

Strength of contraction in isolated bladder strips of rats: effect of chemotherapeutic agents mitomycin C and farmorubicin

Dirk P.J. Michielsens¹, Jean-Jacques Amy², Danny Coomans³, Guy Storme⁴, Jean-Jacques Wyndaele¹

¹Department of Urology, University of Antwerp, Edegem, Belgium

²Department of Gynecology, Andrology and Obstetrics, Vrije Universiteit Brussel, Brussels, Belgium

³School of Mathematical and Physical Sciences, James Cook University, Townsville, Australia

⁴Department of Radiotherapy, Vrije Universiteit Brussel, Brussels, Belgium

Submitted: 3 May 2005

Accepted: 22 July 2005

Arch Med Sci 2005; 1, 2: 89-93

Corresponding author:

Dirk P.J. Michielsens, MD

Department of Urology

AZ-VUB

Laarbeeklaan 101

1090 Brussels, Belgium

E-mail: dirk.michielsens@az.rub.ac.be

Abstract

Objectives: The smooth muscle is an important component of the lower urinary tract, as normal voiding depends on its ability to respond appropriately to stimulation. Impairment of the contractile capacity of the vesical smooth muscle may be reflected in the global voiding function. Repeated intravesical instillations with mitomycin C or farmorubicin have a prolonged effect on bladder volume and compliance. The present study was undertaken to determine whether previous intravesical instillations with mitomycin C and farmorubicin influence the contractile responses of the smooth muscle of the bladder in rats.

Material and methods: Forty Wistar rats were divided into four groups. Rats in three of the groups had intravesical instillations of either mitomycin C, farmorubicin, or saline. Rats belonging to the 4th group had no intravesical instillations, and served as normal controls. The doses of drugs administered were based on those used for therapeutic purposes. After an induction course of three weeks, each bladder was cut in four strips (ventral, Elbadawi line, cranial and dorsal). Thereafter 136 bladder strips underwent electrical field stimulation.

Results: Contractile responses did not differ significantly depending on bladder location. The most intense contractile responses, however, were noticed in the dorsal bladder strips, and the weakest responses in the cranial pole strips. Previous intravesical instillations with chemotherapeutic agents did not influence the contractile response of the smooth muscle of the bladder.

Conclusion: Repeated intravesical instillations with mitomycin C or farmorubicin have no prolonged effect on the contractile strength of the isolated smooth muscle of the bladder.

Key words: bladder, mitomycin C, farmorubicin.

Introduction

Approximately 80% of all bladder cancer patients first present with superficial tumors of which 10 to 15% eventually become invasive [1, 2]. The initial therapy for superficial bladder cancer is transurethral resection of all visible tumoral growth. To improve local control and to reduce the risk of progression, the majority of patients with high grade or recurrent malignancy receive adjuvant intravesical chemotherapy or immunotherapy. The most

Table I. Bladder strip length, weight and CSA for each location

	ventral	el-Badawi	cranial	dorsal
length (mm)	12.4±1.7	12.2±1.4	8.1±2.0	12.4±1.6
weight (mg)	9.4±3.0	9.6±2.7	5.0±2.0	8.4±2.6
CSA (mm)	0.6±0.2	0.7±0.2	0.5±0.1	0.5±0.1

values are means ± SEM of single strips

commonly used chemotherapeutic agents are mitomycin C (MMC) and farmorubicin. The latter may occasionally cause mild to severe systemic side effects such as skin rash, chills, fever or malaise. Local bladder toxicity gives rise to dysuria, cystitis, frequency, and hematuria [3]. The incidence of local complications according to the literature ranges from 6% to 41% [3].

Experimental chemical cystitis has been studied both histologically and radiologically [4-7]. Urodynamic investigations have shown prolonged changes in bladder volume and compliance [8]. Either destruction of the glycosaminoglycan (GAG) layer, edema of the musosa or a dysfunction of the smooth muscle of the bladder wall causes these changes. So far, no research has been carried out to assess the effect of various intravesically instilled chemotherapeutic agents on the contractile response of the smooth muscle after electrical field stimulation of Wistar rat bladder strips.

