Eight hours of cold static storage with adenosine and lidocaine (Adenocaine) heart preservation solutions: Toward therapeutic suspended animation

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Objective: Most cardiac preservation solutions provide safe cold ischemic storage times for 4 to 5 hours. Our aim was to investigate the effects of 8 hours of cold static storage (4°C) using 2 normokalemic, polarizing adenosine-lidocaine (Adenocaine; Hibernation Therapeutics Global Ltd, Kilquade, Ireland) solutions and to compare their functional recovery with hearts preserved in gold standard histidine-tryptophan-ketoglutarate (Custodiol-HTK; Essential Pharma, Newtown, Pa) and Celsior (Genzyme, Cambridge, Mass) solutions.

Methods: Male Sprague–Dawley rats (350–450 g) were randomly assigned to 1 of 4 groups (n = 8): (1) adenosine-lidocaine cardioplegia with low Ca^{2+}/high Mg^{2+}; (2) 2× adenosine-lidocaine cardioplegia, low Ca^{2+}/high Mg^{2+}, melatonin, and insulin (2× adenosine, lidocaine, melatonin, and insulin); (3) histidine-tryptophan-ketoglutarate solution; or (4) Celsior. Hearts were perfused in working mode, arrested (37°C), removed, stored for 8 hours at 4°C, reattached in Langendorff mode and rewarmed for 5 minutes (37°C), and switched to working mode for 60 minutes. Myocardial oxygen consumption, effluent lactates, and troponin T levels were measured.

Results: Hearts preserved for 8 hours in adenosine-lidocaine and 2× adenosine, lidocaine, melatonin, and insulin returned 50% and 76% of aortic flow and 70% and 86% of coronary flow, respectively, at 60 minutes of reperfusion. In contrast, Custodiol-HTK and Celsior hearts returned 2% and 17% of aortic flow and 11% and 48% of coronary flow, respectively, at 60 minutes of reperfusion. Hearts preserved in adenosine-lidocaine and 2× adenosine, lidocaine, melatonin, and insulin returned 90% and 100% of developed pressures and 101% and 104% of heart rate, respectively. Hearts preserved in histidine-tryptophan-ketoglutarate failed to increase systolic pressure greater than 14 mm Hg (11% baseline) and diastolic pressure greater than 10 mm Hg (17% baseline), and recovered only 16% of heart rate. Hearts preserved in Celsior developed 70% of baseline systolic pressures and 86% recovery of heart rate. At 5 minutes of rewarming after cold storage, the myocardial oxygen consumption for hearts preserved in adenosine-lidocaine, 2× adenosine, lidocaine, melatonin, and insulin, Custodiol-HTK, and Celsior was 23.0 ± 5, 20 ± 4, 15 ± 1, and 10 ± 2 μmol O_2/min/g dry wt, respectively, with corresponding lactate outputs of 1.8 ± 0.8, 1.5 ± 0.7, 2.6 ± 0.7, and 3.2 ± 1.4 μmol lactate/min/g dry weight. Troponin T was not detected in the coronary effluent of adenosine-lidocaine or 2× adenosine, lidocaine, melatonin, and insulin hearts, whereas Custodiol-HTK and Celsior hearts had troponin T levels of 0.08 and 0.24 μg/mL, respectively.

Conclusions: We report a 78% return of cardiac output, 90% to 100% return of developed pressures, and 101% to 104% return of heart rate after 8 hours of cold static storage using normokalemic, adenosine, lidocaine, melatonin, and insulin preservation solution in the isolated rat heart compared with 55% cardiac output with polarizing adenosine-lidocaine cardioplegia alone, 4% cardiac output with Custodiol-HTK, and 25% cardiac output in Celsior preservation solutions. (J Thorac Cardiovasc Surg 2011;142:1552-61)

From a scientific standpoint, depolarizing potassium concentrations of 10 mEq/L and above in surgical cardioplegia or heart preservation solutions may not afford optimal arrest and protection.[1] In 2004, we introduced a new concept of polarized arrest for surgical cardioplegia using a composition of adenosine and lidocaine (Adenocaine; Hibernation Therapeutics Global Ltd, Kilquade, Ireland) in a physiologic Krebs–Henseleit solution.[1,2] We reported that Adenocaine in a normokalemic solution arrested the heart by ''clamping'' the myocyte's diastolic membrane potential at or approximately –80 mV with an accompanying decrease in myocardial oxygen consumption (MVO_2) of more than 95%.[2] The idea was borrowed from natural hibernating animals (or summer estivators) who do not flood their cells with high potassium and depolarize their cell membranes as they

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Abbreviations and Acronyms

AF	= aortic flow
AL	= adenosine-lidocaine
ALMI	= adenosine, lidocaine, melatonin, and insulin
ANOVA	= analysis of variance
CF	= coronary flow
CO	= cardiac output
Custodiol-HTK	= Custodiol histidine-tryptophan-ketoglutarate
MVO_2	= myocardial oxygen consumption
RPP	= rate-pressure product

decrease their body's "basal" metabolic rate to pilot-light.[3] The question posed was "Could the human heart in cardiac surgery be pharmacologically manipulated to operate more like a heart from a natural hibernator?" The early objective was to inhibit the voltage-dependent Na^+ fast channels responsible for the phase O upstroke (lidocaine) and *simultaneously* decrease the action potential duration (open K^+ channels) of atria, Purkinje fibers, and ventricles (adenosine), which would theoretically arrest the heart in a more "natural" polarized, diastolic state compared with hyperkalemic depolarized arrest.

