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ORIGINAL ARTICLE

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Intravesical administration of doxorubicin to swine bladder using magnetically targeted carriers

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Abstract *Purpose*: The feasibility of using magnetic targeted carriers (MTC) to deliver doxorubicin intravesically was studied in normal swine bladder. MTCs are microparticles consisting of metallic iron and activated carbon. Doxorubicin is adsorbed to the activated carbon component of the MTCs (MTC-DOX) while the iron component provides magnetic susceptibility. This technology is designed for site-specific delivery of a drug to a tumor in the presence of an externally applied magnetic field in order to achieve prolonged release of high localized drug concentrations by retention of MTCs in the region of interest. An intravesical route of administration was evaluated as intravesical chemotherapy is used in the treatment of bladder cancer. *Methods*: The urethras of six swine were catheterized and Foley catheters were placed in their bladders. The effects of doses ranging from 10 to 80 mg doxorubicin adsorbed onto 300 to 800 mg MTCs were studied. A 30-min period of magnetic targeting immediately followed dosing, in which an external magnet was placed on the skin surface over a predetermined site on the bladder. The subsequent retention and distribution of test material was evaluated by measurement of doxorubicin levels in plasma and histopathological examination of the bladder following treatment. Blood samples were taken prior to treatment and at 15 and 30 min after infusion for measurement of doxorubicin. The bladder was drained and rinsed thoroughly following the procedure. *Results*: Plasma doxorubicin concentrations were less than the assay limit of detection (10 ng/ml) during the 30 min following dosing. MTCs were found within the bladder walls, predominantly at the targeted site where they were present at greater depths within the layers of the epithelium. The study results show that MTC-DOX can be targeted and retained within specific locations in the bladder using magnetic targeting. *Conclusions*: MTC delivery may allow greater exposure and specific deposition of drug at a defined site over intravesical administration of doxorubicin alone. The feasibility of this novel method of drug delivery was demonstrated and the results support further study for its potential use in treating bladder cancer.

Keywords Magnetic targeting · Doxorubicin · Intravesical · Delivery · Bladder

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Introduction

Bladder cancer is the sixth most common cancer in the United States with an estimated 56,500 new cases and 12,600 deaths in 2002 [1]. Cancer arising from the transitional cells of the bladder is known as transitional cell carcinoma (TCC) and accounts for more than 90% of urothelial cancers [9]. It can be further divided into superficial tumors (i.e., Ta tumors located in the urothelium, T1 tumors located in the lamina propria, and/or carcinoma in situ) and muscle-invasive tumors. Cancer that begins as a superficial tumor may grow through the lining and into the muscular wall of the bladder or it can be metastatic if it progresses into the lymph nodes or other organs. TCC is typically treated by transurethral resection (TUR). TUR is restricted to patients with superficial disease; patients with invasive tumors are commonly treated by cystectomy. Tumors often reappear in a large percentage of patients after

TUR. Chemotherapy has been used in conjunction with TUR to treat superficial bladder cancer to decrease the likelihood of recurrence [4, 5]. By placing chemotherapeutic agents directly in the bladder, drug effects can be concentrated at the tumor site to maintain high exposure of tumor tissue to the drug. According to a meta-analysis of 11 randomized trials involving 3703 patients, administration of intravesical chemotherapy after complete TUR in patients with newly diagnosed bladder cancer significantly reduces the risk of tumor recurrence [8]. The rate of tumor recurrence fell by 30–80% after intravesical chemotherapy, depending on whether recurrence was examined at 1, 2 or 3 years after TUR. Intravesical chemotherapy appears to have a major impact on decreasing the chance of recurrence of superficial bladder cancer according to the findings of this study.

Compared with intravenous administration of chemotherapeutics, the intravesical route permits direct contact between tumor and drug and is associated with reduced systemic exposure. Doxorubicin is one of the chemotherapeutic agents commonly administered intravesically. There are side effects associated with its use that include bladder wall irritation, genital area skin rash, bone marrow suppression and bladder contracture. Doxorubicin has been studied extensively with magnetic targeted carrier (MTC) drug delivery technology [6, 7]. MTCs are composite microparticles formed from metallic iron and activated carbon in a high-energy milling process with a particle size range from 0.5 to 5 μm. Doxorubicin is adsorbed to the activated carbon component while the iron provides magnetic susceptibility. Targeting is achieved with the use of a 5 kG magnet placed on the skin over a predetermined target site prior to and following dosing. Currently MTC-DOX is delivered via selective hepatic arterial catheterization to patients with liver cancer.

