Intravesical Treatments of Bladder Cancer: Review

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Abstract. For bladder cancer, intravesical chemo/immunotherapy is widely used as adjuvant therapies after surgical transurethral resection, while systemic therapy is typically reserved for higher stage, muscle-invading, or metastatic diseases. The goal of intravesical therapy is to eradicate existing or residual tumors through direct cytoablation or immunostimulation. The unique properties of the urinary bladder render it a fertile ground for evaluating additional novel experimental approaches to regional therapy, including iontophoresis/electrophoresis, local hyperthermia, co-administration of permeation enhancers, bioadhesive carriers, magnetic-targeted particles and gene therapy. Furthermore, due to its unique anatomical properties, the drug concentration-time profiles in various layers of bladder tissues during and after intravesical therapy can be described by mathematical models comprised of drug disposition and transport kinetic parameters. The drug delivery data, in turn, can be combined with the effective drug exposure to infer treatment efficacy and thereby assists the selection of optimal regimens. To our knowledge, intravesical therapy of bladder cancer represents the first example where computational pharmacological approach was used to design, and successfully predicted the outcome of, a randomized phase III trial (using mitomycin C). This review summarizes the pharmacological principles and the current status of intravesical therapy, and the application of computation to optimize the drug delivery to target sites and the treatment efficacy.

KEY WORDS: bladder cancer; computational modeling; intravesical therapy; pharmacokinetic/pharmacodynamic; regional therapy.

INTRODUCTION

Bladder cancer is the fifth most common cancer in the United States, with an estimated 67,160 newly diagnosed cases and 13,750 deaths in the United States in 2007. The 5-year survival rate is 82% for all stages combined (1). The standard of treatment for patients with superficial bladder cancer is surgical transurethral resection (TUR) of tumors, with an 80% early success rate. However, nearly 70% of these patients will develop tumor recurrence, with 25% showing progression to muscle-invading disease, within 5 years with TUR (2). Intravesical chemotherapy and immunotherapy are widely used as adjuvant therapies after TUR, to prevent recurrence and progression of superficial disease. Systemic therapy is typically reserved for higher stage, muscle-invading, or metastatic diseases.

The urinary bladder is an ideal organ for regional therapy. The urethra provides easy access of therapeutic agents to the urinary bladder. The presence of the specialized asymmetric unit membrane on the urothelium serves as a barrier and limits the absorption of molecules or particulates into the systemic circulation. For most small molecule drugs, less than 5% of the dose is absorbed into the systemic circulation. The rationale for intravesical therapy is to maximize the exposure of tumors located in the bladder cavity to therapeutics agents while limiting the systemic exposure and thereby limiting the host toxicities; the primary goal is to eradicate existing or residual tumors through direct cytoablation or immunostimulation. The unique properties of the urinary bladder render it a fertile ground for evaluating novel experimental approaches to regional therapy, including iontophoresis/electrophoresis, local hyperthermia, co-administration of permeation enhancers, bioadhesive carriers, magnetic-targeted particles and gene therapy.

This review consists of five parts. Part I summarizes the current status of intravesical treatments of bladder cancer. Part II discusses the drug disposition in bladder tissues during intravesical therapy. Part III discusses the application of...
PART I. CURRENT STATUS OF INTRAVESICAL TREATMENTS

**Immunomodulators.** Bacille Calmette-Guérin (BCG) is the most adopted first-line immunotherapeutic and is the most effective treatment for prophylaxis and treatment of carcinoma in situ (CIS). Phase I and II trials have shown that other immunoregulators such as interferons (IFN) (6–8), interleukin-2 (IL-2) (9–11), interleukin-12 (IL-12) (12,13), tumor necrosis factor (TNF) (14–16), keyhole limpet hemocyanin (KLH) (17, 18) and rubratin (19,20) have activity in BCG-refractory patients, albeit with low durable remissions (<20%).

**Chemotherapy.** Multiple chemotherapeutic agents, such as mitomycin C (MMC) (21–25), doxorubicin (26–28), epirubicin (29–32), thiotepa (33,34), ethoglucid (35–37), valrubicin (38–40), cisplatin (41–43), gemcitabine (44–46), suramin (47,48) or their combinations (49–52) have been evaluated in patients; meta-analyses did not show apparent superiority of a particular treatment. The addition of intravesical chemotherapy to TUR yields, on average, a further reduction of recurrence by 14–17%, but has limited benefits against disease progression (53).

