CUSTODIOL VERSUS BLOOD CARDIOPLEGIA: COMPARISON OF MYOCARDIAL PROTECTION IN ADULT CARDIAC CASES

By

Daniella Boros

A Thesis Submitted to the Faculty of the

DEPARTMENT OF MEDICAL PHARMACOLOGY

In Partial Fulfillment of the Requirements

For the Degree of

MASTERS OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

2013

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that an accurate acknowledgement of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Daniella Boros

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

_____	_____12/11/2013____
Douglas F. Larson Professor of Surgery	Date

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my advisor, Professor Doug Larson, PhD for his encouragement, guidance and support during the pursuit of my Master degree. My accomplishments would not have been possible without him. I would also like to thank Sreekumar Subramanian, MD for his help in designing this research study and Ed Meister, PhD for all of his expertise and assistance analyzing the data for this project. Lastly, I would like to thank my thesis committee members, Professors Ed French, PhD and Michael Ossipov, PhD for their help with this thesis.

TABLE OF CONTENTS

ABBREVIATIONS

IR	Ischemia reperfusion
ATP	Adenosine triphosphate
MI	Myocardial infarction
HTK	Histidine-tryptophan-ketoglutarate
NAD	Nicotinamide adenine dinucleotide
STS	Society of thoracic surgeons
CPB	Cardiopulmonary bypass
RV	Right ventricle
TAPSE	Tricuspid annular plane systolic excursion
CABG	Coronary artery bypass grafting
AVR	Aortic valve repair/replacement
MVR	Mitral valve repair/replacement
EF	Ejection fraction
Cr	Creatinine
HCT	Hematocrit
CVA	Cerebrovascular event
IAC	Intermittent aortic cross-clamping

ABSTRACT

Objectives: When used as a cardioplegic solution, Custodiol® HTK solution is typically administered in a single-dose, allowing the operation to be performed continuously. This is an advantage over alternative cardioplegic solutions that may have to be re-administered every 20-30 minutes. Although Custodiol is widely used as a cardioplegic solution in Europe, its use for myocardial protection remains an off-label indication in the United States. Thus, the aim of this study is to compare the efficacy of Custodiol to standard 4:1 blood cardioplegia in adult cardiac cases.

METHODS: This study was a single-center retrospective review of prospectively collected data. Adult cardiac cases performed between November 2011 and August 2013 using Custodiol® were compared to cases using standard Plegisol® 4:1 blood cardioplegia. Twenty-six primary intra-operative and post-operative endpoints were compared including 30-day mortality, 30-day hospital readmission, prolonged mechanical ventilation time, and renal failure.

RESULTS: Of the 229 cases identified, 63 cases used Custodiol and 166 used 4:1 blood cardioplegia. Demographics were similar in both groups with a mean patient age of 65.27±15.07 years for Custodiol and 66.72±12.85 years for 4:1 blood cardioplegia. The average cardiopulmonary bypass time for Custodiol and 4:1 blood cardioplegia was 124.76±61.45 and 137.93±54.05 minutes respectively. The Custodiol group had a greater incidence of prolonged ventilation (>24 hours), 20.6% versus 15.1% respectively, and this approached statistical significance with a p value of 0.052. Intra-operative blood

usage was significantly higher in the Custodiol group compared to the blood cardioplegia group, with 44.4% of patients receiving fresh frozen plasma during the operation compared to only 25.3% in the blood cardioplegia group (p=0.005). The results revealed no statistically significant difference in 30-day mortality, 30-day hospital readmission, renal failure, and stroke.

CONCLUSION: Despite the distinct advantage of long-term ischemic tolerance, Custodiol use was associated with an increased requirement for fresh frozen plasma during the perioperative period when compared to blood cardioplegia.

INTRODUCTION

Myocardial ischemia reperfusion (IR) is defined as myocardial damage resulting from the restoration of blood flow to ischemic tissue. Ischemia causes a pattern of metabolic responses that result in a reduction of cellular adenosine triphosphate (ATP), a transition from aerobic to anaerobic energy utilization, and an accumulation and secretion of anaerobic metabolic products. Reperfusion can produce an array of events such as; damage to cellular and organelle membranes, oxidative stress, endothelial damage and vasoconstriction, and cellular and non-cellular pro-inflammatory immune responses (1). In the context of cardiac surgery, myocardial protection refers to the strategies and techniques used during the operation to prevent IR injury. These strategies help lower the heart's metabolic demands, minimizing myocardial stunning and perioperative necrosis. Myocardial stunning can lead to an increase requirement for inotropic support and is associated with a 5-fold increase in mortality in the high-risk patient population.

Perioperative necrosis, or myocardial infarction (MI) results from an imbalance in myocardial oxygen supply versus demand. The effects of perioperative necrosis can be experienced months to years after the surgery (2). Despite the importance of myocardial protection, controversies remain regarding the most clinically effective method to prevent IR.

One strategy for myocardial protection is the use of cardioplegia, administered at the time of myocardial arrest. Cardioplegic solutions function to lower the metabolic demand and protect the myocardium by preserving metabolic substrates, preventing electrolyte imbalances and minimizing changes in pH. Cardioplegic solutions are classified into two groups according to electrolyte content: extracellular solutions and intracellular solutions. Extracellular solutions contain high levels of potassium, magnesium and sodium, while intracellular solutions contain low electrolyte levels. Blood cardioplegia, an extracellular cardioplegia developed by Gerald Buckberg over 20 years ago, remains the preferred strategy for myocardial protection in the United States and parts of Western Europe. Standard 4:1 blood cardioplegia utilizes the patient's own blood as the vehicle for delivery. Blood cardioplegia contains endogenous proteins for oncotic pressure and enzymatic functions, oxygen free-radical scavengers, substrates for ATP production, and buffers to maintain pH. Blood cardioplegia can be delivered at varying temperatures allowing the surgeon to customize the delivery for each patient (3). Despite the advantages, blood cardioplegia must be administered every 20 minutes to maintain its protective properties.