The feasibility of using MTC-DOX in the bladder was investigated following intravesical administration. Since magnetic targeting allows site-specific delivery of drugs, it seems plausible that this technology could confer an advantage over the way chemotherapeutic agents are currently delivered to the bladder. Presently, patients treated with intravesical chemotherapy are rotated to ensure either complete exposure of the entire bladder wall or a specific area to the medication. Magnetic targeting may facilitate exposure within the bladder. The site-specificity and drug retention associated with delivery are major advantages of magnetic targeting and this aspect was explored in the study.

Materials and methods

Materials

Sterile MTCs packaged in vials each containing 100 mg were used in these experiments. MTC controls were suspended in a final volume of 20 ml/vial (5 mg/ml) with vehicle comprising 10% mannitol and 0.5% sodium carboxymethylcellulose solution. The resulting solution was sonicated for 30 s and used within 2 h of preparation. The MTC preparation was infused into the bladder with contrast medium (Omnipaque, 350 mg/ml) and sufficient saline to result in a total volume that was equal to the bladder capacity. MTC-DOX was prepared by incubating up to 10 mg doxorubicin (2 mg/ml; Fujisawa, Deerfield, Ill.) in vials containing 100 mg of MTCs at room temperature for 30 min. The vials were then diluted to a final volume of 20 ml/vial with vehicle. The solution was sonicated and used within 2 h of preparation. Doxorubicin alone was administered (no MTCs) by mixing 50 mg with contrast medium and saline so that the solution infused through the catheter syringe was equivalent to the bladder capacity to allow contact with all sides of the bladder. The catheters used for intravesical administration of test material included a 5 French pigtail angiogram catheter for use in the one male animal studied. Either an 8 French Bardex pediatric Foley catheter or a 6 French Rusch pediatric Foley catheter were used to infuse test material into the female swine bladders. Magnetic targeting utilized a rare-earth NdFeB permanent 5 kG magnet housed in a flexible magnet holder that allowed precise positioning of the magnet.

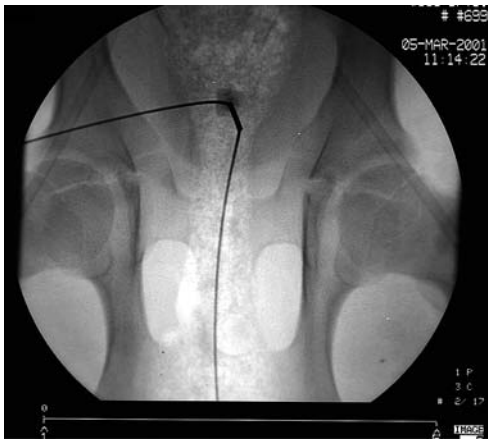

Fig. 1 Pretreatment radiograph showing catheter placement in the bladder

Study design

Six Yorkshire domestic swine (18–30 kg) were treated in this study. Five females and one male were anesthetized with halothane following ketamine/xylazine induction. An intravenous injection of 30 ml contrast material allowed visualization of the bladder using fluoroscopy. In order to catheterize the bladder, an arterial puncture needle (Cook 18 G×7 cm) was used for percutaneous entry into the bladder through the abdomen. A guide wire (0.035 inch angled Glidewire; Boston Scientific Corporation, Natick, Mass.) was threaded through the bladder and into the urethra. A catheter was introduced into the bladder using the wire extending from the urethra for guidance. Fluoroscopy (Fig. 1) was used to verify catheter placement in the bladder and to determine the depth of the bladder from the skin. Bladder volume was determined for each animal by draining urine through the catheter and filling the bladder with saline until it was full. The volume of saline was measured so that the subsequent infusion of test material would be equivalent to each animal's bladder capacity, thus allowing contact with all sides of the bladder. The saline was then drained from the bladder.

