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Extravesical cryosurgical approach for VX2 bladder tumor in rabbits

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Abstract This study characterized the VX2 bladder cancer model in rabbits and tested the feasibility of treating bladder cancer by extravesical cryosurgery. After the growth characteristics of the VX2 bladder tumor model were determined, the VX2 tumor was inoculated into rabbits at the dome of the bladder. One week later, three freeze/thaw cycles were followed by immediate surgical repair. The control group underwent a sham operation without freezing. When the VX2 tumor is injected into the bladder wall, invasion and central necrosis occurred within 1 week, lymphatic metastases by 2 weeks, and lung metastases by 3 weeks after inoculation. By 4 weeks, all control rabbits had large VX2 tumors in their bladders and advanced lung metastases. Nine of the ten rabbits in the cryosurgical group had mild to moderate degrees of lung metastases, and six of them had relatively small local recurrences. One rabbit had no tumor in the bladder and only microscopic lung metastasis. The extravesical approach to cryosurgery employing bladder inversion is well tolerated. Cryosurgery exhibits modest efficacy in treating local tumors and delaying lung metastasis in this aggressive tumor model.

Keywords Cryosurgery · Rabbit bladder cancer model · VX2 tumor

Introduction

The choice between cystectomy and bladder sparing procedures in patients with locally advanced bladder cancer is controversial [5, 12, 23, 31, 33]. Combined-modality therapy aimed at organ preservation has been employed successfully in selected patients [13, 17, 27]. Nevertheless, complete bladder excision by radical cystectomy remains the most common treatment for locally advanced bladder cancer [10, 19]. Although radical cystectomy has the theoretical advantage of complete eradication of local disease, it is also associated with significant morbidity, mortality and functional compromise. Partial cystectomy offers the opportunity for bladder preservation but is associated with the complications of tumor seeding, and local recurrences may result from either residual disease or second primaries [18].

Cryosurgery can provide tissue destruction without the immediate disruption of the integrity of the treated tissue [21, 26] and has been used to treat both benign and malignant diseases [1, 2, 14, 32, 36]. Transvesical cryosurgical treatment of bladder cancer has been described [20, 28], but this open approach carries the risk of tumor seeding as a result of tumor manipulation in an open bladder.

In this study, we characterize a rabbit bladder tumor model using the VX2 tumor and describe an extravesical cryosurgical approach for the treatment of bladder cancer. All animals received humane care in accordance with the requirements of the United States Animal Welfare Act. The VX2 tumor is a highly malignant Shope virus-induced squamous cell carcinoma, that consistently produces tumors in the bladders of rabbits [15, 16, 24, 25]. To prevent delayed bladder rupture following cryosurgery, we used a bladder inversion technique. This extravesical technique avoids opening the bladder, thereby minimizing the risk of tumor seeding.
Materials and methods

Preparation of VX2 tumor cell suspension

The VX2 tumor was obtained from the thighs of New Zealand White rabbits 14 days after transplantation. The tumor was minced and treated with 1% collagenase for 1 h. The digested tumor was then successively passed through 18-gauge, 20-gauge, and 22-gauge needles. The cells were concentrated by centrifugation and resuspended at a concentration of \(1\times10^7\) cells/ml in minimum essential medium with glutamine, non-essential amino acids, and 10% fetal calf serum.

Tumor model

The rabbits were anesthetized by mask induction of 4% isoflurane in oxygen using high flow techniques. They were then endotracheally intubated, and anesthesia was maintained with 2–3% isoflurane in oxygen employing a semiclosed system. Isotonic saline was administered at a rate of 5 ml/kg per hour throughout surgery and during recovery from anesthesia.

The bladder was exposed through a low midline incision, and 100 μl of VX2 cell suspension (10^7 cells) was injected into the dome of the bladder using an extravesical approach with a 22-gauge needle. A successful injection produced a small bleb at the dome.

