

## IS THE RELEASE OF UROTHELIAL ATP, ACETYLCHOLINE AND PROSTAGLANDIN E<sub>2</sub> AFFECTED BY THE CHEMOTHERAPEUTIC AGENT DOXORUBICIN?

### Hypothesis / aims of study

Intravesical chemotherapy is a common approach used in the treatment of superficial bladder cancer to prevent recurrence and progression of the disease. This treatment method has the benefit of direct exposure of the target cancer cells to effective concentrations of cytotoxic agents such as doxorubicin, while also limiting systemic exposure and associated systemic adverse effects. The effects of chemotherapeutic drugs on the tumour have been extensively studied but little is known of their effects on normal bladder function despite evidence of significant adverse effects including dysuria, and increased urinary frequency and urgency.

Stretch of the urothelium during bladder filling causes the release of a number of mediators that influence the activity of sensory nerves and also the detrusor smooth muscle. These mediators are required for the maintenance of normal bladder function and include ATP, acetylcholine (ACh) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). Given the direct contact between the urothelium and high concentrations of the cytotoxic agents during cancer treatment it is likely that the observed symptoms of overactive bladder are due, at least in part, to changes in urothelial function. The objective of this study was to determine the effects of doxorubicin on basal and stretch-induced mediator release from a human urothelial cell line (RT4) immediately and 24 hours following treatment.

### Study design, materials and methods

RT4 human urothelial cells were treated with a range of doxorubicin concentrations up to the clinical dose of 1mg/ml for 1 hour at 37°C. Either immediately or 24 hours following treatment, samples were prepared for analysis of basal and stimulated mediator release by incubating cell cultures in normal Krebs (280 mOsm/L) or in hypotonic (180 mOsm/L) Krebs solution (mimics cell stretch) respectively for 15 minutes. The level of ATP, ACh and PGE<sub>2</sub> in these samples was then measured using commercially available kits and compared to release from vehicle-treated control cultures.

### Results

Immediately following 1 hour treatment there was a significant increase in basal ACh release from urothelial cells treated with doxorubicin at its clinical concentration (1 mg/mL). A concentration dependent decrease in stimulated ACh release was observed at the same time-point with no observable stimulated release at the highest concentration tested. Basal ATP release was unchanged immediately following treatment however stimulated release of ATP was completely abolished at doxorubicin concentrations  $\geq 1\mu\text{g/mL}$ . Basal PGE<sub>2</sub> release was unaffected immediately following treatment, while a significant increase in stimulated release was observed in cells treated with 1 mg/mL doxorubicin.

Twenty-four hours following doxorubicin pre-treatment there was a significant increase in basal ACh and PGE<sub>2</sub> release even at a drug concentration of only 0.01mg/mL, while basal ATP release remained unchanged at all doxorubicin concentrations tested. These changes were accompanied by a significant decrease in ACh and ATP and increase in PGE<sub>2</sub> release in response to hypotonic stimulation at 0.01mg/mL doxorubicin.

### Interpretation of results

Urothelial-derived ATP plays an important role in the perception of pain as well as communicating the degree of bladder distension to the sensory nerves. An inhibitory effect of doxorubicin on stimulated release of ATP was observed in this study, a response which exhibited partial recovery 24 hours following treatment. This would suggest that pain induced by doxorubicin treatment in the bladder cancer patients may be due to increased sensitivity of C afferent pain fibres rather than enhanced urothelial ATP release. PGE<sub>2</sub> is thought to play an important role in the modulation of nerve and detrusor function. Thus, the increase in stimulated release of PGE<sub>2</sub> observed following doxorubicin treatment may lead to a sensitization of bladder sensory nerves and thus the micturition reflex, possibly causing increased urinary frequency as well as perception of pain which are adverse effects commonly seen in bladder cancer patients treated with doxorubicin.

### Concluding message

These findings indicate that urothelial mediator release is affected by treatment with doxorubicin. The urothelium is known to play an important role in maintaining normal bladder function through release of mediators which communicate with underlying nerves and detrusor muscle. The adverse effects associated with intravesical chemotherapy may therefore be due to the impact of the treatment on urothelial function, particularly the release of PGE<sub>2</sub>.

### Disclosures

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