**BCG vs chemotherapy.** Compared to several chemotherapeutic agents (thiotepa, epirubicin and doxorubicin), BCG is more effective in preventing tumor recurrence (14–47% lower recurrence rate) and disease progression (54). In patients with stage Ta or T1 carcinoma, BCG showed a lower recurrence rate (38.6% versus 46.4%) and lower disease progression (34% lower), compared to standard albeit suboptimal MMC treatments (i.e., no pharmacological interventions to improve the MMC delivery to tumor cells, see Part III) (55). Patients who failed BCG and desired avoiding radical cystectomy have been treated with intravesical IFN-α (56–59), valrubicin (60) or gemcitabine (61,62) with some limited success.

PART II. DRUG DISPOSITION DURING INTRAVESICAL THERAPY

Drug disposition in the bladder during intravesical therapy is affected by several attributes, i.e., physicochemical properties of the drug (molecular weight, hydrophilicity or lipophilicity, water/lipid partition coefficient), urine volume and pH, patient hydration status, and integrity of urothelium. Our laboratory has provided the first pharmacokinetic models to describe drug disposition in urine and bladder tissues. These models enable the prediction of changes in drug concentration in different parts of bladder wall as a function of physiological, pathological or pharmacological parameters (63).

The first set of equations describes the urine pharmacokinetics during treatment:

\[ C_u = \frac{\text{Dose}}{V_u} e^{-(K_o+K_i)t} \]  

\[ V_u = V_0 + K_o t + V_{\text{res}} \]

where \( C_u \) is the urine drug concentration at time \( t \), \( V_u \) is the volume of the urine, \( K_o \) is the first order rate constant describing drug absorption into the systemic circulation, \( K_i \) is the hybridized first order rate constant describing degradation, metabolism, and tissue binding, \( V_o \) is the dosing volume, \( K_0 \) is the zero order rate constant describing urine production, and \( V_{\text{res}} \) is the post-catheterization residual urine volume.

The urine pharmacokinetic model provides the tool to depict changes in urine drug concentrations due to changes in physiological parameters that can vary from patient to patient (e.g., residual urine volume, urine pH, urine production rate) and changes in drug-related parameters (e.g., dose, dosing volume, degradation in acidic or basic environment). It is noted that most of the small molecule drugs used in intravesical chemotherapy have pH-dependent stability, e.g., MMC is unstable in pH<5 or pH>8 (64, 65), thiotepa and its active metabolite are unstable in acidic pH (<5) and stable at alkaline pH (8.4) (66, 67), whereas doxorubicin is more stable in acidic pH (5.4) than in alkaline pH (8.1) (68). pH also affects the antitumor activity, e.g., MMC is more active at acidic pH in monolayer cultures (but no pH dependent-effect in 3-dimensional cultures) (69), epirubicin is more active at alkaline pH (8.0) than at lower pH (6.0) (70). The urine production rate and residual urine volume can be altered by patients’ hydration status, e.g., dehydration for 6 hr decreased the average volume of urine production from 209 to 143 ml, which is further reduced to 103 ml by co-administering an antidiuretic desmopressin (71).

For a drug-of-interest, the urine pharmacokinetic model can be established by defining the values of the rate constants in the equations. The values for the physiological parameters in patients can be readily obtained from the literature. The values for the drug-related parameters are either defined by investigators (e.g., dose, dosing volume) or by measuring the degradation rate in buffers with different pH values. Successful development of the urine pharmacokinetic model for a drug-of-interest would enable the prediction of the effects of changing the various parameters on the urine drug concentrations and, because the urine concentration is the driving force for delivering the drug to bladder tissues, on the drug concentrations in the urothelium layer.

