Experimental and Clinical Observations on Hepatic Cryosurgery for Colorectal Metastases

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ABSTRACT

Cryosurgery, using liquid nitrogen at −196°C, was explored for treating colorectal liver metastases in an experimental study of rat colon cancer and in a clinical investigation of patients with unresectable liver tumors. The viability of rat colon cancer isografts showed that while two or three freeze-thaw cycles were 100% effective in controlling established isografts and preventing isograft take, one freeze-thaw cycle was suboptimal. In these animals cryosurgery was as effective as surgical resection in controlling established experimental liver metastases.

Cryosurgery by operative liver exposure and intraoperative ultrasound monitoring were used to treat liver metastases from colorectal cancer in 24 patients. At a median follow-up of 2 years (range, 5 months to 5 years), seven patients (29%) were disease free, eight (33.5%) are alive with recurrent tumors, and nine (37.5%) have died. The patterns of failure were: remaining liver and extrahepatic sites, ten patients (59%); remaining liver only, six patients (35%); and extrahepatic only, one patient (6%). These data demonstrate that cryosurgery is a useful modality for treating unresectable primary and metastatic liver cancers. Addition of systemic adjuvant therapy may improve the tertiary failure following the control of liver metastases.

INTRODUCTION

About 157,000 new cases of cancers of the colon and rectum will be diagnosed in 1991, and colorectal cancer accounts for the second highest cancer-related mortality rate in the United States (1). Among the 60,000–70,000 patients dying from this disease each year, the liver remains the sole site of metastatic spread in about 20% (2, 3). Surgical resection, the only curative therapeutic option for liver metastases from colorectal cancer, is feasible in only about 5–10% of these patients (4). Systemic chemotherapy has not made any impact on the overall survival of patients with colorectal liver metastases. Fluorinated pyrimidines, the commonly used agents, produce a response rate of 20% at best, and the addition of modulators such as leucovorin has not improved the results (5). Despite the impressive response rates of 29–88% with the use of hepatic intraarterial 5-fluorodeoxyuridine, several prospective randomized trials have not shown any survival benefit when compared with systemic chemotherapy with 5-fluorouracil (6–9). Therefore, there is an urgent need for effective regional therapeutic options for the management of liver metastases from bowel malignancies.

Several novel strategies are being explored for the treatment of liver metastases, such as selective tumor ablation (using radioactive seeds, alcohol injection, cryoablation, monoclonal antibody infusion), hepatic irradiation, regional chemoperfusion, and liver transplantation. In situ ablation of tumors using subzero temperatures, called cryosurgery, has been in vogue for several decades as a treatment for surface malignancies (10, 11). The advances in technology and the sophistication of our ability to deliver liquid nitrogen and other cryogens deep into tissues with precise monitoring have evoked interest in applying this principle to treating hepatic malignancies (12, 13). Our previous studies have demonstrated the technical feasibility, safety, and antitumor effect of hepatic cryosurgery (14, 15). In this article, we report the results of an experimental study in a rat colon cancer model designed to establish the ground rules for performing cryosurgery in the management of human colorectal liver metastases. Furthermore, we have analyzed our data on the first 24 patients with liver metastases from colorectal cancer who have undergone hepatic cryosurgery, with respect to survival and patterns of failure.

MATERIALS AND METHODS

Animals and Colon Cancer Cell Lines. Male w/fu rats, 8–9 weeks of age, were used. The two colon cancer cell lines used in this study were poorly differentiated adenocarcinomas induced by 1,2-dimethylhydrazine, syngeneic to w/fu rats. W-1779 is a locally growing tumor, and W-1756 is a tumor exhibiting local growth and distant metastases.

