Treatment of Murine Intraperitoneal Ovarian Ascitic Tumor with Hematoporphyrin Derivative and Laser Light


Radiation Oncology Branch, Division of Cancer Treatment, National Cancer Institute, NIH [Z. T., J. B. M., F. S. H., D. T. R., A. R.], and Biomédical Engineering and Instrumentation Branch, Division of Research Services, NIH [P. S.], Bethesda, Maryland 20205.

ABSTRACT

An ascitic murine tumor, ovarian embryonal carcinoma, was used as a model of human ovarian carcinoma restricted to the peritoneum. The tumor was treated with a combination of hematoporphyrin derivative (HPD) and i.p. 514-nm laser light. Mice were given injections of $2 \times 10^6$ tumor cells, and by 9 days, there were 2 to 4 g of ascitic tumor/mouse. On Day 9, mice (68) were treated as follows: (12) no treatment; (12) only HPD (50 mg/kg i.p.); (12) laser only (9.6 J for 16 min); and (32) HPD (50 mg/kg i.p.) and 2 h later, laser treatment. On Day 15, a second treatment with HPD and laser was given to 15 mice. All mice not receiving HPD-laser treatment died between Days 20 and 23. The response rate as determined by decrease in weight and abdominal size for HPD-laser-treated mice was 90%, but the response was short, and all but one animal died by Day 34. However, 6 of 15 of the twice-treated group are alive at 90 days and considered cured. Photoradiotherapy with HPD for i.p. ascitic tumors appears to have promise as a treatment modality.

INTRODUCTION

During the last decade, there has been considerable interest in the use of PRT as a cancer treatment modality. This approach consists of the administration of photosensitizing drugs and subsequent introduction of light into the tumor to trigger the dye’s cytotoxicity. HPD is an incompletely defined mixture of porphyrin compounds (17, 21). It is an effective, well-tolerated photosensitizing drug that is retained longer in malignant cells than in normal tissues (12, 22). These properties have made HPD the most commonly used photosensitizing agent in experimental and clinical PRT (17).

The mechanisms of tumor cytotoxicity by PRT have not been fully elucidated. There is substantial evidence to suggest that the cytotoxicity results from light-induced sensitizer excitation (25) followed by transfer of energy to ground-state triplet oxygen resulting in the reactive excited species, singlet oxygen (9, 20, 27). Singlet oxygen is a reactive species for which the cell has little detoxifying machinery; consequently, multiple sites of cellular damage are possible. The 2 predominant sites of singlet oxygen damage are cell membranes (1, 16) and intracellular organelles (3, 10). However, local hyperthermic effects (19) and vascular damage (4) may contribute to the in vivo cytotoxicity of PRT. Porphyrins typically absorb strongly in the region of 400 nm (18); however, in PRT of solid tumors, 630 nm wavelength is commonly used for better tissue penetration and greater tumoricidal effect (13, 17, 26).

PRT has been demonstrated to be effective in the treatment of a number of human (2, 5, 6, 14, 15, 28) and experimental animal tumors (7, 11). In all of these studies, only solid tumors have been treated, the HPD was administered 24 to 48 h prior to treatment to take advantage of apparent preferential tumor sequestration, and the light beam was directed to the tumor to minimize normal tissue exposure.

The successful treatment of human ovarian carcinoma is an ongoing oncological challenge. Although this tumor almost invariably responds to chemotherapeutic drugs, response is often only partial or of brief duration (29). The eradication of the microscopic or small residuals of peritoneal disease following aggressive frontline chemotherapy remains one of several unresolved problems. Since deposits of ovarian carcinoma are usually limited to the peritoneal cavity, there have been several trials studying the efficacy of i.p. chemotherapy, either as part of initial therapy in patients with small residual disease following surgical extirpation or following second-look laparotomy (29). Results of i.p. chemotherapy for removing superficial ovarian carcinoma from the peritoneum are considered encouraging, and trials are ongoing.

The present study was initiated to determine the therapeutic potential of PRT in treating the peritoneal cavity of mice containing OECC, an ascitic tumor usually limited to the peritoneal cavity (8). In order to treat such tumor deposits dispersed throughout the peritoneal cavity with local phototherapy, invasive laser fiber optic technology was necessary. We report here our results with PRT in the treatment of a murine tumor limited to the peritoneal cavity.

MATERIALS AND METHODS

Animals and Tumors. Two- to 3-month-old female C3HeB/FeJ mice (The Jackson Laboratory, Bar Harbor, ME) were used. A spontaneous embryonal ovarian carcinoma originating in a C3H mouse (6) has been maintained by serial i.p. passages every 3 weeks. This is an ascitic tumor that rarely metastasizes and has tumor deposits on the peritoneal surface which are always smaller than 3 mm diameter. Ascitic fluid was withdrawn from the abdomen through a 19-gauge needle, cells were counted, and the fluid was diluted with F12 nutrient mixture containing 10% fetal calf serum. Prior to the i.p. injection of tumor, $2 \times 10^6$ cells were suspended in 0.25 ml nutrient mixture and injected into the abdomen through a 25-gauge needle. On Day 9 after transplantation, all mice had clinically evident ascites and weighed 2 to 4 g more than the control mice. By Day 25 after transplantation, all untreated mice died from tumor; on the day of death, the ascitic tumor weighed from 20 to 25 g.