Test material was administered into an empty bladder through a syringe connected to the catheter. Four animals received infusion solutions comprising doxorubicin, MTCs, vehicle, 20 ml contrast medium and saline to volume (Table 1). One animal received doxorubicin, contrast medium and saline. Another animal received MTCs, vehicle, contrast medium and saline. The test material was administered as a single bolus. The catheter was then

Table 1 Treatment groups used in the study of intravesical administration to swine bladder

Swine	MTCs administered (mg)	Doxorubicin administered (mg)	Targeted site	Study duration
1	300	10	Ventral bladder dome	30 min
2	500	50	Dorsal wall	30 min
3	800	80	Trigone	30 min
4	500	50	Ventral wall	44 days
5	0	50	–	30 min
6	500	0	Ventral wall	30 min

closed off following infusion so that the solution remained in the bladder. The north pole of the magnet was placed on the skin surface over the bladder. The magnet was positioned over a different region of the bladder for each animal so that targeting to the ventral wall, dorsal wall, and trigone could be evaluated. In order to target the dorsal wall, the animal was rolled to one side and the magnet was positioned on the back. A 30-min magnetic retention period followed the dosing procedure. The test material was then drained from the bladder and irrigated multiple times with saline. All swine were then killed except for animal no. 4 that was kept for 44 days to evaluate longer-term effects. After recovery, animal no. 4 was returned to her cage and observed daily for clinical signs. At necropsy the entire bladder was carefully removed from each animal and inflated with 10% formalin until full. The neck of the bladder was tied off to keep the formalin in place until paraffin embedding, sectioning and hematoxylin/eosin staining had been carried out. A suture was placed in the bladder at the site where the magnet had been positioned so that the site of targeting could be identified. Animal experiments complied with the NIH publication no. 85-23, revised 1985.

Blood samples were collected in EDTA-containing Vacutainer tubes from all animals prior to dosing, and at 15 and 30 min following dosing and magnet retention. The samples were inverted several times, centrifuged to collect the plasma, and stored at −20°C until analysis. Plasma doxorubicin levels were quantified by HPLC coupled with tandem mass spectrometry. In order to analyze the plasma samples, 0.025 ml aclarubicin was added to a 0.1-ml aliquot of plasma as an internal standard. The doxorubicin and aclarubicin were extracted from the plasma using a plasma precipitation procedure in which samples were treated with a combination of 0.5 ml acetonitrile and 0.5 ml of a 0.1% solution of formic acid. The samples were vortexed to achieve thorough mixing. They were then centrifuged to separate the precipitated plasma proteins from the extracted supernatant. The supernatants were transferred to HPLC vials for analysis using reversed-phase chromatography with a CN column (150×2 mm, 5 μm, Phenomenex, Torrance, Calif.) maintained at 40°C. The mobile phase was nebulized using heated nitrogen in a Z-spray source/interface and the ionized compounds were detected using a triple quadruple mass spectrophotometer. The instrument was set up using a Luna C18 column to achieve separation of the solution components. The mobile phase used included a 0.1% solution of formic acid as the aqueous phase, and acetonitrile as the organic phase. The ratio of aqueous to organic for the analysis was 45:55. The flow rate was set to 0.3 ml/min. This method gave a doxorubicin retention time of 2.06 min, and an aclarubicin retention time of 3.92 min. The quantitation range of the assay was 10 to 5000 ng/ml, with 10 ng/ml as the limit of detection.

Results

There were no adverse events associated with the treatments. Fluoroscopy was used to assist in catheter placement. Radiographs taken after instillation and magnetic targeting of test material showed no abnormalities. Bladder volumes of the animals varied from 150 ml up to 850 ml. The distance between the magnet on the skin surface and the bladder was between 5 and 15 cm depending on the site targeted. The depth averaged 11 cm in most instances. Upon necropsy, distinct dark-colored particle-containing areas were observed in the bladder. When the bladder was inflated with formalin for subsequent tissue processing, the particles were seen more clearly in the translucent bladder. They were present as gray patches a few centimeters in diameter. Particles were most concentrated at the site of magnet placement. They were also found in the bladder wall directly opposite the targeted site to a lesser degree.

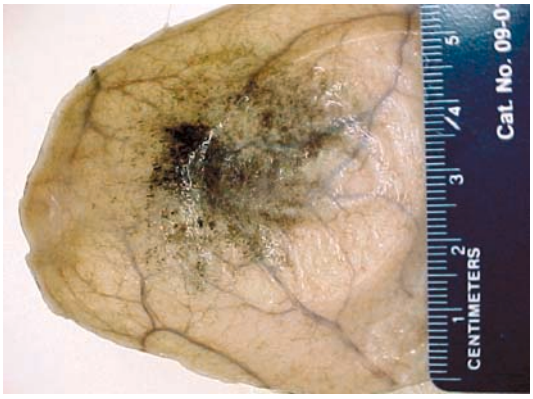

Fig. 2 Ventral bladder surface following intravesical delivery of MTC-DOX and magnetic targeting to this region

