Am J Transl Res 2020;12(1):153-161
www.ajtr.org /ISSN:1943-8141/AJTR0096545

Original Article

Use of modified Custodiol-N as perfusion solution in ex vivo lung perfusion

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Received May 6, 2019; Accepted July 26, 2019; Epub January 15, 2020; Published January 30, 2020

Abstract: Objectives: Ex vivo Lung Perfusion (EVLP) is a promising tool to increase the donor pool for lung transplantation. Custodiol-N solution was originally designed for organ preservation during cold static preservation (CSP) and was successfully used for machine perfusion in kidneys. It was the aim of this study to compare the lung functional outcomes after 4 hours of EVLP using modified Custodiol-N or STEEN Solution™ as perfusion solution. Methods: In a porcine DCD model, lungs were perfused either with STEEN Solution™ (Standard SS, n=8) or modified Custodiol-N with added 1.1 g/l glucose monohydrate and 50 g/l dextran 40 (CD, n=8). For a third group 7 g/l albumin was supplemented to modified Custodiol-N (CDA, n=8). During four hours of EVLP pulmonary gas exchange and activities of lactate dehydrogenase (LDH) and alkaline phosphatase (AP) in perfusate were recorded. Results: Lungs that underwent EVLP with modified Custodiol-N showed significantly higher oxygen capacity (ΔpO_2 averaged over four hours of EVLP: SS: 236.28 ± 47.26 mmHg, CD: 402.79 ± 30.33 mmHg, CDA: 414.86 ± 9.77 mmHg) than lungs perfused with STEEN Solution™. The addition of albumin did not have a significant effect on lung function but these lungs showed lower wet/dry ratio. Conclusion: In a porcine DCD model of 9 hours CSP followed by four hours of EVLP the use of modified Custodiol-N as perfusion solution was feasible and associated with higher oxygen capacity than STEEN Solution™. The addition of albumin seems to further stabilize lung function.

Keywords: Marginal donor lungs, lung transplantation, organ preservation, ex-vivo lung perfusion, Custodiol-N

Introduction

Lung transplantation (LuTx) remains the therapy of choice for patients suffering from terminal pulmonary disease [1]. However, the number of LuTx is limited by the lack of donor lungs. Ex vivo Lung Perfusion (EVLP) technology could increase the lung donor pool by evaluation and eventual reconditioning of extended criteria donor lungs [2-7]. The successful use of EVLP is influenced by the composition of the perfusion solution especially by osmotic pressure and antioxidative properties [8-10]. Established EVLP protocols (Lund and Toronto) use STEEN Solution™ (XVIVO Perfusion, Goteborg, Sweden) for lung perfusion [2, 3] which is essentially composed as a modified low-potassium dextran glucose solution (Perfadex™) with 70 g/l human albumin (HA) as the major additional constituent [3]. The third available EVLP protocol, the Organ Care System™, uses the cellular OCS™ Solution (Transmedics, Andover, MA, USA), a low-potassium dextran 40-based solution without additional HA [3]. Recent studies have already shown that in Steen Solution™ a concentration of 7 g/l HA has a noticeable positive effect of cold stored organs [8]. As the EVLP procedure is a typical model of postischemic reperfusion, the effects of the perfusion solution on lung-ischemia-reperfusion-injury (LIRI) are important for its success. During reperfusion, as well as under ischemic hypothermic conditions, reactive oxygen species (ROS) formation is probably responsible for lung injury [8, 10-13]. Although the mechanisms of ROS induced LIRI are still under investigation, it is known that iron-dependent cell damage represents the main pathway of cold-induced tissue injury during reperfusion [8, 10-16]. Custodiol-N was originally designed by Rauen and de Groot

Table 1. Composition of perfusion solutions

	CD (Custodiol-N plus dextran)	CDA (Custodiol-N plus dextran plus albumin)	Steen™
Sodium	16	16	86
Potassium	10	10	4.6
Magnesium	8	8	0.8
Calcium	0.02	0.02	1.5
Chloride	30.04	30.04	
Histidine	124	124	
N-Acetylhistidine	57	57	
Sucrose	33	33	
α-Ketogluterate	2	2	
Aspartate	5	5	
Glycine	10	10	
Alanine	5	5	
Tryptophan	2	2	
Arginine	3	3	
Deferoxamine (μmol/l)	15.3	15.3	
LK 614 (μmol/l)	6.2	6.2	
Dextran 40 (g/l)	50	50	5
Albumin (g/l)		7	70
Glucose			11
Phosphate			1.2
pH	7.0	7.0	7.4*
Osmolarity (mosm/l)	306	306	

