145 joule/sq cm. In the latter part of the series, the gold metal vapor laser was used. This laser produced a power of up to 2.2 W at the fiber tip, enabling higher doses of light to be delivered in a practical time period (1 hour).

Photoradiation Therapy Dose

We have used a light intensity of up to 2.2 W at the fiber tip and a tumor dose of up to 230 joule/sq cm,\(^{68,95}\) although the laser power in previous series has varied from 12.5 mW\(^{104}\) to 400 mW\(^{78}\) and the dose of light has varied from 0.5 to 9 joule/sq cm,\(^{103,104}\) 8 to 68 joule/
Photoradiation therapy in the management of neurological tumors

sq cm, and 100 mW/sq cm. The apparent reasons for use of a lower irradiation dose included fear of additional toxicity (mainly cerebral edema), and instrumentation that allowed only low powers to be administered. The numbers of patients are too small and the follow-up period too short in our series to determine if the higher doses of light (> 12 joule/sq cm) and the pulsed light delivered by the gold metal vapor laser were more effective than the lower doses of continuous light previously used, although a trend favoring higher doses of light in gold metal vapor lasers was observed in our series. There is considerable difficulty in determining the exact dose of laser light that is administered as a function of the surface area irradiated. The problem is in measuring the surface area accurately, estimating the dose of light lost from the tumor cavity, and ensuring a uniform administration of light. Muller and Wilson have reported the use of an inflatable balloon to aid in achieving an even distribution of light. However, this method would not be appropriate for uneven cavities or for resections such as a frontal, temporal, or occipital lobectomy that results in a flat surface after tumor excision. For these types of resections we use a grid pattern to ensure an even distribution of light.

Side Effects of Photoradiation Therapy

Increased cerebral edema has been reported following PRT, although this complication was not observed in our series of 28 patients. From the laboratory studies it is evident that increased cerebral edema occurs with HpD at doses greater than 20 mg/kg and a 200-joule/sq cm dose of red light from an argon rhodamine pumped-dye laser.

It is highly probable that the selective uptake of the porphyrin sensitizer is related to the peculiarities of the tumor vasculature. It is possible that steroid administration does affect the blood-tumor barrier, and prior steroid administration may result in diminished photosensitizer uptake. However, we have noted no change in HpD uptake in the C6 model as measured by fluorescence, following the prior administration of steroids (AH Kaye, unpublished data, 1987).

Skin photosensitization has been a major problem associated with PRT. We have instructed patients to remain out of direct sunlight for the initial 4 weeks after treatment. Skin testing is then performed to determine if direct sunlight can be tolerated, and the patients are instructed to gradually increase their daily exposure to the sun. The longest period of significant sensitization in 56 patients treated with HpD at 5 mg/kg at the Royal Melbourne Hospital has been 12 weeks, with a median period of 4 weeks. It is possible that the use of lower doses of Photofrin II would produce a lower degree of skin photosensitization. Although carotene-containing substances might theoretically diminish skin sensitization there have been no clinical reports supporting their use.

A major concern has been the possible interaction of postoperative radiotherapy with a porphyrin sensitizer. Schwartz et al. reported both a radioprotective and a radioenhancing effect, depending on the conditions of use. Although it is highly improbable that the photosensitizer does cross an intact blood-brain barrier, the HpD may remain in small vessels for some time. Consequently, it has been our policy not to commence radiotherapy until 4 weeks after the administration of PRT. There have been no reported side effects in 15 patients treated with conventional radiotherapy (45 Gy in 20 divided doses) 4 weeks following phototherapy. The PRT has been well tolerated. There was no increase in the toxicity of postoperative x-irradiation therapy. This is the standard dose used to treat cerebral gliomas in Victoria, Australia. We do not know if there would be additional toxicity if a higher dose of radiotherapy was used.

Photoradiation Therapy Series

The follow-up periods in the reported series are short. Although the early results were disappointing there have been some long-term survivors. We have treated 28 patients with malignant cerebral tumors using up to 5 mg/kg HpD and up to 230-joule/sq cm doses of red light produced by either an argon dye laser or gold metal vapor laser. The patients ranged in age from 18 to 73 years (median age 45 years). Preoperatively, the Karnofsky status was less than 50 in four patients, 50 to 70 in 11 patients, and 70 to 90 in 13 patients. Twenty-seven patients had high-grade gliomas as graded by the Ringertz system (13 glioblastoma, 11 recurrent glioblastoma, two anaplastic astrocytoma, and one recurrent anaplastic astrocytoma) and one patient had a metastatic tumor from the lung. All patients who presented with recurrent gliomas had previously received radiotherapy (45 Gy in 20 divided doses). Patients with new gliomas underwent postoperative radiotherapy (45 Gy in 20 divided doses).

The patients have been followed for between 2 and 22 months. Four of the 15 patients with primarily treated gliomas had clinical and computerized tomography evidence of recurrence at 3, 6, 12, and 13 months following therapy. A further patient died of an acute myocardial infarction 15 days postoperatively. The other 10 patients have been followed clinically for 1 to 20 months (median 8 months) with no clinical evidence of tumor recurrence. Six of the 12 patients with recurrent gliomas have suffered a further recurrence and died. The clinical recurrences developed in all cases between 10 and 15 weeks after PRT. The remaining three patients are clinically disease-free at 4, 10, and 16 months after treatment. The number of patients in this study is too small and the follow-up period too short to draw conclusions concerning the efficacy of the therapy.