Material and methods

Animals: Young adult female Wistar rats (age 13 weeks; median weight 217 g, range 187-255 g) were obtained from IFFA CREDO (France). They were selected as the experimental model because of their availability and easiness to catheterize. The animals were treated in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals (NIH Publication 86-3, revised 1985). The local ethical committee approved of the protocol. The rats received food and water ad libitum, and were weighed regularly for assessment of their general health.

The animals were randomized into four groups of 10 rats each: the normal control group received no instillations; the placebo-group was instilled with the solvent (NaCl 0.9%) of the chemotherapeutic agents; the MMC group and the farmorubicin group were submitted to instillations of solutions containing 1 mg/ml of these substances.

Intravesical drug administration: The two compounds used were MMC (Christiaens Pharma, Belgium) and farmorubicin (Pharmacia & Upjohn, Belgium).

Experimental design

Instillations: In rats belonging to three of the groups instillations were performed weekly for three

consecutive weeks. Heretofore the animals were anesthetized with 3% enflurane (Abbott) in a 1:3 mixture of oxygen and nitrous oxide. For induction of anesthesia the animal was placed in a sealed box in which the anesthetic gas was delivered by means of a Boyle apparatus. When the rat was anesthetized it was transferred to a specially designed instillation table. The bladder was emptied using a 22-gauge catheter (Vasculon). The drugs were given in a volume of 0.1 ml; the rats belonging to the NaCl group received an equal volume of saline, whereas the normal controls had no instillations. The procedure was repeated every week during a 3-week study period.

Tissue preparation: Rats were weighed and brought under anesthesia with sodium pentobarbital (50 mg/kg IP). The abdomen was opened, the aorta was transected and the bladder was dissected out with a segment of the urethra and both ureters, and freed of any adjacent structures. The bladder was placed in 4°C oxygenated Krebs-Ringer solution (pH 7.35) for one night [9]. The bladder was then transferred to a dissection plate, opened by a longitudinal incision, pinned out mucosal side up, and submerged in oxygenated Krebs-Ringer solution. Four bladder strips were carefully cut with known orientation and position in the bladder as described in Figure 1. The strips were weighed and measured before being suspended to avoid fluid absorption during experimentation. The strips were suspended on No. 00 noncapillary silk sutures in organ baths (20 ml) containing Krebs buffer at 37°C, bubbled with 95% O₂ – 5% CO₂, the caudal end fixed and the cranial part connected, via a slight stainless rod, to a force transducer (EMKA force displacement transducer – EMKA Technologies, France EJL SO45-2009 EMKA R 7389). The strips underwent stress relaxation under a load for 30 minutes, were washed every 15 minutes, and adjusted to a 2.5 g tension.

Electrical stimulation: For electrical stimulation a four-channel stimulator of EMKA technologies (EMKA Technologies, France) was used. Tissues were stimulated by means of platinum electrodes. A rectangular biphasic current of 400 µs duration with a frequency of 33.0 Hz was applied. The amplitude of the current ranged from 400 to 800 mA. Stimulation lasted until the contraction reached a plateau phase. Contractile responses were recorded with an adapted software program (Loslab, EMKA Technologies, France). In the interval between stimulations, the strips were washed for 10 minutes.

Chemicals: The Krebs-Ringer buffer contained 4.7 mM KCl, 1.2 mM MgSO₄·7H₂O, 2.5 mM CaCl₂·2H₂O, 0.03 mM CaEDTA, 118 mM NaCl, 25 mM NaHCO₃, 11.1 mM glucose, and its pH was 7.35.

Statistical analysis: All data are expressed as mean \pm 1 standard error of the mean. Cross-sectional area (CSA) of each strip was calculated as weight/(length x density). Statistical analysis was done using one-way ANOVA but for multicomparisons t-tests with Bonferroni corrections were resorted to. $P < 0.05$ was considered significant. For statistical computations SPSS release 10.0.7 (SPSS Inc, Chicago, 2001) was used.

Results

Six rats did not survive the study period; deaths were related to the anesthesia, and occurred in all groups. Thus upon completion of the study there remained 9 rats in the normal control group, 9 in the NaCl 0.9% group, 9 in the MMC group, and 7 in the farmorubicin group.