The second set of equations describes the drug transport in bladder tissues, as a function of time and distance from the urine compartment. For this purpose, the bladder wall is
divided into two sections, the urothelium (mucosa) that is not blood-perfused and the submucosal and muscle layers of the bladder that contain blood vessels and lymphatics. Drug transport from the urine compartment across the urothelium (about 7–10 cell layer or 200 μm thick in man [72]) is depicted by diffusion across a single homogeneous diffusion barrier and is described by Fick’s first law (Eq. 3). Drug transport across the submucosa and superficial muscle (200–4,000 μm) is described by the distributed model (Eq. 4) [5,63,73,74]. The distributed model integrates drug diffusion through a porous structure and drug removal/absorption through the blood vessels.

Urothelium (0–200 μm):

\[ C_{\text{depth}} = C_u - \frac{C_u - C_{\text{uro}}}{{200}} \times \text{depth} \]  

Submucosa/superficial muscle layer (200–4,000 μm):

\[ C_{\text{depth}} = (C_{\text{uro}} - C_b) \times e^{-\left(\frac{\text{depth}}{200}\right)^{0.5}} + C_b \]  

In the urothelium, \( C_u \) is the concentration of unionized drug in the bladder cavity and is the link between the urine and tissue pharmacokinetics. \( C_{\text{uro}} \) is the concentration at the interface between the urothelium and the submucosa, or about 200 μm away from the surface of the urothelium lining. \( C_{\text{depth}} \) is the concentration at a particular tissue depth. In the urothelium, \( C_{\text{depth}} \) declines linearly with increasing depth because diffusion is not dependent on the depth. In the submucosa and superficial muscle layer, \( C_{\text{depth}} \) is determined by \( C_{\text{uro}} \), the plasma drug concentration \( C_b \) in blood vessels, the tissue depth away from the urothelium and the half-width \( W_{1/2} \). The latter is the thickness of tissue over which the drug concentration declines by 50%. Because the number of vessels encountered by the drug molecule increases with increasing tissue depth (assumed to be evenly distributed), the decline of \( C_{\text{depth}} \) in submucosa and superficial muscle layers is first order with respect to tissue depth.

We have experimentally determined the pharmacokinetic model parameters for several drugs, i.e., MMC, doxorubicin, 5-fluorouridine and paclitaxel. The first three less lipophilic drugs show comparable penetration across the urothelium with a \( C_{\text{uro}}/C_u \) ratio of 0.02 to 0.03, whereas the more lipophilic paclitaxel shows a significantly higher \( C_{\text{uro}}/C_u \) ratio of about 0.5. These findings, together with the earlier finding of the extensive systemic absorption of the lipophilic small molecule thiotepa (MW, 189 Da) [75], indicate that lipophilicity is a key determinant of drug penetration across the urothelium. Another consideration is systemic absorption; the absorption of MMC, doxorubicin, 5-fluorouridine and paclitaxel after intravesical therapy is consistently lower compared to thiotepa (<5% vs 20%) [3,4,73,76]. We infer that an ideal drug for intravesical therapy would be a lipophilic compound that can readily penetrate the urothelium and remain localized in bladder tissue (as opposed to being rapidly absorbed into the systemic circulation). Note that the model for drug transport across the urothelium enables the computation of effects of altering the urothelium barrier function (e.g., compromised urothelium due to surgery or treatment with surfactant or absorption enhancers).

A second generation bladder tissue kinetic model was recently described [77]. The major modification is the arbitrary subdivision of the bladder wall into 30 serially interconnected compartments (4 within the urothelium and 26 within the deeper, capillary-perfused tissues). This model was used to generate simulations of the concentration-depth profiles of a hydrophilic drug pipemidic acid (PPA), for the purpose of studying its systemic absorption rate, and the effects of co-administering bioadhesive permeation enhancers (chitosan and polycarrophil). The model parameters such as tissue partition coefficient and diffusion rate constants were obtained using in vitro drug transport data generated using the isolated porcine bladder. The mathematical expansion of the bladder tissues from 2 compartments into 30 compartments enables the inclusion of inter-compartmental drug transfer and drug absorption from each sub-compartment (e.g., different transport rates at different tissue locations) and thereby enhances the data fitting capability. It is noted, however, the anatomical and physiological relevance of the 30 sub-compartments is unclear and the model performance under in vivo conditions cannot be ascertained because the model parameters were obtained under in vitro conditions, i.e., without intact blood perfusion.