Gross observations

A 3×4-cm dark focus was visible through the serosa on the ventrocranial portion of the bladder mucosa following the magnetically targeted delivery of MTC-DOX to the ventral dome of the bladder (Fig. 2). The nontargeted surface directly opposite the target site had the second greatest amount of particles in a 6×3.5-cm area (Fig. 3). MTC-DOX delivery to the dorsal bladder wall resulted in a 6×7-cm area containing dark particles that encompassed the central to distal bladder mucosa. There were no particles observed in either the right or the left lateral segments. When MTC-DOX was targeted to the trigone region at the lower dorsal wall of the bladder, the majority of particles accumulated about 6 cm distal to the trigone and covered a 4×7-cm area. Examination of the bladder from animal no. 4 taken 44 days after the intravesical administration of MTC-DOX showed MTCs primarily in the central to lower ventral bladder wall where they were originally targeted. They were also found on the opposite dorsal wall across from the targeted site in the form of fine black streaks running parallel to the length of the bladder. The largest area of dark pigment measuring 2×3 cm was located near the neck of the bladder in the right ventrolateral portion,

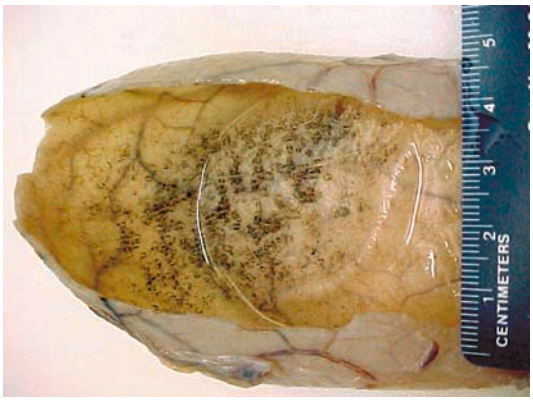

Fig. 3 Dorsal bladder surface following intravesical delivery of MTC-DOX and magnetic targeting to the ventral bladder

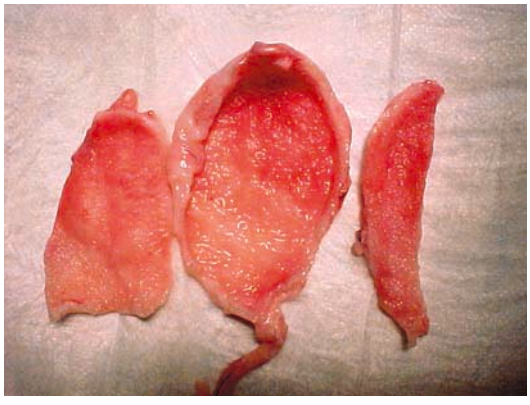

Fig. 4 Bladder divided into three segments following intravesical administration of 50 mg doxorubicin without MTCs

showing that the particles were retained at the target site. MTCs and doxorubicin were also administered separately for comparison. A dark-gray patch of particles was noted on the ventral wall of the bladder following the delivery of 500 mg MTC to this region of the bladder. When doxorubicin was administered intravesically without MTCs, most of the bladder lining was stained red, the color of doxorubicin (Fig. 4). The bladder surface exposed to doxorubicin was also visibly weathered or eroded after a 30-min exposure period.

Microscopic observations

Microscopic evaluation of the bladders showed that MTCs were located to the greatest extent in the superficial layers at the targeted sites. However, particles were also present within the superficial and deep layers of epithelium and within sloughing cells.

When the magnet was positioned on the abdomen above the ventral bladder dome, the particles deposited at the targeted site were larger, more clumped and often reached a greater depth within the layers of the epithelium than those particles present in the bladder wall

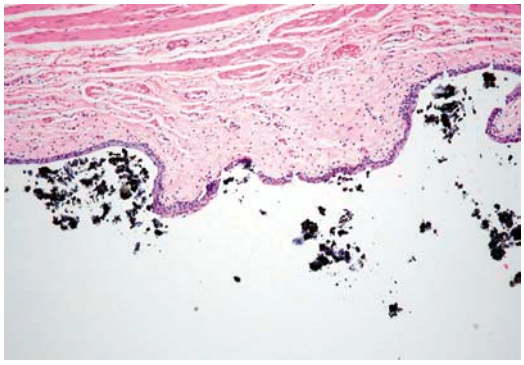

Fig. 5 Loose particles in sloughed cells following administration of 80 mg doxorubicin plus 800 mg MTCs to the trigone region of the swine bladder

directly opposite the targeted site. The epithelial cells were ruptured or sloughing into the bladder lumen in places. There were some areas of erosion and denudation of the basement membrane. The particles in the nontargeted area were smaller, tended to be composed of a single layer of granules and did not show evidence of cellular rupture or sloughing. The particles rarely penetrated below the superficial layers of epithelial cells. The superficial epithelial cells readily picked up the fine granular black MTCs that were distributed throughout the cytoplasm.

