PhD Thesis

by

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INTRAVESICAL GEMCITABINE TREATMENT:
REPERCUSSIONS ON NORMAL BLADDER FUNCTION

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ABSTRACT

Intravesical treatment for non-muscle invasive bladder cancer involves the direct instillation of immunotherapy or chemotherapy into the bladder. While this approach limits systemic absorption, patients undergoing this localised treatment frequently report significant urological side effects, including increased frequency and urgency of urination, haematuria and dysuria. A relatively new drug used for bladder cancer is gemcitabine, which has shown an improved efficacy and toxicity profile with comparison to the first-line chemotherapy mitomycin C in patients. Elucidating the effects of gemcitabine on the normal cells and changes in the normal function of the bladder may reveal possible targets for preventing, alleviating or treating the adverse urological effects associated with this treatment.

The cytotoxic effect of gemcitabine alone and in combination with hyperthermia treatment was examined on cultured non-malignant and malignant human urothelial cells, and compared to mitomycin C. Luminal gemcitabine treatment on full thickness porcine bladder sections examined the immediate histological and functional effects on the urothelium and detrusor muscle. Finally, single and repeated intravesical instillations of gemcitabine in mice examined the changes in voiding behaviours and ex vivo bladder function. Chemical, mechanical and electrical stimuli were used to investigate and compare the responses of control and treated tissues.

The potency of gemcitabine on malignant urothelial cells was >10,000-fold greater than its potency on non-malignant cells. This effect is attributed in part to the enhanced reactive oxygen species production induced by gemcitabine, and the enhanced presence of the human equilibrative nucleoside transporters in malignant cells with comparison to non-malignant urothelial cells. Gemcitabine also induced increased release of inflammatory cytokines from cultured urothelial cells, and these effects were not potentiated by hyperthermia. Luminal gemcitabine sloughed urothelial cells from porcine tissue, resulting in decreased ATP but enhanced prostaglandin E\textsubscript{2} release from the urothelium. Repeated intravesical gemcitabine treatment with subsequent recovery periods in mice increased voiding frequency, enhanced urothelial ATP and prostaglandin E\textsubscript{2} release but depressed detrusor contractile responses mediated by efferent nerve stimulation. Taken together, these results suggest that intravesical gemcitabine induces a painful and overactive bladder phenotype in patients through a combination of enhanced urothelial and inflammatory mediators and altered efferent nerve activity, which may sensitise afferent nerves and reduce detrusor muscle contraction respectively.
DECLARATION

This thesis is submitted to Bond University in fulfilment of the requirements of the degree of Doctor of Philosophy by Research.

I declare that the research presented within this thesis is a product of my own original ideas and work, and contains no material which has previously been submitted for a degree at this university or any other institution, except where due acknowledgement has been made.

Stefanie Elizabeth Farr

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Abstracts as a result of this thesis:


**Stefanie Farr**, Russ Chess-Williams, Catherine McDermott. (2014) Selective cytotoxicity of gemcitabine on superficial malignant vs. normal human urothelial cells and the effects of hyperthermia. 5\(^{th}\) National Symposium on Advances in Gastrointestinal & Urogenital Research, Gold Coast 2014

**Farr, S.,** McDermott, C., Chess-Williams, R. (2014) Gemcitabine enhanced release of ATP from bladder urothelial cells but is selectively cytotoxic to bladder cancer cell lines. Gold Coast Health and Medical Research Conference Gold Coast 2014


**Farr, S.,** McDermott, C., Chess-Williams, R. (2013) Administration of luminal gemcitabine and combined chemohyperthermia treatment depress contractile and relaxant responses of the bladder tissue. Gold Coast Health and Medical Research Conference Gold Coast 2013


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ABBREVIATIONS

α,β-mATP: alpha, beta methylene ATP
ACh: acetylcholine
AChE: acetylcholinesterase
ADP: adenosine 5'-diphosphate
AP-1: activator protein-1
AR: adrenoceptor
ATP: adenosine 5'-triphosphate
Ca²⁺: calcium
cAMP: 3'-5'-cyclic adenosine monophosphate
CGRP: calcitonin gene-related peptide
COX: cyclooxygenase
DAG: diacylglycerol
dCK: deoxycytidine kinase
DFV: discoidal fusiform vesicle
DRG: dorsal root ganglia
EFS: electrical field stimulation
EGF: epidermal growth factor
EGFR: epidermal growth factor receptor
GAG: glycosaminoglycans
GSH: glutathione
H₂O₂: hydrogen peroxide
IL: interleukin
IP₃: inositol trisphosphate
K⁺: potassium
KCl: potassium chloride
L-NNA: L-N⁵-Nitroarginine
MIBC: muscle invasive bladder cancer
NA: noradrenaline
Na⁺: sodium
NANC: non-adrenergic, non-cholinergic
NGF: nerve growth factor
NMBPR: S-(4-nitrobemzyl)-6-thioinsine
NMIBC: non-muscle invasive bladder cancer
NO: nitric oxide
NOS: nitric oxide synthase
PGE₂: prostaglandin E₂
PLC: phospholipase C
PMC: pontine micturition centre
ROS: reactive oxygen species
RT-PCR: reverse transcription polymerase chain reaction
SEM: standard error of the mean
TCC: transitional cell carcinoma
TK2: thymidine kinase 2
TNF: tumor necrosis factor
TRP: transient receptor potential
TTX: tetrodotoxin
TUR: transurethral resection
UDIF: urothelium derived inhibitory factor
UP: uroplakin