Cryosurgery

After anesthesia was induced, the bladder was exposed through a low midline incision. The VX2 bladder tumor was measured in two dimensions. The bladder was emptied by manual compression and then filled with 40–50 ml of air using a 25-gauge needle. A total of three freeze/thaw cycles were performed through an extravesical approach. The cryoprone containing liquid nitrogen was directly applied to the area of the bladder harboring the tumor. A freezing cycle was completed when the tissue was grossly frozen. Purse string sutures of 3–0 chromic were used to outline the frozen area after the first freeze. These sutures were used as a guide to mark the extent of the subsequent two freezes. After the three freeze/thaw cycles, the treated portion of the bladder was inverted using the previously placed purse string suture. The inverted tissue was reinforced with additional sutures of 3–0 chromic.

Sham operation

In the control rabbits, all the procedures including bladder inversion were the same as for the rabbits receiving cryosurgery except that the cryoprobe did not contain liquid nitrogen.

Pathological studies

Four New Zealand white rabbits were used to determine the growth characteristics of the tumor in the VX2 model. The rabbits were killed at 7, 14, 21, and 28 days after tumor implantation, and both local tumor growth and distant metastases were evaluated.

Four rabbits were used for acute pathological studies to determine the early effects of cryosurgery. One week after the animals were inoculated with the VX2 tumor, extravesical cryosurgery was performed. The rabbits were killed 6 h, 24 h, 48 h and 1 week after cryosurgery. Detailed pathological changes were investigated.

Evaluation of cryosurgery

Twenty rabbits were inoculated with VX2 tumor cells in the bladder dome (10^8 cells/100 μl). The rabbits were randomized into control and cryosurgery groups (n = 10 each), and both groups underwent surgery 7 days after tumor cell implantation. The animals were killed 4 weeks after tumor implantation and examined for local and metastatic disease. Bladder tumor volumes were calculated using the formula of a sphere \((4/3\pi r^3)\). Lung metastases were estimated using transparent grid paper covering the surface of the lung. Statistical analysis was by non-paired Student’s t-test.

Results

VX2 bladder tumor model

One week after bladder inoculation, the VX2 tumor cell suspension formed a 11×8 mm tumor in the dome of the bladder that invaded the bladder muscle and extended through the full thickness of the bladder wall. The iliac lymph nodes were free of metastasis on both gross and microscopic examination. The liver, kidneys, and lungs were all normal on gross examination.

Two weeks after bladder inoculation, the bladder tumor was 16×15 mm in diameter and exhibited central necrosis and invasion of adjacent tissue. The iliac lymph nodes were 2×3 mm in diameter and contained VX2 tumor. The liver, spleen, and lung remained grossly free of metastasis.

Three weeks after bladder inoculation, areas of necrotic tumor were noted along with ascites. Metastases were seen in the lymph nodes, the small and large intestines, the mesentery, and the lungs. The liver and spleen remained grossly free of disease.

At 4 weeks, the VX2 tumor was easily visible. The bladder tumor grossly invaded the anterior abdominal wall and the intestines. Multiple metastases were noted at the serosal surface of the small and large intestines and in the omentum. Extensive lung metastases were seen.

Acute changes after extravesical cryosurgery

Four rabbits were killed 6 h, 24 h, 48 h and 1 week after cryosurgery to observe acute changes following cryosurgery. The acute changes at 6 and 24 h are depicted in Figs. 1 and 2. Grossly, the infolded portion of the bladder appeared dusky within 2 days of cryosurgery and at 7 days was pale white. The reconstructed bladder wall on top of the inverted tumor appeared viable. Inversion of the treated part of the bladder resulted in the formation of a cystic mass within the bladder containing the necrotic VX2 tumor. Blood clots were seen in this necrotic tissue in some of the rabbits. Histopathologically, congested blood vessels, hemorrhage, and edema were noted in the areas with coagulative necrosis and in the adjacent tissue. There was no evidence of viable tumor on multiple histologic sections. No metastases were seen.

Evaluation of cryosurgery

At the time of treatment, mean tumor sizes in the experimental and control groups were 6.9 ± 0.2 and