

Enhanced urothelial ATP release and contraction following intravesical treatment with the cytotoxic drug, doxorubicin

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Abstract Intravesical administration of the cytotoxic drug doxorubicin is a common treatment for superficial carcinoma of the bladder, but it is associated with significant urological adverse effects. The aim of this study was to identify doxorubicin-induced changes in the local mechanisms involved in regulating bladder function. As a model of intravesical doxorubicin administration in patients, doxorubicin (1 mg/mL) was applied to the luminal surface of porcine bladders for 60 min. Following treatment, the release of urothelial/lamina propria mediators (acetylcholine (Ach), ATP and prostaglandin E₂ (PGE₂)) and contractile responses of isolated tissue strips was investigated. Doxorubicin pretreatment did not affect contractile responses of detrusor muscle to carbachol, but did enhance neurogenic detrusor responses to electrical field stimulation (219 % at 5 Hz). Contractions of isolated strips of urothelium/lamina propria to carbachol were also enhanced (30 %) in tissues from doxorubicin pretreated bladders. Isolated strips of urothelium/lamina propria from control bladders demonstrated a basal release of all three mediators (Ach>ATP>PGE₂), with increased release of ATP when tissues were stretched. In tissues from doxorubicin-pretreated bladders, the basal release of ATP was significantly enhanced (sevenfold), while the release of acetylcholine and PGE₂ was not affected. The application of luminal doxorubicin, under conditions that mimic intravesical administration to patients, affects urothelial/lamina propria function (increased contractile activity and ATP release) and enhances efferent neurotransmission without

affecting detrusor smooth muscle. These actions would enhance bladder contractile activity and sensory nerve activity and may explain the adverse urological effects observed in patients following intravesical doxorubicin treatment.

Keywords Doxorubicin · Bladder cancer · Urothelium · Detrusor · Intravesical · Complications

Introduction

Intravesical administration of cytotoxic agents for the treatment of superficial bladder cancer has the advantage of direct exposure of the target cancer cells to cytotoxic agents, while also limiting systemic exposure. This method of delivery is used successfully for a number of cytotoxic agents. While the effects of these treatments on the tumour are well documented, little is known of their effects on normal bladder function despite evidence of significant adverse effects following intravesical treatment including dysuria, increased urinary frequency and urgency (Koya et al. 2006; Thrasher and Crawford 1992). Doxorubicin is one such cytotoxic drug that is used intravesically for superficial bladder cancer, but has been shown to cause bladder toxicity in 13–56 % of patients (Thrasher and Crawford 1992). Despite the large number of patients receiving this agent intravesically and the high percentage suffering urological adverse effects, there have been no investigations of the actions of this agent on the non-cancerous tissues of the bladder.

Intravesical treatment brings the administered drug into close proximity with the urothelium and cells of the underlying lamina propria, and some drug may also diffuse into the detrusor muscle itself (Wientjes et al. 1996). The urothelium acts as a significant barrier to the movement of drugs from the

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bladder lumen, but this layer and the underlying lamina propria also play important roles in the regulation of sensory mechanisms and bladder contraction. The bladder urothelium and lamina propria exhibits spontaneous contractile activity (Moro et al. 2011, 2012; Sadananda et al. 2008) and develops tonic contractions in response to agonists such as neurokinin A and carbachol (Sadananda et al. 2008). The contractile mechanisms involved are unclear but may involve myofibroblasts (Sadananda et al. 2008) and/or smooth muscle (Heppner et al. 2011) located in the lamina propria. This tissue also releases a number of chemical mediators that influence sensory nerve activity (ATP, acetylcholine, prostaglandin E₂, PGE₂) and releases a factor that inhibits detrusor contraction (urothelium-derived inhibitory factor, UDIF) (Chaiyaprasithi et al. 2003; Hawthorn et al. 2000; Templeman et al. 2002).

Stretch of the urothelium stimulates the release of ATP (Burnstock 2011), acetylcholine (ACh) (Yoshida et al. 2006, 2008) and PGE₂ (Aizawa et al. 2010), and all three chemical mediators have been implicated in bladder sensory mechanisms (Birder et al. 2010; Cockayne et al. 2000; Maggi et al. 1988b). It is well established that ATP in particular activates P2X_{2/3} receptors on sensory nerves in the lamina propria and mutant mice lacking this receptor have micturition dysfunction (Vlaskovska et al. 2001). The importance of this purinergic system in the human lower urinary tract has been highlighted by the findings that urothelial ATP release is enhanced in patients with idiopathic or neurogenic bladder overactivity (Kumar et al. 2010) and is greatly increased from the urothelium of patients with painful bladder syndrome (Kumar et al. 2007).