Values for strip weight, length and cross-sectional area are given in Table 1. The length and the weight of strips from the cranial pole were significantly different from those of strips taken from other sites. However, cross-sectional areas did not differ. Results are presented as contraction force (g) and contraction strength divided by the cross-sectional area (g/mm^2).

All groups exhibited a similar response pattern to electrical field stimulation. The contractile responses reached a peak force at 720 mA.

Effect of strip location on contractile responsiveness

The bladder strips of the untreated rats were used to compare the contraction force of the strips taken from the four different locations. The absolute contractile responses to field stimulation were similar (Figure 2). However, the strongest contractile responses were observed in the dorsal bladder strips, and the weakest responses in the cranial pole strips. Similarly, there were no differences between the contractile responses of these strips after correction for cross-sectional area (Figure 3).

Effect of chemotherapeutic agents on contractile responsiveness

The absolute contractile responses to field stimulation of bladder strips were similar, although the responses of the strips exposed to NaCl were somewhat less intense than those of the strips exposed to either MMC or farmorubicin ($P=0.15$ and $P=0.06$, respectively) (Figure 4). There was no difference between the normal-strips, on the one hand, and the MMC and farmorubicin strips, on the other hand ($P=0.9$ and $P=0.5$, respectively). Similarly, there were no differences between MMC and farmorubicin-treated strips ($P=0.86$). Even after

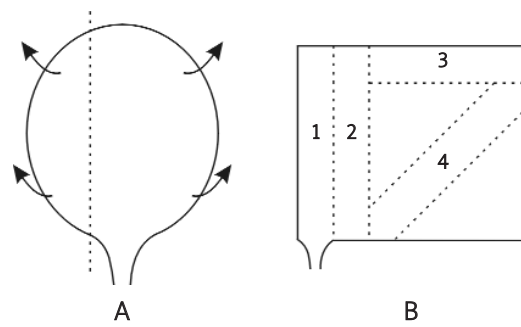


Figure 1. A. Anterior view. The bladder cut ventrally right from midline and spanned open like a rectangle; B. Four strips are chosen. 1) ventral, 2) el-Badawi's line, 3) cranial, 4) dorsal

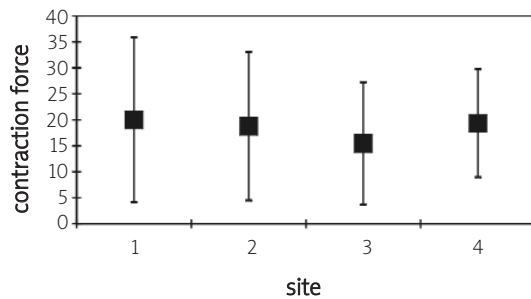


Figure 2. Effects of strip location on contractile responsiveness. 1) ventral strip, 2) el-Badawi's line, 3) cranial strip, 4) dorsal strip

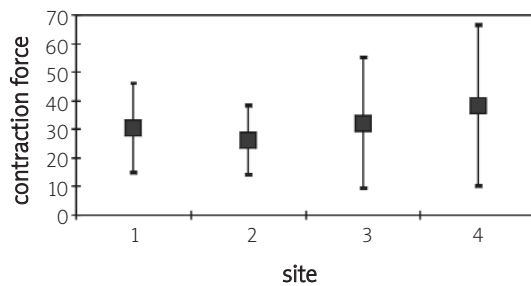


Figure 3. Effects of strip location on contractile responsiveness after correction for strip cross sectional area. 1) ventral strip, 2) el-Badawi line, 3) cranial strip, 4) dorsal strip

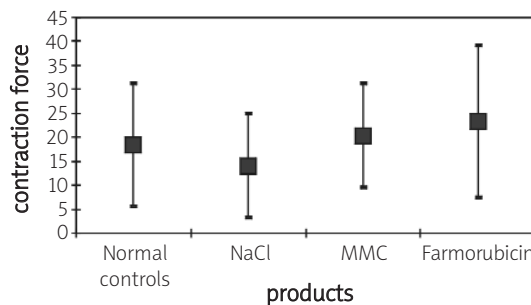


Figure 4. Effects of chemotherapeutic agents on contractile responsiveness

correction for strip cross sectional area, no statistical significant difference was found.

Discussion

Toxicity is the main dose-limiting factor in cancer therapy. Therefore it is important to assess qualitatively and quantitatively induced side effects when comparing different treatments.

