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Research Report

Single Instillation of Hypertonic Saline Immediately Following Transurethral Resection of Bladder Tumor for Recurrence Prevention – A Phase I Study

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Abstract.
BACKGROUND: Urologic guidelines recommend perioperative instillation of chemotherapy after transurethral resection of bladder tumor (TURBT) to decrease tumor recurrence, yet implementation of this recommendation is partial due to associated morbidity. Hypertonic saline destroys cells by osmotic dehydration and might present a safer alternative.
OBJECTIVE: To evaluate the safety of 3% hypertonic saline (Hypersal) intravesical instillation following TURBT in rats and in humans.
METHODS: In 8 rats whose bladders were electrically injured, intravesical blue-dyed Hypersal was administered. We measured serum sodium levels before and after instillation and pathologically evaluated their pelvic cavity for signs of inflammation or blue discoloration. Twenty-four patients were recruited to the human trial (NIH-NCT04147182), 15 comprised the interventional and 10 the control group (one patient crossed over). Hypersal was given postoperatively. Serum sodium was measured before, 1 hour and 12–24 hours after instillation. Adverse effects were documented and compared between the groups.
RESULTS: In rats, average sodium levels were 140.0 mEq/L and 140.3 mEq/L before and following instillation, respectively. Necropsy revealed no signs of inflammation or blue discoloration. In humans the average plasma sodium levels were 138.6 mEq\L, 138.8 mEq\L and 137.7 mEq\L before, 1 hour and 12–24 hours after instillation, respectively. During the postoperative follow-up there was one case of fever. A month after the surgery, dysuria was reported by 5 patients while urgency and hematuria were reported by one patient each. The most severe adverse events were grade 2 on the Clavien-Dindo scale. Adverse events were similar in the control group.

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CONCLUSIONS: Hypersal instillation is safe and tolerable immediately after TURBT.

Keywords: Bladder tumor, intravesical instillation, hypertonic saline solution, clinical trial, phase 1, animal research

INTRODUCTION

Transurethral resection of bladder tumor (TURBT) is the gold standard procedure for the treatment and diagnosis of bladder tumors. While TURBT usually achieves adequate short-term local control, tumor recurrence is common with reported rates ranging from 27–71% [1]. To decrease recurrence, several bladder instillation protocols are commonly used. Perioperative instillation of chemotherapy has the proposed mechanism of action of destroying free-floating tumor cell and preventing their implantation after TURBT [2–4]. Studies evaluating this method have found that the recurrence rate decreased by 24–56% [5, 6]. However, even though immediate postoperative single instillation of chemotherapy is recommended by the American and European urological guidelines, the implementation of these recommendations is partial due to cost, logistic complexity involved with the treatment, and reports of uncommon but severe adverse effects [7–14].

Cell membranes are completely permeable to free water passage but are selectively permeable to various solutes. As a result, exposure of cells to hypertonic solutions forces water out of the intracellular space causing cell destruction by osmotic dehydration. Hypertonic saline is easily available, less toxic, and cheaper than intravesical chemotherapy. Therefore, should perioperative hypertonic saline instillation be proven safe and effective, it may become a more appealing alternative to chemotherapy instillation thereby increasing urologist compliance with postoperative single instillation treatment.

We conducted an animal trial followed by a human phase I clinical trial to evaluate the safety of immediate single instillation of 3% hypertonic saline (Hypersal) after TURBT.

MATERIALS AND METHODS

The animal model part of our research was approved by the Weizmann Institute of Science (Rehovot, Israel) ethical committee for trials in animals (approval number 28820716-2). A group of 8 female rats were anesthetized, a 22-gauge cannula was inserted into their bladders through which a 27-gauge wire was passed and used to electrically injure the bladder using the coagulation setting on 10 volts intensity simulating the injury done to the bladder during TURBT. One milliliter of blue dyed 3% hypertonic saline (Hypersal) was then immediately administered into the bladder and retained for 40 minutes. We measured the rats' serum sodium levels before Hypersal instillation and after bladder drainage. The rats were sacrificed 72 hours later, and their abdominal and pelvic organs were inspected for blue discoloration indicating bladder perforation and possible organ injury. Tissue samples from abdominal organs were examined by light microscopy for evidence of inflammation.