Little is known of the action of doxorubicin on bladder function, despite the fact that it is a common treatment administered repeatedly for superficial bladder cancer and is known to exert significant urological adverse effects. Thus, the aim of the present study was to identify changes in the local bladder mechanisms (e.g. mediator release and/or tissue sensitivity) that are known to be involved in maintaining normal bladder function and which may explain the bladder dysfunction noted by patients following intravesical treatment with doxorubicin.

Materials and methods

Fresh bladders from Large White-Landrace pigs (6 months old, 80 kg) were obtained from a local abattoir and immediately immersed in cold Krebs-bicarbonate solution (composition in mM: NaCl 118, NaHCO₃ 24.9, CaCl₂ 1.9, MgSO₄ 1.15, KCl 4.7, KH₂PO₄ 1.15 and D-glucose 11.7) [5]. The bladders were opened longitudinally and sheets of full thickness anterior wall from the dome region were set up in chambers containing Krebs-bicarbonate solution gassed with 5 % CO₂ in oxygen. Separated solutions of gassed Krebs solution bathed the luminal and serosal surfaces (see Fig. 1), allowing

doxorubicin to be administered to the luminal surface only. The tissues were incubated at 37 °C for 1 h with a therapeutic concentration (1 mg/mL) of doxorubicin applied to the luminal surface. Control bladders were incubated for 1 h without the addition of doxorubicin. Following this pretreatment, tissue strips were isolated and set up under 100 mN resting tension in EZ-Bath tissue baths (GlobalTown Microtechnology, FL, USA) containing Krebs-bicarbonate solution at 37 °C to allow the examination of tissue responses. Four sets of tissues were examined as follows:

- (i) Full-thickness bladder wall with an intact urothelium and lamina propria
- (ii) Denuded detrusor strips with the urothelium and lamina propria removed
- (iii) Strips of urothelium and lamina propria for recording of tissue contraction
- (iv) Strips of urothelium and lamina propria for the measurement of stretch-induced ATP, acetylcholine and PGE₂ release.

Isometric contractions of isolated tissue strips were recorded using a Powerlab data acquisition system (AD Instruments). ATP was measured using a luciferase-luciferin assay kit (Molecular Probes) according to the manufacturer's instructions. Luminescence was measured using a Modulus microplate reader (Promega). Acetylcholine was measured using a fluorescence-based Amplex[®] Red Acetylcholine Assay kit (Molecular Probes) according to the manufacturer's protocol. Fluorescence was measured on a Modulus Microplate reader (Ex. 540/Em. 590 nm). Prostaglandin E₂ was measured using a monoclonal antibody based assay (Cayman Chemicals) performed according to manufacturer's protocol with a Modulus microplate reader (420 nm).

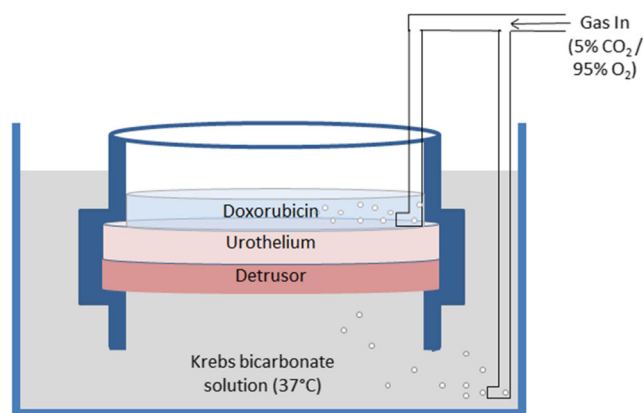


Fig. 1 Schematic figure of the modified Ussing chamber. Full-thickness sheets of bladder dome were sandwiched between two separated bathing solutions, each containing gassed (5 % CO₂/95 % O₂) Krebs-bicarbonate solution at 37 °C. Tissues were incubated with doxorubicin (1 mg/mL) applied to the luminal side only, for 60 min before isolation of the various tissues for pharmacological analysis

