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Comparable renal effects of histidine-tryptophan-ketoglutarate and DelNido cardioplegia in a porcine model of cardiac arrest

Maja-Theresa Dieterlen[#], Jagdip Kang[#], Paul Schütte, Kristin Klaeske, Susann Oßmann, Philipp Kiefer, Marcel Vollroth, Michael A. Borger and Alexandro Hoyer

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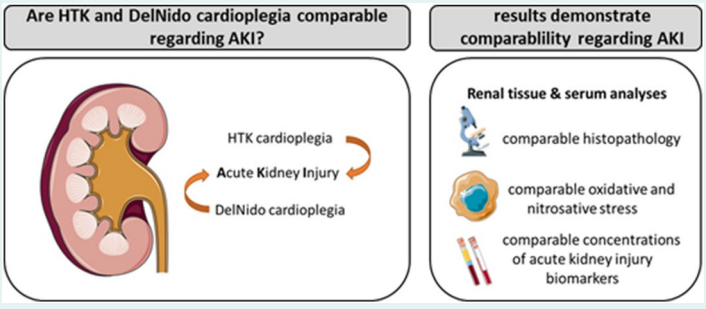
ABSTRACT

Acute kidney injury (AKI) is a serious complication following on-pump cardiac surgery. Choosing the cardioplegic solution with the most appropriate myocardial protection and lowest grade of AKI could improve patient outcome. We compared the crystalloid histidine-tryptophan-ketoglutarate (HTK) solution and a Jonosteril®-based DelNido blood cardioplegic solution regarding AKI in a porcine model. Therefore, German landrace pigs (50–60kg) underwent median sternotomy, cardiopulmonary bypass at 34 °C and 90 min of cardiac arrest. Animals were randomized to single-shot cardioplegia of HTK ($n=9$) or DelNido ($n=9$) cardioplegic solution followed by 120 min of reperfusion. This study demonstrated that DelNido cardioplegia induced less hemoglobin ($p<0.01$) and electrolyte imbalances of blood sodium, chloride, and calcium levels (all $p<0.01$) after aortic cross-clamp than HTK cardioplegia. Renal biopsy analysis after 120 min of reperfusion revealed that histomorphological changes, oxidative and nitrosative stress as well as the cytosolic release of pro-apoptotic molecules in different nephron structures were comparable in HTK and DelNido cardioplegia. The comparability of both cardioplegic solutions was supported by measurements of the urine AKI biomarkers of L-type fatty acid-binding protein 1 ($p = 0.38$), neutrophil gelatinase-associated lipocalin ($p = 0.34$), and cystatin C ($p = 0.46$), which could not detect any differences between the groups. This large animal study demonstrated superiority of the DelNido solution regarding hemoglobin and blood electrolyte concentrations, but comparability of the HTK and DelNido blood cardioplegic solution regarding AKI for surgical interventions requiring cardiac arrest of 90 min. Patients with a higher risk for adverse events, due to either complex, prolonged surgery or a multitude of comorbidities, could especially benefit from the more physiological arrest conditions with DelNido cardioplegia.

GRAPHICAL ABSTRACT



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1. Introduction

Cardioplegic solutions induce cardiac arrest for a resting operation field and provide the best possible myocardial protection during the ischemic period [1]. The choice of the cardioplegic solution is often surgeon- or center-specific, and rarely considers the possible effects on end-organs, such as the kidney.

Cardiac surgery-induced acute kidney injury (AKI) affects up to 30% of the patients who undergo a surgical intervention with cardiopulmonary bypass (CPB) [1]. AKI is associated with prolonged hospitalizations and higher mortality rates [1,2]. Approximately, 2–6% of the patients require hemodialysis after cardiac surgery with CPB [3]. Meta-analyses reported a lower risk of AKI [4] or comparability of renal failure [5] by using the DelNido cardioplegia; however, they included only 1 study [4] or none [5] that specifically addressed this question. Retrospective studies, including patients undergoing coronary artery bypass or minimally invasive mitral valve surgery, reported higher creatinine kinase isoenzyme MB (CK-MB) levels on the 2nd postoperative day with HTK cardioplegia [6] or identified HTK as a risk factor for AKI [7] when compared to DelNido cardioplegia.

Intraoperative parameters have a significant impact on the development of AKI in cardiac surgery patients, as the duration of CPB and cross-clamp time have been identified as significant risk factors for AKI [8–10]. Furthermore, optimizing intraoperative hemodynamics can prevent AKI [8]. The high relevance of intraoperative parameters on AKI development also includes the choice of the cardioplegic solution with the most appropriate myocardial protection and the lowest grade of AKI, which could improve patient outcomes and quality of life.

The European Association for Cardio-Thoracic Surgery (EACTS) recommends considering the use of blood cardioplegia in patients with anemia, chronic kidney disease, or for complex procedures to reduce hemodilution, bleeding complications, and transfusion requirements [11]. To improve knowledge about kidney injury following cardiac arrest induced by different cardioplegic solutions, we conducted a randomized comparison of the crystalloid histidine-tryptophan-ketoglutarate (HTK) solution (Custodiol®) and the Jonosteril®-based DelNido blood cardioplegic solution regarding their renal effects in a large animal model. This study aims to identify and compare perioperatively induced renal damage on a molecular and histopathological basis, which is currently unknown, that could lead to AKI during the mid- and long-term postoperative course. Therefore, a large animal study comparing HTK- and DelNido-induced changes in renal tissue and the release of early AKI biomarkers should provide more certainty in clinical decision-making for the optimal cardioplegic solution.

2. Methods

2.1. Animals

The study was authorized (vote no. TVV 23/19) by the local animal welfare agency (Landesdirektion Leipzig, Germany) and the institutional ethics review board, and was conducted in accordance with Directive 2010/63/EU of the European Parliament regarding animal protection. Animal studies do not require an informed consent. Female 4- to 5-month-old landrace pigs (50–60 kg) were included in this study.