Several clinical studies report moderate to severe acute complications following intravesical administration of either MMC or farmorubicin [4, 10].

Systemic absorption is not the problem. The plasma uptake of any drug administered intravesically depends on its molecular weight and, to a lesser degree on its lipid solubility. Systemic absorption of drugs with a molecular weight greater than 300 is minimal [11]. Because of the relatively high molecular weight of MMC (MW 334) and farmorubicin (MW 342), the plasma uptake after intravesical administration is minimal. Plasma uptake after intravesical instillations of MMC has been investigated by several authors and generally found to be lower than 60 ng/ml. Absorption of farmorubicin instilled into the bladder is generally discrete, and plasma levels do not exceed 0.5 ng/ml [12-15].

The acute bladder symptoms are usually transient and recede when administration of the drug is interrupted.

Recently, unlike earlier data had indicated, we observed changes in bladder volume and compliance after intravesical instillations with MMC and farmorubicin in rats, which persisted three weeks after cessation of therapy [8]. In the medical literature there are even reports of permanently contracted bladders after repeated instillations [16]. In this study we assessed whether functional damage of the smooth muscle cells occurs after repeated intravesical chemotherapy with MMC and farmorubicin.

Before studying the contractility after intravesical instillations, we investigated the contractile force of the vesical smooth muscle taken from different sites. The influence of the strip location is important to know if several different rats are needed for comparative purposes or if several strips from the ventral and dorsal region of the same bladder can be used.

Two different opinions prevail pertaining to the structural organisation of the bladder in rats. According to Gabella and Uvelius, the muscle fibres in the ventral and dorsal walls of the bladder tend to run in a preferential direction. These authors found that the ventral aspect of the bladder in female rats had a large component of longitudinally

oriented muscle fibres, whereas the dorsal side had a large sheet-like component of circular muscle. Laterally and at the cranial pole, the bundles criss-cross in every direction and are probably oriented at random [17]. Elbadawi stated that the muscle bundles of the body unit have a "plexiform" organisation without discernible "layers". According to him, the ventral surface of the detrusor has a vertical midline bundle that extends from its dome to near its junction with the base region: short, transversely oriented bundles derived from the outlying detrusor muscularis are inserted on each side of the midline bundle [18].

Longhurst et al. investigated the contractile force of the dorsal and ventral strips of the urinary bladder of male Sprague-Dawley rats. Both types of bladder strips responded to electrical field stimulation in a similar manner. These investigators also concluded that the contractile response is independent of strip length if data are expressed as tension/CSA [9]. We evaluated the response to electrical stimulation of four different regions of the bladder. The ventral strips were cut with the smooth muscle bundles in their preferential direction. Two ventral strips were taken according to Elbadawi's description of the ventral surface of the bladder with a thin bundle in the midline extending from the dome to near the junction. We found no statistically significant difference in the contractile strength between the different sites.

Observations from normal bladder strip preparations do not necessarily apply to preparations obtained from hypertrophic bladders such as those from diabetic animals [9]. In the latter, both the relative concentrations of connective tissue and contractile proteins, and/or the orientation of the smooth muscle fibres in the bladder may be different. Also biochemical changes may have an impact on energy utilisation. Therefore, the cross-sectional area of each strip is calculated and taken into account to determine the absolute contractile responses. However, after correction for length and weight, the overall results were the same. This is in agreement with observations made by Longhurst et al. [9]. Therefore the benefit of CSA in this kind of studies is questionable.

Post and Stewart observed muscle fibrosis in mice after irradiation of the bladder that resulted in a decreased bladder capacity and increased frequency [19, 20]. The fact that the whole bladder wall is irradiated causes these histologic and urodynamic changes. Intravesical instillations with MMC and farmorubicin also decrease vesical capacity and compliance. These urodynamic alterations persisted three weeks after the end of the instillation period [8]. However, during the present study we observed

no changes in the contractile forces of the smooth muscle of these bladders when assessed in vitro. Therefore, the exclusive role of the smooth muscle in these urodynamic changes can be excluded. Changes such as destruction of the GAG-layer or edema of the bladder mucosa must be investigated in the future.