Drugs. HPD-Panotrin was purchased from Photofrin Medical Inc., Cheektowaga, NY. Each animal received i.p. 2 h before treatment with light 50 mg HPD/kg body weight dissolved in 0.25 ml aqueous solution such that the injection volume was constant. For animals not treated...
with HPD, 0.25 ml of phosphate-buffered saline without HPD was injected i.p. 2 h before treatment.

PRT Procedures. Tumor was transplanted into 68 mice. On Day 9, two groups of 16 mice were randomly selected to be treated. Two h after the administration of HPD, an anesthetized mouse was placed on its back in a transparent acrylic holding device. The holder was built to support the animal's back and steady the abdomen by restraining the rear legs and the chest. An opening in the holder enabled easy access to the anterior abdominal wall. Light was introduced into the peritoneal cavity by a single 125 μ-diameter optical fiber (Corning Glass Works, Corning, NY). A Lexel Model 65 35-mwatt argon ion laser (Lexel, Palo Alto, CA) operating at 514 nm was focused through a ×10 microscope objective onto the fixed proximal end of the optical fiber. The free distal end of the optical fiber was enclosed in a 15-cm sleeve of 30-gauge stainless steel hypodermic tubing. The distal end of the optical fiber was cemented into place within the tubing such that the distal tip of the fiber was flush with the distal end of stainless steel tubing. To the utmost distal tip of the stainless steel tubing was applied a dome of scattering epoxy. The distal end of the tubing was cleansed with 95% ethanol before each insertion of the optical fiber into the peritoneal cavity. The anterior abdominal wall was cleansed with 95% ethanol prior to insertion of a 22-gauge syringe needle. Once the peritoneal cavity had been entered by puncture with the 22-gauge needle, the 30-gauge stainless steel tubing containing the optical fiber was inserted into the peritoneum through the 22-gauge needle. Following this, the optical fiber was left alone in the peritoneum by withdrawing the 22-gauge syringe needle over the 30-gauge stainless steel tubing. The stainless steel tubing allowed for ready mobility of the optical fiber approximately 1 cm within the mouse peritoneum. The abdomen was arbitrarily divided into octants. Each octant was irradiated with laser light for 2 min. Total time of laser treatment to the entire peritoneum was 16 min. Typically, 10 mwatt total output prior to spherical dispersion, as measured by a Spectra-Physics meter, Model 401 (Spectra-Physics Inc., Mt. View, CA) were delivered to the animal through the optical fiber (total energy delivered was 9.6 J for 16 min). The average time for restraining the mouse in the holder, laser treatment, and removal of the treated mouse was 25 min. Thirty-two mice were treated on Day 9 after tumor transplantation; of this group, 15 were retreated on Day 15. The control for this study consisted of 3 groups; each group contained 12 tumor-bearing mice. One group received no treatment, one group received HPD alone, and one group received laser light treatment without HPD. All mice were weighed and counted daily.

RESULTS

Survival. Chart 1 shows the survival of the treated mice in comparison to the 3 control groups. The mice that had no PRT, just HPD, or laser treatment started to die from tumor on the 20th day after tumor transplantation, and by the 23rd day, all were dead in these control groups. HPD or light alone did not alter the natural growth of the tumor as seen by the identical survival in the 3 groups. Eight of 32 mice treated with both HPD and laser light also died by the 23rd day. Four of these deaths were procedure-related deaths. From Days 24 to 34, an additional 17 mice from the PRT group died from tumor. Although these 17 mice survived longer than the control group, they had only a partial response to PRT, and no attempt was made to salvage them by retreatment. This approach resulted in cure of one mouse in the single PRT group; but by contrast to the control and the once-treated mice, 6 of 15 mice that were treated twice with PRT had a complete response and have had no evidence of recrudescence of tumor 3 months after completion of the second course of PRT.