2.2. Anesthesia management and surgical procedure

Anesthesia management and the surgical procedure were performed as described previously [12]. Before transportation, pigs were premedicated with intramuscular injection of 0.5 mg/kg midazolam, 0.02 mg/kg atropine, and 15 mg/kg ketamine. Thereafter, a bolus of 1–3 mg/kg propofol and 50 mg/kg metamizol were administered intravenously *via* the lateral ear vein or intramuscular, respectively. The pigs were intubated and mechanically ventilated (Cato, Draeger, Lübeck, Germany) with 33–100 Vol% oxygen, a respiratory rate of 15–30/min and a tidal volume of 6–10 mL/kg. Anesthesia was maintained by intravenous administration of 2% propofol and 50 µg/mL sufentanil. Pigs were heparinized by intravenous application of 300 IU/kg heparin after they underwent median sternotomy and pericardiotomy. A 6 Fr sheath was placed in the carotid artery to introduce a CD Leycom pressure volume loop catheter into the left ventricle (Hengelo, NL). Pursestring sutures were placed in the aortic arch and the right atrial appendage to allow arterial and

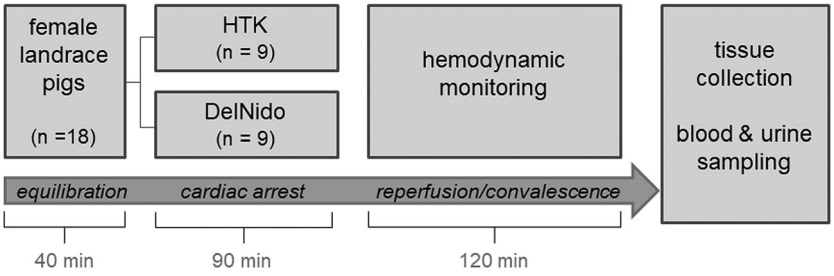

Figure 1. Study design. The 40-min equilibration period was defined as the time between the induction of anesthesia and aorta cross-clamping. Female landrace pigs were randomly assigned to receive either HTK ($n = 9$) or DelNido cardioplegia ($n = 9$) to induce cardiac arrest for 90 min. Following 120 min reperfusion, renal tissue, blood, and urine were collected. HTK: histidine-tryptophan-α-ketoglutarate.

venous cannulation (Edwards Lifesciences, Irvine, CA). A 12 G needle vent (Medtronic, Minneapolis, MN) was placed in the aortic root for application of cardioplegic solution. The total time between induction of anesthesia and cross-clamping of the aorta was constantly set at 40 min and referred to as the equilibration period (Figure 1). At the end of the equilibration period, blood serum and urine were collected.

Priming of the CPB system was performed with 500 mL Jonosteril® crystalloid solution containing 10,000 IU heparin. The activated clotting time (ACT) was greater than 330 s. Afterwards, animals were randomly assigned ($n = 9$ per group) to receive either 1800 mL Custodiol (HTK group; Köhler-Chemie, Alsbach-Henlein, Germany) or 1300 mL modified DelNido cardioplegic solution (DelNido group) over 6–8 min with a pump flow rate of 0.3 L per min. The DelNido cardioplegia solution consisted of one part porcine red cell concentrate received from a porcine blood donor and four parts of crystalloid solution based on Jonosteril® crystalloid solution (Supplementary Table 1). The crystalloid DelNido cardioplegic solution has higher concentrations of Na^+, K^+, Cl^-, Mg^{2+}, and Ca^{2+} than the HTK cardioplegic solution and was supplemented with the sodium channel blocker lidocaine. The crystalloid HTK cardioplegic solution was supplemented with the eponymous components histidine, tryptophan and α-ketoglutarate, and has a higher mannitol concentration than the DelNido cardioplegic solution.

Hearts were arrested for 90 min at 34 °C body temperature. The temperature was regulated *via* the CPB device. The mean arterial pressure (MAP) was set to 40–60 mmHg, and the aortic cannula pressure was 150–300 mmHg. Subsequently, 30 min following reperfusion, the body temperature was restored to physiological conditions. Thereafter, the animals were weaned from extracorporeal circulation. After a total reperfusion time or 120 min, blood serum, urine, and renal tissue were collected using standardized procedures. Hemodynamic and respiratory parameters were monitored *via* Infinity Delta (Draeger). Blood gases were analyzed using the ABL 90 (Radiometer GmbH, Krefeld, Germany). MAP and cardiac output were recorded *via* a PiCCO® catheter (PiCCO®*plus,* PULSION Medical Systems, Feldkirchen, Germany) in the femoral artery. A Swan-Ganz catheter was inserted through the jugular vein for measuring artery pressure.

At the end of the study, all animals were sacrificed by terminal exsanguination, followed by the harvest of organs and tissues (e.g., kidneys and hearts). Blood donors were sacrificed by application of 15 mL T61/ebutramid (MSD, Unterschleißheim, Germany) under general anesthesia.

2.3. Sample preparation

Urine was aliquoted and frozen at −20 °C. Serum samples were centrifuged at 2000 × *g* for 10 min at 4 °C, aliquoted, and frozen at −20 °C. Renal biopsies from the upper third of the left kidney were snap-frozen in liquid nitrogen or fixed in 4% formaldehyde/phosphate-buffered saline (PBS, pH 7.4) for 24 h and then transferred to 70% ethanol for histological evaluation. Investigators were blinded for the laboratory analysis.

2.4. Histological evaluation

Hematoxylin/Eosin staining of paraffin-embedded renal sections was performed as described previously [13]. Histological analysis included twenty glomeruli and ten proximal tubules per animal to determine the glomerular area, glomerular capsule space, as well as the lumen and cell size of the proximal tubules.