Induction of sc. Isografts and Liver Tumors in Rats. Syngeneic tumor isografts were induced by placing 5-mm cubes of viable nonnecrotic tumors (W-1779 and W-1756) into sc. pockets fashioned over the medial aspects of the thighs in rats. The skin incision was closed by metal clip. For liver metastases experiments, W-1756 tumor (1 x 10⁶ cells) in 0.05 ml phosphate-buffered saline was injected under the liver capsule, after the liver was exposed by laparotomy. For all these experiments, the rats were anesthetized by ether inhalation.

Experimental Cryosurgery. Cryoablation was achieved by liquid nitrogen (−196°C) circulated through insulated probes (CS76 system; Frigitronics, Inc., Shelton, CT). Disc or trocar pointed probes were placed either on or into the tumors, respectively, for cryoablation. An 8-min freeze followed by complete thawing of the tumor was used for each freeze-thaw cycle. Tumor growth in thigh isografts was measured twice weekly, and volumes were calculated as described by Attia and Weiss (16). For liver metastases experiments, 1 week following subcapsular liver injection of tumor cells, the rats' livers were exposed by laparotomy. In the resection group, a wedge of liver tissue containing the isografted tumor was resected, and hemostasis was accomplished by approximating the liver edges with 3-0 chronic catgut sutures. In the cryoablation group, two freeze-thaw cycles were carried out to cryoablate the tumor and a rim of surrounding normal liver.

Patients and Eligibility. For all patients considered for cryosurgery, a complete history was taken, a physical examination was performed, and baseline laboratory tests were done within 4 weeks of the surgical procedure. These tests included a complete blood cell count; coagulation profile; liver function tests and quantitation of blood electrolytes; urea nitrogen, glucose, and creatinine; and carcinoembryonic antigen. CT scans of the abdomen and chest and a chest X-ray were also performed. All patients were over 18 years of age and had histologically confirmed colon or rectal cancer with radiographically evident liver metastases. There was no evidence of extrahepatic tumors by preoperative evaluation. The hepatic tumors were considered unresectable because of bilaterality, anatomical location, or the patient's comorbid conditions precluding a high-risk liver resection.

Operative Technique. A right subcostal or "chevron" incision was used for liver exposure. After the standard exploration to rule out...
extrahepatic disease, the liver was examined bimanually and released of its igamentous attachments. The entire liver was scanned using an Intraoperative Ultrasound Unit (OR 330 Technicare; or Toshiba Sonolayer, SSA26), with 5.0- and 7.5-MHz linear array transducers. Hepatic cryoablation was achieved by probes of trocar or disc design, of 8-, 10-, or 12-mm diameter. Our probes have insulated shafts, with cold tips of varying sizes for the creation of iceballs of different diameters. The cryogen used was liquid nitrogen, circulated by a cryosurgical system (CE4; Frigitronics, Inc.; or ERBE, Tubingen, Germany; or LCS 2000 System; Cryotech, Ltd., Derbyshire, England). The probe was placed into the tumor under ultrasound guidance, avoiding injury to major blood vessels or bile ducts. The liver was then insulated from surrounding structures to protect contiguous viscera from accidental freezing and necrosis. The freeze-thaw process was then carried out under ultrasound guidance. The freezing took approximately 8 min to encompass the tumor, and the thawing process took approximately 10–20 min. Two to three freeze-thaw cycles were carried out for each lesion. At the completion of treatment for each lesion, the probe was withdrawn, and the tract was packed with Surgicel or Gelfoam to minimize bleeding.

Follow-up Response Evaluation. In addition to the routine postoperative care, the therapeutic response to cryosurgery was monitored by CT scan, ultrasound examination of the liver, and carcinoembryonic antigen/liver function tests at 1 week, 6 weeks, 3 months, and every 3 months thereafter; CT scan and ultrasound were performed at 1 week, 3 months, 6 months, and every 6 months thereafter.

Statistical Methods. Statistical analysis of animal experiments was carried out using the χ² test and analysis of variance. Kaplan-Meier survival curves were used to evaluate patient survival following hepatic cryosurgery.