Tumor Response. Response of the mice to the treatment was defined daily based on the size of the abdomen and weight of the animal. Within 72 h of the first treatment, 3 mice died of treatment-related deaths. Of the 29 mice that survived beyond 72 h after treatment with both HPD and laser light, 26 of 29 had a marked reduction in abdominal girth. One half of these returned to weights similar to those of normal mice that had no tumor transplant. In most of the mice that initially responded to HPD and light, minimal relapse was detected between the third and fourth day after treatment. By the fifth day, clear progression of the tumor was obvious in all but 3 mice. By the sixth day, 23 of 29 mice had between 2 to 4 g of tumor/mouse. Each of the 3 mice that had not responded initially had between 7 to 10 g tumor. Three mice had normal weights, and as noted above, 3 mice had died within 72 h of treatment. On Day 15 after inoculation of tumor and 6 days after the first PRT, one group was treated a second time. Using the same technique, 15 mice were included in this group (the 16th mouse in this group had died after the first treatment). Seven mice of this twice-treated group achieved a complete remission; one of this group of complete responders died 3 days after the second treatment. Eight mice of the twice-treated group had minimal or no response. None of the 24 mice within the 2 control groups that received either HPD or laser alone showed any difference in response when compared to the group of mice that received no treatment.

Toxicity. In this experiment, i.p. administration of light was given to 44 mice. The treatment resulted in death in 5 mice. There were 4 postoperative deaths in the mice given injections of 50 mg/kg followed by HPD and light. Three of these died within 72 h after the first treatment; the fourth died 72 h after the second treatment with light and HPD. Autopsy was performed in only one of these 4 mice and revealed perforation of small bowel as the probable cause of death. One of the control mice that had laser treatment alone also died 48 h after treatment. None of the mice died from treatment with HPD alone.

DISCUSSION

Ovarian carcinoma continues to be a clinical challenge. In the United States, it is the leading gynecological cancer, the fourth cause of cancer-related deaths in women, and each year kills more than 11,000 women (29). The 5-year survival is considerably worsened for that group of women who are discovered to have ascites or penetration of the ovarian capsule at the time of initial diagnosis. Usually, this tumor responds initially to aggressive systemic chemotherapy (29); unfortunately, the responses are often only partial or ephemeral. Minimal early distant metastasis and frequent peritoneal spread are 2 aspects of the unique biology of this tumor that have led to a search for an effective means of local treatment of the peritoneum. Clinical trials of i.p. cytotoxic chemotherapy for ovarian cancer are ongoing. So far, only minimal benefit has been demonstrated, and at this time, more effective agents are being sought. Since early peritoneal spread of human ovarian carcinoma is characterized by tumor cells suspended in ascitic fluid and small surface implants, we have chosen to study OECCs which have similar characteristics (8). As an alternative to simple i.p. chemotherapy, we have explored the possibility of utilizing a chemical-physical means of treatment using HPD and laser light. Our results with PRT, showing a 90% response with a single treatment and a 38%
Certain components of PRT are critical to its success. A treatment modality for eradication of ascitic or plaque-forming tumors of the peritoneal cavity, 3 basic requirements must be met: (a) the light used should be delivered in such a manner that all the volume space inside the abdomen, including those cloistered regions which are apparently secluded behind and between internal structures, will be exposed to light; (b) the amount of light and HPD used in one treatment should be sufficient to destroy all viable tumor cells. Even if this ideal situation were not attained within one treatment, substantial tumor killing should have occurred, allowing for complete eradication of the remaining viable tumor by a second course of PRT delivered soon after the first; and (c) there should be minimal, or no, normal tissue injury.

In order to achieve success in the treatment of an ascitic tumor, an integration of the different PRT components is required. The variables that must be dealt with are: (a) delivery of the sensitizer; (b) tumor versus normal tissue uptake and sequestration of sensitizer; (c) timing of light treatment after the drug has been given; (d) wavelength of light to be used; (e) intensity of illumination; (f) duration of illumination; (g) elastic versus inelastic scattering of light within the peritoneum; (h) normal tissue injury secondary to puncturing of the abdominal wall and intraabdominal organs; (i) aseptic techniques; (j) ability to immobilize the animal for the duration of light treatment; and (k) the number and frequency of treatments needed to eliminate all tumor cells. Any of these components may be critical.

Since OECC is an ascitic tumor, cells are in suspension, and exposure to higher concentration of dye sensitizer can be achieved with the use of i.p. rather than with i.v. drug administration (24). In the case of a large solid tumor in the peritoneal cavity, i.v. administration or a combination of both i.p. and i.v. administration may well be preferable. A cautionary note should be made. In addition to potential drug toxicity with higher concentration of the sensitizer, the dye sensitizer concentration becomes extremely high, the complex issue of dye aggregation may cause light extinction, thus decreasing the generation of singlet oxygen and cytotoxicity. Simply stated, the dictum of chemotherapy that the more drug that can be given safely the greater its efficacy does not necessarily hold true for i.p. PRT.