For immunohistochemical analyses of hypoxia-inducible factor-1α (HIF-1α), apoptosis-inducing factor (AIF), and nitrotyrosine, paraffin-embedded, 3 µm-thick sections of kidney tissue were deparaffined in xylene and hydrated in ethanol (100%, 96%, 70%, and 50%) and distilled water. Following incubation in Tris-buffered saline (TBS; 0.05 M Tris, 0.5 M NaCl) for 10 min, sections were boiled in 0.01 M sodium citrate for 30 min. Endogenous peroxidases and nonspecific binding sites were blocked with 0.1% H_2O_2 in methanol and TBS for 50 min, followed by incubation in 2% bovine serum albumin (BSA) in TBS for 60 min. Primary and secondary antibodies, as well as chromogen incubation, are listed in Supplementary Table 2. Nuclei were stained with hematoxylin. Twenty glomeruli and 10 proximal, intermediate, and distal tubules, as well as collecting ducts, were analyzed per animal. The histological evaluation was performed at 200× magnification using an Axio Plan 2 microscope (Carl Zeiss AG, Oberkochen, Germany) and AxioVision Release version 4.8.2 SP3 software, as well as ImageJ version 2.0.0 software (U.S. National Institutes of Health, Bethesda, MD).

2.5. Enzyme activity measurements

The measurement of reactive oxygen species (ROS)-producing and -degrading enzyme activities have been previously used as surrogate parameters for quantifying the renal ROS burden. The enzyme activities of the ROS-producing nicotinamide-adenine dinucleotide phosphate (NOX) and the ROS-degrading enzymes catalase and superoxide dismutase (SOD) were measured in protein extracts of renal tissue biopsies. Protein extraction and quantification, as well as assays for determining enzyme activities, are reported in detail in Supplement A.

2.6. Enzyme-linked immunosorbent assay

Troponin I and CK-MB were quantified in serum samples obtained after 120 min of reperfusion using the enzyme-linked immunosorbent assays (ELISAs) (both antibodies-online GmbH). Cytochrom C release into the cytosol was quantified in the cytosolic fraction of renal tissue homogenates using an ELISA from antibodies online GmbH. AKI markers were quantified in urine samples using the neutrophil gelatinase-associated lipocalin (NGAL; Wuhan Fine Biotech, Wuhan, China), L-type fatty acid-binding protein 1 (FABP-1; Cloud-Clone, Wuhan, China) and cystatin c (Pig Cystatin 3; Biomatik, Ontario, Canada) according to the manufacturer's instructions. The NGAL concentration in the urine after 120 min of reperfusion was the primary endpoint of this study. Measurements were performed using the microplate reader Infinite™ 200 PRO and i-control™ software (both Tecan, Männedorf, Switzerland).

2.7. Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics 28 (IBM Corporation, Armonk, NY). Values were expressed as mean and with the 95% confidence interval (CI) unless specified otherwise. The Shapiro–Wilks test was used to check the normality of the data. The Levene test was used to test for homogeneity of variance. Unpaired t-tests were performed for two-group comparisons of metric data. p Values ≤0.05 were considered statistically significant.

3. Results

3.1. Hemodynamic measurement and blood gas analyses

The study groups did not differ in hemodynamic parameters, including heart rate, systolic and diastolic blood pressure, MAP, central venous pressure, systolic and diastolic pulmonary artery pressure, systemic vascular resistance index, and heart index, during equilibration and after 120 min reperfusion (Table 1). Two animals in the HTK group required a norepinephrine administration after weaning from CPB, while none of the animals in the DelNido group required a norepinephrine administration ($p = 0.47$). Additional information about the norepinephrine infusion for an exact measurement of the left ventricle performance in this animal study was reported previously [6]. The hemoglobin content was lower in the HTK

Table 1. Hemodynamic parameters in the HTK and Del Nido group during equilibration and after 120 min reperfusion.

	Equilibration			120 min reperfusion		
	HTK	DelNido		HTK	DelNido	
	($n=9$)	($n=9$)	p Value	($n=9$)	($n=9$)	p Value
HR (bpm)	95 [81–109]	95 [81–109]	0.99	124 [108–139]	112 [102–123]	0.18
RR_{sys} (mmHg0	93 [78–109]	97 [82–113]	0.69	74 [62–86]	80 [72–88]	0.34
RR_{dias} (mmHg)	51 [41–60]	53 [45–60]	0.75	41 [39–44]	44 [41–48]	0.14
MAP (mmHg)	68 [56–80]	69 [59–80]	0.81	54 [49–60]	59 [55–64]	0.12
CVP (mmHg)	13 [9–16]	13 [13–14]	0.58	18 [16–19]	16 [15–17]	0.08
CO (L/min)	6.0 [5.1–6.9]	6.5 5.6-7.3]	0.36	5.3 [4.1–6.5]	5.4 [4.4–6.5]	0.85
SVRI (dyne*s/cm^5/m^2)	961 [746–1176]	877 [790–965]	0.42	760 [525–994]	830 [732–928]	0.53
PA_{sys} (mmHg)	29 [22–36]	30 [23–36]	0.74	41 [32–50]	43 [33–53]	0.75
PA_{dias} (mmHg)	21 [16–26]	23 [20–27]	0.42	26 [21–32]	27 [21–32]	0.96
PA_{mean} (mmHg)	25 [20–30]	27 [22–32]	0.60	34 [26–41]	34 [26–41]	0.99
CI ([L/min]/m^2)	4.7 [4.0–5.3]	5.1 [4.5–5.6]	0.30	4.1 [3.2–5.0]	4.3 [3.4–5.2]	0.77

