JCU ePrints

This file is part of the following reference:

Canyon, Sarah J. (2003) ***A novel cardioprotective therapy : adenosine and lidocaine solution in an in vivo rat model of acute myocardial ischemia-reperfusion.*** **PhD thesis, James Cook University.**

Access to this file is available from:

http://eprints.jcu.edu.au/1642

JCU
JAMES COOK UNIVERSITY

A Novel Cardioprotective Therapy:

Adenosine and Lidocaine Solution in an *In Vivo* Rat Model of Acute Myocardial Ischemia-Reperfusion

Thesis submitted by Sarah J. Canyon BSc (Hons) JCU
in October 2003

For the degree of Doctor of Philosophy
School of Biomedical sciences
Department of Physiology and Pharmacology
James Cook University of North Queensland, Australia

ELECTRONIC COPY

I, the undersigned, the author of this work, declare that the electronic copy of this thesis provided to the James Cook University Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

_____ _____

Signature Date

Statement of Access

Declaration

I, the undersigned, the author of this thesis, understand that James Cook University of North Queensland will make it available for use within the University Library and, by microfilm or other means, allow access to users in other approved libraries. All users consulting this thesis will have to sign the following statement:

> In consulting this thesis, I agree not to copy or closely paraphrase it in whole or in part without the written consent of the author; and to make proper public written acknowledgment for any assistance which I have obtained from it.

Beyond this, I do not wish to place any restriction on access to this thesis.

…… ……

(Signature) (Date)

Statement of Sources

Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

…… ……

(Signature) (Date)

Acknowledgements

Immense gratitude is owed to Associate Professor Geoff Dobson for his outstanding knowledge, guidance and infectious enthusiasm regarding scientific discovery. He is a true lover of the fundamentals of basic science from its philosophical roots to its application in education and the laboratory. In our lab, Geoff treats his students and technicians as colleagues and creates an atmosphere of encouragement so great that the smallest achievements are praised with the same zeal as the larger ones. The effect of this is to enhance performance and respect for the power of science and its limitations.

Much appreciation goes to Sam Hitchins for his willingness to come in and provide support with various aspects of the *in vivo* model and especially the NMR and to Uwe Himmelreich who also went out of his way to help me gain NMR confidence. Thanks to the other talented postgrads, Michael Jones, Donna Rudd and Kathy Sloots for friendship, laughs, company and often guidance. Furthermore, special thanks to Mr. John Atkinson, whose special aptitude for solving mechanical puzzles enriched many of the experiments presented in this work.

Further acknowledgements are owed to the School of Biomedical Sciences and School of Pharmacy and Molecular sciences, especially administrative and technical staff, for continued friendly support throughout the course of my doctorate.

Lastly, I wish to acknowledge the tremendous support, understanding and seemingly endless patience of my husband, Deon Canyon and my three children, Chauncy, Kestrel and Rowan. Moreover, I want to thank my father, Professor Cooper Lansing, for it was trust in his wisdom that steered me towards science and academic pursuits early on in life.

Abstract

Background: Recently, our laboratory demonstrated that an adenosine and lidocaine (AL) non-depolarizing cardioplegic arrest solution conferred superior protection during arrest and recovery compared with the hyperkalemic depolarizing St. Thomas' Hospital cardioplegic solution in isolated rat hearts. The aim of this thesis was to extend those findings by applying an adenosine and lidocaine (AL) solution at nonarresting concentrations before and during ischemia in an *in vivo* rat model of acute myocardial ischemia. No study has investigated the effect of AL combination treatment to reduce ischemic injury. Yet, previous studies in the 1990s have used the sequential and separate administration of lidocaine (2 mg/kg i.v.) and adenosine (150 μg/kg/ml/min i.c) as reperfusion therapy with conflicting results.

Methods: In all four studies, ischemia-reperfusion was achieved by placing a reversible tie around the left coronary artery of anaesthetized (sodium pentobarbital, 60 mg/ml/kg i.p.) and ventilated male Sprague-Dawley rats (300 - 400 g). The ischemic period lasted 30 min while reperfusion times were maintained for either 40 min or 120 min. Where applicable, a lead II electrocardiogram, heart rate, and systolic and diastolic pressures were recorded and mean arterial pressure and rate-pressure product calculated. The primary end points included infarct size, episodes and durations of ventricular arrhythmias, pH and changes in the concentration of ATP ([ATP]) and phosphocreatine ([PCr]) during ischemia-reperfusion recorded every 5 min. The level of statistical significance was $P < 0.05$.