The above urine and tissue pharmacokinetic models provide the basis for computing drug delivery to the targeted, tumor-residing sites in the bladder as a function of treatment conditions (e.g., dose, drug concentration, volume of dosing solution, patient hydration status, treatment duration), during intravesical therapy. Integration of drug delivery with pharmacodynamic data such as the effective drug concentrations in preclinical models provides a means to rational design of intravesical treatments (see Part III).

**PART III. APPLICATION OF COMPUTATIONAL MODELING TO OPTIMIZE INTRAVESICAL TREATMENTS**

Response of a patient to a treatment is largely determined by whether his/her tumor cells are sensitive to the selected chemotherapy and whether sufficient drug is delivered to tumor cells. Tumor sensitivity is a biological/genetic property that cannot be readily controlled or manipulated. On the other hand, as discussed in Part II, the delivery of a therapeutic agent to tumor cells located in different parts of bladder wall can be depicted as mathematical relationships with controllable variables such as dose, volume of dosing solution, urine production rate during treatment, residual...
urine volume at the time of dose instillation, and urine pH. Drug concentration in the urine compartment is directly affected by the first four variables (e.g., a lower dose or a larger urine volume diminishes the drug concentration), whereas the pH affects the drug stability and thereby indirectly determines the concentration of the intact drug. The following example demonstrates the application of computational modeling to clinical trial design (please see reference (63) for a more detailed discussion). First, we used the urine pharmacokinetic model described in Equations 1 to 4 to evaluate how changes in the variables or treatment conditions alter the drug delivery (63). The simulated drug amounts, equivalent to (concentration) x (treatment time) product or CXT, were then compared to the drug concentrations found active in patient bladder tumors (n>60), in order to determine the fraction of patients likely to receive sufficient drug amount to produce a therapeutically meaningful response under various treatment conditions. Computation was used to evaluate seven changes in treatment conditions, including increasing the dose from 20 to 40 mg or to 60 mg, decreasing the dosing volume from 40 to 20 ml, increasing instillation time from 120 to 240 minutes, decreasing urine production rate from 1.48 to 0.63 ml/min, reducing residual volume from 32.4 ml to 0 ml and increasing pH from 5 to 7. The simulations predicted that changes of the above parameters would affect the treatment outcome in the following rank order: dose > urine residual volume > urine production rate > dosing volume > urine pH > instillation time.

The three major findings of the computer simulation results are as follows. (a) Changing one parameter at a time would yield small incremental improvements (i.e., <8% higher 13-month recurrence free rate) such that at least 450 patients would be required to demonstrate the benefit of changing a single individual treatment parameter. This finding suggests that the inconclusive results for the earlier trials that attempted to evaluate the benefits of changing various individual treatment parameters were likely due to inadequate patient sample size. (b) In contrast, simultaneous changes in five treatment parameters (see below) would increase the 13-month recurrence rate by 18–20%, a difference that is large enough to be detected with a relatively small number of patients (230 patients showing 116 events). (c) The simulation results further showed that two additional changes in treatment parameters (using the maximally tolerated dose of 60 mg or increasing the treatment time from 2 to 4 hr) would not produce additional benefits.

The computer simulation results were used to synthesize an optimized MMC treatment protocol that was subsequently tested in a multi-center, two-arm phase III clinical trial (78). In this trial, superficial bladder cancer patients with histologically proven transitional cell carcinoma (Ta, T1, CIS) and at high risk for recurrence were randomized to optimized or standard treatment arms. Patients in the optimized arm received 40-mg dose of MMC, with pharmacological manipulations to maximize the drug delivery (ultrasound-guided bladder emptying, voluntary dehydration, urine alkalinization by oral sodium bicarbonate). In the standard arm, patients received a 20-mg dose and 20 ml dosing volume without manipulations. All patients received intravesical treatments weekly for 6 weeks. The primary endpoint was time to recurrence and the secondary endpoint was recurrence-free rate. The results showed that in evaluable patients, the median time to recurrence was 29.1 months in the optimized arm, significantly higher than that in standard arm (11.8 months) (p<0.001). The projected 5-year recurrence-free rate with a median 11.7 months follow-up was 42.6% in optimized arm and 23.5% in standard arm, or a 19.1% increase. The extent of improvement is identical to the simulated prediction (18–20% increase). To our knowledge, this is the first efficacy trial designed based on computational results that shows good agreement between the model-prediction and observed results.

