

DEPRESSED CONTRACTILE RESPONSES OF THE BLADDER DETRUSOR MUSCLE FOLLOWING LUMINAL GEMCITABINE AND COMBINED CHEMOHYPERTHERMIA TREATMENT

Hypothesis / aims of study

Local hyperthermia in combination with chemotherapy as a treatment for superficial bladder cancer is currently undergoing Phase III trials. Temperatures between 40-44°C exhibit a tumour cell killing effect, and enhance the cytotoxicity of commonly used chemotherapeutics [1]. Intravesical administration of cytotoxic therapies for the treatment of non-invasive bladder cancer often cause adverse urological side effects including increased urgency and frequency of urination, coupled with dysuria and haematuria. We have shown doxorubicin enhances neurogenic detrusor responses and ATP release which may explain the significant adverse effects observed with this agent [2]. Intravesical treatment with gemcitabine (GEM) is associated with fewer side effects. This study investigated the effects of intravesical gemcitabine treatment in combination with hyperthermia therapy on the normal responses of the bladder detrusor muscle and urothelium/lamina propria and release of ATP.

Study design, materials and methods

Isolated full thickness sheets of bladder wall from the dome of the porcine bladder were set up in gassed (5%CO₂/95%O₂) Krebs-bicarbonate solution and incubated for 60min at 37°C with or without gemcitabine at the clinical dose of 40mg/mL applied to the luminal surface. The effects of this gemcitabine treatment were also examined after the 60min treatment was performed at 42°C (hyperthermia). Following this treatment, strips of intact bladder wall, detrusor strips denuded of the urothelium and lamina propria and strips of isolated urothelium and lamina propria were set up in organ baths and responses were obtained to the muscarinic agonist carbachol and the β -adrenoceptor agonist isoprenaline. Neurogenic contractions of detrusor strips to electrical field stimulation (20V, 0.5ms pulse width, applied for 5s every 100s) with and without the presence of the muscarinic antagonist atropine were also examined. ATP release from isolated urothelium and lamina propria was determined at basal and at approximately 50% stretch.

Results

Carbachol induced a contraction in both the detrusor and urothelium. The responses of the detrusor pre-incubated with gemcitabine at 37°C and 42°C were significantly depressed (**P<0.01, n=8 and ^^^P<0.001, n=8 respectively) compared to the untreated control (n=8) (Figure 1). The contraction of the urothelium/lamina propria pre-incubated with gemcitabine at 37°C (n=12) and 42°C (n=7) was depressed compared to the control, however this was only statistically significant between the pretreated 37°C gemcitabine and control (P<0.05, n=12). The potency of carbachol was not different between tissues pre-incubated with gemcitabine compared to the control (detrusor pEC₅₀ values of -5.93±0.36 for control, -5.72±0.17 for 37°C gemcitabine and -5.91±0.27 for 42°C gemcitabine; urothelium pEC₅₀ values of -5.92±0.24 for control, -5.53±0.23 for 37°C gemcitabine and -5.40±0.22 for 42°C gemcitabine)

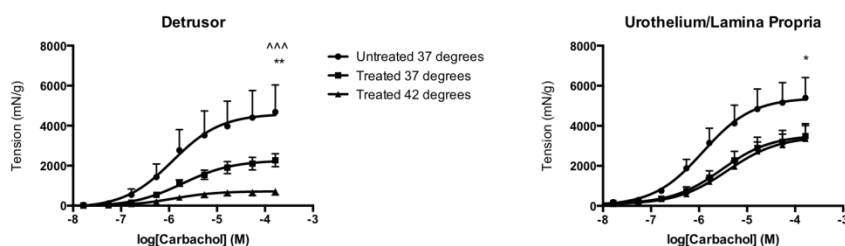


Figure 1: Effect of gemcitabine treated porcine detrusor smooth muscle and urothelium/lamina propria at 37°C and 42°C on responses to carbachol