Conclusion

Repeated intravesical instillations with MMC or farmorubicin have no effect on the contractile forces of the smooth muscle of the bladder.

References

- Lum BL, Torti FM. Adjuvant intravesical pharmacotherapy for superficial bladder cancer. *J Natl Cancer Inst* 1991; 83: 682-94.
- Donat SM. Evaluation and follow-up strategies for superficial bladder cancer. *Urol Clin North Am* 2003; 30: 765-6.
- Thrasher JB, Crawford ED. Complications of intravesical chemotherapy. *Urol Clin North Am* 1992; 19: 529-39.
- Shelley MP, Coort JB, Kynaston H, Wilt TJ, Coles B, Mason M. Intravesical bacillus Calmette-Guerin versus mitomycin C for Ta and T1 bladder cancer. *Cochrane Database Syst Rev* 2003; (3): CD 003231.
- Chang SY, Yu DS, Wang J, Ma CP. Histopathological effects and recovery rate of rat bladders following intravesical instillation of thiotepa, doxorubicin and mitomycin C. *Br J Urol* 1990; 66: 623-7.
- Kumar S. Effect of intravesical formalin on the urothelium. *Br J Urol* 1979; 51: 375-9.
- Maggi CA, Abelli L, Giuliani S, Santicioli P, Geppetti P, Somma V, et al. The contribution of sensory nerves to xylene-induced cystitis in rats. *Neuroscience* 1988; 26: 709-23.
- Michielsen D, Amy JJ, Coomans D, Storme G, Wyndaele JJ. Mitomycin C and farmorubicin: functional bladder damage in rats after repeated intravesical instillations. *J Urol*, accepted for publication.
- Longhurst PA, Leggett RE, Briscoe JA. Influence of strip size and location on contractile responses of rat urinary bladder body strips. *Gen Pharmacol* 1995; 26: 1519-27.
- Onrust, SV, Wiseman LR, Goa KL. Epirubicin: a review of its intravesical use in superficial bladder cancer. *Drugs Aging* 1999; 15: 307-33.
- Kowalkowski, TS, Lamm DL. Intravesical therapy of superficial bladder cancer. In: *Current Trends in Urology*. MI Resnick (ed.). Baltimore: Williams & Wilkins, 1988; vol. 4: pp 89-107.
- Bracken RB, Swanson DA, Johnson DE, De Furia D, von Eschenbach AC, Crooke S. Role of intravesical mitomycin C in management of superficial bladder tumors. *Urology* 1980; 16: 11-15.
- Wientjes MG, Dalton JT, Badalament RA, Drago JR, Au JL. Bladder wall penetration of intravesical mitomycin C in dogs. *Cancer Res* 1991; 51: 4347-54.
- Kurth KH, De Wall JG, Van Oosterom AT, De Jong EA, Tjaden UR. Plasma levels during intravesical instillation of mitomycin-C. In: *EORTC Genitourinary Group Monograph 2, part B: Superficial bladder tumors*. F Schroeder, B Richards (eds). New York: Alan R. Liss, Inc., 1985: pp. 81-93.
- Mishina T, Watanabe H. Mitomycin C instillation therapy for bladder tumors. In: *Mitomycin C: Current Status and New Developments*. SK Carter, ST Crook (eds). New York: Academic Press, 1979: pp. 193-203.
- Eijsten A, Knonagel H, Hotz E, Brusch HP, Hauri D. Reduced bladder capacity in patients receiving intravesical chemoprophylaxis with mitomycin C. *Br J Urol* 1990; 66: 386-8.
- Gabella G, Uvelius B. Urinary bladder of rat: fine structure of normal and hypertrophic musculature. *Cell Tissue Res* 1990; 262: 67-79.
- el-Badawi A, Shenk EA. A new theory of the innervation of bladder musculature. 1. Morphology of the intrinsic vesical innervation apparatus. *J Urol* 1968; 99: 585-7.
- Post JG, te Poele JA, Oussoren Y, Stewart FA. Radiation tolerance of normal mouse bladders after intravesical chemotherapy. *Radiother Oncol* 1995; 34: 30-8.
- Stewart FA. Mechanism of bladder damage and repair after treatment with radiation and cytostatic drugs. *Br J Cancer Suppl* 1986; 7: 280-91.