Photochemotherapy of Glioma Cells by Visible Light and Hematoporphyrin

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SUMMARY

Malignant tumors take up and retain hematoporphyrin to a much greater extent than do normal tissues. Porphyrins are photodynamic agents that sensitize cells so that they are damaged by exposure to light. Treatment with hematoporphyrin followed by irradiation with light can destroy glioma cells in culture in less than 8 min and gliomas growing s.c. in rats in about 40 min. Photochemotherapy may become useful in the management of malignant tumors that are resistant to current methods of treatment.

INTRODUCTION

Several porphyrins are preferentially taken up and retained by malignant tumors (1, 7, 10—12, 14, 18, 19, 25, 26). In humans, porphyrins have been used as a diagnostic tool to help delineate the periphery of malignant tumors from normal tissue (1, 7, 11, 12, 14, 18, 19, 26). The porphyrin-containing cancers exhibit a characteristic red fluorescence when excited with UV, while normal surrounding tissues are not activated.

Porphyrins are powerful photodynamic agents that render cells vulnerable to light that corresponds to the absorption spectra of the porphyrins (13, 15). Because hematoporphyrin may persist in malignant tumors for long periods of time, it should be possible to deliver visible light to porphyrin-containing tumors when normal surrounding cells are depleted of the injected pigment. We have determined that administration of hematoporphyrin followed by light therapy is lethal to glioma cells in culture and produces massive destruction of porphyrin-containing gliomas transplanted s.c. in rats. Treatment with either light or hematoporphyrin alone is without effect. A preliminary report concerning some of this work has been published (3).

 MATERIALS AND METHODS

Hematoporphyrin-free base (Sigma Chemical Co., St. Louis, Mo.) was used without purification. Thin-layer chromatography of this material on silica gel (benzene: methanol:formic acid, 85:15:1.3) showed a major band along with several other red fluorescent bands. The major component (hematoporphyrin) comprised about 50% of the total, and the minor peaks consisted of other porphyrins and possibly hematoporphyrin aggregates. The pigment was suspended in 0.9% NaCl solution, dissolved by drop-wise addition of 1 N NaOH, neutralized to pH 7.4 with HCl, and used immediately for in vitro and in vivo studies. A glioma induced in rats by methyl nitrosourea (2) and maintained as actively growing monolayer cultures was used in this study. Although a definite tendency to sarcomatous change was observed in this tumor, the glial element remained a significant component of the tumor cell population throughout this study.

In Vivo Studies. In vivo experiments were performed with 19 Fisher 344 male rats (150 to 170 g) bearing tumors produced by implanting 10⁶ glioma cells s.c. in the right flank. Twenty-one days after inoculation, the animals were given hematoporphyrin (20 mg/kg) in a 1-mL dose by tail vein injection under light ether anesthesia. Twenty-four hr after injection the tumors showed red fluorescence when examined with a Wood’s lamp, while surrounding tissues and normal serum did not. Therefore, treatment with light was begun at this time. Tumors in animals were treated with white light for varying periods of time. The light source for in vivo irradiation consisted of a 1600 watt high-pressure xenon arc lamp (Model X-16; Strong Electric Co., Toledo, Ohio). To remove infrared radiation, light was passed through a dichroic dielectric heat filter (range of transmission 400 to 700 nm) (Optical Coating Laboratories, Inc., Santa Rosa, Calif.) placed 71 cm from the lamp. To remove UV radiation and diffuse the light, the beam was next passed through ground glass (3 mm) placed 75 cm from the light source. Next, the light was directed through 2 clear solid Lucite cylinders. The 1st (91 x 4.5 cm) was placed 85 cm from the light source and the 2nd (165 x 4.1 cm) was placed 198 cm from the light source. Maximal irradiation to the cancer was achieved by applying the end of the 2nd cylinder directly to the s.c. tumor through a skin incision made at least 5 cm from the tumor. This incision did not compromise the blood supply of the tumor. The 2nd cylinder could be removed easily for cleaning without altering the stationary arrangement of the remainder of the light system. Control animals were treated with light without hematoporphyrin. In earlier studies (3), we have found that injection of hematoporphyrin without
HP(20 mg/Kg IV), light

Chart I. The effect of hematoporphyrin (HP) and light on the viability of rat glioma in vivo. Gliomas growing s.c. in 19 animals were irradiated with light for varying periods of time. Fifteen animals received hematoporphyrin (20 mg/kg) 24 hr before irradiation, and 4 control animals were treated with light alone. The percentage of cell viability was estimated by light microscopy; vertical lines range.

Chart 2. Photosensitizing effect of hematoporphyrin (HP) on the viability of glioma cells in culture. Glioma cultures were exposed to hematoporphyrin in the presence or absence of varying periods of light, as described in "Materials and Methods." The percentage of viable cells was estimated by the exclusion of trypan blue 6 hr after irradiation. Each point is a separate culture.

RESULTS

Twenty-one days after implantation of tumor cells, the diameter of the glioma growing s.c. was found to range from 1.5 to 2.0 cm by external measurement with calipers. At this time a combination of light and hematoporphyrin therapy produced massive coagulation necrosis of tumor cells which was nearly complete after only 40 min of irradiation (Chart 1). When surviving cells were present, they were usually located along the rim of the tumor farthest from the light source. Light without hematoporphyrin was without effect.