Isolated tissue responses

To assess the effects of doxorubicin pretreatment on tissue responsiveness, cumulative concentration-response curves to carbachol were obtained on tissues (i), (ii) and (iii) described above. Tissues (iv) above were used to examine the effects of doxorubicin on basal and stretch-induced release of mediators from the urothelium/lamina propria. These tissues were washed, and 2 min later, a sample of the bathing medium was collected and frozen for later assay of mediators (basal release). The tissues were then stretched, increasing length by 50 % and the bathing medium again collected and frozen for assay of mediators (stimulated release). To investigate the effects of doxorubicin on nerve-mediated responses, detrusor strips denuded of urothelium and lamina propria (tissues (ii) above) were set up under 100 mN resting tension in organ baths and electrically field stimulated via silver electrodes placed either side of the tissue. Tissues were stimulated at 1, 5, 10 and 20 Hz using 5 s trains of pulses (20 v, 0.5 ms pulse-width) delivered every 100 s. Contractile responses and the release of mediators for tissues from doxorubicin pretreatment bladders were compared with those of tissues from control incubated bladders.

Bladder histology

Sections of control and doxorubicin pretreated intact bladder dome were fixed (4 % neutral-buffered formalin), processed and embedded in paraffin. Tissues were sectioned at 6 μ m and placed on uncharged slides. Sections were stained using haematoxylin and eosin to assess urothelial integrity and examined using an Olympus CX31 microscope (Olympus Australia Pty. Ltd.) equipped with an Infinity 2 camera and Infinity Capture software. Image J software was used to measure relative urothelial thickness in control and doxorubicin pretreated tissues. At least ten urothelial thickness measurements were obtained from each bladder section, with six bladders examined from each group.

Drugs, chemicals and reagents

Carbachol (carbamoylcholine chloride) and atropine sulphate were obtained from Sigma-Aldrich (Castle Hill, New South Wales, Australia). Doxorubicin was obtained from Tocris (Bristol, UK). Solutions were prepared in deionised water and further diluted in Krebs-bicarbonate solution.

Data analysis and statistical procedures

Mean (\pm SD) increases in force developed in response to carbachol or electrical field stimulation were calculated. For response to carbachol, individual $-\text{LogEC}_{50}$ (pEC₅₀) values were determined from the concentration-response curves by use of

GraphPad “PRISM” software and mean (\pm SD) pEC₅₀ values and maximum responses calculated. Similarly for the mediator release study, mean (\pm SD) concentrations were determined before and after stretch and data for doxorubicin and control pretreated bladders compared. Data were analysed using a paired Student *t* test or one-way ANOVA with Dunnett multiple comparisons test, using Graphpad InStat3 software (SanDiego, CA). Significance levels were defined as $P < 0.05$.

Results

Contractile responses to carbachol

Isolated strips of intact bladder and strips of urothelium/lamina propria exhibited spontaneous phasic activity (3.2 ± 1.1 cycles/min, $n=6$ for intact bladder and 2.9 ± 1.6 cycles/min for urothelial strips, $n=6$), while this activity was rarely observed in isolated strips of detrusor muscle. Doxorubicin pretreatment reduced the spontaneous phasic rates of contraction in both intact bladder strips and strips of urothelium/lamina propria (Fig. 2a), without significantly affecting the amplitude of contractions (Fig. 2b).

All tissue strips (detrusor smooth muscle strip denuded of urothelium/lamina propria, bladder strips with an intact urothelium/lamina propria and isolated strips of urothelium/lamina propria) from control and doxorubicin pretreated

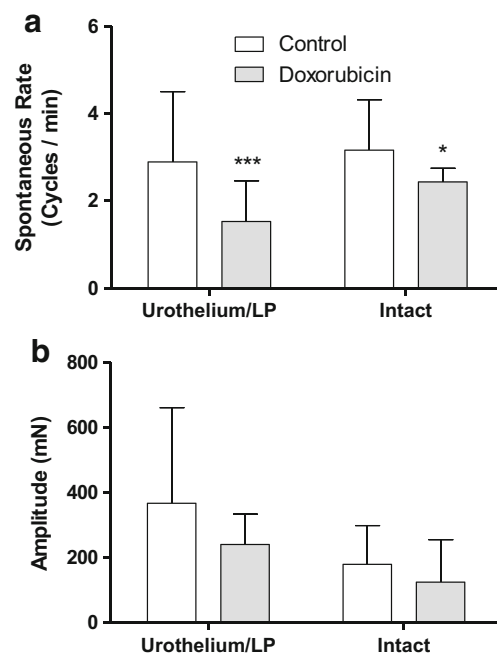


Fig. 2 Mean (\pm SD) **a** frequency and **b** amplitude of basal spontaneous contractile activity in intact bladder strips and urothelium/lamina propria strips from control (*open columns*) and doxorubicin-pretreated bladders (*shaded columns*). * $P < 0.05$; *** $P < 0.001$ for pretreated vs control tissues