*Adjust to pH 7.4 with sodium hydroxide.

as improved modification of the former HTK solution including the iron chelators LK 614 and Desferoxamine [13, 14]. It has already been used for organ preservation or hypothermic machine perfusion with promising results [14, 17-20]. Recent studies in experimental lung preservation have also shown that an antioxidative effect of STEEN Solution™ is important for EVLP's success [8, 10]. It was the aim of this study to compare lung functional outcomes during four hours of EVLP using standard STEEN Solution™ and modified Custodiol-N as a perfusion solution. According to the electrolyte composition STEEN is an extracellular perfusion solution, whereas Custodiol-N is of intracellular type. Furthermore, we investigated the effect of the addition of 7 g/l bovine albumin in Custodiol-N used as perfusion solution.

Materials and methods

Animals

The University of Duisburg-Essen's central animal laboratory supervised all aspects of the present study. All animals received human care in compliance with the "Principles of Laboratory and Animal Care" and the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NH Publication No. 86-23, revised 1996). Because none of the animals underwent medical treatment prior to euthanasia, the present study is an organ procurement only, which was reported to the local authorities (Landesamt für Natur, Umwelt und Verbraucherschutz NRW) according to applicable law (§ 1 VTMVO).

Chemicals

Perfadex™ and STEEN Solution™ were acquired from XVIVO Perfusion (Gothenburg, Sweden). Custodiol-N was provided by Dr. F. Köhler Chemie (Bensheim, Germany). For the modified Custodiol-N solution pyrogen-free dextran 40, molecular weight approximately 40.000 g/mol (CAS No. 9004-54-0; AppliChem, Darmstadt, Germany), was added. In the CDA-group also bovine serum albumin, molecular weight approximately 66,000 g/mol (CAS No. 90604-29-8; Carl Roth, Karlsruhe, Germany), was supplemented. To see the composition of the used solutions, please refer to **Table 1**. Subsequently, the solution was sterilized by filtration through a 0.22-μm filter (Filtropur BT25, Sarstedt, and Nümbrecht, Germany). Immediately for use 10 ml of 10% glucose (G-10, B.Braun, Melsungen, Germany) was added to 1 liter of CD and CDA solution.

Experimental groups, surgical process and porcine EVLP

24 mature domestic male hybrid pigs (weight 35 ± 5 kg) underwent sedation with ketamine (30 mg/kg, i.m.) and azaperon (0.05 mg/kg, i.m.) until they tolerated manipulation on the ear and were then anesthetized with midazolam (0.1 mg/kg, i.v.) and ketamin (30 mg/kg,

i.v.). In deep anesthesia the animals were euthanized with potassium chloride (14.9%/kg, i.v.). None of the pigs were ventilated during anesthesia or received any medication like heparin in advanced of the euthanasia. After 5 minutes, cardiac arrest death was certified and sternotomy performed. After 45 minutes, warm ischemia lungs were harvested using a standard operative technique as previously described [2, 3, 7] and then randomized into the three experimental groups. Trometamol (10.9 g) and heparin (100 IU) were added to 4 liters of 4°C cold Perfadex™ to flush the lungs antegrade and retrograde starting at one hour warm ischemia. In all three groups, lungs were stored in one liter of low potassium dextran solution (LPD, Perfadex™) at 4°C in a standard preservation bag for nine hours. Temperature of 4°C was monitored hourly. All lungs were perfused and stored in LPD solution to eliminate the influence of different preservation solutions. The subsequent EVLP was performed according to the Toronto Protocol using the XVIVO perfusion system XPS™ (XVIVO Perfusion, Gothenburg, Sweden) [2, 3, 7]. In the standard group EVLP was performed with gold standard STEEN Solution™ (SS, n=8), while two groups used modified Custodiol-N for perfusion, without albumin (CD, n=8) and with supplemented 7 g/l of bovine albumin (CDA, n=8). The ventilation strategy was adjusted to weight according to the Toronto Protocol.