The adenosine-lidocaine (AL) polarizing arrest and protection concept has subsequently received proof-of-concept in the canine model of cardiopulmonary bypass[4] and is used clinically in a number of US centers as Adenocaine all-blood microplegia.[5] In 2008, Jin and colleagues[6] carried out a 134-patient pediatric safety trial and showed that AL crystalloid "one shot" with moderate hyperkalemia (10 mmol/L) was more protective than AL with 20 mmol/L K^+ or 20 mmol/L K^+ alone. In 2010, we confirmed the importance of keeping potassium within its normokalemic limits for optimal AL polarized protection at 32°C to 33°C in isolated rat hearts and showed that higher (depolarizing) or lower (hyperpolarizing) extracellular potassium arrest resulted in significantly higher coronary vascular resistances, slower times to first beat (stunning), and lower cardiac outputs (COs) with lower contractility.[1,7]

AL cardioplegia also appears versatile as a preservation solution at both cold static storage (4°C) and warmer intermittent perfusion (28°C–30°C) compared with Celsior (Genzyme, Cambridge, Mass).[8] In 2011, we reported that reperfusing the isolated rat heart for 5 minutes with warm, oxygenated polarizing AL arrest *after* 6 hours of cold storage in AL cardioplegia or Celsior led to significantly higher recoveries and may offer a new paradigm of polarizing protection for rewarming and implantation.[9] The aim of the present study is to investigate the effects of 8 hours of cold static storage (4°C) using 2 modified AL (Adenocaine) preservation solutions and compare them with Custodiol histidine-tryptophan-ketoglutarate (Custodiol-HTK; Essential Pharma, Newtown, Pa) and Celsior solutions.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (350–450 g, n = 40) were obtained from James Cook University's breeding colony. Animals were fed ad libitum and housed in 10/14 hour light/dark cycle. On the day of experiment, rats were anesthetized with an intraperitoneal injection of thiopentone sodium (Thiobarb; 60 mg/kg body wt [Lyppard, Queensland, Australia]), and the hearts were rapidly excised as described by Dobson and Jones.[2] Rats were handled in compliance with James Cook University Guidelines (ethics approval numbers A1084 and A1515) and the "Guide for Care and use of Laboratory Animals" from the National Institutes of Health (Publication No. 85-23, revised 1985, and PHS Publication 1996). Adenosine (A9251 > 99% purity), histidine, histidine-HCl, tryptophan, alpha-ketoglutarate, mannitol, and melatonin (N-acetyl-5-methoxytryptamine) were obtained from Sigma Chemical Company (Castle Hill, NSW, Australia). Lidocaine hydrochloride (2% solution, ilium) and insulin (40 IU/mL) were obtained from Lyppard (Queensland, Australia). Celsior was purchased as a Food and Drug Administration-approved product from Clifford Hallam Healthcare (Agent for Genzyme, North Ryde, NSW, Australia).

Arrest Solutions for Normothermic Induction and Cold Static Storage

Adenosine-lidocaine solution with low Ca^{2+}/high Mg^{2+}. The AL cardioplegia contained 200 μmol/L adenosine plus 500 μmol/L lidocaine in 10 mmol/L glucose containing modified Krebs–Henseleit buffer (pH 7.7 at 37°C) with low Ca^{2+}/high Mg^2 (0.22 mmol/L $CaCl_2$ and 2.6 mmol/L $MgCl_2$). The solution was filtered using 0.2-μm filters and maintained at 37°C. These AL concentrations have been used in previous cardioplegia and preservation studies.[8,9] The arrest solution was not actively bubbled with 95% O_2/5% CO_2, thus the higher pH. The average Po_2 of the AL solution at the beginning of storage was 140 mm Hg and the Pco_2 was 5 to 10 mm Hg.

2× adenosine-lidocaine solution with low Ca^{2+}/high Mg^{2+}, melatonin, and insulin. The composition was the same as the AL solution (above) with the following additions: twice the concentration of A and L (400 μmol/L and 1000 μmol/L), 100 μmol/L melatonin, and 0.01 IU/mL insulin. The reason for doubling AL was from previous 6-hour cold static storage studies that showed a moderate but significant 1.3-fold increase in left ventricular function (aortic flow [AF]) and improved electrical stability (n = 6; D.M.R. and G.P.D. unpublished data, 2010). Higher AL concentrations may also improve protection because lidocaine is known to have a reduced ability to block sodium fast channels at lower temperatures[10] (and perhaps the same exists for adenosine and adenosine receptors). The naturally occurring pineal gland hormone melatonin was chosen because it is a free radical scavenger (5 times more effective in neutralizing OH^- radicals than glutathione) and is a powerful antioxidant with cardioprotective properties.[11] Insulin was chosen because of its antioxidant and cardioprotective properties (see "Discussion").[12] Preliminary experiments showed that melatonin and insulin added to AL arrest solution, singly or in combination, improved functional recoveries after 6 hours of cold static storage. The concentrations of each drug were chosen from their cardioprotective properties in the isolated perfused heart.[11,12]

Celsior solution. The Celsior solution contained 100 mmol/L NaOH, 15 mmol/L KCl, 13 mmol/L $MgCl_2$, 0.25 mmol/L $CaCl_2$, 20 mmol/L glutamic acid, 80 mmol/L lactobionic acid, 30 mmol/L histidine, and 3