Data are represented as mean and the 95% confidence interval in squared brackets.
bpm: beats per minute; CI: cardiac index; CO: cardiac output; CVP: central venous pressure; HR: heart rate; HTK: histidine-tryptopha n-α-ketoglutarate; MAP: mean arterial pressure; $PA_{dias/mean/sys}$: diastolic/mean/systolic pulmonary artery pressure; $RR_{dias/sys}$: diastolic/systolic blood pressure; SVRI: systemic vascular resistance index

group at aortic cross clamp due to the application of the cardioplegic solution (HTK: 3.7 mmol/L [95% CI: 3.5–4.0], DelNido: 4.2 mmol/L [95% CI: 4.0–4.5], $p<0.01$), but returned to comparable values in both groups after 90 min ischemia (HTK: 5.7 mmol/L [95% CI: 5.5–6.0], DelNido: 5.7 mmol/L [5.4–6.0], $p=0.75$) and after 120 min reperfusion (HTK: 5.3 mmol/L [95% CI: 5.0–5.6], DelNido: 5.3 mmol/L [95% CI: 5.0–5.5], $p=0.90$) (Supplementary Figure 1(A)). Blood lactate levels increased in both groups until 90 min ischemia and remained high during reperfusion (Supplementary Figure 1(B)).

Blood sodium, chloride and calcium decreased in the HTK group at the time point of aortic cross clamp after application of the cardioplegic solution compared to the DelNido group ($p_{sodium} < 0.01$, $p_{chloride} < 0.01$, $p_{calcium} < 0.01$) (Figure 2(A–D); Supplementary Table 3). The difference between the groups persisted until the end of the ischemic period for calcium ($p=0.03$) and until the end of the 120 min reperfusion period for sodium ($p<0.01$) and chloride ($p=0.04$). Blood potassium levels were comparable between the study groups throughout the cardiac arrest and the reperfusion period (Figure 2(D)).

3.2. Measurement of cardiac injury

The cardiac injury markers troponin I (HTK: 422 pg/mL [95% CI: 83–761], DelNido: 362 pg/mL [95% CI: 148–576], $p=0.36$) and CK-MB (HTK: 165 mU/mL [95% CI: 12–318], DelNido: 125 mU/mL [95% CI: −25-275], $p=0.34$) were comparable between the HTK and the DelNido group after 120 min reperfusion, indicating a comparable cardiac protection with the different cardioplegic solutions.

3.3. Histomorphological analysis of renal tissue

Histomorphological analysis of kidney structures comprised measurements of proximal tubules, glomeruli, and Bowman's capsules. Glomeruli area (HTK: 37,523 µm^2 [95% CI: 34,532–40,514], DelNido: 40,282 µm^2 [95% CI: 34,660–45,904], $p=0.34$), glomerular capsule space length (HTK: 11.6 µm [95% CI: 9.4–13.9], DelNido: 13.4 µm [95% CI: 10.2–16.5], $p=0.31$), proximal tubules cell height (HTK: 21.9 µm [95% CI: 20.7–23.1], DelNido: 22.7 µm [95% CI: 21.4–24.0], $p=0.34$) and diameters of proximal tubules (HTK: 102.9 µm [95% CI: 95.9–110.0], DelNido: 104.3 µm [95% CI: 94.9–113.6], $p=0.80$) were comparable between both groups (Figure 3).

