Intraperitoneal Hyperthermic Treatment of Implanted Peritoneal Cancer in Rats

Man H. Shiu and Joseph G. Fortner

ABSTRACT

The feasibility and efficacy of treating peritoneal cancer implants by applying heat to the peritoneal surfaces were studied in inbred Buffalo A rats given i.p. injections of Morris hepatoma 5123TC tumor cells. Heat was delivered to the peritoneum by contact with a heated physiological salt solution (Normosol-R) in the peritoneal cavity. A treatment temperature of 43.3 ± 0.3°C was maintained for 30 min by an immersed stainless steel coil through which hot liquid circulated. Rats implanted with 0.5 to 1.0 × 10⁶ tumor cells were treated at 1 to 4 hr (Group I), 4 to 5 days (Group II), and 22 to 24 days (Group III) after tumor implantation to simulate treatment for the clinical conditions of surgically spilled cancer cells, established microscopic cancer implants, and macroscopic cancer implants, respectively. A statistically significant improvement in survival was observed in Groups I and II compared with sham-treated control animals; 58% of the heat-treated animals were cured. Only a slight but statistically insignificant improvement was noted in Group III. These observations indicate that i.p. surface heat treatment of peritoneal implanted cancer is feasible and effective.

INTRODUCTION

Peritoneal implants of cancer are frequently encountered in the clinical management of intraabdominal cancer. In patients who undergo surgical resection for cancers of the stomach, pancreas, colon, and ovary, such implants may be the only cause of treatment failure. These implants may develop from microscopic foci of transperitoneal metastases already present, but not detectable at the time of initial therapy, or from cancer cells spilled during surgical resection of the primary neoplasm when numerous vascular and lymphatic channels are transected. Once established, peritoneal implants result in considerable morbidity and eventual mortality as they lead to intractable ascites, gastrointestinal obstruction, and further tumor proliferation. Currently used chemotherapy and radiation therapy have shown little success in preventing or treating such implants. Because in their incipiency these implants are microscopic and located entirely on, or immediately under, the peritoneal surface, direct contact treatment with heat would seem to be feasible.

The selective killing effects of heat on many types of tumor in animals (1, 3–6) and in humans (1, 9, 11, 13, 16) have been demonstrated. The topical application of heat in the form of a wet bath has also been used in the treatment of human bladder cancer (2) and tumors of the skin and s.c. tissues (11).

In order to study the feasibility of surface heat treatment of peritoneal cancer implants, we devised a simple mechanical heat delivery system and tested its efficacy using a transplantable tumor model in the rat.

MATERIALS AND METHODS

Heat Delivery System. A thermostatically regulated water bath (50–55°C) served as the heat source, while a separate closed system of fluid (tap water) delivered the heat as it circulated by pump through tubing and stainless steel coils (Chart 1). The peritoneal cavity of the treated animal was filled with a balanced physiological salt solution (Normosol-R; Abbott Laboratories, Chicago, Ill.); each liter of the solution contained 140 mEq sodium, 5 mEq potassium, 3 mEq magnesium, 98 mEq chloride, 27 mEq acetate, and 23 mEq gluconate. This solution had a pH of 6.2 which promptly rose to a physiological range upon contact with the peritoneal surfaces prior to heat application. A plastic collar sutured to the abdominal opening permitted the entry and exit of tubing and temperature probes and prevented the escape of the solution. The stainless steel coils acted as heat exchangers; the circulating fluid picked up heat in the water bath and released it into the solution which bathed the peritoneal cavity, thus heating the peritoneal surfaces. Rhythmic massage of the abdomen was necessary to ensure even heat distribution inside the abdominal cavity. By adjusting the depth of immersion of the large coil in the water bath, adjusting the pump speed, and the occasional use of an auxiliary cooling coil, the temperature of the i.p. solution could be maintained within ±0.3°C of the selected treatment temperature.

Temperature Measurement. The i.p. fluid temperature of treated animals was continuously monitored in 2 locations by a multichannel electronic thermometer using copper-constantan thermocouples (Model BAT-8c; Bailey Instruments, Saddle Brook, N. J.). This thermometer had a resolution of 0.1°C and an accuracy of 0.1°C as checked against a National Bureau of Standards thermometer in the range of 42–44°C.