There were no direct complications from the phototherapy in this study. No significant increase in neurological deficit was observed following surgery and PRT; there was no evidence of increased cerebral edema and...
no increase in the toxicity of postoperative x-irradiation therapy.

**Future Applications**

The clinical studies have shown that, when used as an adjuvant to tumor resection, PRT using HpD doses of 5 mg/kg and red light up to 230 joule/sq cm delivered by a gold metal vapor laser is safe and can be followed by conventional radiotherapy.\(^7^8,9^5\) Further follow-up monitoring is required to determine if PRT is a beneficial adjuvant to surgery and x-irradiation therapy. A problem with testing PRT as an adjuvant is that tumor responses to photoradiation are difficult to measure. However, since the depth of tumor kill of PRT is probably only 0.5 to 1.0 cm, it can be expected that PRT has little to offer as a sole treatment for large tumors. A properly instituted randomized trial of PRT for cerebral gliomas is required. However, as the trial would probably require approximately 200 patients to reach statistical significance, there are obvious problems with logistics and organization.

At the present time the major limiting clinical factors are the selectivity of tumor uptake, particularly relating to the brain adjacent to the tumor and the penetration power of the porphyrin-activating system, such as the laser light. The tissue transmission spectra are characteristic for each tissue and depend on factors such as hemoglobin and water content and the degree of myelination in the brain.\(^1^1^8\)

A number of new sensitizers are being tested in the laboratory, including new porphyrins such as porphyrin C, 81,114,116,117 meso-tetra (hydroxyphenyl) porphyrins, 13 phthalocyanines, 10,29,112 and rhodamine-123. 106 Porphyrin C can be synthesized in pure form and is highly selectively localized to the intracerebral tumor; its maximum uptake is at 2 hours with no significant fluorescence remaining at 8 hours after administration. It is rapidly excreted from the body and does not produce skin sensitization. It can be radiolabeled with sulfur-35, so that accurate radiotracer studies can be performed in the laboratory. Porphyrin C is localized in the extra-cellular region of the tumor and has a photosensitizing power approximately one-half that of HpD.

Chloroammonium phthalocyanine has been found to be an efficient photosensitizer of mammalian cells in culture. 10,29 Because phthalocyanines have a strong absorption band in the red section they should theoretically be able to utilize red laser light to kill tumor cells more efficiently than HpD. Rhodamine-123 is a lipophilic cationic dye that is specifically taken up by mitochondria of living cells. 106 Although rhodamine-123 is bound to all mitochondria of a wide variety of cells, different types of cells differ widely in their intensity of staining and their ability to retain dye. It has been suggested that mitochondrial staining by rhodamine-123 is related to the internal negative membrane potential of the mitochondria and the positive charge on rhodamine-123 that causes it to be drawn into the mitochondria. Positively charged, lipophilic dyes can concentrate across membrane potentials into mitochondria and up to a 10,000-fold concentration equivalent is theoretically possible. 106 Powers, et al., 106 have shown selective retention of rhodamine-123 in cultured U-251 glioma cells, its exclusion from normal cells, and a photoactivated cytotoxicity when exposed to blue-green light between 488 and 514.5 nM using a continuous-wave argon laser. The difficulty with using rhodamine-123 as a photosensitizing agent is that it is only activated by blue-green light. In solution, rhodamine-123 has an absorption maximum of 502 nM whereas the absorption maximum shifts to 512 nM upon interaction of the rhodamine-123 with mitochondria. The penetration power of blue-green light through most biological tissues is significantly less than the longer wavelengths, and this would severely limit its clinical application.

The ideal sensitizer would have the potential localizing selectivity demonstrated by rhodamine-123 and have the absorption spectrum of the phthalocyanines. At the present time, HpD is a compromise for both of these properties.

Improved laser systems are required to increase the penetration of light through brain and tumor. The gold metal vapor laser is a substantial improvement on the argon dye laser due to its portability, higher laser power, and the theoretical advantage of the pulsed dose. The wavelength required from the laser depends on the sensitizer used, and the power that is required is limited by the tolerance of normal brain. The excimer-dye laser systems 59 have the potential for developing very high powers up to 120 W but at present they are not sufficiently developed for clinical use.

The use of phototherapy in combination with stereotaxic equipment 44 is an exciting possibility for the treatment of small deep-seated previously unresectable tumors. It is only a minor technical problem to shield the fiber tip and prevent local charring. Although most of the tumors treated to date have been supratentorial gliomas or metastases, PRT has been used for posterior fossa tumors and pituitary tumors (E Laws Jr, personal communication, 1987). It is probable that PRT could be used safely to treat tumor remnants in the floor of the fourth ventricle that are invading the brain stem. However, careful dosimetry would be required to avoid swelling the brain stem. No studies have been performed on the uptake of sensitizer by pineal tumors. However, it is likely that these tumors should accumulate the photosensitizer, and phototherapy might provide a useful adjuvant in the treatment of these difficult tumors.

At the present time, a dose rate dependence of tumor kill has not been demonstrated. This may be a reflection of the inadequacies of the experimental technique. However, if a dose rate dependence is expected then long-term implantation and irradiation in the tumor cavity could be performed. The future application of PRT will depend upon clinical trials showing its usefulness in the treatment of cerebral tumors. The present
Photoradiation therapy in the management of neurological tumors

sensitizer, irradiation systems, and techniques of delivery can all be improved so that the technique can become a useful adjuvant in the therapy of a wide range of cerebral tumors.

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