Monitoring and measurements

Lung function: The difference between pulmonary arterial and venous oxygen pressure (ΔpO_2) and the lactate concentration was measured by blood gas analysis of the perfusate hourly during EVLP (ABL 700, Radiometer, Copenhagen, Denmark) at an FiO_2 of 1.0. The peak airway pressure (Paw_{peak}), pulmonary artery pressure (PAP), pulmonary vascular resistance (PVR) and the dynamic compliance (C_{dyn}) were measured continuously by XVIVO PGM Disposable Sensors™ (XVIVO Perfusion, Gothenburg, Sweden) and recorded hourly.

Lactate dehydrogenase: As a marker of cell damage we measured the extracellular lactate dehydrogenase (LDH) photometrically by a standard assay using a clinical chemistry analyzer (VITALAB Selectra E, Vital Scientific NV, Dieren, NL) [11-14]. These values were corrected by subtraction of the LDH activity released by destroyed erythrocytes, which were identified by released hemoglobin by uv/vis spectral analysis (CLARIOstar, BMG LABTECH, Ortenberg, Germany). For the latter, a calibration curve was obtained by lysing pig erythrocytes with Triton-X-100 (1%).

Alkaline phosphatase: As a marker of pneumocyte type II injury the alkaline phosphatase (AP) activity in the perfusate was measured by the common reference method (zAP, ADVIA Clinical Chemistry, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany) [21, 22].

Wet/dry-ratio: Wet/dry-ratio was used to capture the water content of the lung tissue. A sample of about 3 cm^3, taken from the left lower lobe, was weighed immediately after removal for wet weight determination and then dried at 65°C. For 72 hours before it was weighed again. The wet/dry-ratio is the quotient of the two values.

Statistical analysis

Comparisons between the groups were made hourly during the EVLP and at the end of the EVLP (wet/dry-ratio). Outcomes were compared using the students t-test for normally distributed values (validated by the Kolmogorov-Smirnov-test) while the Mann-Whitney-U-test was used for non-normally distributed values. Moreover, variance analysis with repeated measurements was used to evaluate the differences between the groups concerning the functional outcomes. All results are expressed as mean ± standard deviation, and differences were considered significant at the level of $P < 0.05$ (SPSS Statistics 22, IBM, Armonk, New York, US).

Results

General macroscopic consideration

When modified Custodiol-N was used as perfusion solution, lungs showed a typically spongy consistency and looked well ventilated after four hours of EVLP (**Figure 1**). Comparing the standard group with the both groups using modified Custodiol-N, intratracheal fluid and partially big liquid-filled bubbles of pleural excrescence could be detected in four of eight standard perfused lungs, while it appears only