Tumor Model. Transplantable Morris hepatoma 5123TC (7, 14, 17) was obtained from Dr. K. Sugihira, Sloan-Kettering Institute, Rye, N. Y. and maintained by serial passage every 5 to 7 weeks in inbred male Buffalo A rats purchased from Simonsen Laboratories, Gilroy, Calif. This hepatoma is capable of hematogenous dissemination and kills a rat at 100 to 120 days after i.p. implantation with massive tumor growth and intraabdominal hemorrhage. Prior studies in our laboratory had established the in vitro and in vivo heat sensitivity of this tumor in the range of 42–44°C. Implants s.c. of tumor made from cell suspensions incubated for 1 hr at 42°C showed delayed tumor development and no growth if incubated at 43°C. Established tumors on the dorsum of the foot, with a maximum diameter of...
3 mm, were destroyed by immersion in hot water at 43° for 1 hr, whereas larger tumors, up to 1 cm in diameter, showed incomplete but pronounced regression after treatment at 44° for 1 hr. To produce peritoneal implants in the rats of this study, a fresh tumor suspension was made for each set of experiments. Growing s.c. tumors not larger than 1 cm in diameter were harvested, minced in cold Eagle’s minimal essential medium, and passed through a No. 80 sieve. Cell clumps were gently dispersed by repeated drawing through a tuberculin syringe, and any remaining clumps were discarded by decantation. After centrifugation and resuspension in medium, the cells were examined for viability (10 to 20%) as indicated by trypan blue exclusion, counted, and adjusted to a concentration of 10⁸ per ml. Each rat was given an i.p. injection of 5 ml of 154 mM NaCl followed by 0.5 to 1.0 x 10⁸ tumor cells. The abdomen was massaged to promote the distribution of cells within.

**Heat Treatment and Aftercare.** The rats were maintained on standard laboratory feed and used for experiments when a weight of 300 to 400 g was attained. Prior to undergoing peritoneal heat treatment, the animal was anesthetized with ether and intermittent i.m. injections of ketamine hydrochloride (10 mg/kg). The abdominal cavity was opened through a midline incision and filled with 30 to 50 ml of Normosol-R solution after inserting the small stainless steel heat coil and the thermocouple probes. A separate low-rectal temperature probe inserted to a distance of 1 cm into the rectum registered the i.p. temperature whenever it tends to occur. The auxiliary cooling coil (Aux Cool) is used to prevent overshooting of the temperature whenever it tends to occur.

**RESULTS**

**Function of the Heat Delivery System.** This relatively simple mechanical setup proved to be sufficiently powerful and flexible in providing the heat transfer necessary to maintain the selected treatment temperature in the peritoneal cavity. Complete immersion of the stainless steel coil in the water bath heat source was needed to obtain the maximum quantity of heat during the first few min of treatment in order to reach the desired temperature. Afterwards, the coil had to be raised considerably to prevent excessive heat transfer and overshooting of the i.p. temperature. From time to time during treatment, perhaps from periodic intensification of the physiological vascular response to heat, the temperature would drop, and more heat had to be delivered to bring it back to the desired range. The process can be automated, but for the purposes of the experiments herein reported, the temperature was regulated manually and without difficulty within the stated range of 43.3 ± 0.3°. Constant massage of the abdomen was found to be very important in preventing uneven heating within the peritoneal cavity; without this effort, the peritoneal recesses would rapidly show a temperature difference of 1° or more as compared with the central abdomen. Using a fine flexible temperature probe inserted under the peritoneal surface through an 18-gauge needle, limited observations were made on the temperature gradient beneath the heated surface. It was found that the temperature drop varied considerably with the site and anatomic structure tested, as well as the general trend of the intraabdominal temperature. In general, however, a drop of as much as 1° at 1 mm deep was observed in the abdominal wall and of as little as 0.2° in the intestinal wall.