Upon delivery and targeting of MTC-DOX to the dorsal wall in which the magnet was positioned on the animal's back, fewer particles appeared to be present overall as compared to studies where the ventral wall was targeted. MTCs were deposited predominantly in the targeted area and the greatest amount was in the form of a fine dust present only within the most superficial layers in which the MTCs were noticeably smaller. Multifocal hemorrhage was noted in the bladder and was present more in the epithelium than in the submucosa. The hemorrhage was likely traumatic in origin, possibly induced during the manipulation of the bladder. There were no changes in the epithelium to suggest that this was associated with the instillation of MTC-DOX.

The highest dose of MTC-DOX administered contained 80 mg doxorubicin and 800 mg MTCs. Upon delivery to the trigone areas of ulceration, denudation or attenuation of the epithelium were seen. The majority of these areas were not accompanied by an inflammatory infiltrate. The submucosa was edematous in the most severely ulcerated areas. There were larger numbers of particles within the superficial and sloughing epithelial cell layers (Fig. 5). Finer, smaller particles were distributed throughout the most superficial layers of the epithelium. Microscopic examination of tissue 44 days after treatment from animal no. 4 showed MTCs located to the greatest extent in the targeted region and the area opposite the target site. The majority of particles were present within the superficial submucosa. The smaller particles in the targeted region tended to be in the epithelium and the larger particles were in the superficial

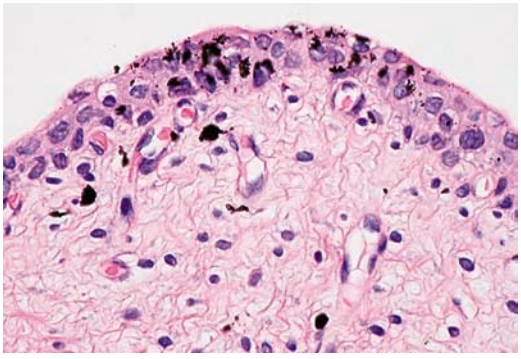

Fig. 6 Particles in bladder mucosa and submucosa 44 days after magnetically targeted delivery of MTC-DOX (higher magnification)

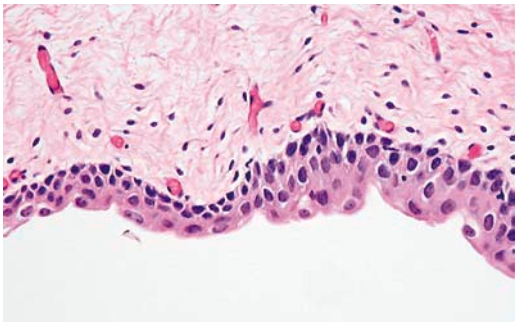

Fig. 7 Normal mucosa following intravesical administration of doxorubicin

submucosa (Fig. 6). In the submucosa, the particles were either free or associated with endothelial cells or fibroblasts. There did not appear to be signs of injury to the bladder due to the presence of MTCs several weeks following treatment as shown in animal no. 4.

Animal no. 6 was killed 30 min after delivery of 500 mg MTCs and magnetic targeting. At this time there was evidence of submucosal hemorrhage, edema and neutrophil recruitment in the targeted region where MTCs were more distinctly concentrated. While the bladder mucosal epithelium appeared to slough the MTCs, some particles reached the basement membrane with less of an epithelial reaction to the presence of particles than that seen in the superficial layers. Upon microscopic examination of tissue exposed to doxorubicin alone, cystitis was the primary finding. There was minor submucosal hemorrhage. It did not transmigrate the epithelium and was likely associated with removal of the bladder. The bladder mucosa did not demonstrate any alteration in thickness, cellular orientation or conformation. Overall, the effects on the bladder mucosa were unremarkable (Fig. 7).