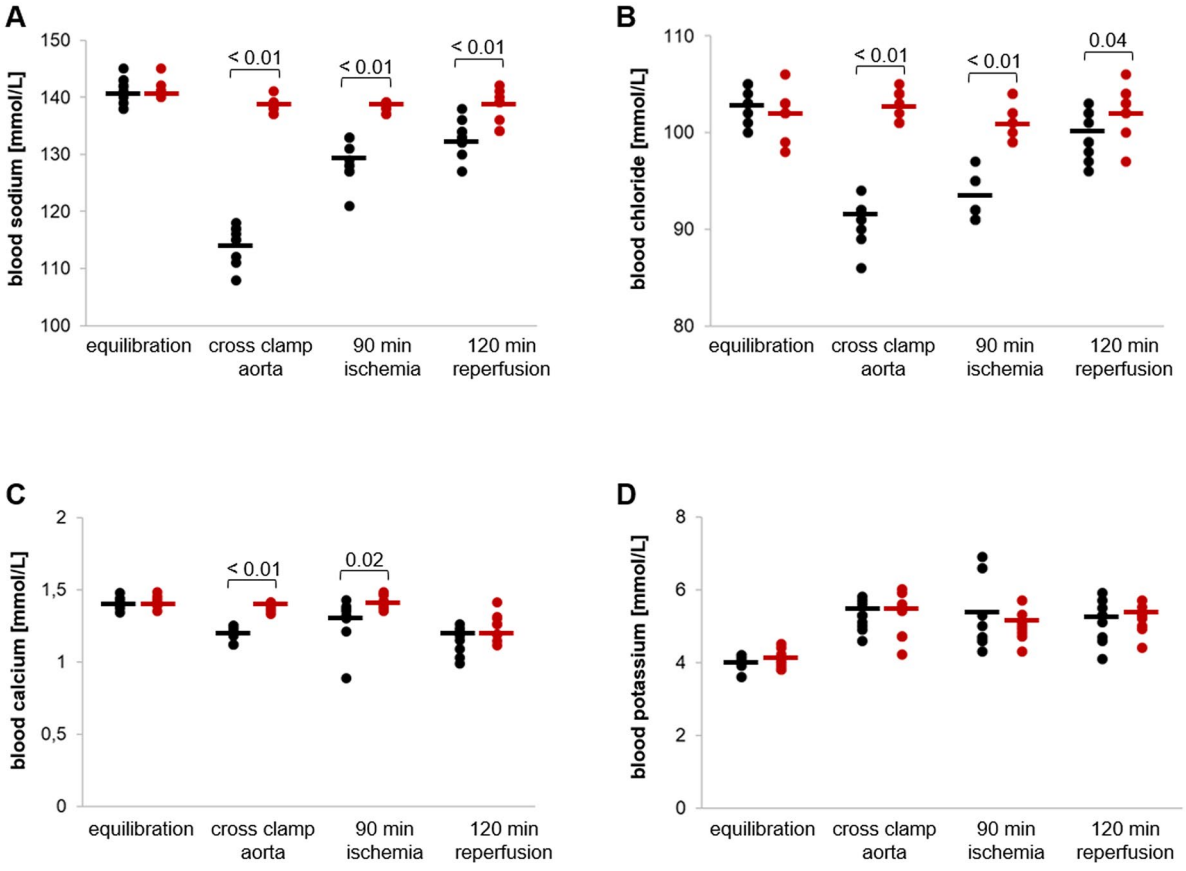

Figure 2. Blood parameters measured during equilibration, at aortic cross clamp, after 90 min ischemia and after 120 min of reperfusion. Blood concentration of sodium (a), chloride (B), calcium (C) and potassium (D) have been quantified in the HTK group (black color) and the DelNido group (red color). The mean is displayed as a line in the dot plot. HTK: histidine-tryptophan-α-ketoglutarate.

3.4. Oxidative and nitrosative stress in renal tissue

Oxidative stress due to ischemic events or altered oxygen conditions was quantified by measuring of enzyme activities of NOX, SOD, and catalase. The investigated enzyme activities serve as surrogate parameters for oxidative stress, while NOX is a ROS producer, and SOD and catalase are enzymes that degrade ROS. In renal tissue, the enzyme activities of NOX (HTK: 15.2 mU/mg [95% CI: 5.9–24.4], DelNido: 11.8 mU/mg [95% CI: 3.8–19.7], $p = 0.53$), SOD (HTK: 0.73 U/mg [95% CI: −0.25-1.71], DelNido: 0.35 U/mg [95% CI: 0.22–0.48], $p = 0.39$) and catalase (HTK: −0.09 U/mg [95% CI: −6.63–6.45], DelNido: 5.30 U/mg [95% CI: −2.82–13.41], $p = 0.25$) were comparable between the HTK and DelNido group.

A further marker of oxidative stress is the nuclear translocation of the transcription factor HIF-1α, which corresponds to its activation. HIF-1α translocation in the renal structures: glomeruli ($p = 0.76$), proximal tubules ($p = 0.92$), intermediate tubules ($p = 0.62$), distal tubules ($p = 0.45$) and collecting ducts ($p = 0.67$) was comparable between the HTK and DelNido group (Figure 4(A–C); Supplementary Table 4). The highest degree of nuclear HIF-1α translocation was detected in the proximal tubules (HTK: 33.0% [95% CI: 10.1–55.8], DelNido: 31.7% [95% CI: 12.7–50.7]).

Nitrosative stress was quantified by analyzing the expression of nitrotyrosine in cells of different renal structures. Nitrotyrosine expression was present in more than 90% of the renal cells. It was comparable between the HTK and DelNido groups in the different renal structures (glomeruli: $p = 0.26$, proximal tubules: $p = 0.12$, intermediate tubules: $p = 0.54$, distal tubules: $p = 0.74$, collecting ducts: $p = 0.78$) (Figure 4(D–F); Supplementary Table 4).

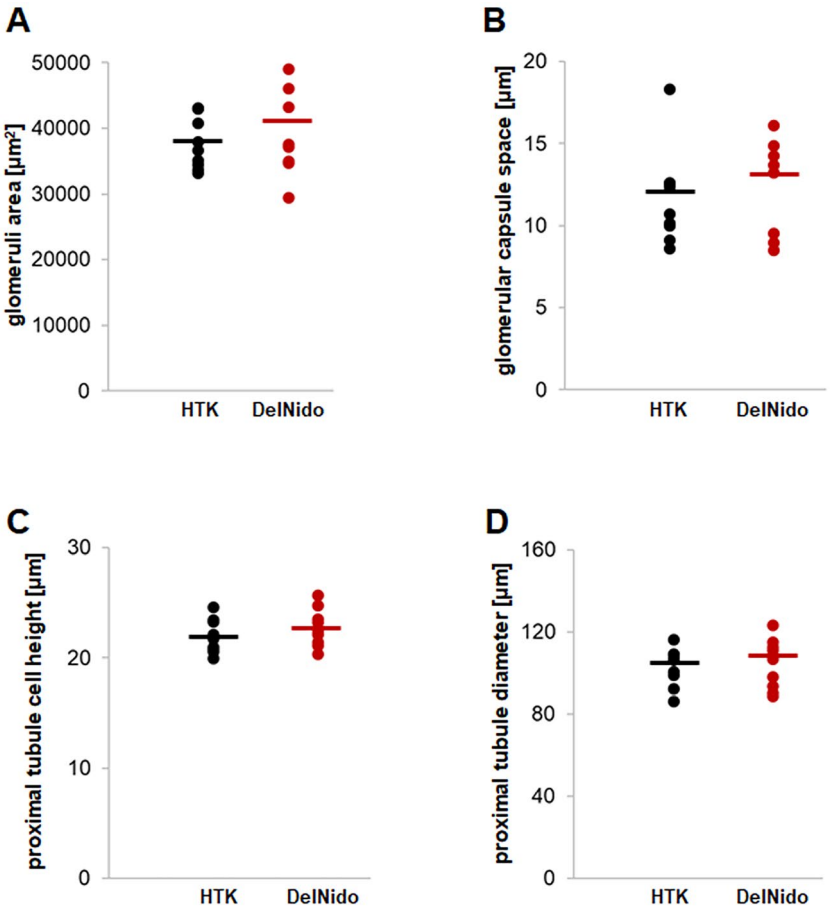

Figure 3. Histomorphological evaluation of renal tissue after cardioplegia with HTK (black color) or DelNido (red color). Hematoxylin and eosin staining was used to evaluate the morphological changes of the glomeruli, including their area (A) and the glomerular capsule space (B), as well as of proximal tubules, including the proximal tubule cell height (C) and the proximal tubule diameter (D). The mean is displayed as a line in the dot plot. HTK: histidine-tryptopha n-α-ketoglutarate.