**Complications of Peritoneal Heat Treatment.** Eleven % of the heat-treated rats died during treatment, 20% died within the next 24 hr, and another 11% died without visible signs of tumor growth from 1 to 21 days after treatment. The deaths within 24 hr were associated with external features suggestive of circulatory collapse and internal findings of splanchic congestion. No attempt was made to ascertain the cause and mechanism of death in these studies. Other investigators have commented on the poor tolerance of rodents subjected to systemic whole-body or large-volume regional heat treatment (5, 6). Such a peculiarity may be the result of unusual thermoregulatory and pathophysiologic responses in these animals; preliminary, unpublished observations in our laboratory indicate that the dog can withstand peritoneal heat treatment much better.

**Survival and Cure after Heat Treatment.** The survival data of the 3 groups of rats are presented as survival curves in Chart 2. Each pair of curves shows the cumulative results of 2 separate batches of experiments, each with randomly selected treatment and control animals. Rats that died as a result of the treatment procedure and without visible signs of tumor develop-
temperature was not reached in the center of these nodules. With microscopic implants, 58% of the heat-treated rats (Groups I and II) were cured. The lack of complete efficacy against microscopic implants in this study suggests that while a therapeutically effective temperature was achieved for a critical duration of time in the entire peritoneal surface of the animals that were cured, for those that did not survive cancer cells must have survived because of nonuniform heat distribution in the peritoneal cavity. Despite vigorous abdominal massage during treatment, temperature differences of ±0.5°C were commonly observed in the various peritoneal compartments and recesses.

Selection of a higher treatment temperature could theoretically increase the cure rate by elevating the temperatures in even the most remote peritoneal recesses to above "threshold" level. An attempt was made to test this hypothesis, but the rats could not tolerate treatment at or above 44°C. In several pilot studies with various strains of rats including the Buffalo variety, we have found that few of these animals could survive such treatment at temperatures above 43.5°C, although dogs could.

An alternative approach might be to use chemotherapy or radiation therapy as an adjunct to heat. Because of synergism between heat and these modalities in their oncolytic properties (10, 12), it may be possible to obtain higher cure rates with temperatures lower than otherwise necessary if heat alone was used. Such synergism has been found with a number of animal and human tumors tested in vitro and has been applied to clinical use in the management of tumors of the skin and the extremity (11, 16). Work needs to be done to ascertain if this synergism can be similarly exploited for i.p. heat therapy to improve the cure rate and decrease the complications of treatment.

Manipulation of the pH of the i.p. fluid during heat treatment also merits investigation as an adjunctive measure. A low pH milieu has been shown to sensitize mammalian cells to heat damage (8). In the experiments herein reported, the instilled i.p. fluid had a pH of 6.2. Although this promptly equilibrated to a physiological level, some degree of heat sensitization could have occurred. Further studies with sustained i.p. acidity during heat treatment are necessary to define the possible value of such manipulations.

Heat therapy i.p. holds promise of possible clinical application in a variety of conditions: for the destruction of cancer cells spilled during abdominal cancer surgical procedures; as a method of adjunctive therapy after resection of cancers of the stomach, pancreas, colon, and ovary, which tend to develop peritoneal implant recurrences; and as definitive therapy for abdominal carcinomatosis with small cancer implants or pseudomyxoma peritonei. However, it would seem inappropriate at this time to consider a clinical trial of this experimental technique, as direct extrapolation of observations made in a single rat tumor model to the treatment of patients cannot be justified. We need detailed data on the heat sensitivity of human cancers that are suitable for such treatment. Extensive physiological studies are also needed to determine the short- and long-term side effects of peritoneal heat therapy of the various organ systems and anatomic structures inside the abdomen, preferably in large laboratory animals, so that safe time-temperature relationships can be established for normal tissues to serve as a guide to safe and ethical experimentation in a clinical setting. Furthermore, the prototype heat treatment apparatus which we have developed for the rat must be refined and

**DISCUSSION**

The observations made in these experiments indicate that the technique of i.p. heat therapy is feasible and that it is capable of destroying heat-sensitive cancer cells implanted on the peritoneal surface. The method was ineffective against macroscopic nodules of tumor, probably because a therapeutic
modified for use in a large subject. In order to obtain adequate and safe heat delivery with uniform heat distribution on a much larger peritoneal surface, electronic automation and more efficient means of heat transfer will be necessary.

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