bladders contracted to carbachol (Fig. 3, Table 1). The potency (pEC₅₀ values) of carbachol was similar on all three tissues, but the magnitude of responses varied between the tissues. When expressed as the force developed per gramme of tissue, the largest contractions to carbachol were produced by the urothelium/lamina propria tissues, which were significantly ($P<0.01$) greater than the denuded detrusor tissues, which in turn produced contractions significantly ($P<0.01$) greater than the intact bladder tissues (Table 1). Pretreatment with doxorubicin (1 mg/mL) for 1 h did not alter subsequent responses of the detrusor muscle, either intact or denuded to carbachol (Fig. 3; Table 1). However, doxorubicin pretreatment resulted in enhanced subsequent responses of the urothelium/lamina propria to carbachol (Fig. 3b) with no change in potency (Table 1).

Detrusor responses to electrical field stimulation

Electrical field stimulation induced frequency-dependent contractions of denuded detrusor muscle strips that in preliminary experiments were abolished by the neurotoxin tetrodotoxin (1 μ M), thus confirming their neurogenic origin. When these nerve-mediated contractile responses were examined after drug treatment, the responses to electrical field stimulation were significantly enhanced in the doxorubicin pretreated tissues (Fig. 3d). At the lowest stimulation frequency of 1 Hz, control tissues failed to respond ($n=5$), but after doxorubicin pretreatment, tissues contracted by 31 ± 13 mN ($n=6$). With increasing stimulation frequency, responses increased, but at

every frequency examined (1, 5, 10 and 20 Hz) responses were significantly greater ($P<0.05$) in tissues that had received the doxorubicin pretreatment (Fig. 3d). The presence of atropine (1 μ M) depressed detrusor contractions to EFS (20 Hz) by 76.4 ± 11.1 % in control tissues ($P<0.001$, $n=5$). The inhibition of responses to EFS (20 Hz) by this muscarinic antagonist was similar after doxorubicin pretreatment (79.2 ± 4.3 % inhibition, $n=5$).

Urothelial mediators

Isolated strips of urothelium/lamina propria from control-incubated tissues released all three mediators, but the basal release of Ach was approximately 1000-fold greater than that of ATP or PGE₂ (Fig. 4). Stretching the tissues produced a 5-fold increase in ATP release, but had no effect on either Ach or PGE₂ release. In tissues from bladders pretreated with doxorubicin (1 mg/mL for 60 min), the basal release of Ach and PGE₂ was similar to control tissues. However the basal release of ATP in pretreated tissues was significantly increased compared to tissues from control bladders and these elevated levels were not significantly different to those of the stimulated values for controls. Similar to tissues from control bladders, stretching doxorubicin-pretreated tissues increased ATP levels without affecting Ach or PGE₂ release. The release of ATP during stretch was similar to that observed in tissues from control bladders, although the stretch-induced increase in ATP was less than that observed in controls due to the higher initial basal levels (Fig. 4).

Fig. 3 Cumulative concentration-response curves to carbachol of **a** intact strips of bladder wall and **b** strips of urothelium/lamina propria from control (*closed squares*) and doxorubicin-pretreated (*closed circles*) bladders. Also shown are responses to carbachol **c** and electrical field stimulation at 1, 5, 10 and 20 Hz **d** of detrusor muscle strips denuded of urothelium/lamina propria from control-pretreated (*closed squares*) and doxorubicin-pretreated (*closed circles*) bladders. Data are mean \pm SD values. * $P<0.05$ compared to control-incubated tissues