Unlike standard blood cardioplegia, the Bretschneider histidine-tryptophan-ketoglutarate solution, known as Custodiol (Custodiol HTK, Köhler Chemie GmbH, Bensheim, Germany), minimizes disruption to the technical flow of the operation and is administered with as a single dose infusion. Custodiol is an intracellular crystalloid solution commonly used to prevent long-term ischemia in solid organ preservation. Custodiol contains a low sodium concentration and a high concentration of buffers, histidine and histidine HCl (4). Histidine is a naturally occurring buffer, effective under hypothermic conditions (5). In addition to its buffering components, Custodiol contains mannitol, ketoglutarate and tryptophan. Mannitol is a free radical scavenger that decreases cellular edema (6). Ketoglutarate is an intermediate in the krebs cycle and a precursor to nicotinamide adenine dinucleotide (NAD). Ketoglutarate increases energy production upon reperfusion, while tryptophan stabilizes cell membranes (4).

Although Custodiol is intended for perfusion and flushing donor kidney, liver, heart, and pancreas during organ transplants, its buffering capacity make it an attractive option for myocardial protection. Custodiol is approved for myocardial protection in several countries; however, its use for myocardial protection has not been adopted universally and remains an off-label indication in many countries including the United States. Table 1 provides an overview of the current literature describing the use of Custodiol for myocardial protection in adult cardiac cases. As evidenced in the table, relatively few studies exist comparing the effect of Custodiol with other widely used cardioplegic solutions. Furthermore, most studies are limited to patients undergoing mitral valve repair/replacement or coronary artery bypass grafting. Thus, the aim of this study is to

compare the efficacy of Custodiol to standard 4:1 blood cardioplegia in adult cardiac cases.

METHODS

This study was a single-center retrospective review of prospectively collected data. Adult cardiac cases performed at the University of Arizona Medical Center between November 2011 and August 2013 were included in this study. Operations using Custodiol (Custodiol® HTK Bretschneider) and standard Plegisol® 4:1 blood cardioplegia were identified using the Society of Thoracic Surgery (STS) database. The STS database is a national database, established in 1989 to promote quality improvement. As a participant in the STS database, all cardiac cases performed at the University of Arizona Medical Center are entered into the national database. For this study, demographics, pre-operative risk factors, operative techniques, and clinical outcomes were collected from the STS database. The local Institutional Review Board approved this protocol and a waiver of informed consent was granted.

Cardioplegic Delivery

Patients were divided into two groups according to the type of cardioplegic solution used during the operation: Group 1: Custodiol® versus Group 2: Plegisol® 4:1 blood cardioplegia. The composition of each cardioplegic solution used in the study is shown in Table 2. Patients were placed on cardiopulmonary bypass (CPB) according to the standard procedure at the University of Arizona Medical Center. At the initiation of the aortic cross-clamp, cardioplegic arrest was induced. For patients in Group 1, Custodiol

was cooled to 4-8˚C and administered at 200-250cc/min for 6 minutes. Perfusion pressure was maintained at 100-120 mmHg. Custodiol was administered as a single dose with repeat administration every 60 minutes for cases with the cross-clamp time exceeding 60 minutes. For patients in Group 2, blood cardioplegia was cooled to 4-8˚C and perfused every 20 minutes at a rate of 200-250cc/min while maintaining a perfusion pressure of 100-120 mmHg. Blood cardioplegia was mixed at a ratio of 4:1 (blood: Plegisol® solution). At the induction of cardiac arrest, a higher level of potassium was used, 25 mmol/L versus 9 mmol/L during all maintenance doses thereafter.

Study Endpoints

Twenty-six primary intra-operative and post-operative endpoints were compared including blood product usage, 30-day mortality, 30-day hospital readmission, prolonged mechanical ventilation time, renal failure, and multi-organ failure. Blood usage was defined as the incidence of patients receiving blood products, regardless of the amount of units delivered. The primary endpoints were as follows: 30-day mortality, 30-day hospital readmission, prolonged mechanical ventilation time (>24 hours post-op), renal failure (newly required dialysis), and stroke (perm > 24 hours).

Statistical Analysis

Data was analyzed using IBM SPSS for Windows, version 21.0 (IBM Corp, Armonk, New York). Descriptive statistics were calculated for all variables. Continuous data is presented as mean ± standard deviation and categorical data as percentages and counts. For analysis of statistical significance of continuous variables, a two-tailed Student's

independent *t* test or the Mann-Whitney U test was used. The Levene's Test was used to assess equality of variances for all significance tests for continuous measure variables. When the Levene's F test was statistically significant, equal variances were assumed in reporting t-test results for comparisons between groups. Categorical variables were evaluated using Fisher's exact tests or the Chi-square test. A p-value ≤ 0.05 was considered statistically significant based on an alpha of 5%.

RESULTS

A total of 229 surgical cases were identified, of which 63 cases (28%) used Custodiol and 166 cases (72%) used 4:1 blood cardioplegia for myocardial protection. The pre-operative clinical characteristics for each group are presented in Table 3. Demographics were similar in both groups with a mean patient age of 65.27±15.07 years for Custodiol and 66.72±12.85 years for 4:1 blood cardioplegia. The average pre-operative ejection fraction for Custodiol and 4:1 blood cardioplegia was 57.28±10.67% and 55.13±11.23% respectively. The majority of cases (41.3%) performed using Custodiol were aortic valve replacements (AVR), while only 22.3% of cases performed using blood cardioplegia were AVRs. In the blood cardioplegia group, 27.7% of cases were coronary artery bypass grafts (CABG), while no CABG cases were performed using Custodiol. Both groups had a high incidence of hypertension and dyslipidemia. The average pre-operative Creatinine value was significantly higher in the blood cardioplegia group compared to the Custodiol group, 1.246±0.912 µmol/L versus 0.989±0.317 µmol/L, respectively.