Isoprenaline caused relaxation of pre-contracted detrusor and urothelium/lamina propria strips (Figure 2). The responses of the detrusor pre-incubated with gemcitabine at 37°C and 42°C were significantly depressed (**P<0.01, n=9 and ^P<0.001, n=8 respectively) compared to the untreated control (n=10) (Figure 2). The relaxation of the urothelium/lamina propria pre-incubated with gemcitabine at 37°C (n=12) and 42°C (n=7) was less compared to the control, however this was only statistically significant between the pre-treated 37°C gemcitabine tissue and control (*P<0.05, n=12). The potency of isoprenaline was not different between tissues pre-incubated with gemcitabine compared to the control (detrusor pEC₅₀ values of -6.95±0.70 for control, -6.82±0.22 for 37°C gemcitabine and -7.30±0.52 for 42°C gemcitabine; urothelium pEC₅₀ values of -6.34±0.44 for control, -6.14±0.26 for 37°C gemcitabine and -6.25±0.59 for 42°C gemcitabine)

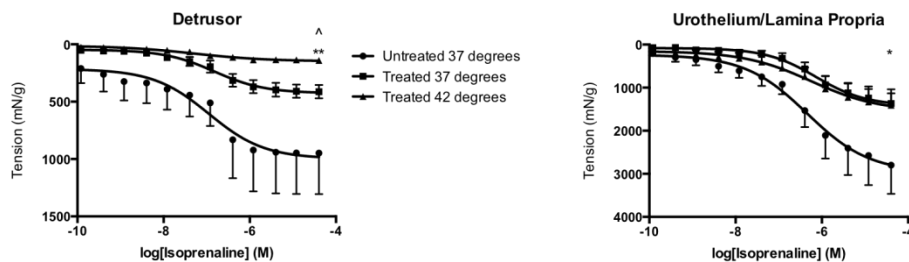


Figure 2: Effect of gemcitabine treated porcine detrusor smooth muscle at 37°C and 42°C on responses to isoprenaline

Contraction due to neurogenic stimulation was significantly depressed ($P < 0.05$, $n = 7$) in the gemcitabine pre-treated detrusor muscle at 42°C compared to the control ($n = 12$) at 5Hz and 10Hz (Figure 3). In the presence of atropine, the response to EFS in gemcitabine pre-treated tissue at 37°C ($P < 0.05$, $n = 10$) and 42°C ($P < 0.05$, $n = 7$) was significantly depressed at 1Hz and 10Hz and all frequencies respectively.

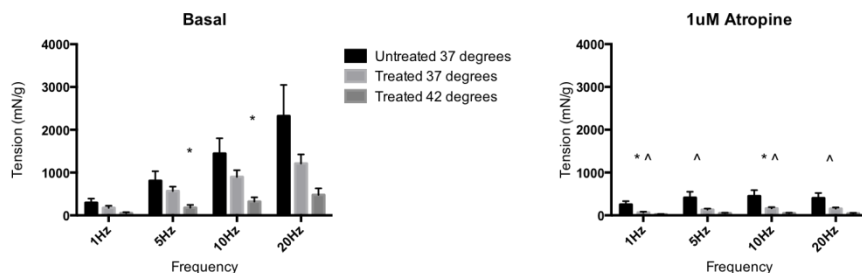


Figure 3: Effect of gemcitabine treated porcine detrusor smooth muscle at 37°C and 42°C on responses to neurogenic stimulation in the absence and in the presence of atropine.

There was no difference found in urothelial ATP release between control tissues and gemcitabine pre-incubated tissues.

Interpretation of results

Gemcitabine at a clinical dose applied to the luminal surface of the bladder significantly depresses subsequent responses of the detrusor smooth muscle and urothelium/lamina propria to muscarinic and adrenergic receptor stimulation and detrusor muscle responses to electrical field stimulation. If the pre-incubation was performed at 42°C the depression of responses was significantly enhanced.

Concluding message

The enhancement of detrusor responses and urothelial ATP release that we have previously observed with doxorubicin was not seen with gemcitabine. This may explain why fewer urological adverse effects are reported with this agent. However gemcitabine did depress detrusor and urothelial contractile and relaxation responses and these effects were enhanced when the gemcitabine treatment was performed at 42°C.

References

1. RAMPERSAUD, E. N., VUJASKOVIC, Z. & INMAN, B. A. 2010. Hyperthermia as a treatment for bladder cancer. *Oncology (Williston Park)*, 24, 1149-55
2. Chess-Williams R, Kang S, Farr S, McDermott C 2012 'Intravesical doxorubicin enhances neurogenic responses of detrusor smooth muscle' Abstract ICS Pharmacology

Disclosures

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