In a typical solid tumor, transport of dye sensitizer is mainly dependent on the vascular supply, and usually, PRT is delayed for 24 to 48 h to maximize tumor sequestration and minimize normal tissue retention (12). In contrast to a solid tumor, an ascitic tumor, drug delivery is probably independent of vascular supply, and in many ways closely resembles an in vitro Petri dish tissue culture system. Since OECC used in this study resembled our in vitro tissue culture model (23), a 2-h time period after drug exposure was chosen as ideal for laser light treatment. The choice of laser treatment 2 h after i.p. injection was further supported by evaluation of OECC intracellular porphyrin concentrations (data to be published elsewhere).

In most studies of solid tumors, the wavelength of light used in PRT is 630 nm (17). The reason for this choice is that longer wavelengths of light provide better penetration of light into the tumor bed. For this ascitic tumor, it is nonvascular and mainly in suspension, and the depth of tissue penetration is not as important as it is in solid-tumor therapy; on the other hand, light dispersion and strong HPD absorbance are important. The greater the quantity of light absorbed by HPD, the greater the production of singlet oxygen; hence, the greater the cytotoxicity. The OECC because of its rapid rate of tumor growth necessitates large tumor population kill with each treatment. If large quantities of tumor are not killed with each treatment and the time of the second treatment is delayed, the tumor will have grown to at least the population of cells that were present initially; hence, no progress will be made.

Absorbance of light by the tumor is directly proportional to the intensity and overall time used for treatment of the animal's
peritoneum. The limiting factor in the application of high-intensity light is the heat generated by increasing laser power. This increase in heat increases the chance to damage normal tissue. A means of minimizing this damage is to use lower light intensities for longer periods. Obviously, there is a practical limit to the time that any one mouse can be treated. We arrived at several parameters used in this study by trial and error and feel that for ease of use and expenditure of time and money, an argon laser emitting light at 514 nm delivers sufficient light to kill suspended tumor cells in the peritoneum.

A plausible explanation for the initial failure of 3 mice to respond to PRT treatment is that the light intensity was not great enough for the volume space treated. In the growth of ascitic tumors, for a linear increase in the abdominal radius, the volume space increases cubically. Since diminution of light intensity through a nontransparent medium is a function of the light path, there appears to be a limited time window for optimal tumor treatment. Beyond this given time point, the increasing density of cells within the ascitic fluid may severely restrict light transmission. Two other possibilities for the lack of response in the 3 mice in the HPD-plus-light group are (a) the drug was not injected into the peritoneum but rather into s.c. space or into the mouse’s viscera, and (b) the tumor population may have outgrown its oxygen supply and become hypoxic. Hypoxia would result in both the lack of oxygen (a necessary component for photodynamic action) (23) and the possibility of changes in pH that might alter the pharmacodynamics of HPD sequestration or HPD intracellular metabolism.

In a peritoneum containing ascites, both inelastic and elastic light scattering are possible. Inelastic scattering diminishes the energy of the light and hence lessens the efficacy of treatment. On the other hand, elastic scattering does not lessen light energy, but it allows the light to scatter into regions that could not otherwise be reached. Examples of these cloistered regions are the hepatic diaphragmatic surface and the area distribsing the intestines. In addition to our depending on the light-scattering properties of the ascitic fluid, a dispersive compound affixed to the distal end of the optical fiber allowed for large arcs of radiant light. We have not studied thoroughly the effect of varying geometry of the distal tip dispersion. If the scattering properties of the ascitic fluid were sufficient, there would be little need for a dispersing tip.

In this study, there were several animal deathsc associated with treatment of the peritoneum with laser light. The only animal suitable for autopsy revealed bowel perforation as the probable cause of death. The PRT technique used in this study required a 22-gauge needle to be inserted blindly into the peritoneal space. It is also possible that movement of the 30-gauge hypodermic tubing damaged internal organs. If the intestine were to be punctured by the 22-gauge needle or torn by the fiber-optic device, the fluid would provide an ideal milieu for bacterial growth and subsequent sepsis.

This preliminary study demonstrates a clear improvement in both response and survival of mice bearing the OECC tumor. Although all but one responder ultimately relapsed after a single treatment of PRT, we have demonstrated that 2 spaced PRT treatments significantly improve cure rate. We believe that the moderate number of treatment-related deaths were caused by mechanical injury to the internal organs, especially bowel perforation, rather than to toxic effects of the HPD and/or the laser light; these technical problems will be more easily circumvented in larger experimental animals or human beings. PRT studies that are in progress or planned include further tests with mice aimed at optimizing light wavelength and intensity, duration of treatment, number and frequency of treatments, and with larger animals the determination of normal i.p. tissue tolerance. We believe that PRT has potential as part of a combined modality approach in the treatment of human ovarian cancer and gastrointestinal cancers confined to the peritoneal cavity, and mesothelioma of the pleural space. We intend to investigate these possibilities upon completion of our planned animal experiments.

ACKNOWLEDGMENTS

We thank Terence R. O’Donovan of the Animal Resources Program, Division of Cancer Biology and Diagnosis, NIH, for advice and care of animals used in this study.

REFERENCES


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