Table 4 displays the intra-operative characteristics across the two groups. The average cardiopulmonary bypass time for Custodiol and blood 4:1 cardioplegia was 124.76±61.45 minutes and 137.93±54.05 minutes respectively. The cross-clamp time for Custodiol was 89.02±42.26 minutes, compared to 93.21±37.52 minutes for blood cardioplegia. The lowest core temperature under CPB was 32.73±3.74ºC versus 33.45±2.95ºC for Custodiol and blood cardioplegia respectively. Intra-operative blood usage was higher in the Custodiol group compared to blood cardioplegia. The amount of patients receiving fresh frozen plasma during the operation was significantly higher in the Custodiol group compared to blood cardioplegia, 44.4% versus 25.3% respectively. This did reach statistical significance with a p-value of 0.005.

The post-operative outcomes are displayed in table 5. The average ICU time for Custodiol was 104.54±122.95 hours compared to 98.32±132.28 hours for 4:1 blood cardioplegia. The Custodiol group had a greater incidence of prolonged ventilation (>24 hours), 20.6% versus 15.1% respectively, and this approached statistical significance with a p-value of 0.052. Post-operative blood product usage was equivalent between groups and reached statistical significance. The results revealed no significant difference in 30-day mortality, 30-day hospital readmission, renal failure (newly required dialysis), and stroke (perm > 24 hours).

Due to the unequal distribution of operations conducted in each group, a sub-analysis was conducted to compare aortic valve replacements between Custodiol and 4:1 blood cardioplegia groups. The results are presented in tables 6-8. Pre-operative patient characteristics were similar in both groups. The average cardiopulmonary bypass time

was notably shorter for the Custodiol group compared to the blood cardioplegia group, 100.00±37.53 minutes versus 120.22±44.27 minutes respectively. This difference trended toward statistical significance with a p-value of 0.063. Intra-operative red blood cell usage was also notably different, with 61.5% of Custodiol patients receiving blood compared to only 9.6% in the blood cardioploegia group. This reached statistical significance with a p-value was 0.048. No other statistically significant differences were noted for the other endpoints evaluated when comparing the Custodiol with 4:1 blood cardioplegia groups. When determining statistical significance using the p-value, several were indicated as N/A status, which generally reflects the low or inadequate sample required to determine a differential effect if such an effect exists. The sub-analysis is not part of the overall power of the study, and was conducted as exploratory for further investigation.

DISCUSSION

Custodiol is an intracellular cardioplegic solution used worldwide in over 700,000 cases of open-heart surgery (7). A single administration of Custodiol provides up to 2 hours of myocardial protection (8, 9), providing a distinct advantage over alternative cardioplegic solutions requiring re-administration every 20 minutes. With the growing interest in minimally invasive valve procedures and other technically complex operations, cardioplegic solutions providing longer ischemic tolerance shorten the patient's duration on cardiopulmonary bypass by minimizing interruption to the operation (10). However, few studies exist to evaluate the efficacy of long lasting cardioplegic solutions such as Custodiol used for myocardial protection. Thus, this study was conducted to compare

clinical outcomes obtained by two different myocardial protection strategies: single shot administration with Custodiol and multi-dose administration using 4:1 blood cardioplegia. Despite the advantage of long-term ischemic tolerance, Custodiol use was associated with an increased requirement for fresh frozen plasma during the perioperative period when compared to blood cardioplegia. After cardiopulmonary bypass, fresh frozen plasma is often required to alleviate persistent bleeding (11). The requirement for fresh frozen plasma can also be attributed to disruptions in homeostasis including hemodilution (12), hypothermia (13), acidosis (13, 14), and electrolyte imbalance (13). Unlike blood cardioplegia, myocardial protection provided with Custodiol requires a large volume of crystalloid solution at the initiation of the cross-clamp (15), often resulting in hemodilution and a disruption in osmolarity (16). The distinct differences in delivery and electrolyte composition, could explain the significant increase in the intra-operative requirement for fresh frozen plasma. Although there were no statistically significant differences noted in post-operative outcomes for the sample population receiving Custodiol, increased use of fresh frozen plasma has been associated with prolonged ICU times (17, 18), increased risk for multiple organ failure (19) and increased hospital costs (18).

Additional risks have been identified relating to the homeostatic imbalance caused by Custodiol administration. While Custodiol composition allows for an increased concentration of biological buffers, namely histidine and histidine HCl, (4), the low sodium concentration contained within Custodiol can cause hyponatremia (16). Severe hyponatremia adversely affects the central nervous system and is associated with seizures

(20, 21). Under anesthesia, hyponatremia causes cerebral edema and disrupts the blood brain barrier function (21). This risk is further increased for patients on cardiopulmonary bypass. Hyponatremia in the presence of hypoxia decreases cerebral perfusion and impairs the brains adaptive mechanisms (22). In pediatric cardiac cases, Custodiol was associated with a significantly higher incidence of post-operative seizures compared to del Nido solution (16). As an intracellular solution, Custodiol causes fluctuations in the patient's sodium levels, often requiring acute correction with hypertonic saline. The acute normalization of sodium levels is associated with brain shrinkage and may damage cerebral vessels (16). It is unknown as to whether adults are also susceptible to increased post-operative seizures as seen within the pediatric population. Further studies are required to determine the full impact of the acute hyponatremia experienced following Custodiol delivery.