Experimental design: The first two studies examined the cardioprotective potential of adenosine and lidocaine using the following treatment strategies: i) three AL solutions with varying concentrations of adenosine (A: 152, 305 and 457 μg/kg/min

plus L: 608 μg/kg/min i.v., n = 18) compared to saline controls (0.9% saline, n = 12), adenosine only (adeno-only, 305 μg/kg/min i.v., n = 8) and lidocaine only (lido-only, 608 μg/kg/min i.v., n = 8); all of these treatments were given 5 before ischemia and continued throughout 30 min ischemia but not reperfusion; and ii) the separate and sequential administration of adenosine (150 μg/kg/min i.v.) and lidocaine (2 mg/kg i.v) during reperfusion (n = 7); the sequential administration of AL solution (A: 305 μg/kg/min plus L: 608 μg/kg/min i.v.) of 5 min pretreatment and again 5 min before and during 30 min reperfusion, n = 6 and a 5 min pretreatment of AL solution (A: 305 μg/kg/min plus L: 608 μg/kg/min i.v.) continued throughout ischemia and 30 min of reperfusion (n = 6). In the third study, ^{31}P nuclear magnetic resonance was used to investigate the changes in [ATP], [PCr] and pH during ischemia (30 min) and reperfusion (40 min) with AL solution treatment (n = 6) or in controls (n = 7). In the fourth study, AL solution (A: 305 μg/kg/min plus L: 608 μg/kg/min i.v.) was compared to that of ischemic preconditioning (three 3 min cycles of ischemia-reperfusion) (IPC) (n = 6), the adenosine receptor A_1 agonist, 2-chloro-N6-cyclopentyladenosine (CCPA) (5 μg/kg i.v.) plus lidocaine (n = 6), and CCPA alone (n = 7).

Results: Seven of the 12 saline-control rats and 4 of the 8 Adeno-only treated rats died during the ischemic period from an episode of ventricular fibrillation. No deaths occurred in the Lido-only treated rats (n = 6) or in any group where AL solution was infused. Ventricular tachycardia (VT) occurred in 100% of saline controls (18 ± 9 episodes), 50% of the adeno-only group (11 ± 7 episodes), and 83% of lido-only treatment (23 ± 11 episodes). VT was also experienced in 60% of low-dose AL treated rats (2 ± 1 episodes) ($P < 0.05$), 57% of mid-dose AL (2 ± 1 episodes) ($P < 0.05$), and 67% of high-dose AL treated rats (6 ± 3 episodes). Ventricular fibrillation

(VF) occurred in 75% of saline controls (4 ± 3 episodes), 100% of adeno-only (3 ± 2 episodes), and in 33% lido-only treated rats (2 ± 1 episodes). Low-dose AL and mid-dose AL completely prevented VF from occurring during ischemia. The mean infarct size of mid dose-AL (38 ± 6%) was significantly reduced from saline controls (61 ± 5%), adeno-only (56 ± 4%), and lido-only (66 ± 8%) ($P < 0.05$) but not from low-dose AL (45± 9%) and high-dose AL animals (45 ± 6%).

The separate and sequential administration of lidocaine and adenosine resulted in 2 out of 7 deaths and ischemia-induced VT (6 ± 3 episodes, 4 ± 2 sec) was not prevented while VF (1 ± 0 episodes, 1 ± 0 sec) was reduced. Infarct size (52 ± 5%) was not significantly different from saline-controls (61 ± 5%). When AL was given at pretreatment, stopped for ischemia and resumed 5 min before reperfusion, infarct size reduction (67 ± 8%) and protection from ventricular arrhythmias (VT: 39 ± 23 episodes, 84 ± 49 sec; VF: 2 ± 1 episodes, 21 ± 8 sec) were lost; though, there were no deaths in this group. AL solution given continuously from pretreatment through ischemia and reperfusion provided similar protection to AL infusion during pretreatment and ischemia (41 ± 10% vs. 38 ± 6%, 2 ± 1 VT and 0 VF).