**PART IV. EXPERIMENTAL APPROACHES**

Various experimental approaches in two general categories have been evaluated. Approaches in the first category share a common theme of improving the total drug exposure, by enhancing the delivery of agents to bladder tissues (e.g., using permeation enhancers, iontophoresis/electrophoresis, hyperthermia, disrupting the extracellular matrix), prolonging the exposure (e.g., using bioadhesive), or enhancing cell membrane permeability (intravesical hyperthermia). The second category is gene therapy, with the goal of either correcting the mutated and malfunctioned genes responsible for tumor formation and progression or as a means to deliver intrinsic or extrinsic signals for cell destruction.

**Permeation enhancers.** Approaches to enhance urothelium permeability or drug transport in extracellular matrix include chemical and physical methods, as follows.

The chemical methods use permeation enhancers such as dimethyl sulfoxide (DMSO), which has been used to treat interstitial cystitis (79). Co-administration of DMSO (10–50%) promoted the penetration of water-soluble drugs (e.g., cisplatin, pirarubicin, doxorubicin) (80–82) and a lipophilic drug paclitaxel (83) across the urothelium in dogs or rats. However, DMSO also promoted the urine production rate and the absorption of paclitaxel from bladder tissues into systemic circulation, thereby partially negating the benefits of improved drug partition into bladder tissues (83).

Other permeability enhancers have been evaluated. Chitosan is a cationic polysaccharide that, by binding to the negative charges on cell membrane, causes rearrangement of cellular tight junctions and enhances paracellular drug transport. Polycarbophil is a mucoadhesive polyacrylic acid cross-linked with divinyl glycol that, by chelating with extracellular calcium ions, causes opening of cellular tight junctions in isolated porcine bladder (in vitro), co-administration of chitosan (0.05% to 1% w/v) or polycarbophil (1% w/v) promoted the penetration of moxifloxacin (pKa values of 6.4 and 9.5) or pipemidic acid (pKa values 5.4 and 8.2) by 3- or 4-fold (84,85).

Hyaluronan or hyaluronic acid, a glycosaminoglycan, is a major component of extracellular matrix. Hyaluronidase, an enzyme that hydrolyzes the hyaluronan network, can be safely administered to humans and has been approved for treating vitreous hemorrhage (86,87). Co-administration of hyaluronidase improves drug diffusion into bladder mucosa in rats (88). In humans, hyaluronidase did not improve the efficacy of cisplatin in 33 patients (89), but significantly...
Gene therapy poses several theoretical properties; it acts as either a tumor suppressor or promoter depending on the cell type and concentration (92,93). Low concentration of hyaluronidase (14–40 milliunits/10^6 cells) stimulated tumor growth whereas high concentration (>100 milliunits/10^6 cells) induced apoptosis and inhibited tumor formation. These properties indicate the need of carefully fine-tuning the hyaluronidase treatment conditions.

Physical methods to disrupt the urothelium include electromotive therapy (iontophoresis/electrohoresis) and hyperthermia. Both methods are under clinical evaluations. Electromotive administration, by temporary breaching of the urothelium, increases the transport of MMC across urothelium in human bladders (six to nine times higher compared to MMC alone) and absorption into the plasma (~5 times higher peak plasma concentration); the peak concentration was 43 ng/ml or about 1/10 of the threshold toxic concentration of 400 ng/ml (94, 95). An earlier study in 28 patients with superficial bladder tumors suggests that adding electromotive administration to intravesical MMC did not improve the complete response rate at 10 weeks post-treatment (40% for electromotive MMC vs 41% for MMC alone), but improved he reduction in the recurrence rate (from 60% to 33%) and the duration of disease-free interval (from 10.5 to 14.5 months) (96). A more recent three-arm study compared MMC alone, MMC plus electromotive administration, and BCG in 117 high risk patients. The results show that, compared to MMC alone, electromotive MMC significantly improved the recurrence-free rate (58% vs 31% at 6 months, p=0.012) and significantly prolonged the median time to recurrence (35 vs 19.5 months, p=0.013) (97). The combination of MMC and electromotive administration produced similar benefits as BCG (64% recurrence-free at 6 months and median time to recurrence of 266 months, p>0.05). Another recent randomized trial in 212 high risk patients further demonstrated a greater efficacy for a combination of BCG followed by MMC plus electromotive administration, compared to single agent BCG (prolongation of disease-free interval from 21 to 69 months, reduction of recurrence rate from 57.9 to 41.9%, reduction of progression rate from 21.9 to 9.3%, decrease of overall death rate from all causes from 32.4 to 21.5% and decrease of disease-specific death rate from 16.2 to 5.6%) (98). These results compare favorably to the earlier data that showed no benefits in combining MMC and BCG relative to single agents (99–102).