Also relating to Custodiol delivery is an increased concern for myocardial re-warming. Hypothermia is often employed as part of the myocardial protection surgery, as it lowers myocardial metabolism during periods of ischemia (23). Custodiol is delivered at 4-8°C, lowering ATP consumption and contributing to ischemic tolerance (4). While a single shot administration minimizes the interruption to the surgery, the prolonged time between doses can be associated with myocardial re-warming, especially in the right ventricle (9). During cardiopulmonary bypass, suboptimal myocardial protection is associated with right ventricle dysfunction postcardiotomy. Following mitral valve operations, right ventricle function is a major determinant of clinical outcomes (24, 25). Suboptimal myocardial protection with Custodiol cardioplegia was reported in a randomized

prospective study of patients undergoing mitral surgery. Patients with depressed pre-operative right ventricle function had a higher incidence of post-operative complications when Custodiol was administered compared to warm blood cardioplegia. Custodiol use was correlated with prolonged mechanical ventilation times and extended hospitalization. In a sub-study, the addition of topical cooling to Custodiol statistically lowered post-operative complications compared to Custodiol in the absence of topical cooling (9). It is arguable that the risk of myocardial re-warming is reduced with cardioplegic solutions dosed more frequently, like cold blood cardioplegia.

When compared to blood cardioplegia, the intracellular composition of Custodiol is also unique in that it contains mannitol (26). Mannitol is an osmotic diuretic that reduces cellular edema and acts as a free-radical scavenger (27). Mannitol does not undergo tubular reabsorption and is excreted by the renal glomeruli (28). Because mannitol is freely filtered by the glomerulus, it is often used during cardiopulmonary bypass to preserve renal function and prevent endothelial swelling during low blood flow and perfusion pressure (29). Despite its benefits, mannitol can lead to serious clinical disturbances in patients with preexisting renal failure (28). When administered to patients with renal failure, mannitol is retained in the extracellular space, causing an increase in the plasma osmolarity. The increase in plasma osmolarity is followed by the movement potassium and water to the extracellular space, causing extracellular volume expansion, hyponatremia, and hyperkalemia (30). Currently, there are no studies examining the impact of Custodiol in patients with renal dysfunction and further research is needed to

determine if the concentration of mannitol contained within Custodiol (30 mmol/L) is appropriate for patients with renal failure.

Within the past decades, many different concepts have contributed to improving myocardial protection. Custodiol, developed by Bretschneider et al was first reported in clinical use in 1977 (4) and its efficacy has been reported in several clinical trials (6-8). As the mechanism of ischemia reperfusion injury is becoming more clearly understood, myocardial protection strategies are evolving to assure improved clinical outcomes. Recently, HTK-N46b, a novel cardioplegic solution based on the classic Custodiol HTK solution described in this study, was developed to improve post-ischemic hemodynamic recovery by reducing iron-independent injury. The new HTK-N46b solution was modified from the original Custodiol solution to contain the addition of amino acids aspartate, L-arginine, L-alanine, and L-glycine. N-a-acetyl-L-histidine has been added, and the new solution contains a lower concentration of chloride compared to the classic solution (31). In the new HTK-N46b solution, the concentration of L-histidine contained in HTK has been partially replaced by N-a-acetyl-L-histidine. N-a-acetyl-L-histidine is a naturally occurring derivative of L-histidine with little to no toxicities (32). L-histidine has been associated with increased levels of intracellular iron contributing to the generation of reactive oxygen species. By partially replacing L-histidine with N-a-acetyl-L-histidine, the new formulation is intended to reduce iron-dependent toxicities, while maintaining the buffering capabilities of histidine under hypothermic conditions (31). The new HTK-N solution is currently being studied in a phase III clinical trial in Germany, comparing HTK-N to the classic HTK solution in coronary artery bypass

surgery. It is unknown as to how the myoprotective properties of the new HTK-N formulation will compare to alternative cardioplegic solutions, like standard 4:1 blood cardioplegia evaluated in this study.

LIMITATIONS

This study is retrospective in nature. Although the study was limited to a single institution, the operations included in this study were performed by multiple surgeons and therefore, lack standardization. The purpose of this study was to compare the efficacy of Custodiol used for myocardial protection. However, the primary endpoints were based on clinical outcomes experienced within 30 days of the operation. Post-operative necrosis, a common complication resulting from inadequate myocardial protection is often delayed, occurring months to years after the operation. Therefore, the primary endpoints examined in this study do not accurately reflect the long-term effects of the cardioplegic solution. Also relating to the endpoints evaluated in this study, blood product usage was defined as the incidence of patients receiving blood products and was not indicative of the number of units received. To truly determine the extent to which blood product usage is correlated to the type of cardioplegic solution used, the number of units received be each patient must be evaluated. Lastly, the clinical outcomes may not be solely attributed to the cardioplegic solution and instead the patients' disease and or surgical operation. Therefore, further studies that include molecular markers associated with myocardial ischemia reperfusion injury are warranted.

CONCLUSION

Despite the advantage of long-term ischemic tolerance, Custodiol use was negatively correlated with an increased requirement for fresh frozen plasma during the perioperative period when compared to blood cardioplegia. Prospective clinical trials are required to further evaluate clinical efficacy.

CONFLICT OF INTEREST

None declared.