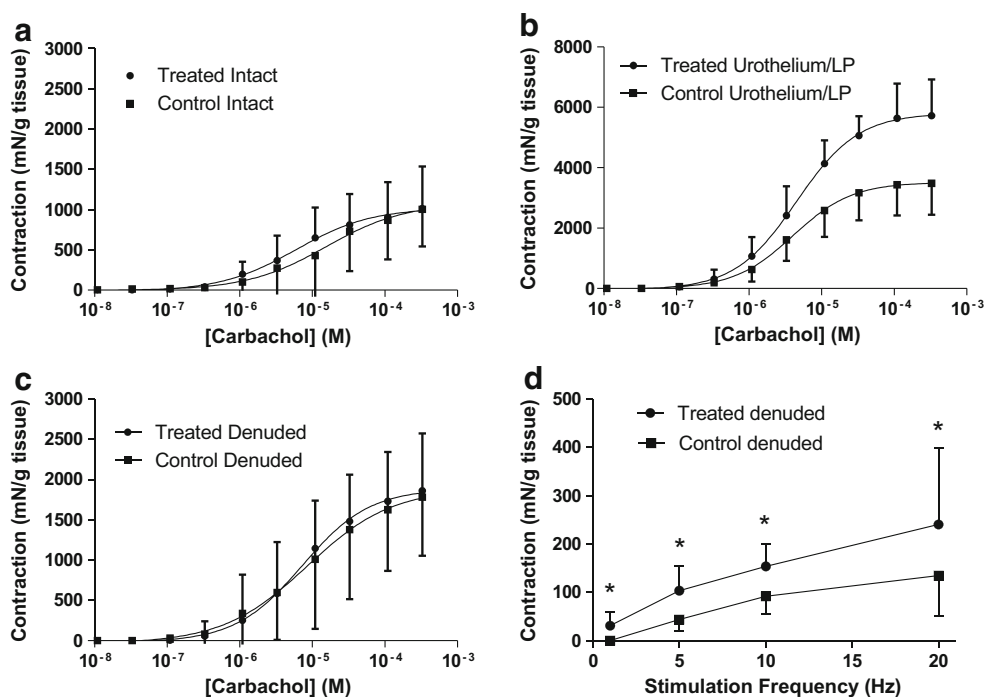


Table 1 Mean (\pm SD) potency (pEC₅₀) values and maximum responses (mN/g tissue) to carbachol of intact bladder strips, detrusor muscle strips denuded of urothelium/lamina propria and strips of isolated urothelium/lamina propria

Tissue	Control (untreated) bladders			Doxorubicin-pretreated bladders		
	Potency	Maximum	<i>n</i>	Potency	Maximum	<i>n</i>
Intact bladder	4.81 \pm 0.73	1082 \pm 610	6	5.23 \pm 0.64	1021 \pm 370	6
Denuded detrusor	5.07 \pm 0.86	1891 \pm 828 ^a	6	5.14 \pm 0.64	1888 \pm 717 ^a	6
Urothelium/lamina propria	5.39 \pm 0.34	3518 \pm 573 ^b	6	5.35 \pm 0.34	5807 \pm 730 ^{bc}	6

n number of bladders examined

^a*P*<0.01 denuded detrusor compared to intact detrusor

^b*P*<0.05 urothelium compared to denuded detrusor

^c*P*<0.001 pretreated urothelium compared to corresponding control urothelium.

Inhibition of detrusor contraction by the urothelium

The presence of an intact urothelium/lamina propria on strips of detrusor smooth muscle inhibited contractions to carbachol