3.5. Acute kidney injury markers

The early kidney injury biomarkers NGAL, FABP-1 and cystatin c were quantified in urine samples obtained at equilibration and after 120 min reperfusion. According to biomarker analysis, kidney injury in the HTK and DelNido groups was comparable at equilibration and after 120 min of reperfusion (Table 2, Figure 5).

3.6. AIF translocation and cytochrome c release

Increased oxidative stress and cellular damage can lead to the induction of caspase-independent apoptosis through the release of mitochondrial cytochrome C, as well as the release and activation of AIF. The nuclear translocation of AIF and the release of cytochrome C into the cytosol were used as surrogate markers for the induction of caspase-independent apoptosis. AIF translocation in the renal structures: glomeruli ($p = 0.26$), proximal tubules ($p = 0.29$), intermediate tubules ($p = 0.38$), distal tubules ($p = 0.51$), and collecting ducts ($p = 0.33$) was comparable between the HTK and DelNido group (Supplementary Table 4).

The analysis of cytochrome C release from mitochondria into the cytosol yielded comparable results, with no detectable difference between the HTK and DelNido groups (HTK: 6.3 ng/mg [95% CI: 4.8–7.8], DelNido: 6.0 ng/mg [95% CI: 3.8–8.1], $p = 0.79$).

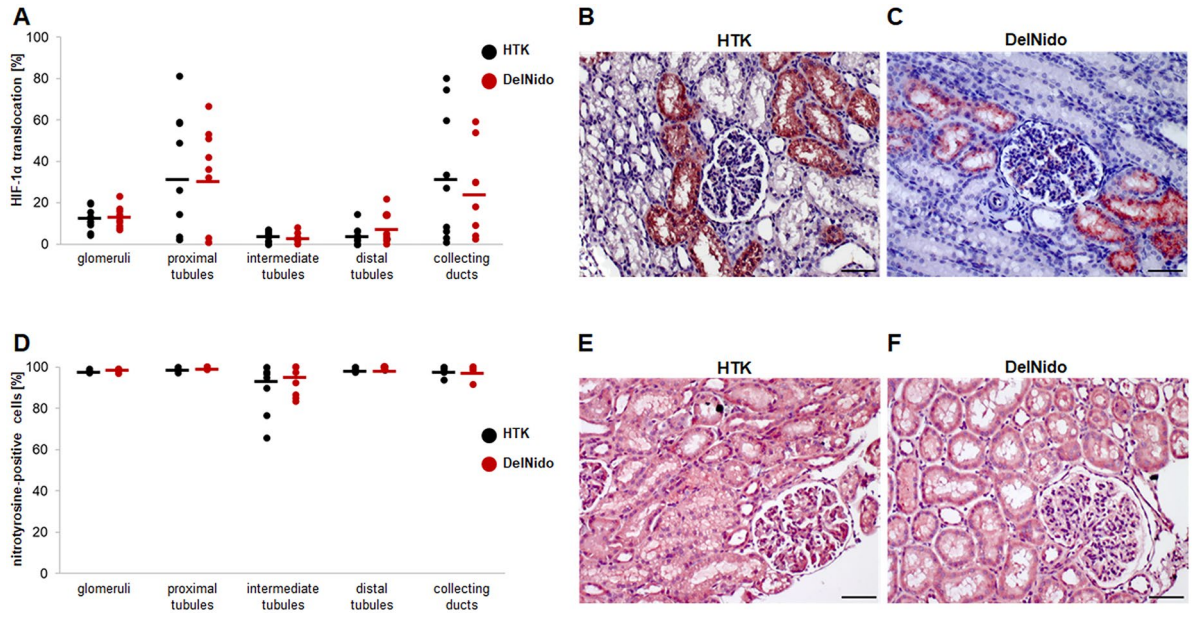

Figure 4. Immunohistochemical analysis of HIF-1α nuclear translocation (A–C) and nitrotyrosine expression (D–F) in renal tissue after cardioplegia with HTK (black color) or DelNido (red color). HIF-1α translocation (A) and nitrotyrosine expression (D) were quantified in different renal structures. The mean is displayed as a line in the dot plot. Exemplary stainings of HIF-1α (B, C) and nitrotyrosine (E,F) of HTK- and DelNido-treated pigs were presented. scale bar = 100 µm. HIF-1α: hypoxia-inducible factor 1α; HTK: histidine-tryptophan-α-ketoglutarate.

Table 2. Urinary concentrations of acute kidney injury markers in the HTK and DelNido group during equilibration and after 120 min reperfusion.