RESULTS

Effects of Cryosurgery in Destruction of Established Rat Tumor Isografts. The aim of the first set of experiments is to evaluate the efficacy of cryoaublation in controlling exponentially growing rat colon cancer isografts. Bilateral thigh isografts were established by implantation of 5-mm cubes of W-1779 tumors in w/fu rats (n = 3). Two weeks after implantation when the tumors were 2.5 cm in diameter, the tumor on one side in each rat was exposed and subjected to 3 freeze-thaw cycles. The skin was closed over the treated tumor. The tumor on the contralateral side served as the control in each animal. Three weeks later (Fig. 1), the tumors on the control and treated sides were harvested and subjected to light microscopy. Complete necrosis was demonstrated in all three tumors subjected to cryoablation, while the contralateral exponentially growing control tumors showed viable tumor cells (Fig. 2).

In an effort to document the effects of cryosurgery in a more aggressively growing tumor, and to evaluate the relative efficacy of various freeze-thaw cycles, W-1756 tumor isografts (n = 9 animals) were used. One week after tumor implantation, when the tumors were 2.5 cm in diameter, the animals were randomized to receive 1, 2, or 3 freeze-thaw cycles for tumors on the experimental side, while the contralateral tumor in each animal served as the control. Three weeks posttreatment, the tumors on both the experimental and control sides were harvested. Light microscopy revealed viable tumor cells in only one tumor subjected to one freeze-thaw. As in the previous experiment, all control tumors exhibited exponential growth and viable tumor cells by light microscopy.

Pretreatment of Tumors and Isograft Growth. In this experiment, freeze-thaw was carried out on viable tumors before implantation, to observe the isograft growth potential. Five-mm cubes of W-1756 tumor were cut from nonnecrotic viable parts of tumor isografts. The tumor cubes were subjected to 1, 2, or 3 cycles of freeze-thaw (by immersing the liquid nitrogen and thawing at room temperature) and isografted s.c. into w/fu rat thighs (n = 9 animals; 3 animals each for 1, 2, or 3 freeze-thaw cycles). Viable tumor isografts of similar size that were not subjected to freeze-thaw were implanted into the contralateral thighs to serve as control tumors in each animal. Isografts were monitored twice weekly for tumor growth. Progressive tumor growth was seen in all control tumors [21.3 ± 4.6 (SD) cm² at 4 weeks]; 8 of 9 cryoablated tumors demonstrated no tumor growth (P < 0.001). Only one tumor (0.8 cm²) was detected in the one freeze-thaw group.

Liver Metastases Experiments. One week after subcapsular liver injection of 1 × 10⁶ cells of W-1756 tumor, the animals were anesthetized and the liver was exposed by laparotomy. The tumors measured 5–8 mm in diameter. The animals were randomized (n = 6 in each group) to (a) no treatment (sham laparotomy); (b) resection of liver tumor; or (c) cryoablation of liver tumor by 2 freeze-thaw cycles. Five weeks after subcapsular injection the rats were sacrificed by euthanasia and examined for local tumor control in the liver and distant metastases to the lung. All six control animals had large liver tumors measuring 5–6 cm in diameter and multiple lung metastases (Fig. 3). Both resection and cryosurgery achieved local control of tumors in the liver in 5 of 6 animals; there was no difference in the incidence of lung metastases (5 of 6 and 6 of 6, respectively) as shown in Table 1.

Patient and Tumor Characteristics. A total of 24 patients with colorectal cancer with subsequent development of liver metastases were subjected to cryosurgery during a 5-year period, 1985–1990. There were 15 males and 9 females. The median age was 67 years, with a range of 36 to 81 years.

Histologically, all tumors were moderately differentiated adenocarcinomas. Twenty-two of twenty-four patients had elevated carcinoembryonic antigen levels. All patients had a normal preoperative liver function profile.