During ischemia, control [ATP] fell to 61% of baseline at 15 min and recovered 68% - 88% of baseline during reperfusion. AL treatment maintained [ATP] in a steady state throughout ischemia and reperfusion with changes ranging of 95 ± 7 % to 117 ± 10% of baseline. Control [PCr] was significantly reduced compared to AL treated hearts during ischemia at 10 min (62 ± 7 vs. 89 ± 9%), 15 min (45 ± 4% vs 81 ± 7%), 20 min (44 ± 9% vs. 92 ± 9%) and 30 min (45 ± 8% vs. 77 ± 7%) and during reperfusion at 10 min (44 ± 19% vs. 92 ± 9%) and 15 min (50 ± 8% vs. 90 ± 7%) ($P < 0.05$). The pH

of AL and control hearts were similar throughout ischemia ranging from pH 7.6 to 6.4 in control and pH 7.5 to 6.8 in AL hearts. Controls maintained a mean pH below baseline for the first 20 min of reperfusion (pH 7.1) while AL hearts pH recovered to baseline within the first 5 min of reperfusion (pH 7.4 $\pm$ 0.1).

Pretreating the heart before and during ischemia with AL or with CCPA plus lidocaine resulted in no deaths, and no lethal arrhythmias. Infarct size reduction in CCPA plus lidocaine treated rats (12 ± 4 %) was similar to ischemic preconditioning (11± 3%), whereas in AL- and CCPA- treated rats, the infarct size was 38 ± 6% and 42 ± 7% respectively.

Conclusions: i) The intravenous infusion of AL solution before or during 30 min was more cardioprotective than adenosine alone, lidocaine alone, or the separate and sequential infusion of adenosine and lidocaine; ii) the AL combination led to no death, virtually no episodes of VF, few episodes of VT, and a significantly reduced infarct size; iii) AL cardioprotection appears to be associated with preservation of high energy phosphates and a better balance between supply and demand during ischemic conditions; iv) low pH was not an indicator of myocardial damage in AL treated rats, and v) when adenosine was substituted with an adenosine A_1 receptor agonist, CCPA, plus lidocaine cardioprotection was significantly enhanced and similar to IPC. In summary, targeting adenosine receptors, especially the A_1 adenosine receptor, with lidocaine Na^+ fast channel modulation may offer a new combination therapy to delay myocardial damage during ischemia and prevent ischemia-induced arrhythmias.

Abbreviations

^{31}P	phosphorus-31
adeno	adenosine
ADP	adenosine diphosphate
AL	adenosine and lidocaine
AL solution	adenosine and lidocaine solution
AMP	adenosine monophosphate
APD	action potential duration
ATP	adenosine triphospate
bpm	beats per minute
Ca^{2+}	calcium ion
CCPA	2-chloro-N6-cyclopentyladenosine
CK	creatine kinase
Cr	creatine
D_2O	deuterium oxide
ECG	electrocardiogram
FID	free induction decay
H^+	hydrogen ion
HR	heart rate
Hrs	hours
i.c.	intracoronary
i.p.	intraperitoneal
i.v.	intravenous
K_{ATP}	ATP sensitive potassium channel
Lido	lidocaine hydrochloride
Map	mean arterial pressure
Mg^{2+}	free magnesium
min	minutes
Mito	mitochondrial
Na_i	intracellular sodium
NMR	nuclear magnetic resonance
o.d.	outer diameter
PCr	phosphocreatine
pH	$-\log_{10} [H^+]$
P_i	inorganic phosphate
PKC	protein kinase C
PPA	phenylphosphoric acid
Ppm	parts per million
PVB	premature ventricular beat
rpp	rate pressure product
Sarc	sarcolemmal
SCF	saturation correction factor
Sec	seconds
solution	solution
T	tesla
TTC	triphenyltetrazolium chloride
VF	ventricular fibrillation
VT	ventricular tachycardia
VT+VF	sum of VT and VF

List of figures

List of illustrations