	Equilibration			120 min reperfusion		
	HTK	DelNido		HTK	DelNido	
	(*n* = 9)	(*n* = 9)	p value	(*n* = 9)	(*n* = 9)	*p* Value
FABP-1 (pg/mL)	160 [160-160]	160 [156–164]	1	146 [103–188]	162 [159–166]	0.38
NGAL (ng/mL)	0.22 [−0.01-0.44]	0.07 [−0.04-0.18]	0.20	0.37 [−0.20-0.94]	0.26 [0.04–0.48]	0.34
Cystatin C (ng/mL)	9.4 [−12.2-31.0]	48.8 [1.5–96.1]	0.11	136.2 [−72.9-345.3]	60.7 [−29.6-151.0]	0.46

Data were represented as mean and the 95% confidence interval in squared brackets.
FABP-1: *L*-type fatty acid-binding protein 1; HTK: histidine-tryptophan-α-ketoglutarate; NGAL: neutrophil gelatinase-associated lipocalin

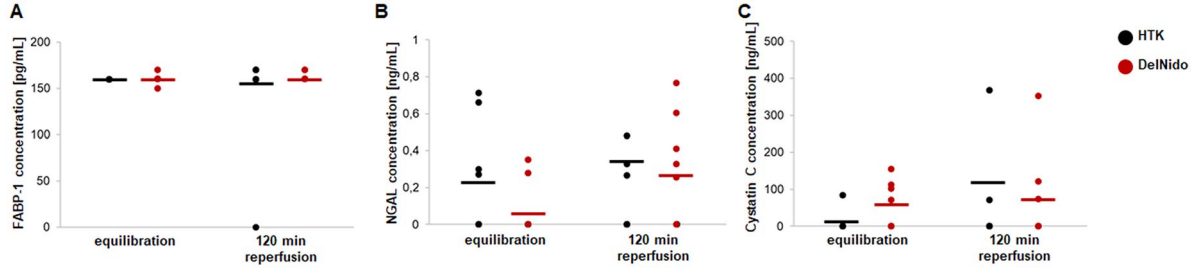

Figure 5. Urinary concentration of early acute kidney injury markers FAPB-1 (a), NGAL (B) and cystatin C (C) after cardioplegia with HTK (black color) or DelNido (red color). The mean is displayed as a line in the dot plot. FABP-1: *L*-type fatty acid-binding protein 1; HTK: histidine-tryptophan-α-ketoglutarate; NGAL: neutrophil gelatinase-associated lipocalin.

4. Discussion

This study compared the renal effects of the crystalloid HTK cardioplegic solution and the Jonosteril®-based DelNido blood cardioplegic solution in a porcine animal model with 90 min cardiac arrest and a 120-min reperfusion period.

In our animal model, DelNido cardioplegia induced fewer electrolyte and hemoglobin imbalances than HTK cardioplegia. Histomorphological changes, oxidative and nitrosative stress, as well as the cytosolic release of pro-apoptotic molecules in the various nephron structures, were comparable between HTK and DelNido cardioplegia. The comparability of both cardioplegic solutions regarding kidney injury in a 90-min cardiac arrest model was supported by measurements of the AKI biomarkers FABP-1, NGAL and cystatin C, which revealed no differences between the groups.

The study results demonstrate a significantly greater decrease in hemoglobin levels in the HTK group compared to the DelNido cardioplegia group. Hemoglobin is a tetrameric protein that binds oxygen and serves as an indirect marker of erythrocyte count. A decrease of hemoglobin in both study groups of our experimental setting could be caused by intraoperative blood loss, CPB-induced hemolysis, or hemodilution during cardioplegia, as reported in previous studies investigating HTK or DelNido cardioplegia [14,15]. Our study design included compensating for intraoperative blood loss with porcine red cell concentrates to mitigate the adverse effects of low hematocrit and hemoglobin concentrations, which are known to reduce AKI in CPB surgery [16–18]. A reduced hemoglobin content in combination with increased lactate has been correlated with the occurence of AKI [19], which explains the risk for AKI in surgical interventions with ischemia–reperfusion issues.

Blood electrolyte measurements revealed hyponatremia and hypochloremia as well as low calcium concentrations as a result of cardioplegia in the HTK group but not in the DelNido group. Electrolyte imbalances, such as hyponatremia, are common following the application of cardioplegic solutions [20]. Hypochloremic conditions with blood chloride concentrations <95 mmol/L are a risk factor for AKI development [21] and have been documented in the HTK group of our study. However, hypoosmolarity is necessary to induce cellular swelling and damage. The hyperosmolar HTK solution should counteract hypoosmolar conditions and lead to an isotonic hyponatremia in our experimental setting.

The HTK solution contains the amino acids histidine and tryptophan, as well as α-ketoglutarate, which is involved in the synthesis of amino acids. The nephroprotective effects of amino acids have been reported by Landoni et al. [22], who investigated the use of amino acid infusion in patients undergoing cardiac surgery in a randomized controlled trial. They reported a lower incidence of AKI after amino acid infusion, with no effect on adverse events. Landoni et al. used Baxter's 10% Isopuramin, which contains about 14 amino acids, including histidine and tryptophan. Therefore, it might be assumed that the composition of the HTK solution is beneficial for nephroprotection in cardiac surgery.

Histomorphological analysis of AKI-induced tubular cell injury and glomerular damage did not reveal differences between the study groups. These findings are supported by the AKI marker measurements in the urine, which show comparable levels of FABP-1, NGAL, and cystatin C for HTK and DelNido cardioplegia. NGAL is more sensitive in detecting renal damage than the proximal tubule-specific injury marker FABP-1 [23]. Cystatin C is a marker for kidney damage and renal inflammation, and it has the potential to serve as a surrogate marker of glomerular filtration rate [24]. All three AKI markers are suitable for detecting renal damage, if present, in the 120-min reperfusion period. Urine NGAL and FABP-1 concentrations peak 2–6 h postoperatively [25–28]. Cystatin C has been reported to be the most suitable predictor of grade I AKI according to the AKI network (AKIN) criteria in the early postoperative period [29].

Changes in oxygen conditions resulting from hemodilution or CPB-induced alterations in hemodynamic parameters can lead to a cellular reaction and the activation of molecules that, in turn, induce cell-protective and anti-apoptotic pathways. One of the most sensitive molecules sensing changes in oxygen conditions is the subunit *α* of the HIF-1 molecule. The nuclear translocation of HIF-1α in different nephron structures revealed a strong but comparable response to changed oxygen conditions in the proximal tubules. Distal tubules and collecting ducts exhibited a lower rate of HIF-1α translocation, followed by the intermediate tubules and glomeruli, and there was no difference between the groups.