Table 1: Myocardial protection in adult cardiac cases using Custodiol

Author	Year	Study Design	Sample	Measures	Results
Guadino et al (9)	2013	Prospective Randomized Study – comparing Custodiol to warm blood cardioplegia in mitral valve surgery	31 – Custodiol 29 – Warm blood 60 – Total Cases	Right ventricular function assessed by echocardiogram and hemodynamic assessment	Statistically lower right ventricle (RV) ejection fraction, end-diastolic volume and fractional area change with HTK compared to warm blood for patients with impaired pre-operative RV systolic function (assessed by tricuspid annular plane systolic excursion (TAPSE)
Viana et al (33)	2012	Retrospective Records Review – Comparing Custodiol to tepid (28°C) blood cardioplegia 4:1 in cardiac procedures	1774 – Blood cardioplegia 126 – Custodiol 1900 – Total Cases (propensity score matching gave 71 Custodiol cases and 71 blood cases)	Primary end pts – 30-day mortality, return to OR, MI, stroke, post-operative requirement for intra-aortic balloon pump, new renal failure, prolonged ventilation, re-admission to hospital within 30 days	No statistical significance after propensity-score matching.
Braathen et al (7)	2011	Prospective Randomized Study – Comparing HTK (cold, single dose, antegrade) to blood cardioplegia (repetitive, antegrade) in elective isolated mitral valve surgery for mitral regurgitation	38 – blood cardioplegia 38 – HTK 76 – Total cases	Troponin-T and CK-MB analyzed at baseline and 7 hours, 1 day, 2 days and 3 days after surgery	No difference in Troponin-T and CK-MB between groups. Significantly more spontaneous ventricular fibrillation after release of cross clamp in HTK group
Scrascia et al (15)	2011	Retrospective Records Review – Elective or emergent thoracic aorta operations comparing Custodiol (HTK)	54 – Custodiol 58 – Cold Blood 112 – Total cases	Primary end pt – post-operative cTnI release and low cardiac output syndrome;	For cross-clamp time >160 min, cold blood had higher post-operative cTnI than HTK; all

		to cold blood multi-dose cardioplegia		Secondary end pt – clinical outcome	other measures similar for both groups
Demmy et al (34)	2008	Multi-center, Randomized Phase 3 Comparing Custodiol to Plegisol; CABG patients randomized into two groups and stratified by EF into 40% or greater or 20-39%.	68 – Custodiol 68 – Plegisol 136 – Total Cases	Primary end pt – cTnI 6 h post-ischemia; Secondary end pt – CK-MB 6 h, 12h, 24 h, 48h following release of cross-clamp	cTN-I 6h post-ischemia was higher for HTK vs Plegisol
Arslan et al (35)	2005	Prospective Randomized Study – Comparing low dosage HTK to cold crystalloid cardioplegia in CABG patients	21 – HTK 21 – Cold Crystalloid 42 – Total Cases	Malondialdehyde, lactate, CK, CK-MB, cTnI	No statistical difference in ischemic serum markers between groups
Savini et al (8)	2004	Retrospective Records Review – Examining early results of HTK used in Minimally invasive mitral valve surgery	8 – Total Cases	CK and its MB fraction; ECG prior to surgery and 1h, 6h, 1d, 2d, 7d post-op	No operative deaths or major post operative complications; no significant changes in serum enzymes and ECG; after cross clamp cardiac rhythm restarted spontaneously in 6 cases, 2 cases required defibrillation
Careaga et al (6)	2001	Prospective Randomized Study – comparing HTK to conventional cold crystalloid cardioplegia in elective open heart surgery	15 – HTK 15 – Crystalloid 30 – Total Cases	Incidence of arrhythmias, inotropic support requirement, length of stay in ICU	During reperfusion, no difference in incidence of arrhythmias; in post-op, HTK had lower incidence of arrhythmias; HTK had lower inotropic support and length of stay in ICU
Sunderdiek et al.	2000	Prospective Randomized Trial comparing HTK to intermittent aortic cross-clamping (IAC) in CABG patients	51 – HTK 52 – IAC 103 – Total Cases	CK and CK-MB; cTnI; ECG changes	HTK group had a longer cross-clamp time than IAC group (117 min vs 107 min); For ischemia time >40 min, IAC had greater

					cTnI and CK-MB levels.
Sakata et al (36)	1998	Retrospective Study – Comparing HTK to cold blood cardioplegia (15°C, 1:1) in mitral valve replacement	20 – HTK 26 - CBC 46 – Total Cases	Dose of inotropic agent, changes in CI pre and post-op; CK 1d and 2d post-op; frequency of spontaneous defibrillation after release of cross-clamp; frequency of temporary pacing	Mean CK value was higher in HTK group at 1d and 2d post-op; Spontaneous defibrillation was higher in HTK group; temporary pacing used less frequently in HTK group after CPB; no change in inotropic agent; changes in CI were non-significant

Table 2: Cardioplegia Composition

	Custodiol®	Blood cardioplegia at Induction*	Blood cardioplegia 4:1 Maintenance*
Sodium (mmol/L)	15.0	105.0	105.0
Potassium (mmol/L)	9.0	25.0	9.0
Magnesium (mmol/L)	4.0	3.0	3.0
Calcium (mmol/L)	0.015	0.7	0.7
Potassium hydrogen 2-Ketoglutarate (mmol/L)	1.0	-	-
Histidine · HCl · H 2 O (mmol/L)	18.0	-	-
Histidine (mmol/L)	180.0	-	-
Tryptophan (mmol/L)	2.0	-	-
Mannitol (mmol/L)	30.0	-	-
Sodium Bicarbonate (mmol/L)	-	18.0	18.0
Glucose (mmol/L)	-	35.0	35.0
Phosphate (mmol/L)	-	0.5	0.5

*The composition of the solution below is before it is mixed with blood in 1:4 ratio. 4:1 Blood to Solution Mixture as Delivered.