Intravesical thermo-chemotherapy, combination of chemotherapy and localized hyperthermia, is under clinical evaluation. Hyperthermia enhances the effects of chemotherapy on inhibition of DNA synthesis and DNA damage, increases cell membrane permeability and alters intracellular drug trafficking and distribution (103–105). Intravesical hyperthermia is delivered using a microwave applicator inserted inside the bladder cavity, where the bladder wall temperature is maintained at 42–45°C. The drug solution is recycled to avoid overheating. In two single arm trials in intermediate-to-high risk patients, this modality showed a recurrence-free rate of 91% after a mean follow up of 289 days (n=22) (105), 86% at 1 year and 75% at 2 year (n=90) (106). A multi-center, randomized trial comparing the combination of intravesical MMC plus hyperthermia with MMC alone in superficial bladder cancer patients shows that adding hyperthermia improved the recurrence-free rate at 24 months (n=83, 83% vs 43%, p=0.0002) (25).

Prolonging residence time. Intravesical therapy is usually given over 2 hr, after which time the drug is drained from the bladder. Sustained-retention delivery platforms such as bioadhesive microparticles or hydrogel systems can serve as drug depots, thereby extending the drug exposure in the bladder cavity beyond the voiding of urine. In a mouse bladder cancer model, bioadhesive, paclitaxel-loaded poly (methylene malonate) microspheres were retained on bladder wall for more than 48 hr, and yielded survival advantage over a solution of paclitaxel in 5% Tween 80 (9-week survival rate of 91% vs 58%) (107). Similarly, a solution-state thermosensitive poly-(ethylene glycol)-poly (lactic acid-co-glycolic acid)-poly (ethylene glycol) polymer, capable of transforming itself into hydrogel matrix at body temperature (37°C), showed sustained drug release/retention in rat bladders over multiple bladder voidings for up to 24 hr (108). Ye et al reported a gelatin–doxorubicin complex that released doxorubicin for up to 12 days (109). None of the above formulations have been evaluated in humans.

Magnetic targeting. Magnetic targeting uses a magnet placed externally on the skin covering a predetermined site in the bladder (typically where tumors reside) to localize drug-containing magnetic particles in tumors, thereby providing continuous exposure to high drug concentrations in tumors. In swine bladders, administration of magnetic doxorubicin-loaded microparticles (10 to 80 mg drug in 300–800 mg magnetic particles) followed by 30-min of external magnetic targeting yielded localization of microparticles in superficial and deep tissue layers of the magnetic-targeted sites (primarily in superficial submucosa) that were retained for at least 44 days (110).

Gene therapy. Gene therapy poses several theoretical advantages over chemotherapy: (a) high selectivity for tumor cells with mutated genes, (b) restore cell growth to normalcy by correcting genetic defects rather than by killing cells, and (c) avoiding the emergence of chemoresistance. Development of gene therapy in bladder cancer has focused on modifying the mutated urothelial cells and restoring normal functions of tumor suppressor genes. A popular target is p53. Mutation of p53 gene or loss of p53 functions leads to uncontrolled cell growth (111). As p53 mutation is found in about 40% of bladder cancer patients with advanced transitional carcinoma (112), p53 gene therapy is an attractive therapeutic approach.