Oxidative stress is one of the crucial drivers of AKI development following cardiac surgery and is induced by the accumulation of cytotoxic ROS [30]. Surrogate markers for cellular ROS content include the enzyme activities of the ROS-producing enzyme NOX and the ROS-degrading enzymes SOD and catalase. The ROS burden, measured by NOX, SOD, and catalase activities, was comparable between HTK and DelNido cardioplegia.

In addition to oxidative stress, nitrosative stress could play a role in the pathogenesis of AKI. Therefore, we detected nitrosative stress by quantification of nitrotyrosine [31]. Nitrotyrosine expression was comparable between HTK and DelNido cardioplegia in renal tissue; however, the presence of nitrotyrosine in more than 90% of the cells in the different nephron structures indicates an increased level of intervention-associated nitrosative stress.

Both oxidative and nitrosative stress could lead to apoptosis *via* the caspase-independent apoptosis pathway. An indicator of apoptosis induction is the cytosolic release of cytochrome C and AIF from the mitochondria, as well as the translocation of AIF into the nucleus. We found comparable levels of cytochrome C release into the cytosol and nuclear translocation of AIF in renal tissue after HTK and DelNido cardioplegia. Nuclear AIF translocation, especially in the proximal tubules, was high, indicating an increased risk of renal tissue damage through apoptosis.

Our study has several limitations. The study results are based on investigations in 18 animals. The number of animals per group was calculated using statistical methods and was strictly controlled and monitored by the animal welfare agency for ethical reasons. The study was conducted with female pigs, which could introduce a potential sex bias. Predictive risk models identified female sex as a risk factor for cardiac-surgery-associated AKI [32], indicating that physiological differences may influence susceptibility to kidney injury and response to cardioplegia.

We included young and healthy pigs, which differ from the multimorbid patients that usually undergo cardiac surgery with a 90-min cardiac arrest. The use of diseased animal models or aged animals should be addressed in the future to enhance the translation of the results. Further, a 120-min reperfusion period limits the number of significant markers that can be detected, making it challenging to investigate structural changes in renal tissue or kidney injury. However, previous investigations have shown that reperfusion lasting more than 120 min resulted in hemodynamic instability during anesthesia of the pigs. An alternative would be a chronic experimental design with a follow-up of 3–5 d or a more extended period of 4 weeks. Long-term assessment of renal injury and recovery would be an essential aspect of a comprehensive evaluation of the effects induced by the HTK and DelNido cardioplegic solutions. However, a mid- or long-term setting bears the risk of additional influencing factors that could bias the results and reduce the validity of this study.

Blinding of the surgical and veterinary teams was impossible due to the composition of the CPB apparatus and the surgical setting (e.g., necessary vents differed between the both groups). However, blinded investigators performed laboratory and statistical analyses.

We employed several surrogate parameters to assess molecular changes in renal tissue. For example, we have concluded oxidative stress level by measuring the activities of ROS-producing and ROS-degrading enzymes, which increase with higher ROS burden.

The DelNido cardioplegic solution has shorter application times due to the lower volume, and induces less hemodilution than the HTK cardioplegic solution. The observed superiority of the DelNido cardioplegia, in terms of more stable hemoglobin and electrolyte concentrations, appeared to have no measurable effect on the renal outcomes. Hemoglobin is recognized as a significant factor in kidney injury. For example, perioperative hemoglobin content is a risk factor for AKI [33], and free hemoglobin triggers oxidative stress, promoting AKI in cardiac surgery patients [34]. Nevertheless, the detected differences between the DelNido and HTK solution did not result in measurable differences of AKI biomarkers, histopathological changes, oxidative stress, and apoptosis markers within 120 min after ischemia. This finding is supported by a clinical study comparing 197 HTK- and 158 DelNido-treated cardiac surgery patients, which could not detect a difference in the postoperative renal insufficiency grade during a mean hospital stay of 12 d [6].

Our results confirm the findings of clinical studies comparing HTK and DelNido cardioplegia, which have demonstrated comparability between the two solutions regarding renal protection, even with up to 90-min cross-clamp times [6,35]. The meta-analysis of Fresilli et al. [4] reported a lower risk for postoperative AKI with DelNido cardioplegia, but does not contradict our findings because the underlying clinical trials compared DelNido with warm blood, cold blood, or St. Thomas cardioplegic solutions.

The present large animal study demonstrated the superiority of the DelNido solution regarding hemoglobin and blood electrolyte concentrations, but comparability of the crystalloid HTK solution and the Jonosteril®-based DelNido blood cardioplegic solution regarding their renal effects for surgical interventions requiring cardiac arrest up to 90 min. Patients with a higher risk for adverse events, due to either

complex, prolonged surgery or a multitude of comorbidities, could especially benefit from the more physiological arrest conditions with DelNido cardioplegia. Future studies should investigate longer perfusion periods of approximately 120 min and utilize comorbid or aged animal models to enhance the translation of the results.

Author contributions

CRediT: **Maja-Theresa Dieterlen**: Conceptualization, Formal analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing; **Jagdip Kang**: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing; **Paul Schütte**: Data curation, Formal analysis, Methodology, Writing – original draft; **Kristin Klaeske**: Formal analysis, Methodology, Writing – review & editing; **Susann Oßmann**: Conceptualization, Data curation, Methodology, Project administration, Writing – original draft, Writing – review & editing; **Philipp Kiefer**: Conceptualization, Formal analysis, Methodology, Writing – review & editing; **Marcel Vollroth**: Formal analysis, Methodology, Writing – review & editing; **Michael A. Borger**: Conceptualization, Formal analysis, Project administration, Writing – review & editing; **Alexandro Hoyer**: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing.

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Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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