Based on encouraging results from our animal model, we continued with a phase I clinical trial in humans which was approved by our local institutional review board (approval number 0116-17-KMC) and registered in the national institute of health (NIH) clinical trial registry (NCT04147182). A total of 24 patients who underwent TURBT for low grade bladder tumors were included after obtaining their signed informed consent. We excluded patients with a history of high-grade urothelial carcinoma, with tumors suspected of being high-grade according to cystoscopy, with serum sodium abnormality preoperatively, and when bladder perforation was suspected during TURBT. Fifteen patients were treated post-operatively with Hypersal and included in the interventional group and 9 patients received no intravesical treatment and were included in the control group. One patient in the intervention group had a recurrence 21 months after being recruited and agreed to cross over to the control group for the second operation, rounding the control group to 10 patients total. Upon TURBT completion, the bladder of patients in the interventional group was drained and 40 ml of intravesical Hypersal were instilled and the urethral catheter was plugged. The bladder was drained 1 hour after instillation. Patients in the control group were left with a catheter at the end of surgery. None of the patients in both the interventional and control groups received any perioperative chemotherapy following resection, and further intravesical installations were given only upon completion of the study. Serum

sodium levels were measured immediately before, 1 hour and 12–24 hours after Hypersal instillation. Adverse effects in both groups were documented and graded by Clavien-Dindo Classification during post-operative hospital recovery and on outpatient follow up visit a month after the surgery. Pain and dysuria were evaluated using a visual analogue scale (VAS) for pain. Pathology results from the resection and recurrence data were recorded.

We used paired Mann Whitney U test to evaluate differences in serum sodium levels from baseline with a p-value of less than 0.05 considered significant. All statistical analysis was done using IBM SPSS version 25.

RESULTS

In the animal model, the average serum sodium levels were 140.0 mEq\L and 140.3 mEq\L before and after Hypersal instillation, respectively ($p = 0.451$). Following the intervention, all animals recovered well. Necropsy revealed neither macroscopic nor microscopic signs of inflammation or blue discoloration in the pelvic and abdominal cavities. Upon pathologic inspection, the bladders showed signs of focal thermal necrosis and hemorrhage, consistent with the electrical injury that was inflicted before Hypersal instillation.

In the human patients, TURBT was done for a solitary papillary lesion in 4 patients in the interventional group and in 3 patients in the control group. Multiple lesions were resected in 11 patients in the interventional group and 7 patients in the control group. None of the lesions were greater than 3 cm in diameter. The average serum sodium levels in the interventional group were 138.6 mEq\L, 138.8 mEq\L and 137.7 mEq\L (normal values 135–145 mEq\L) before instillation, 1 hour and 12–24 hours after installation, respectively ($p = 0.567$, $p = 0.183$ respectively). No cases of hypernatremia were observed. Three adverse events were reported in the postoperative hospital recovery period in the interventional group including: one case of fever treated with antibiotics and two cases of catheter or surgery related symptoms which resolved spontaneously. These adverse events were graded 1-2 on the Clavien-Dindo scale. Four patients in the control group suffered an adverse event in the postoperative hospital stay, all of which were catheter or surgery related. All symptoms were of grade 1 on Clavien-Dindo scale and all resolved spontaneously. At the follow up visit one month after surgery, 5 patients in the interventional group reported dysuria that persisted for 1–4 weeks postoperatively. Dysuria was mild to moderate in most cases, with a single patient who reported a grade 4/5 dysuria on the VAS score. One of the patients with dysuria also reported hematuria which resolved spontaneously without further intervention. One patient complained of urinary urgency which resolved with anti-muscarinic therapy. In the control group there were two cases of late adverse events recorded. One patient reported of dysuria VAS grade 2\5 which persisted for 2 weeks and resolved spontaneously while the other had urinary urgency requiring anti-muscarinic therapy. The patient who crossed over from the interventional group to the control group had no adverse event after being instilled with Hypersal but suffered from urinary urgency requiring treatment after the second operation, even though the tumor characteristics were similar in both operations. As shown in Table 1, the most severe adverse events in both groups were grade 2 on the Clavien-Dindo scale.