The two major types of gene delivery systems are viral and nonviral vectors. Viral vectors include adenovirus and vaccinia. The advantages of adenoviral vector include: (a) it is not integrated into host chromosomes, (b) ease of obtaining recombinant proteins and producing vectors in high titers, and (c) high viability of host cells post-infection. Adenovirus normally enters a cell via its membrane receptor, coxsackie/adenovirus receptor (CAR) (113,114). The luminal surface of the urothelium is coated with a glycosaminoglycans (GAG) layer (115), which retards the adherence of adenoviral vectors to CAR and the subsequent gene transfection (116). The GAG layer can be disrupted by ethanol (117), surfactants (e.g., dodecyl-beta-D-maltoside or sodium dodecyl sulfate) (118), Syn3 (a synthetic polyamide) (119) and low concentra-
tion of hydrochloric acid (60 mM). Hydrochloric acid was able to promote the transfection efficiency of intravesical E. Coli LacZ gene in adenoviral vector from 10% to 80% in rat urothelial cells (120). Syn3 acts as a transfer enhancer that circumvents the need for CAR binding (119).

A comparison of intravesical instillation (single administration) of viral IFN gene therapy (Ad-IFN/Syn3, recombination replication-deficient adenovirus containing human IFN alpha-2b gene and Syn3) to intravesical IFN showed that IFN gene therapy resulted in much higher IFN concentrations (1000-times higher peak level) that were sustained for a much longer duration (96 hr or longer vs undetectable at 24 hr) in urothelial tissues (121). This same study further showed that a second dose of viral gene therapy on day 3 was sufficient to maintain the IFN level in urine. Another study demonstrated that intravesical treatment with Ad-IFN/Syn3 for 1 hr on two consecutive days resulted in more than 40% shrinkage of murine bladder tumors; the extent of antitumor effect was correlated with the dose size of Ad-IFN and the IFN concentration in urine (122). Other replication-deficient adenovirus-mediated gene therapy targeting p53 (123), bFGF (124), IL-8 (125) also demonstrated successful gene delivery and effective inhibition of tumor growth in orthotopic (123) or subcutaneous (124,125) mouse bladder tumor models.

Another approach is the replication-competent oncolytic adenovirus, which, through self replication, results in lysis of tumor cells. CG0070, an oncolytic adenovirus encoding granulocyte macrophage colony stimulating factor (GM-CSF), preferentially replicates in retinoblastoma protein-defective bladder cancer cells and produces GM-CSF that activates host immune response (126). The tumor selectivity of CG0070 was indicated by the 100-fold higher replication and 1000-fold greater cytotoxicity in bladder transitional cell carcinoma cells compared to normal cells. CG0070 showed significant activities against orthotopic and subcutaneous human xenograft bladder tumor model in mice (126), and is undergoing phase 1 clinical trial in patients with recurrent bladder cancer after BCG treatment (127).

Vaccinia is a large, double-stranded DNA virus used for vaccination against smallpox (128). The advantages of recombinant vaccinia virus (rVV) include application in a wide range of hosts, rapid infection and efficient expression of inserted transgenes (112). Early experiments demonstrated that rVV effectively introduced functional genes into bladder tumor cells in vitro and in vivo (129). In a syngeneic, orthotopic mouse bladder tumor model, delivery of p53 gene using rVV (rVV-TK-53) resulted in 33% survival on day 70, whereas the delivery using phosphate buffer or empty vector showed no survival benefits (112).

Several phase I trials demonstrated that intravesical gene therapy can be safely administered to bladder cancer patients; no dose-limiting toxicity was observed for Dryvax vaccinia viral vectors (no therapeutic gene, 4 patients) (130) and adenoviral vector containing wild-type p53 gene with a gene-transfer enhancer (Big CHAP, 12 patients, single treatment) (131). The latter treatment resulted in the induction of RNA and protein levels of p21/WAF1 (downstream target of p53) in patient tumors, and the expression of vector DNA uniformly throughout the urothelium and submucosal tumors, indicating successful transgene expression. A third study in 13 patients receiving repeated doses of adenoviral vector-mediated p53 (Ad5CMV-p53) on a 28-day cycle also showed good tolerability at the highest dose of 1012 viral particles (132). Specific transgene expression was observed in 2 of 7 patients that yielded biopsy samples. Two recently initiated studies are evaluating intravesical Ad-IFN/Syn3 (133) and Ad-GM-CSF (127) in patients with recurrent, BCG-refractory bladder cancer.