Table 3: Pre-operative Patient Characteristics

	Custodiol (n=63)	Blood Cardioplegia (n=166)	P value
Age (years)	65.27±15.07	66.72±12.85	0.502
Females (no)	25 (39.7%)	52 (31.3%)	0.002
Body Surface Area (m2)	1.97±0.24	1.95±0.25	0.444
Surgical Status			
Elective	38 (60.3%)	106 (63.9%)	<0.001
Urgent	21 (33.3%)	54 (32.5%)	<0.001
Emergent	4 (6.3%)	6 (3.6%)	0.527
Redo Operation	14 (22.2%)	22 (13.3%)	0.182
Operation			

AVR	26 (41.3%)	37 (22.3%)	0.166
MVR	5 (7.9%)	23 (13.9%)	0.001
CABG	0 (0%)	46 (27.7%)	N/A
AVR/CABG	4 (6.3%)	15 (9.0%)	0.012
MVR/CABG	2 (3.2%)	2 (1.2%)	N/A
Other	26 (41.3%)	43 (25.9%)	0.041
EF%	57.28±10.67	55.13±11.24	0.202
Hypertension	49 (77.8%)	142 (85.5%)	<0.001
Diabetes	13 (20.6%)	52 (31.3%)	<0.001
Hematocrit (g/dL)	38.80±5.68	40.95±6.14	0.193
Creatinine (µmol/L)	0.989±0.317	1.246±0.912	0.020
Chronic Lung Disease			
None	48 (76.2%)	140 (84.3%)	<0.001
Mild	5 (7.9%)	12 (7.2%)	0.090
Moderate	8 (12.7%)	8 (4.8%)	1.00
Severe	2 (3.2%)	6 (3.6%)	0.157
Dyslipidemia	46 (73.0%)	123 (74.1%)	<0.001
Myocardial Infarction	12 (19.0%)	40 (24.1%)	0.415
Peripheral Artery Disease	1 (1.6%)	10 (6.0%)	0.007
Cerebrovascular event	7 (11.1%)	20 (12.0%)	0.012
Arrhythmia			
None	43 (68.3%)	125 (75.3%)	<0.001
Recent	13 (20.6%)	23 (13.9%)	0.096
Remote	7 (11.1%)	18 (10.8%)	0.028
Aortic Insufficiency			
None	22 (34.9%)	76 (45.8%)	<0.001
Trace/trivial	3 (4.8%)	12 (7.2%)	0.020
Mild	20 (31.7%)	42 (25.3%)	0.005
Moderate	11 (17.5%)	14 (8.4%)	0.549
Severe	7 (11.1%)	22 (13.3%)	0.005

Table 4: Intra-operative Characteristics

	Custodiol (n=63)	Blood Cardioplegia (n=166)	P value
CPB (min)	124.76±61.45	137.93±54.05	0.114
X-clamp (min)	89.02±42.26	93.21±37.52	0.467
Lowest core temp under CPB (ºC)	32.73±3.74	33.45±2.95	0.176
Blood products			
Red blood cells	38 (60.3%)	82 (49.4%)	0.140
Fresh frozen plasma	28 (44.4%)	42 (25.3%)	0.005
Platelets	35 (55.6%)	76 (45.8%)	0.186

Table 5: Post-operative Outcomes

	Custodiol (n=63)	Blood Cardioplegia (n=166)	P value
ICU time (hours)	104.54±122.95	98.32±132.28	0.746
Ventilation time (hours)	36.00±82.68	32.26±100.91	0.793
Prolonged ventilation (>24 hours)	13 (20.6%)	25 (15.1%)	0.052
Reintubation	7 (11.1%)	15 (9.0%)	0.634
Blood products			
Red blood cells	19 (30.2%)	56 (33.7%)	<0.001
Fresh frozen plasma	15 (23.8%)	41 (24.7%)	0.001
Platelets	10 (15.9%)	26 (15.7%)	0.008
Creatinine (µmol/L)	1.47±1.29	1.66±1.64	0.410
Renal failure	4 (6.3%)	11 (6.6%)	0.940
Multi-organ failure	0 (0%)	3 (1.8%)	0.238
Sepsis	1 (1.6%)	3 (1.8%)	0.910
Stroke	1 (1.6%)	1 (0.6%)	0.474
Pacemaker	2 (3.2%)	4 (2.4%)	0.746
Atrial fibrillation	15 (23.8%)	42 (25.3%)	0.816
Operative Death	3 (4.8%)	9 (5.4%)	0.841
30-day mortality	3 (4.8%)	9 (5.4%)	0.841

30-day hospital readmission	8 (12.7%)	33 (19.9%)	0.206

Table 6: Sub-analysis AVR Pre-operative Patient Characteristics

	Custodiol (n=26)	Blood Cardioplegia (n=37)	P value
Age (years)	69.53±15.64	71.73±11.74	0.549
Females (no)	10 (40.0%)	9 (24.3%)	0.189
Body Surface Area (m2)	2.02±0.29	1.95±0.25	0.338
Surgical Status			
Elective	16 (64.0%)	31 (83.8%)	0.029
Urgent	9 (36.0%)	6 (16.2%)	0.317
Emergent	0 (0%)	0 (0%)	N/A
Redo Operation	7 (28.0%)	7 (18.9%)	0.402
EF%	54.88±12.30	54.66±13.12	0.947
Hypertension	19 (76.0%)	30 (81.1%)	0.630
Diabetes	7 (28.0%)	10 (27.0%)	0.933
Hematocrit (g/dL)	38.80±5.67	40.95±6.14	0.166
Creatinine (µmol/L)	0.99±0.32	1.25±0.91	0.119
Chronic Lung Disease			
None	18 (72.0%)	30 (81.1%)	0.116
Mild	1 (4.0%)	2 (5.4%)	N/A
Moderate	6 (24.0%)	2 (5.4%)	N/A
Severe	0 (0%)	3 (8.1%)	N/A
Dyslipidemia	22 (88.0%)	32 (86.5%)	0.862
Myocardial Infarction	6 (24.0%)	6 (16.2%)	0.447
Peripheral Artery Disease	0 (0%)	2 (5.4%)	0.237
Cerebrovascular event	1 (4.0%)	3 (8.1%)	0.518
Arrhythmia			
None	18 (72.0%)	31 (83.8%)	0.090
Recent	5 (20.0%)	5 (13.5%)	>0.05
Remote	2 (8.0%)	1 (2.7%)	N/A