Table 1
Adverse Events Grading

Clavian-Dindo grade	Number of immediate complications		Number of delayed complications	
	Control group	Interventional group	Control group	Interventional group
1	4	2	1	4
2	0	1	1	2
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0

The pathology results following the TURBT in the interventional group included low-grade urothelial carcinoma in 14 patients and a high-grade carcinoma in another. In the control group, pathology revealed low grade urothelial carcinoma in 7 patients, no evidence of tumor in 2 patients and high-grade urothelial carcinoma in one patient. In all patients the resected tumors proved to be superficial with no invasion to either lamina porpria or muscle layer.

Of the 15 patients enrolled in the interventional group, 12 patients returned for follow up cystoscopy within 3 months of surgery, 14 returned within 6 months and one patient refused cystoscopic examination. 4 recurrences were found, one of them in the fossa navicularis. Two recurrences, including the one seen in the fossa navicularis, were treated with transurethral resection with low grade tumors found on pathologic examination in both cases, and 2 were small and were treated with BCG.

DISCUSSION

Because progression to muscle-invasive bladder cancer is rare in patients with low-grade tumors, the primary treatment endpoint in this group is recurrence risk reduction [1]. Perioperative single dose intravesical chemotherapy instillation has been associated with a 24–56% recurrence risk reduction in patients with low grade tumors [5, 6]. As a result, both the European and American Urological guidelines recommend the administration of a single dose of chemotherapy within 24 hours after TURBT (strong recommendation, evidence strength Level 1a and moderate recommendation, evidence strength grade B, respectively) [15, 16]. Despite this, compliance with guideline recommendations is partial, ranging from 0.33% to 44.3% in Europe and the United States [7, 17]. Reasons for non-compliance include fear from infrequent but significant side effects, logistic complexity involved with giving chemotherapy and its related cost [7–14]. Intravesical chemotherapy is typically associated with few side effects manifesting mainly with local irritative symptoms. This is due to the low solute absorption across intact urothelial lining. Transurethral resection of bladder tumors may compromise bladder wall integrity causing extravasation of the cytotoxic agent either through unobserved bladder wall perforations or through a large surface area of injured bladder wall. Despite its overall safety, perioperative intravesical chemotherapy has been associated with significant side effects including chronic pelvic pain, chemically induced peritonitis, fat necrosis, bowel obstruction and rare mortality [8–14]. These risks and the resulting low adherence to guidelines compelled us to seek for a safer alternative to perioperative chemotherapy. If found, not only would such treatment be safer, but it might improve surgeon adherence with guidelines leading in turn to better patient outcomes.

The putative mechanism of action of perioperative intravesical chemotherapy is the destruction of free-floating tumor cells released into the urine during TURBT [2–4]. Such free-floating tumor cells have been shown to implant into injury sites in the bladder wall, such as those created by TURBT [18]. Exposure of cells, especially free-floating cells, to either hypotonic or hypertonic solutions causes a shift in the intracellular water-solute equilibrium and causes their destruction through cell swelling and rupture or osmotic dehydration, respectively. This effect has been used during surgical removal of echynococcal cysts, wherein hypertonic saline is injected into the cyst to eradicate the live scolices within it and prevent possible dissemination during inadvertent spillage [19]. Several trials evaluating the efficacy of hypotonic saline irrigation after TURBT on bladder cancer recurrence showed promising results but these trials were small or retrospective, and so the treatment has not been accepted as a mainstream option [20–22]. One trial reported that continuous irrigation with hypotonic saline caused transient and clinically insignificant hyperkalemia, a result that might hint that continuous exposure to hypotonic saline leads to its absorption and results in hemolysis. We failed to find any clinical trials evaluating the effectiveness of hypertonic saline in the treatment of bladder cancer but did find several *in vitro* cell line studies that found hypertonic saline caused downregulation of adhesion molecules in lung and colon cancer cell, potentially inhibiting their ability to implant into new sites [23–25]. One study found that injecting hypertonic saline directly into hepatic tumors in a rabbit animal model improved overall survival and decreased tumor size [26].