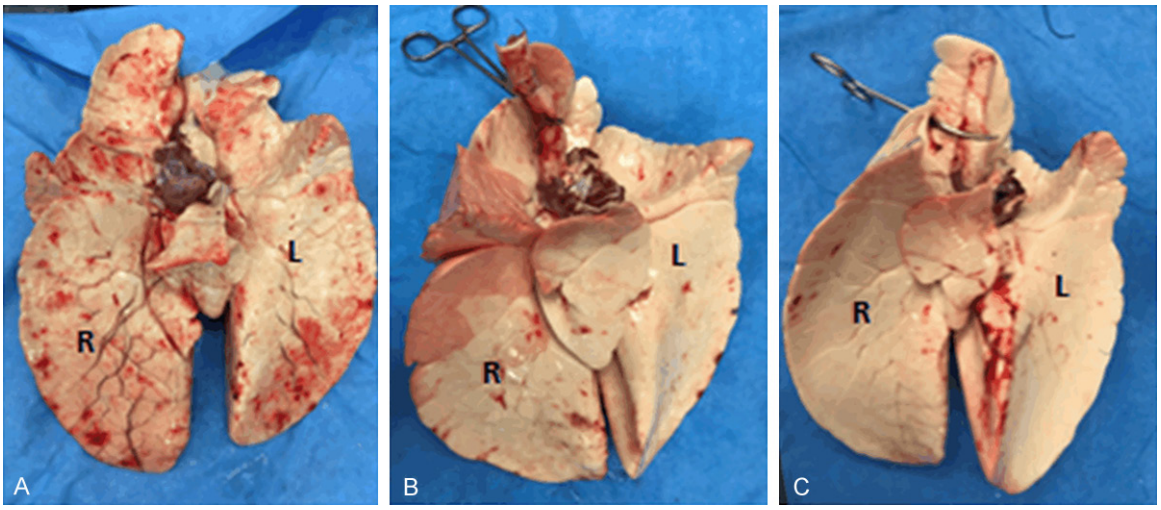

Figure 1. Macroscopic appearance of retrieved pig lung with right (R) and left (L) lower lobe with improved gross appearance, although reddening of lobe tips and alterations like circumscribed interlobular edema formation could regularly be detected. Especially the lung perfused with the standard STEEN Solution™ (SS, A) showed macroscopically detectable edema, while the CD (B) and the CDA (C) lungs looked well ventilated.

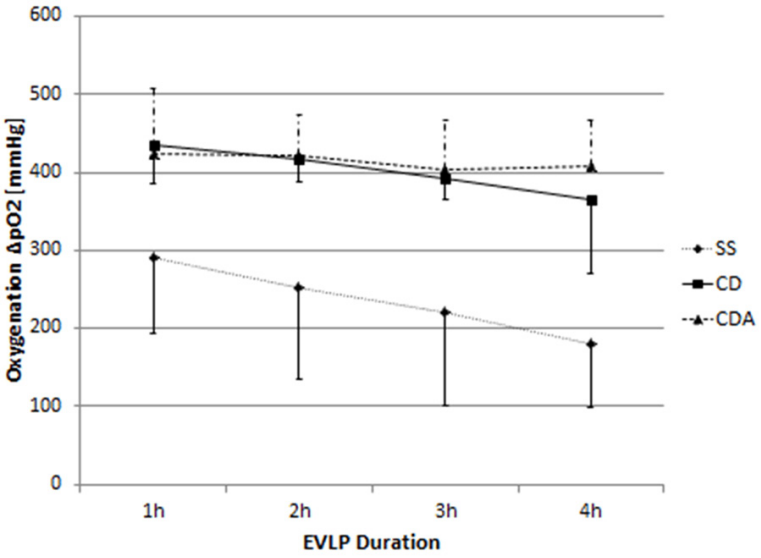

Figure 2. Oxygenation capacity during 4 hours of EVLP. Results are expressed as arithmetic mean ± SD. Only one side of the error bar is marked to increase the clarity of the diagram. The values decreased over the period of EVLP with significant higher values in the CD and CDA group compared with the SS group ($P < .001$). There are no significant differences between both groups using Custodiol-N for perfusion (CD, CDA) but a trend towards higher values in the fourth hour of EVLP in the CDA group.

in one of the Custodiol-N perfused lungs (CDA-group). Especially the left lower lobe showed a high grade of damage with edema and atelectasis as well as discoloration and haemorrhages immediately after lung retrieval. There were no macroscopically detectable changes after nine hours of cold storage. Intravascular clot formation was hardly detected.

Lung function

Lungs of both modified Custodiol-N groups achieved significant higher values of ΔpO_2 (pulmonary venous-pulmonary arterial pO_2) compared with the standard perfused lungs: mean values for all four measurements per group; standard: 236.28 ± 47.26 mmHg; CD: 402.79 ± 30.33 mmHg; CDA: 414.85 ± 9.77 mmHg ($P < .001$). Regardless of the perfusion solution, the course of ΔpO_2 slightly decreased over four hours of EVLP (**Figure 2**). The two Custodiol-N groups did not differ significantly. However, values in the CDA group remained higher after three hours of EVLP.

Peak airway pressures (PAW_{peak}) and pulmonary vascular resistance (PVR) were lower in CD and CDA than in the standard group (**Figure 3**). In all groups the PAW_{peak} remained stable during 4 hours of EVLP but with significantly lower values in the CD and CDA group compared with the standard group (standard: 32 ± 0.7