Aortic Insufficiency			
None	5 (20.0%)	7 (18.9%)	0.564
Trace/trivial	1 (4.0%)	1 (2.7%)	N/A
Mild	11 (44.0%)	17 (45.9%)	0.564
Moderate	2 (8.0%)	2 (5.4%)	N/A
Severe	6 (24.0%)	10 (27.0%)	0.317

Table 7: Sub-analysis AVR Intra-operative Characteristics

	Custodiol (n=26)	Blood Cardioplegia (n=37)	P value
CPB (min)	100.00±37.53	120.22±44.27	0.063
X-clamp (min)	76.69±25.64	81.95±24.94	0.419
Lowest core temp under CPB (ºC)	34.03±2.62	33.96±2.13	0.897
Blood product usage			
Red blood cells	16 (61.5%)	16 (9.6%)	0.048
Fresh frozen plasma	8 (30.8%)	8 (21.6%)	1.00
Platelets	10 (38.5%)	15 (41.7%)	0.317

Table 8: Sub-analysis AVR Post-operative Outcomes

	Custodiol (n=26)	Blood Cardioplegia (n=37)	P value
ICU time (hours)	63.76±46.45	82.32±69.06	0.238
Ventilation time (hours)	17.51±41.71	14.03±21.46	0.666
Prolonged ventilation (>24 hours)	3 (12.0%)	5 (13.5%)	0.862
Reintubation	1 (4.0%)	1 (2.7%)	0.777
Blood products			
Red blood cells	6 (23.1%)	13 (35.1%)	0.305
Fresh frozen plasma	5 (19.2%)	6 (16.2%)	0.756
Platelets	4 (15.4%)	4 (10.8%)	0.591
Creatinine (µmol/L)	1.54±1.55	1.72±1.75	0.682

Renal failure	0 (0%)	2 (5.4%)	0.228
Multi-organ failure	0 (0%)	0 (0%)	N/A
Sepsis	0 (0%)	0 (0%)	N/A
Stroke	1 (3.8%)	1 (2.7%)	0.799
Pacemaker	0 (0%)	0 (0%)	N/A
Atrial fibrillation	8 (30.7%)	9 (24.3%)	0.570
Operative Death	2 (7.7%)	2 (5.4%)	0.714
30-day mortality	2 (7.7%)	2 (5.4%)	0.714
30-day hospital readmission	1 (3.8%)	7 (18.9%)	0.077

REFERENCES

1. Bae YS, Kim Y, Kim JH, Lee TG, Suh PG, Ryu SH. Independent functioning of cytosolic phospholipase A2 and phospholipase D1 in Trp-Lys-Tyr-Met-Val-D-Met-induced superoxide generation in human monocytes. Journal of Immunology. 2000;164(8):4089-96.
2. Mentzer RM, Jr. Myocardial protection in heart surgery. Journal of Cardiovascular Pharmacology and Therapeutics. 2011;16(3-4):290-7.
3. Martin J, Benk C. Blood cardioplegia. Multimedia Manual of Cardio-Thoracic Surgery. 2006;2006(1009).
4. Bretschneider HJ. Myocardial protection. The Thoracic and Cardiovascular Surgeon. 1980;28(5):295-302.
5. Triana JR, Yanagihashi M, Larson DF. Mathematical modeling of buffers used in myocardial preservation. Perfusion. 2007;22(5):353-62.
6. Careaga G, Salazar D, Tellez S, Sanchez O, Borrayo G, Arguero R. Clinical impact of histidine-ketoglutarate-tryptophan (HTK) cardioplegic solution on the perioperative period in open heart surgery patients. Archives of Medical Research. 2001;32(4):296-9.
7. Braathen B, Jeppsson A, Schersten H, Hagen OM, Vengen O, Rexius H, et al. One single dose of histidine-tryptophan-ketoglutarate solution gives equally good myocardial protection in elective mitral valve surgery as repetitive cold blood cardioplegia: a prospective randomized study. The Journal of Thoracic and Cardiovascular Surgery. 2011;141(4):995-1001.
8. Savini C, Camurri N, Castelli A, Dell'Amore A, Pacini D, Suarez SM, et al. Myocardial protection using HTK solution in minimally invasive mitral valve surgery. The Heart Surgery Forum. 2005;8(1):E25-7.
9. Gaudino M, Pragliola C, Anselmi A, Pieroni M, De Paulis S, Leone A, et al. Randomized trial of HTK versus warm blood cardioplegia for right ventricular protection in mitral surgery. Scandinavian Cardiovascular Journal : SCJ. 2013.
10. Fannelop T, Dahle GO, Salminen PR, Moen CA, Matre K, Mongstad A, et al. Multidose cold oxygenated blood is superior to a single dose of Bretschneider HTK-cardioplegia in the pig. The Annals of Thoracic Surgery. 2009;87(4):1205-13.
11. Stanworth SJ, Brunskill SJ, Hyde CJ, McClelland DB, Murphy MF. Is fresh frozen plasma clinically effective? A systematic review of randomized controlled trials. British Journal of Haematology. 2004;126(1):139-52.
12. Maegele M, Lefering R, Yucel N, Tjardes T, Rixen D, Paffrath T, et al. Early coagulopathy in multiple injury: an analysis from the German Trauma Registry on 8724 patients. Injury. 2007;38(3):298-304.
13. Ferrara A, MacArthur JD, Wright HK, Modlin IM, McMillen MA. Hypothermia and acidosis worsen coagulopathy in the patient requiring massive transfusion. American Journal of Surgery. 1990;160(5):515-8.
14. Brohi K, Cohen MJ, Davenport RA. Acute coagulopathy of trauma: mechanism, identification and effect. Current Opinion in Critical Care. 2007;13(6):680-5.