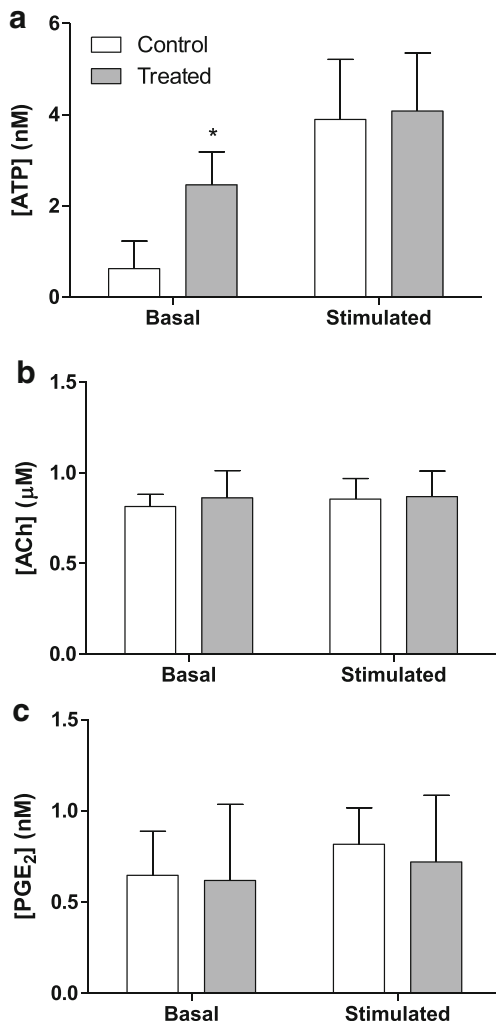


Fig. 4 Mean (\pm SD) basal and stretch-induced release of **a** ATP, **b** ACh and **c** PGE₂ from strips of urothelium/lamina propria from control- (*open columns*) and doxorubicin-pretreated (*closed columns*) bladders. **P*<0.05 compared to basal release in control tissues

in tissues from both control and doxorubicin-pretreated bladders (Table 1, Fig. 3). However pretreatment with the cytotoxic drug did not alter the amount of the inhibition. In control tissues, the potency of carbachol was similar in intact and denuded tissues, but maximum responses were significantly depressed in the intact compared to urothelium/lamina propria denuded tissues. Similar results were obtained in tissues from doxorubicin-pretreated bladders, with the potency of carbachol being similar in intact and denuded tissues, but with the maximum responses being significantly reduced (*P*<0.01) in the tissues with an intact urothelium/lamina propria (Table 1, Fig. 3).

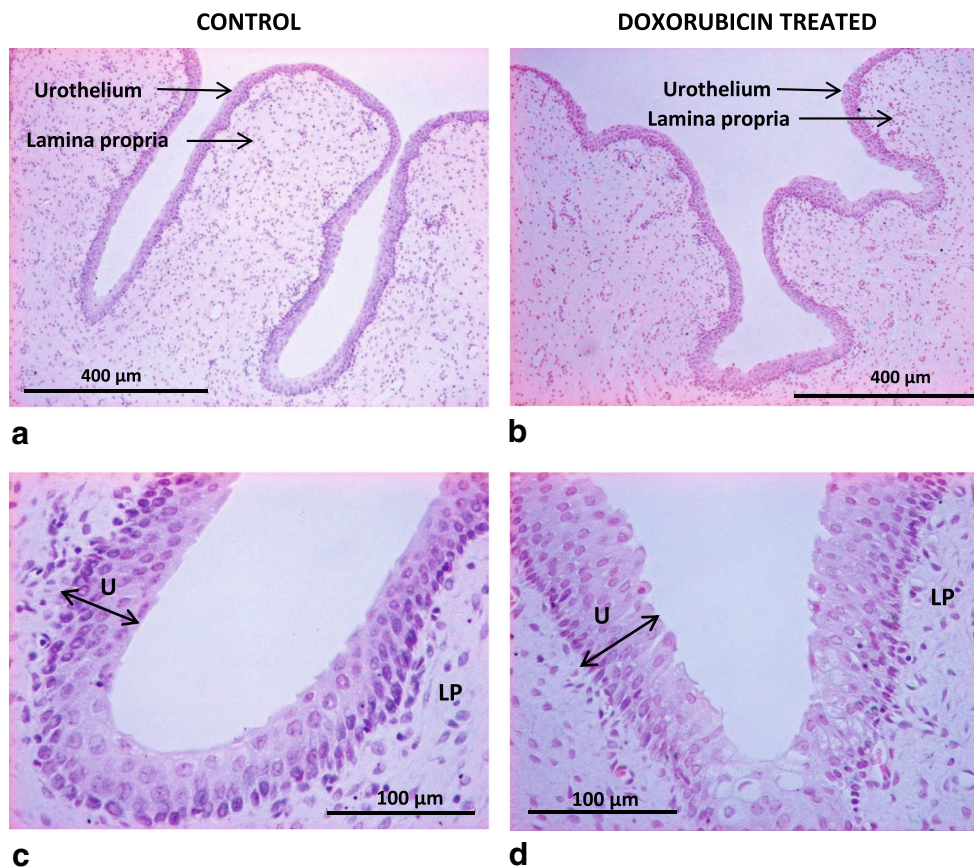
Bladder histology

Typical histological features were clearly identifiable in sections of control-incubated bladder, with the urothelium and lamina propria thrown into folds and overlying a deeper smooth muscle layer (Fig. 5). Histologically, the bladder structure was not affected by pretreatment with doxorubicin. Urothelial thickness was similar in control (52.3 \pm 9.5 μ m, *n*=4 bladders) and doxorubicin-treated bladders (52.8 \pm 7.6 μ m, *n*=4 bladders).

