Vascular reactivity in sepsis and platelet dysfunction in septic shock

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THESIS DECLARATION

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THESIS ABSTRACT

Sepsis remains an important global cause of morbidity and mortality. Sepsis can be complicated by pathological vasodilation causing cardiovascular septic shock. The present study identifies that dysfunction of the RhoA/Rho-kinase (ROK) signaling pathway in vascular smooth muscle cells contributes to vasomotor dysfunction in sepsis. ROK inhibits myosin light chain phosphatase (MLCP) through Thr855 phosphorylation of MYPT, the 130 kDa myosin binding regulatory subunit of MLCP. MLCP dephosphorylates myosin light chain (LC_{20}) inhibiting the acto-myosin cross-bridge cycling underpinning vasoconstriction or platelet contraction. ROK dependent MLCP inhibition therefore favours vasoconstriction and can be indexed by Thr855-MYPT phosphorylation. Western blot analysis identified that Thr855 phosphorylation of MYPT was reduced in arterial segments isolated from a murine caecal ligation and puncture model of sepsis. Wire myography yielded data consistent with reduced contractile responses to thromboxane A_2 receptor stimulation, high [K^+] mediated depolarisation and direct PKC stimulation. α_1-adrenergic receptor mediated vasoconstriction was similar in septic and non-septic animals, possibly reflecting the multiple mechanisms by which α_1- adrenergic agonists elicit vasoconstriction. Certain bacterial toxins and inflammatory mediators have the potential to attenuate ROK signaling; our data suggest therapeutic benefit of agents that promote MLCP inhibition or which vasoconstrict independent of the RhoA/ROK pathway.

Current vasopressor strategies for septic shock primarily rely upon catecholamine therapy. However, there is interest in administration of vasopressin, an

endogenous vasopressor inappropriately suppressed in septic shock. It is proposed that vasopressin mediates Ca^{2+} sensitisation through ROK mediated inhibition of MLCP, however, neither vasopressin dependent Ca^{2+} sensitisation nor Thr855 MYPT phosphorylation have been directly identified. In permeabilised rat caudal artery Ca^{2+} sensitisation was observed and found to depend at least partly upon PKC signaling. In contrast, stimulation with arginine vasopressin (AVP) was not associated with Thr855 MYPT phosphorylation despite the ROK inhibitor Y27632 attenuating vasopressin dependent vasoconstriction. These data support clinical evaluation of vasopressin therapy targeted to cases of septic shock arising from organisms capable of producing toxins, which neutralise RhoA/ROK. Furthermore, the data suggest either an MLCP independent vasoconstrictor role for ROK or ROK independent action of Y27632.

Sepsis is also complicated by coagulopathy promoting both thrombosis and haemorrhage. Data regarding platelet function in sepsis is equivocal and absent in the specific subset of patients with septic shock. Recognising the importance of platelet contraction in thrombus formation and suggested similarities between vascular smooth muscle and platelet contraction we aimed to identify whether platelet contractile dysfunction contributed to impaired platelet aggregation in septic shock. Whole blood impedance aggregation was impaired in patients suffering from septic shock; deficits in aggregation correlated with illness severity. Impaired platelet aggregation was not associated with biochemical evidence of contractile dysfunction: neither Ser19-LC_{20} nor Thr855-MYPT phosphorylation differed between septic shock and non-septic patients. These data indicate that

therapeutic strategies to restore platelet function in septic shock might more profitably focus on platelet adhesion and secretion.

These studies identify MLCP inhibition as a potential therapeutic avenue to ameliorate vascular smooth muscle, but not platelet, function in septic shock. Vasopressin might provide particularly effective vasoconstriction when targeted to cases of septic shock associated with disrupted ROK/MLCP integrity.