Given the potential absorption of the irrigation fluid, as hinted by the transient hyperkalemia found in the study of P Bijalwan et al. [21], we wanted to limit the exposure of the patient to the agent chosen and decided to evaluate it in a single instillation protocol, as is commonly accepted with Mitomycin C. When considering whether to evaluated hypotonic or hypertonic saline as potential treatment option, we chose the latter for two main reasons. One consideration was that urine produced during treatment will change the solutions osmolarity, an effect that will be more pronounced when using hypotonic saline. The second reason was that if a dose-effect relation could be proven, hypertonic saline has virtually no upper limit to the osmolarity that can be achieved while hypotonic saline can only be as low as distilled water.

We therefore decided to evaluate immediate intravesical instillation of Hypersal after TURBT as an alternative to the current practice of perioperative intravesical chemotherapy. The initial phase in the development of any new treatment is to prove its safety. Consequently, this was the objective of our current research.

Our results in both the animal and human models prove that intravesical instillation of Hypersal into a recently operated bladder is safe. No significant absorption of Hypersal into the blood stream was documented, no patient or animal had hypernatremia and there were no severe adverse events or

high-grade complications neither in the rats nor in the human patients. Some of the side effects observed in humans, including infection and dysuria, could be related either to the exposure to Hypersal or to the TURBT. In addition, Hypersal was well-tolerated by our 15 patients and no unusual pain or discomfort were reported. When compared to patients who had undergone TURBT with no intravesical treatment given post-operatively, early and late complications were similar in character and severity.

Cystoscopic follow up revealed tumor recurrence in 4 patients who received Hypersal however, our sample is small, and this study was not designed to determine efficacy. While tumor recurrence at the fossa navicularis in uncommon, its relationship to Hypersal instillation is uncertain.

In conclusion, our results provide evidence that postoperative intravesical instillation of Hypersal is both safe and well tolerated. As such, we feel encouraged to proceed and explore the efficacy of this therapy in non-muscle invasive bladder cancer.

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AUTHORS CONTRIBUTION

Jonathan Modai: data acquisition, data analysis and interpretation, drafting the manuscript, statistical analysis. Alexey Kovalyonok: data acquisition, critical revision of the manuscript, animal testing phase execution. Avigdor Scherz: data acquisition, critical revision of the manuscript, animal testing phase supervision. Dina Preise: data acquisition, critical revision of the manuscript, animal testing phase supervision. Yuval Avda: data acquisition, critical revision of the manuscript, patient recruitment. Igal Shpunt: data acquisition, critical revision of the manuscript, patient recruitment. Keren Sasson: data acquisition, critical revision of the manuscript, animal testing phase supervision. Morad Jaber: data acquisition, critical revision of the manuscript, patient recruitment. Yamit Peretz: data acquisition, critical revision of the manuscript, patient recruitment. Roy Croock: data acquisition, critical revision of the manuscript, patient recruitment. Yaniv Shilo: data acquisition, critical revision of the manuscript, patient recruitment. Sergey Ikher: data acquisition, critical revision of the manuscript, pathological evaluation. Uri Lindner: data acquisition, critical revision of the manuscript, patient recruitment. Dan Leibovici: conception and design, data acquisition, data analysis and interpretation, critical revision of the manuscript, patient recruitment, clinical trial phase supervision.

CONFLICT OF INTEREST

Jonathan Modai, Alexey Kovalyonok, Avigdor Scherz, Dina Preise, Yuval Avda, Igal Shpunt, Keren Sasson, Morad Jaber, Yamit Peretz, Roy Croock, Yaniv Shilo, Sergey Ikher, Uri Lindner and Dan Leibovici have no conflict of interest to report.

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