15. Scrascia G, Guida P, Rotunno C, De Palo M, Mastro F, Pignatelli A, et al. Myocardial protection during aortic surgery: comparison between Bretschneider-HTK and cold blood cardioplegia. Perfusion. 2011;26(5):427-33.
16. Kim JT, Park YH, Chang YE, Byon HJ, Kim HS, Kim CS, et al. The effect of cardioplegic solution-induced sodium concentration fluctuation on postoperative seizure in pediatric cardiac patients. The Annals of Thoracic Surgery. 2011;91(6):1943-8.
17. Lei Q, Chen L, Jin M, Ji H, Yu Q, Cheng W, et al. Preoperative and intraoperative risk factors for prolonged intensive care unit stay after aortic arch surgery. Journal of Cardiothoracic and Vascular Anesthesia. 2009;23(6):789-94.
18. Magee G, Peters C, Zbrozek A. Analysis of inpatient use of fresh frozen plasma and other therapies and associated outcomes in patients with major bleeds from vitamin K antagonism. Clinical Therapeutics. 2013;35(9):1432-43.
19. Johnson JL, Moore EE, Kashuk JL, Banerjee A, Cothren CC, Biffl WL, et al. Effect of blood products transfusion on the development of postinjury multiple organ failure. Archives of Surgery. 2010;145(10):973-7.
20. Arieff AI. Hyponatremia, convulsions, respiratory arrest, and permanent brain damage after elective surgery in healthy women. The New England Journal of Medicine. 1986;314(24):1529-35.
21. Castilla-Guerra L, del Carmen Fernandez-Moreno M, Lopez-Chozas JM, Fernandez-Bolanos R. Electrolytes disturbances and seizures. Epilepsia. 2006;47(12):1990-8.
22. Ayus JC, Armstrong D, Arieff AI. Hyponatremia with hypoxia: effects on brain adaptation, perfusion, and histology in rodents. Kidney International. 2006;69(8):1319-25.
23. Drescher C, Diestel A, Wollersheim S, Berger F, Schmitt KR. How does hypothermia protect cardiomyocytes during cardioplegic ischemia? European Journal of Cardio-Thoracic Surgery. 2011;40(2):352-9.
24. Haddad F, Couture P, Tousignant C, Denault AY. The right ventricle in cardiac surgery, a perioperative perspective: II. Pathophysiology, clinical importance, and management. Anesthesia and Analgesia. 2009;108(2):422-33.
25. Kaul TK, Fields BL. Postoperative acute refractory right ventricular failure: incidence, pathogenesis, management and prognosis. Cardiovascular Surgery. 2000;8(1):1-9.
26. Hachida M, Ookado A, Nonoyama M, Koyanagi H. Effect of HTK solution for myocardial preservation. The Journal of Cardiovascular Surgery. 1996;37(3):269-74.
27. Ferreira R, Burgos M, Llesuy S, Molteni L, Milei J, Flecha BG, et al. Reduction of reperfusion injury with mannitol cardioplegia. The Annals of Thoracic Surgery. 1989;48(1):77-83.
28. Aviram A, Pfau A, Czaczkes JW, Ullmann TD. Hyperosmolality with hyponatremia, caused by inappropriate administration of mannitol. The American Journal of Medicine. 1967;42(4):648-50.
29. Ziegeler S, Raddatz A, Schneider SO, Sandmann I, Sasse H, Bauer I, et al. Effects of haemofiltration and mannitol treatment on cardiopulmonary-bypass induced immunosuppression. Scandinavian Journal of Immunology. 2009;69(3):234-41.

30. Manninen PH, Lam AM, Gelb AW, Brown SC. The effect of high-dose mannitol on serum and urine electrolytes and osmolality in neurosurgical patients. Canadian Journal of Anaesthesia = Journal Canadien D'anesthesie. 1987;34(5):442-6.
31. Trescher K, Hasun M, Baumgartner A, Dietl W, Wolfsberger M, Hallstrom S, et al. New HTK-N46B cardioplegia provides superior protection during ischemia/reperfusion in failing hearts. The Journal of Cardiovascular Surgery. 2013;54(3):413-21.
32. Baslow MH. A review of phylogenetic and metabolic relationships between the acylamino acids, N-acetyl-L-aspartic acid and N-acetyl-L-histidine, in the vertebrate nervous system. Journal of Neurochemistry. 1997;68(4):1335-44.
33. Viana FF, Shi WY, Hayward PA, Larobina ME, Liskaser F, Matalanis G. Custodiol versus blood cardioplegia in complex cardiac operations: an Australian experience. European Journal of Cardio-thoracic Surgery. 2013;43(3):526-31.
34. Demmy TL, Molina JE, Ward HB, Gorton ME, Kouchoukos NT, Schmaltz RA, et al. Custodiol versus Plegisol: A phase 3 multicentre myocardial protection study. The International Journal of Angiology. 2008;17(3):149-53.
35. Arslan A, Sezgin A, Gultekin B, Ozkan S, Akay T, Uguz E, et al. Low-dose histidine-tryptophan-ketoglutarate solution for myocardial protection. Transplantation Proceedings. 2005;37(7):3219-22.
36. Sunderdiek U, Feindt P, Gams E. Aortocoronary bypass grafting: a comparison of HTK cardioplegia vs. intermittent aortic cross-clamping. European Journal of Cardio-Thoracic Surgery. 2000;18(4):393-9.