The patients tolerated hepatic cryosurgery well without any operative mortality. Significant morbidity was encountered in two patients (8.3%): (a) one patient developed a supphrenic abscess, which was drained percutaneously under CT guidance; (b) a second patient developed partial wound dehiscence on the fifth postoperative day, requiring closure under general anesthesia.
Fig. 2. Photomicrograph of representative resected material from animals with W-1779 isografts. Left, viable tumor on the control side; right, biopsy material from the cryoablated side demonstrating fibrosis and coagulative necrosis with inflammatory cells, without viable tumor. H & E, × 125.

Fig. 3. Experimental rat liver metastases. Representative autopsy pictures of rats 5 weeks after tumor induction. The animal on the left is a control with a large liver tumor. The middle animal has a scar in the liver after cryosurgery but no liver tumor. The animal on the right shows no liver tumor following resection. Note the appearance of lung metastases in all the animals.

Table 1 Experimental rat liver metastases: animals with liver/lung recurrence following therapy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver recurrence (n)*</th>
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</thead>
<tbody>
<tr>
<td>Control sham laparotomy</td>
<td>6/6</td>
</tr>
<tr>
<td>Resection</td>
<td>1/6</td>
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<tr>
<td>Cryosurgery</td>
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* n = 6, each group. P, no significance, resection versus cryosurgery.

Monitoring of Tumor Response, Survival, and Patterns of Failure. The usefulness of intraoperative ultrasound in detecting liver metastases less than 1 cm in diameter, providing a 2-dimensional view of anatomical landmarks for safe cryoprobe placement and monitoring the progress of freeze-thaw cycles during cryosurgery, has been reported previously (14, 17, 18). Fig. 4 provides an example of intraoperative ultrasound documenting the freeze front as a hyperacoustic rim with postacoustic shadowing. Typically, the freezing is continued until the hyperacoustic rim encompasses the tumor margin; the lesion is thereafter allowed to thaw at room temperature. The freeze-thaw cycle is repeated two or three times. Our initial 10 patients were treated using 3 freeze-thaw cycles for each lesion. Subsequently, backed by experimental data, we have used 2 freeze-thaw cycles as a standard. We have not noticed any difference in response rates between these two groups of patients.

The follow-up period ranged from 5 months to 5 years (median, 2 years). Nine patients have died (37.5%). CT scans (GE 8800 scanner with oral and i.v. contrast; General Electric Co., Milwaukee, WI) and blood carcinoembryonic antigen levels were useful in the serial evaluation of tumor response to cryosurgery. Seven patients (29%) are alive and disease free, and eight (33.5%) are alive with recurrent tumors. Failure pattern analysis revealed that only two patients (8%) recurred at the cryoablated site in the liver. One of these two patients underwent retreatment of the recurrence detected at the margin of previously cryoablated tumor at 15 months. He is alive and disease free at 21 months. The second patient is alive, with recurrent disease at the treated site and widespread recurrence in the peritoneal cavity, at 2 years following the initial treatment. Except for these two patients, all other recurrences (n = 17) were noted either in other parts of the liver or in extrahepatic sites. As shown in Table 2, a majority of patients (n = 10, 59%) had tumor recurrences both in the remaining liver and at extrahepatic sites (lung, peritoneum, bone). Appearance of new metastases in the remaining liver only was noted in six patients (35%), while one patient (6%) had extrahepatic failure.

DISCUSSION

Among the 70,000 patients with colorectal cancer at risk for liver metastases as part of their recurrence each year, the liver will be the only site of failure in about 20% (4, 19). Approximately 5000 patients will be potential candidates for surgical resection, which is the only curative regional treatment modality (4). No effective option exists for the remaining 10,000 patients with unresectable liver metastases. Systemic and regional chemotherapy have not been effective. Cryosurgery is one of the novel treatment strategies being explored for the management of unresectable liver tumors. The experimental
Cryosurgery can complement surgical resection, i.e., resection with minimal recovery, low complication rate, and ability to re-treat. Ablation of hepatic metastases from colorectal cancer that are otherwise resectable may be achieved using cryosurgery (21-25). Lowering tissue temperature below ~35°C, maintenance of tissue in the frozen state for several minutes (e.g., 3 min), and slow thawing were the most effective means of achieving a 100% tissue kill rate (24). Thawing should be complete before freezing is repeated; our study supports the view that at least 2 freeze-thaw cycles are necessary. Although we and others monitor the freeze front by ultrasonography, the temperature at the freeze front may be only 0°C to ~10°C. Thermal gradients of 10°C/mm or more are commonly obtained during cryosurgery (26). Future studies may therefore need even more precise monitoring by using thermocouples at the tumor margins to ensure a temperature of ~40°C or lower.