To define further the characteristics of hematoporphyrin photosensitization of malignant tissue, glioma cells were studied in culture under differing conditions. Glioma cell cultures incubated in the dark with added hematoporphyrin, or exposed to light without hematoporphyrin, maintained 99% viability. However, a combination of hematoporphyrin and light produced 100% cell death in 8 min (Chart 2). To determine whether a toxic breakdown product of hematoporphyrin was produced in the medium and caused cell death, one-half of the treated cultures were washed immediately after irradiation with light. This maneuver did not affect the rate of production of cell death (Chart 3). It is also possible that hematoporphyrin uptake was rate limiting in this system or that metabolism of hematoporphyrin was required to produce a lethal effect in the cultured cells. Therefore, glioma cells were examined after preincubation with pigment and compared with controls where hematoporphyrin was added at the time of irradiation. Under these conditions, there was no significant increase in the rate of production of cell death (Chart 4). Finally, to determine whether the photodynamic effect was due to sensitizer that was firmly bound to the malignant cells, the glioma cultures were preincubated with hematoporphyrin and one-half of the cultures to be treated with light were washed immediately before irradiation (Chart 5). After washing, the glioma
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(singlet oxygen) (6, 9, 20) which is toxic to living organisms because it reacts rapidly with subcellular organelles, proteins, and nucleic acids (20).

The earliest attempt to use a photosensitizing agent (eosin) for cancer chemotherapy was carried out in 1903 when Tappeiner and Jesionek (24) treated 3 patients who had skin cancer. In 1942, Auler and Banzer (1) noted that hematoporphyrin appears to localize selectively in tumor tissue, and this observation has been confirmed by many investigators (1, 7, 10–12, 14, 18, 19, 25, 26). In 1972, Diamond et al. (3) demonstrated that hematoporphyrin could serve as a photosensitizing agent for the destruction of tumor cells by exposure to light. Subsequently, Dougherty (4) reported similar results with fluorescein and Tomson et al. (21) showed that acridine orange was also effective with an argon laser.

In this study, administration of hematoporphyrin followed by irradiation with broad-spectrum visible light was lethal to glioma cells in culture or growing s.c. as solid tumors in rats. In our earlier report, several hr of light therapy were used to treat the s.c. gliomas (3). In this study, the time required to produce massive necrosis was reduced to nearly 40 min by the use of a more intense light source and the application of light directly to the tumor through a plastic probe. This result demonstrates that it is possible to manipulate the light source to improve the effectiveness of photochemotherapy and suggests that retreatment would probably produce a high rate of cure of the experimental tumors. In vitro exposure to hematoporphyrin and light produced 100% cell death in only 8 min (Chart 2). Thus, it is possible that the time required for photochemotherapy in vivo might be substantially reduced once optimal conditions are achieved. Moreover, since red light may penetrate tissues more readily than other wavelengths of visible light (23) and hematoporphyrin has significant absorption of

### DISCUSSION

There is a growing interest in utilizing photochemotherapy in the treatment of several disorders including chronic herpes infections (5, 8, 16), psoriasis (17), and malignant tumors (1, 3, 4, 21). In such studies, a photosensitizing agent is taken up selectively by abnormal tissues and activated by light to produce local changes. Most photochemotherapeutic approaches use photodynamic agents, i.e., photosensitizing chemicals that require oxygen to destroy biological preparations when they are exposed to light. Lethal photosensitization probably involves the production of metastable, electronically excited molecular oxygen

![Chart 3](image-url)

Chart 3. Photosensitizing effect of hematoporphyrin (Hp) on the viability of glioma cells in culture. Glioma cultures were treated as in Fig. 2, except that one-half of the treated cultures were washed with medium immediately after irradiation with light. The percentage of viable cells was estimated as described in Chart 2. Each point is a separate culture.

![Chart 4](image-url)

Chart 4. Photosensitizing effect of hematoporphyrin (Hp or HP) on the viability of glioma cells in culture. Glioma cultures were treated as in Chart 2, except that the light source contained 6 bulbs instead of 8, and one-half of the treated cultures were preincubated with hematoporphyrin in the dark for 1 hr. Each point is a separate culture.

![Chart 5](image-url)

Chart 5. Photosensitizing effect of hematoporphyrin (Hp) on the viability of glioma cells in culture. Glioma cultures were treated as in Chart 2, except that all of the treated cultures were preincubated with hematoporphyrin for 1 hr. Immediately before exposure to light, one-half of the treated cultures were washed with medium. Each point is a separate culture.
light in the red region, a selective increase in the intensity of red light might be even more effective in producing photodynamic destruction of the malignant cells.

Photosensitization of glioma cells was probably due to hematoporphyrin which entered malignant cells rapidly and light in the red region, a selective increase in the intensity of photochemotherapeutic agent for the destruction of malignant cells. Our study was carried out to determine whether hematoporphyrin, once inside tumors, can act as a selective photochemical therapeutic agent for the destruction of malignant cells. The results are encouraging and, taken together with recent findings in other laboratories, suggest that photochemotherapy may be useful in the management of several kinds of malignant tumors. In particular, since light penetrates through the skull into the brain of large animals (22) and hematoporphyrin does not cross the blood-brain barrier but is taken up by human intracerebral gliomas (26), it is possible that photochemotherapy may provide a new approach for the treatment of glioblastoma in humans. This might be achieved by delivering light to intracranial tumors through fine fiber optic probes.

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