In intravesical viral gene delivery, the primary safety concern is the inadvertent absorption of the vectors into the systemic circulation, as viral vectors may produce excessive immune response or insertional mutagenesis in host cells. In comparison, nonviral vectors (e.g., Lipoplexes) have the advantages of simple preparation, low-cost, easy manufacturing, low immunogenicity, and can carry larger size genes (134). In mice bearing established orthotopic bladder tumors, intravesical treatments of IL-2 gene therapy (delivered in cationic liposomal formulations, 6 treatments given every other day) resulted in higher survival rate, compared to the reporter gene control (40% vs 0% on day 60) (135). Similarly, IL-12 gene therapy (delivered in cationic liposomes, 6 treatments given every 3 days) was equally effective as high dose BCG (400 µg) in the same mouse model, and both treatments were significantly better compared to the reporter gene control (30% survival on day 60 vs 0%). Interestingly, IL-12 gene therapy was more efficacious than BCG in the surviving mice that were again implanted with orthotopic bladder tumors (100% vs 0% survival on day 60 after the reimplantation of tumor cells), suggesting more durable benefits of IL-12 gene therapy (136). Lipid-based gene vectors have undergone clinical evaluation in other cancer types, with good safety records (137-143).

Other experimental approaches. Small interfering RNA (siRNA) is emerging as novel cancer therapeutic agents (144-146). siRNA silences the expression of the targeted gene in a sequence-specific manner on the mRNA level. Treatment with survivin- or telomerase-targeted siRNA down-regulated the expression of the corresponding protein and suppressed the growth of bladder tumor cells (147-149). Instillation of siRNA/cationic liposomes targeting polo-like kinase-1 or PLK-1, a biomarker of poor prognosis in a murine orthotopic bladder cancer model, resulted in significant inhibition of PLK-1 expression and lowering the PLK-1 protein level in bladder tissues, in a time- and dose-dependent manner (150).

Another emerging experimental approach, still in preclinical evaluation, is using chemosensitizer to enhance the activity of agents with demonstrated clinical benefits. In cultured cells, meglumine-modified eicosapentaenoic acid, an omega-3 fatty acid, enhances the cellular uptake and activity of epirubicin and MMC (151). Our laboratory has shown that suramin, when given at non-cytotoxic doses, enhances the activity of MMC against human transitional cell RT4 xenograft tumor in mice (152). Suramin is a polyanion with over 20 molecular targets, including growth factors and proteins involved in signaling pathways important to cell cycle check points (153).

PART V. PERSPECTIVES

Because of the unique anatomical and physiological properties of the urinary bladder, including easy access for
instilling treatment and limited systemic absorption of the instilled agent, delivery of effective concentrations of therapeutics to bladder tumors during intravesical therapy is more readily accomplished compared to tumors located in systemic organs (e.g., lungs). From this perspective, treatment of bladder cancer bears similarity to treating tumor cells under in situ conditions. Hence, disease eradication or cures are real possibilities and should be the goal of future translational research. In addition, as shown in our work on intravesical MMC therapy, the computational drug transport models enable the prediction of drug concentrations in different parts of the bladder in vivo and, together with the pharmacodynamic data in human bladder tumors, can be used to predict the outcomes of different treatment conditions (e.g., dose, concentration, dwell time) and thereby enable the selection of optimal treatment conditions. From the standpoint of clinical therapy development, such computational approaches enable the projection of the anticipated benefits, including the margins of errors, of the experimental treatments and consequently the selection of appropriate patient sample sizes for detecting the desired statistical significance. Such quantitative approaches have the potential of improving the success rate of the clinical development of new treatments, which currently stands at <6% for cancer therapeutics (154). Finally, additional lessons learned from the use of intravesical therapy may improve the utility of other intracavity or regional therapies such as intraperitoneal treatment of peritoneal cancers or intrathecal treatment of brain cancer.

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