Plasma concentrations of doxorubicin

Doxorubicin was not detected in the circulation of any animals receiving MTC-DOX or doxorubicin alone over the 30-min sampling period. The limit of quantitation for the HPLC with mass spectrometric detection method used was 10 ng/ml. All plasma doxorubicin concentrations were below that level indicating that the drug did not readily enter the circulation. The urothelium is considered an effective barrier to doxorubicin penetration [10]. Therefore, appreciable quantities of doxorubicin were not expected to be found in the systemic circulation following the concentrations of drug administered in this study.

Discussion

This study was designed to assess the feasibility of magnetic targeted delivery of doxorubicin to the bladder via intravesical administration. It focused on identifying the distribution of targeted MTC particles in swine bladder by gross and microscopic examination. Doxorubicin plasma levels were measured to determine whether an appreciable amount of the drug entered the circulation. Tissue drug concentrations were not determined, but the presence of doxorubicin as MTC-DOX was inferred by the distribution of MTCs observed in the bladder.

The swine urological system is similar anatomically and physiologically to that of humans [3]. This species has been used extensively as an animal model for studying the bladder and for assessing MTC effects. A large suburethral diverticulum makes the catheterization of the urethra difficult in the sow [2]. In order to catheterize the swine bladder in this study, an arterial puncture needle was used for percutaneous entry into the bladder through the abdomen. This allowed a guide wire to be threaded through the bladder and into the urethra to position a pediatric Foley catheter in the bladder using the wire extending from the urethra for guidance. Because the bladder is translucent, especially when inflated with formalin, MTC particles can be readily seen without the use of a microscope. Thus, the distribution and retention of particles in the wall can be verified. MTCs were most pronounced at the site of the bladder exposed to the external magnetic field. The contribution of the effect of gravity may have contributed to the relatively lesser accumulation of particles opposite the targeted site. Animals were placed in the supine position for targeting to the ventral bladder wall and rolled over for magnetic targeting to the dorsal wall.

Microscopic examination of tissues showed the distribution of MTCs and a relative lack of histopathology. The bladder mucosa was three to five cells thick and the cells were moderately vacuolated, which is normal. Some cell sloughing and ulceration was present following exposure to the highest dose of MTC-DOX that was not seen with lower doses. However, cell sloughing also occurred with lower doses. The extent of this sloughing appeared to be related to size and number of particles. This response would be expected since rapid sloughing of superficial injured cells is one of the common defense

mechanisms of the bladder. Multifocal hemorrhage was noted in the bladder. It was present more in the epithelium than in the submucosa. The hemorrhage was likely traumatic in origin, possibly induced during the manipulation of the bladder. There were no changes in the epithelium to suggest that this was associated with the instillation of MTC-DOX.

The intravesical delivery of doxorubicin by itself lacks the degree of site-specificity observed with magnetic targeting. Its chemotherapeutic action is limited to where the drug comes into contact with the bladder by gravity. A patient's body must be positioned in such a way as to target a general region. Another drawback to intravesical administration of chemotherapeutics is the repeat treatments involved. The optimal dose and treatment schedules for any of the intravesical agents have not been determined. A 6- to 8-week course is usually recommended. A drug delivery system that gets more drug to the tumor site and provides a more continuous exposure over time could decrease the need for repeat dosing. Less-frequent treatment may be possible with magnetic targeted delivery.

Plasma doxorubicin concentrations in all samples were below the limit of detection of 10 ng/ml. These results suggest that the drug was not released immediately into the circulation, but rather remained localized primarily in the tissues, as is the goal of intravesical administration. The human urothelium is 0–200 μm thick and is not perfused by blood. Therefore, drug is presumed to be transferred by diffusion across the deeper tissues that are perfused by capillary blood flow, and then enters the circulation.

Given the chemosensitivity of urothelial cancer, an improved, site-specific method of delivering chemotherapeutic agents would be advantageous. Toxicity of intravesical chemotherapy remains a problem. However, it may be helpful when administered after TUR to reduce the risk of tumor recurrence. There may also be some benefit from intraarterial delivery of chemotherapeutics as well. Intraarterial infusion chemotherapy is used in the treatment of invasive bladder cancer and most experience with MTC-DOX delivery to date has been by the intraarterial route. While most clinical and laboratory experience with magnetic targeting has involved doxorubicin, other chemotherapeutic agents may be delivered using this same technology. Mitomycin C is more widely used in intravesical therapy and is a good candidate for future study. The feasibility of using MTCs to deliver doxorubicin intravesically was demonstrated and the results support further study of its potential use in treating bladder cancer. While MTCs could clearly be seen in the bladder wall, future studies should focus on quantifying the MTCs and drug in the bladder following magnetic targeted delivery.

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