LIST OF COMMON ABBREVIATIONS

(c)AMP	(cyclic) adenosine monophosphate
(c)GMP	(cyclic) guanosine monophosphate
$[Ca^{2+}]$	calcium concentration
ADP	adenosine diphosphate
ATP	adenosine trisphosphate
AVP	arginine vasopressin
CaM	calmodulin
CLP	caecal ligation and puncture
CPI-17	PKC-potentiated inhibitory protein of 17 kDa
DAG	diacylglycerol
EC_{50}	effective concentration for 50% response
Emax	maximal effective concentration
GPCR	G-protein coupled receptor
GTP	guanosine trisphosphate
IP_3(R)	inositol trisphosphate (receptor)
L-NAME	N_ω-Nitro-L-arginine methyl ester
LC_{20}	20kDa myosin light chain
LTCC	L-type ($Ca_V1.2$) calcium channel
MAP	mean arterial pressure
MLCK	myosin light chain kinase
MLCP	myosin light chain phosphatase
MYPT	myosin phosphatase targeting protein
NO(S)	nitric oxide (synthase)
NSCC	non-selective cation channel
PKA/B/C	protein kinase A/B/C
PLC	phospholipase C
ROK	Rho kinase
SDS PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
Ser	serine
SERCA	sarco-endoplasmic reticulum calcium ATPase
SR	sarcoplasmic reticulum
TBS (- T)	tris buffered saline (with Tween 20)
Thr	threonine
TRAP	thrombin receptor activating protein
TxA_2	thromboxane A_2
VSM(C)	vascular smooth muscle (cell)

STATEMENT OF AUTHORSHIP

Calcium desensitisation is associated with loss of vasopressor sensitivity in a murine model of polymicrobial sepsis
Submitted to Intensive Care Medicine Experimental

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TABLE OF CONTENTS

THESIS DECLARATION ii

ACKNOWLEDGMENTS iii

THESIS ABSTRACT iv

LIST OF COMMON ABBREVIATIONS vii

STATEMENT OF AUTHORSHIP viii

Chapter 1: Research background 3

The clinical problem of septic shock 3

Epidemiology and Definitions 3

Initiating events in septic shock 4

Clinical physiology of septic shock 5

Mechanisms of pathological vasodilation and vasopressor insensitivity in septic shock 8

Excess circulating vasodilators 9

Relative insufficiency of endogenous vasoconstrictors 11

Insensitivity to endogenous / administered vasopressors 13

Current and tested therapies for the restoration of vascular tone and vasopressor responsiveness in septic shock 17

Vasopressors 17

Adjunctive therapies to vasopressors 21

Why aren't current and trialed therapies more effective? 27

Vasomotor regulation in health 28

Control of vasomotor tone (i) calcium dependent mechanisms of vascular smooth muscle contraction 28

Control of vasomotor tone (ii) calcium independent mechanisms of vascular smooth muscle contraction 39

Control of vasomotor tone (iii) mechanisms of smooth muscle relaxation 46

Control of vasomotor tone (iv) the endothelium 48

Control of vasomotor tone (v) mechanisms of action of selected vasoconstrictors pertinent to this study 52

Evidence that Ca^{2+} desensitisation contributes to septic shock 56

Regulation of platelet aggregation in health 61

Platelet activation 61

Platelet contraction 64

Platelet aggregation and secretion 69

Platelet dysfunction in sepsis 69

Research questions addressed in this thesis 71

Study 1 Does Ca^{2+} desensitisation contribute to loss of vasopressor insensitivity in a murine model of polymicrobial sepsis 71

Study 2 Does vasopressin elicit Rho kinase dependent Ca^{2+} sensitisation in vascular smooth muscle? 72

Study 3 Is human septic shock associated with a generalised reduction in platelet aggregation? Is impaired aggregation associated with reduced myosin light chain activity as a result of attenuated RhoA/ROK mediated inhibition of myosin light chain phosphatase? 73

Chapter 2: Calcium desensitisation is associated with loss of vasopressor sensitivity in a murine model of polymicrobial sepsis..... 76
Introduction 76
Materials and Methods 79
Results 84
Discussion 97

Chapter 3: Vasopressin elicits calcium sensitisation in vascular smooth muscle in a PKC dependent manner, but independent of Rho kinase mediated Thr-855 phosphorylation of MYPT 105
Introduction 105
Materials and methods 108
Results 113
Discussion 122

Chapter 4: Attenuated platelet aggregation in patients with septic shock is independent from the activity state of myosin light chain phosphorylation or a reduction in Rho kinase-dependent inhibition of myosin light chain phosphatase.....130
Introduction 130
Materials and Methods 132
Results 139
Discussion 154

Chapter 5: Discussion 160
Main findings 160
Study 1 Does Ca^{2+} desensitisation contribute to loss of vasopressor insensitivity in a murine model of polymicrobial sepsis.....160
Study 2 Does vasopressin elicit Rho kinase dependent Ca^{2+} sensitisation in vascular smooth muscle?.....162
Study 3 Is human septic shock associated with generalised reduction in platelet aggregation? Is impaired aggregation associated with reduced myosin light chain activity as a result of attenuated RhoA/ROK mediated inhibition of myosin light chain phosphatase?.....164
Implications for future therapeutic strategies.....166
Conclusions.....170