Discussion

Doxorubicin is a cytotoxic agent used in the treatment of superficial bladder cancer. It is administered intravesically to maximise delivery to the site of therapeutic action while minimising systemic adverse effects. However, intravesical administration of cytotoxic agents can have local effects within the bladder and for doxorubicin, dysuria, urgency and frequency have been reported to develop in a significant number of patients following treatment (Thrasher and Crawford 1992). The present study investigated mechanisms that may be involved in mediating these adverse effects by using a full-thickness bladder wall model that could best represent intravesical treatment in patients. Thus, the intact

Fig. 5 Haematoxylin and eosin (H and E) staining of control- (a, c) and doxorubicin-pretreated (b, d) bladders. H and E staining at a, b $\times 10$ and c, d $\times 40$ showing the urothelium (U) and lamina propria (LP)



glycosaminoglycans (GAG) layer and uroplakins are present and these limit diffusion of drug into the underlying urothelium, lamina propria and detrusor layers. A previous study has estimated that only 3 % of intravesically administered doxorubicin penetrates the uroplakin/GAG layers to reach the urothelial cells beneath (Wientjes et al. 1996). Also, in our model, drug administration mimicked conditions that the tissues would be exposed to during treatment with a luminal doxorubicin concentration of 1 mg/mL applied for 60 min at 37 °C.

As might be expected, the greatest effect of doxorubicin was observed on the urothelium, but surprisingly, these effects were an enhancement of basal ATP release and increased contractions of the urothelium/lamina propria to muscarinic receptor stimulation. In urothelium and lamina propria from untreated bladders, there was a basal release of ATP, acetylcholine and PGE₂, but only ATP release was increased during stretch. Urothelial cells in culture have also been shown to release ATP in response to stretch (Kang et al. 2012; McDermott et al. 2012) and in the normal bladder the urothelial ATP then initiates a mechanism by which low threshold sensory nerves are stimulated via P2X3 receptors and the micturition reflex is activated (Burnstock 2011). Acetylcholine and PGE₂ were also released from the urothelium/lamina propria, but unlike ATP, stretch did not

increase release. The role of these urothelial mediators is still unclear, but Ach appears to influence sensory nerve activity (Daly et al. 2010; Yu and de Groat 2010) and urothelial contractile activity (Akino et al. 2008; Moro et al. 2011, 2012), while PGE₂ influences bladder contractions (de Jongh et al. 2007) and sensitises some capsaicin-sensitive afferent neurons (Maggi et al. 1988a). A stretch-independent release of this prostaglandin from the urothelium has also been reported (Nile et al. 2010).

Doxorubicin pretreatment altered neither the basal nor the stimulated release of Ach and PGE₂, and it is therefore unlikely that these mediators are involved in the adverse effects induced by intravesical administration of this cytotoxic drug. Basal release of ATP however was enhanced more than 3-fold by doxorubicin pretreatment, and basal levels in treated bladder tissues were not significantly different to those of the stretch-induced levels obtained in control tissues. The finding that doxorubicin affected the mediators differently is not surprising since the release mechanisms for these mediators are very different. Prostaglandins are synthesised fresh by cyclooxygenase; ATP release involves hemichannels and vesicular release, while Ach release involves CFTR channels and intracellular calcium release (McLatchie and Fry 2014; McLatchie et al. 2014).

It is well established that not only ATP plays a central role in initiating the normal micturition reflex, but also stretch-induced ATP release is increased in the bladders of patients with idiopathic or neurogenic overactive bladders (Kumar et al. 2010). Thus, elevated ATP release may provide an explanation for the urgency and frequency observed in patients following treatment with intravesical doxorubicin. In addition, ATP can also activate high threshold, nociceptive (pain) nerve fibres and ATP release is greatly increased in patients with painful bladder syndrome (Kumar et al. 2007). Thus, the dysuria observed after intravesical doxorubicin treatment may be related to the enhancement of urothelial ATP release.

