Cytotoxic drugs and their effects on bladder function

PhD Thesis

By

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Summary of Thesis

This thesis investigates the effects of intravesical agents, doxorubicin, mitomycin C (MMC), and epirubicin on release of mediators and inflammatory cytokines from the urothelium, and tissue responses and morphological integrity of the bladder. Intravesical therapy which administers cytotoxic agents into the bladder lumen is an important approach for treatment of superficial bladder cancer. Despite evidence of significant local adverse effects such as chemical cystitis (dysuria, increased urinary frequency and urgency) following this treatment, few studies have assessed their effects on non-malignant tissues of the bladder and the changes in urinary function resulting from this therapy. A better understanding of the mechanisms of bladder toxicity by these agents may lead to the identification of novel approaches for reducing the severity of reported adverse effects. Moreover, the effects of ageing on bladder function were also investigated in this thesis.

Two in vitro bladder models, human urothelial cell lines (RT4 & UROtsa) and porcine bladder tissues (young & aged) were used to evaluate the effects of the three cytotoxic drugs on bladder function at clinically relevant concentrations and durations of treatment. Tissue responses to carbachol, isoprenaline and electrical field stimulation (EFS) were assessed in isolated bladder tissues, and integrity of bladder structure was also evaluated in terms of changes in urothelial thickness. Urothelial mediators (ATP, ACh and PGE₂) were measured in both cells and tissues under basal and stretch-induced conditions (mechanically or hypo-osmotically). Release of inflammatory cytokines (IL-8, IL-1β, IL-6, IL-10, TNF and IL-12p70) and nitric oxide (NO) were also measured, and recovery studies (24 hour- and 1 week-post treatment, and 1 week-post repeat treatment) were also conducted using urothelial cultures.

The first study comparing function of the bladder between young (4 to 6 months old) and aged (2 to 3 years old) pigs revealed age-associated decreases in contractile responses to muscarinic stimulation and also reduced urothelial thickness, while increases in urothelial mediator release, efferent neurogenic responses and relaxation responses of the urothelium/LP were observed with increasing age. In bladders from both young and aged pigs, pretreatment with doxorubicin enhanced ATP release from the urothelium/LP and also increased contractile responses of the
urothelium/LP to muscarinic stimulation. In bladders from young pigs, doxorubicin pretreatment enhanced efferent neurogenic responses of the detrusor muscle without affecting the muscle response itself, but in bladders from aged pigs, doxorubicin enhanced contractile response (muscarinic) of the detrusor muscle and also depressed neurogenic detrusor contractility.

The second study using bladders from aged pigs alone demonstrated that in tissues pretreated with MMC, efferent neurotransmission in detrusor muscle was depressed, relaxation of the detrusor muscle to adrenergic stimulation was reduced, and urothelial contractility to muscarinic stimulation was decreased, compared to control tissues. Pretreatment with both MMC and epirubicin caused urothelial thinning, abolished the ability of the urothelium to inhibit detrusor contractility, and depressed detrusor contractility to muscarinic stimulation. Also, stretch-induced release of ATP from the urothelium/LP was decreased after MMC and epirubicin pretreatment, while basal release of PGE₂ and ACh was increased by MMC and epirubicin, respectively.

Experiments using urothelial cells demonstrated a decrease in stretch-induced ATP release immediately and 1 week following MMC pretreatment, but recovery was observed 1 week after repeat MMC pretreatment. Basal release of ACh was enhanced immediately and 24 hours following pretreatment, but 1 week following pretreatment, basal ACh release was decreased while stretch-induced release of ACh was increased. Immediately following pretreatment, a decrease in basal PGE₂ release was observed. However, 24 hours and 1 week following pretreatment, an increase in basal PGE₂ release was demonstrated. One week after repeat pretreatment, stretch-induced release of PGE₂ was enhanced. In addition, enhancement in NO release 24 hours and 1 week following MMC pretreatment was shown, with recovery observed 1 week following repeat pretreatment.

Further experiments revealed that immediately and 24 hour following epirubicin pretreatment, both basal and stretch-induced ATP release were enhanced while stretch-induced ATP release was depressed 1 week following pretreatment. An increase in basal ACh release and a decrease in stretch-induced ACh release were observed immediately and 24 hour after pretreatment. One week following pretreatment, an increase in stretch-induced ACh release was observed. The release of PGE₂ was only affected immediately after pretreatment, in which basal release
was increased while stretch-induced release was depressed. NO release was enhanced 24 hour following pretreatment, but a recovery was observed 1 week after pretreatment.

One of the most important findings in this study was the persistent induction of inflammatory cytokines in urothelial cell culture models following pretreatment with the each of the chemotherapeutic drugs tested. All three agents enhanced IL-8 release, while IL-6 and IL-1β were enhanced by epirubicin and doxorubicin, respectively. This suggests that changes in inflammatory response may be the key to pathogenesis of the urological adverse effects reported in bladder cancer patients treated with these intravesical agents.

Thus, these studies have identified a number of changes in bladder function that may contribute to the adverse effects observed following intravesical chemotherapy. A number of pathological changes were observed in muscle, nerve and urothelium/LP that may influence sensory and motor functions of the bladder, but the changes following treatment appear to differ for each drug and even the age of the bladder, the only consistent change being an inflammatory state that was observed with all the drugs tested.
Declaration of Originality

This thesis is submitted to Bond University in fulfilment of the requirements of the degree of Doctor of Philosophy. This thesis represents my own original work towards this research degree and contains no material that has previously been submitted for a degree or diploma at this university or any other institution, except where due acknowledgement is made.

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Co-supervisor: Professor Russ Chess-Williams. Professor of Pharmacology, Faculty of Health Sciences and Medicine, Bond University.
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Publications

Research articles as a result of this thesis:


Conference presentations:


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Introduction

UROtsa cell line

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Effects of epirubicin on mediator release 24 hour post-treatment

Effects of epirubicin on mediator release 1 week post-treatment

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Discussion

ATP

Acetylcholine

Prostaglandin E₂

Nitric oxide

Inflammatory cytokine

Conclusions

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Inflammation and urothelial abnormalities

Inflammation and abnormal detrusor activity

Inflammation and sensitisation of afferent nerves / neuroplasticity

Concluding remarks

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