The predominant patterns of failure in the remaining liver and extrahepatic sites highlight two issues. First, it is not far-fetched to hypothesize that most if not all of these tumor deposits were present at the time of cryosurgery but were not diagnosed by any of our current staging modalities. Further developments in targeting agents (e.g., radiolabeled monoclonal antibodies) and improved imaging modalities (e.g., sophistication in intraoperative ultrasound probes and magnetic resonance imaging with new contrast agents) may enhance the resolution of micrometastases. Second, major improvements in curing patients after either cryosurgery or resection of liver metastases from colorectal cancer will not be made until more effective systemic chemotherapy can be applied in an adjuvant setting after the treatment of a regional recurrence.

In the past, one of the mechanisms purportedly mediating the systemic effects of cryosurgery for tumor control was immunological (27, 28). The release of antigens from necrosing tumors has been said to activate tumor-specific host immune responses. We did not notice any immune-mediated regression of contralateral thigh tumors in our experimental isograft study, nor did we note any reduction in lung metastases following treatment of liver tumors in our randomized liver tumor therapy experiment. It should be noted that our rat model system is not immunogenic, which, we believe, is analogous to human colorectal neoplasia, making neither system amenable to any immunotherapy approach used thus far.

| Table 2 Pattern of failure following hepatic cryosurgery for colorectal metastases |
|---------------------------------|--------|--------|
| Site of failure                  | No. of points | %      |
| Remaining liver and extrahepatic | 10      | 59     |
| Liver only                       | 6       | 35     |
| Extrathepatic only               | 1       | 6      |
| Total                           | 17      | 100    |

Data presented in this article provide some ground rules for hepatic cryosurgery and demonstrate that by extrapolation from the data obtained in animals, cryosurgery is perhaps equivalent to resection in achieving local tumor control in the liver. The clinical study provides the rationale for using this modality to ablate hepatic metastases from colorectal cancer that are otherwise unresectable due to multiplicity, strategic anatomical location, or the patient's comorbid conditions. Rapid postoperative recovery, low complication rate, and ability to re-treat new liver lesions are some of the added advantages. In addition, cryosurgery can complement surgical resection, i.e., resection of one lobe with cryoablation of a tumor in the other lobe of the liver.

Despite the enthusiasm in applying the principles of cryobiology to liver metastasis treatment, slow patient accrual and failure pattern analysis demonstrate that this technology is applicable to only a select group of patients. Failure at the treatment site occurs in 11% of the cases in animal studies and 8% in the clinical trial. Improvement in understanding the mechanisms of cryodestruction and better monitoring of temperature may eliminate local failure in the future. At present, known causes of tumor destruction by freeze-thaw are protean: cold shock injury, reduction of cell volume by osmotic dehydration, denaturation of vital cellular enzymes, perforation of cell membranes by intracellular ice crystals, and destruction of tumor microvasculature (20-23). In experimental systems using normal and tumor tissues, various freeze-thaw conditions have been tested to evaluate their relative efficacy in inducing cell death (21, 24, 25). Thermal gradients of 10°C/mm or more are commonly obtained during cryosurgery (26). Further studies may therefore need even more precise monitoring by using thermocouples at the tumor margins to ensure a temperature of ~40°C or lower.

REFERENCES

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