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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
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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 $\mu\text{g}/\text{kg}/\text{min}$ i.v., $n = 18$) compared to saline controls (0.9% saline, $n = 12$), adenosine only (adeno-only, 305 $\mu\text{g}/\text{kg}/\text{min}$ i.v., $n = 8$) and lidocaine only (lido-only, 608 $\mu\text{g}/\text{kg}/\text{min}$ 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 $\mu\text{g}/\text{kg}/\text{min}$ i.v.) and lidocaine (2 mg/kg i.v.) during reperfusion ($n = 7$); the sequential administration of AL solution (A: 305 $\mu\text{g}/\text{kg}/\text{min}$ plus L: 608 $\mu\text{g}/\text{kg}/\text{min}$ 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 $\mu\text{g}/\text{kg}/\text{min}$ plus L: 608 $\mu\text{g}/\text{kg}/\text{min}$ 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 $\mu\text{g}/\text{kg}/\text{min}$ plus L: 608 $\mu\text{g}/\text{kg}/\text{min}$ 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 $\mu\text{g}/\text{kg}$ 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 ($38 \pm 6\%$) was significantly reduced from saline controls ($61 \pm 5\%$), adeno-only ($56 \pm 4\%$), and lido-only ($66 \pm 8\%$) ($P < 0.05$) but not from low-dose AL ($45 \pm 9\%$) and high-dose AL animals ($45 \pm 6\%$).

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 ($52 \pm 5\%$) was not significantly different from saline-controls ($61 \pm 5\%$). When AL was given at pretreatment, stopped for ischemia and resumed 5 min before reperfusion, infarct size reduction ($67 \pm 8\%$) 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 ($41 \pm 10\%$ vs. $38 \pm 6\%$, 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 $95 \pm 7\%$ to $117 \pm 10\%$ of baseline. Control [PCr] was significantly reduced compared to AL treated hearts during ischemia at 10 min (62 ± 7 vs. $89 \pm 9\%$), 15 min ($45 \pm 4\%$ vs $81 \pm 7\%$), 20 min ($44 \pm 9\%$ vs. $92 \pm 9\%$) and 30 min ($45 \pm 8\%$ vs. $77 \pm 7\%$) and during reperfusion at 10 min ($44 \pm 19\%$ vs. $92 \pm 9\%$) and 15 min ($50 \pm 8\%$ vs. $90 \pm 7\%$) ($P < 0.05$). 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 ± 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 ($12 \pm 4\%$) was similar to ischemic preconditioning ($11 \pm 3\%$), whereas in AL- and CCPA- treated rats, the infarct size was $38 \pm 6\%$ and $42 \pm 7\%$ 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 A₁ receptor agonist, CCPA, plus lidocaine cardioprotection was significantly enhanced and similar to IPC. In summary, targeting adenosine receptors, especially the A₁ 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.

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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 triphosphate
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	$-\log_{10} [\text{H}^+]$
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

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