PhD Thesis
by
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Cyclophosphamide and ifosfamide: mechanisms of cytotoxic action and consequences for normal bladder function

Submitted in total fulfilment of the requirements of the degree of Doctor of Philosophy by Research

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Abstract

This thesis investigates the urotoxic effects of the commonly used cytotoxic drugs cyclophosphamide and ifosfamide. Both drugs are well recognised for causing haemorrhagic cystitis and lasting adverse effects in the bladder including pain, increased urinary frequency and urgency and sensations of incomplete bladder emptying. These adverse effects have been largely attributed to the formation of the toxic metabolite acrolein which is excreted in the urine. However, another urinary metabolite of these drugs is chloroacetaldehyde and its role in urotoxicity has not been explored. Understanding more about what effects these drugs and their metabolites have on the bladder and its function may uncover possible targets for preventing, alleviating or treating the adverse urological effects and could lead to better drug toleration and better treatment outcomes.

The effects of acrolein and chloroacetaldehyde were investigated using cultured human urothelial cells as well as full thickness porcine bladder sections. Systemic administration of cyclophosphamide and ifosfamide was also performed in mice to study the effects of the endogenously produced metabolites on whole bladder and nerve function. A combination of pharmacological agents and chemical, mechanical and electrical stimuli were used to investigate and compare the responses of control and treated tissues.

Experiments measuring mediator release from urothelial cells implicated both acrolein and chloroacetaldehyde in the urotoxicity of cyclophosphamide and ifosfamide as both metabolites caused increased excitatory transmitter release from the cells. It was thought that the increase in excitatory transmitter release may contribute to bladder hyperactivity by activating and/or sensitising afferent nerves. Both metabolites also caused urothelial damage when applied to the luminal side of porcine bladder sections. However, despite loss of urothelial cells, the mediator release was comparable to controls suggesting enhanced release from each cell compensated for overall cell loss. Total afferent nerve activity was found to be increased in mice after treatment with either cyclophosphamide or ifosfamide due to enhanced activity of the low threshold nerve fibres. However, the heightened afferent activity observed in mice was not associated with increased excitatory urothelial mediator release or altered detrusor tone. This suggests that cyclophosphamide or ifosfamide treatment is able to enhance nerve activity via a mechanism independent of bladder function and that the bladder pain and urinary hyperactivity experienced by patients is primarily due to sensitisation of the afferent pathways.
Declaration

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

This research represents my own original work towards this research degree and contains no
material which has been previously submitted for a degree or diploma at this university or any
other institution, except where due acknowledgment has been made.

K Mills

Kylie Mills

2 March 2015
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Publications

Abstracts as a result of this thesis


Mills, K., Chess-Williams, R., McDermott, C. (2013) Chloroacetaldehyde, not just acrolein, may be involved in the uro-toxicity of cyclophosphamide and ifosfamide. Gold Coast Health and Medical Research Conference Gold Coast 2013

Kylie A Mills, Russ Chess-Williams, Catherine McDermott. (2013) The protective effect of N-acetylcysteine and Vitamin C on acrolein toxicity in human urothelial cells. 5th National Symposium on Advances in Gastrointestinal & Urogenital Research, Melbourne 2013


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Abbreviations

ACh: acetylcholine
ACHE: acetylcholinesterase
ADP: adenosine 5'-diphosphate
AP-1: activator protein-1
AR: adrenoceptor
ASIC: acid sensing ion channel
ATP: adenosine 5'-triphosphate
Ca²⁺: calcium
CAA: chloroacetaldehyde
cAMP: 3'-5'-cyclic adenosine monophosphate
cGMP: 3',5'-cyclic guanosine monophosphate
CGRP: calcitonin gene-related peptide
COX: cyclooxygenase
CPO: cyclophosphamide
CYP: cytochrome P450
DAG: diacylglycerol
Deg: degenerin Na⁺ channels
DRG: dorsal root ganglia
EFS: electrical field stimulation
EGF: epidermal growth factor
EGFR: epidermal growth factor receptor
ENaC: epithelial sodium channel
EUS: external urethral sphincter
GAG: glycosaminoglycans
GSH: glutathione
H₂O₂: hydrogen peroxide
i.v.: intravenously
IC: interstitial cells
IC-IM: interstitial cells – intramuscular
IC-LP: interstitial cells – lamina propria
IFO: ifosfamide
IL: interleukin
IP3: inositol trisphosphate
IUS: internal urethral sphincter

K⁺: potassium
KCl: potassium chloride
LDH: lactate dehydrogenase
LP: lamina Propria
NA: noradrenaline
Na⁺: sodium
NAC: N-acetylcysteine
NAD: nicotinamide-adenine dinucleotide
NANC: non-adrenergic, non-cholinergic
NF-κB: nuclear factor-kappaB
NGF: nerve growth factor
NO: nitric oxide
NOS: nitric oxide synthase
ONOO⁻: peroxynitrite
p.o.: per oral
PACAP: pituitary adenylate cyclase activating peptide
PAG: periaqueductal gray
PARP: poly ADP-ribose polymerase
PBS: painful bladder syndrome
PGE₂: prostaglandin E2
PLC: phospholipase C
PMC: pontine micturation centre
RNS: reactive nitrogen species
ROS: reactive oxygen species
RT-PCR: reverse transcription polymerase chain reaction
SEM: standard error of the mean
TNF: tumor necrosis factor
Trks: tyrosine kinase receptors
TRP: transient receptor potential
TTX: tetrodotoxin
UDIF: urothelium derived inhibitory factor
UDP: uridine 5'-diphosphate
UTP: uridine 5'-triphosphate