The urothelium/lamina propria also releases another factor during muscarinic receptor stimulation that inhibits contraction of the underlying smooth muscle in the pig and human bladder (Chaiyaprasithi et al. 2003; Hawthorn et al. 2000; Templeman et al. 2002). This urothelium-derived inhibitory factor (UDIF) is not nitric oxide or a prostaglandin and it remains unidentified. The influence of this factor was noticeable in the present study, where the presence of an intact urothelium/lamina propria significantly depressed maximum contractions of the detrusor muscle to carbachol. After doxorubicin pretreatment, the responses of intact and denuded detrusor tissues to carbachol and the degree of inhibition by the urothelium/lamina propria was similar to tissues from untreated bladders. Thus, doxorubicin did not appear to alter either the release or the actions of UDIF and its effects on urothelial mediator release were limited to an action on ATP release.

Previously, it has been demonstrated that intact bladder strips and urothelium/lamina propria strips exhibit basal spontaneous contractile activity (Moro et al. 2011; Sadananda et al. 2008). Pretreatment with doxorubicin depressed the frequency of these spontaneous contractions in both isolated urothelium/lamina propria and in intact bladder tissues. In the pig urothelium, both Ach and ATP can drive spontaneous contractions (Moro et al. 2011), but changes in these mediators cannot explain the depressed contractile activity. At present, it is not clear whether this contractile activity is mediated via myofibroblasts (Sadananda et al. 2008) or smooth muscle (Heppner et al. 2011) within the lamina propria. The physiological relevance of this activity is also unknown, but it has been suggested that it may be responsible for folding of the bladder urothelium in the empty bladder (Sadananda et al. 2008) or that this contractile activity may drive detrusor contraction. Since doxorubicin pretreatment inhibited the urothelial contractile activity, this mechanism is unlikely to contribute to the side effects observed with this cytotoxic drug. If anything, the reduced urothelial spontaneous contractile activity would offset the effects of increased ATP release and EFS responses on overall bladder activity.

In all tissues examined, the muscarinic agonist carbachol induced contraction. These were greater in urothelial/lamina

propria strips than those produced by the detrusor muscle when responses were expressed relative to weight, (i.e. mN/g tissue), a finding that has been reported previously (Sadananda et al. 2008). Surprisingly, after treatment with the cytotoxic drug, contractile responses of urothelial/lamina propria strips were enhanced. The potency of carbachol was unaltered, but the responsiveness of the tissue was increased and maximum responses were significantly greater following doxorubicin treatment. The relevance of this finding to the development of bladder overactivity is currently unclear. There is evidence that spontaneous membrane potential transients induced by stretch can originate in the urothelium/lamina propria (Kanai et al. 2007) and in feline interstitial cystitis cat bladders, there are increased calcium transients in the urothelial/lamina propria, which can enhance smooth muscle spontaneous contractions (Ikeda et al. 2009). Thus, it is possible that the increase in contractile activity of this layer (urothelium/lamina propria) after doxorubicin treatment may contribute to the overactivity observed in patients following treatment.

The effects of doxorubicin on detrusor smooth muscle contraction were also examined. Less than 1 % of doxorubicin penetrates this far into the bladder wall (Wientjes et al. 1996) and responses of the muscle itself were unaltered by doxorubicin treatment. However neurogenic responses, elicited by nerve depolarisation during electrical field stimulation, were enhanced. These data suggest that doxorubicin does not affect the detrusor smooth muscle directly, but does affect the efferent nerves, increasing transmitter release and thus enhancing neurogenic detrusor contractions. At the lowest frequency of stimulation, control tissues did not respond, but treated tissues produced a significant contraction. The current data suggest that doxorubicin enhances transmitter release and this mechanism may induce detrusor contractions during bladder filling and thus contribute to bladder overactivity. The effects of atropine were not affected by doxorubicin treatment suggesting that doxorubicin affects cholinergic and non-cholinergic neurotransmission equally.

In conclusion, all three changes in bladder function induced by doxorubicin would tend to lead to bladder overactivity. The increase in basal urothelial ATP release will stimulate low threshold sensory nerves and sensitise the afferent arm of the micturition reflex, while activation of high threshold nerve fibres may cause dysuria. Enhanced urothelium/lamina propria contraction to muscarinic stimulation and enhanced neurogenic responses of the detrusor would sensitise the contractile machinery of the bladder, again potentially causing bladder overactivity. The enhancement of urothelium/lamina propria and efferent nerve activity are surprising results following treatment with a cytotoxic drug, but all three changes (increased ATP release, urothelium/lamina propria contraction and enhanced neurogenic detrusor responses) may contribute to the frequency, urgency and dysuria reported by patients after intravesical treatment with doxorubicin.

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