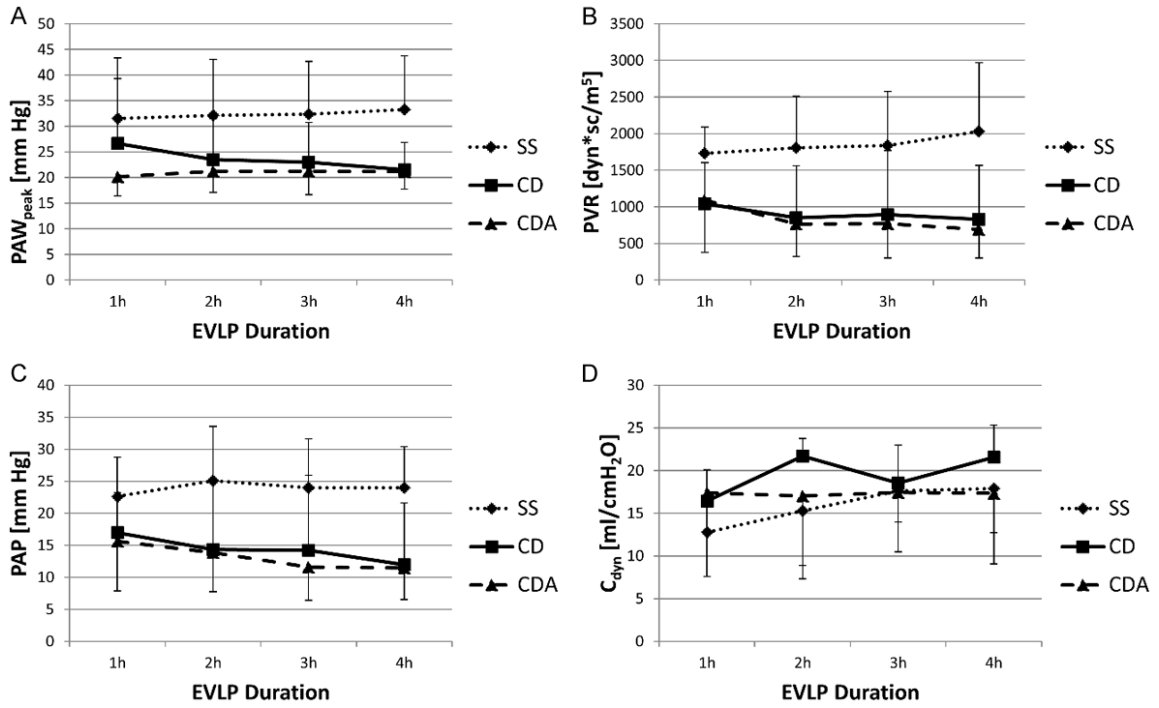

Figure 3. Pulmonary Airway Pressure (A, PAW_{peak}), Pulmonary Vascular Resistance (B, PVR), Pulmonary Artery Pressure (C, PAP) and Dynamic Compliance (D, C_{dyn}) during 4 hours of EVLP. Results are expressed as arithmetic mean ± SD. Only one side of the error bar is marked to increase the clarity of the diagram. The PAW_{peak}, PVR and PAP stay on a constant level in all three groups, with significantly higher values of PVR and PAP in the SS group ($P < .001$). The C_{dyn} showed constant values in the CDA-group, while it increased in the SS group and showed a fluctuating course in the CD-group without any significant differences, but demonstrated a trend towards lower values in the SS group. The two Custodiol-N groups did not differ significantly for PAW_{peak}, PVR, PAP and C_{dyn}.

mmHg; CD 24 ± 2 mmHg; CDA 21 ± 0.5 mmHg, **Figure 3**, a $P < .001$). PVR decreased over time by 21% in the CD group (hour 1: 1044 ± 560 dyn*s/cm^5; hour 4: 829 ± 739 dyn*s/cm^5) and 37% in the CDA group (hour 1: 1093 ± 719 dyn*s/cm^5; hour 4: 693 ± 393 dyn*s/cm^5), while it increased in the standard group by 17% from 1731 ± 358 dyn*s/cm^5 to 2029 ± 943 dyn*s/cm^5 (**Figure 3B**). Regarding mean PVR over 4 hours of EVLP, the CD and CDA showed significantly lower values than the standard group ($P < .001$). Pulmonary artery pressure (PAP) was significantly lower in the CD and CDA group than in the SS group ($P < 0.05$). Mean PAP over 4 hours of EVLP was 23.94 ± 1.02 mmHg in the SS group, 14.41 ± 2.04 mmHg in the CD group and 13.16 ± 1.97 mmHg in the CDA group (**Figure 3C**). The dynamic compliance (C_{dyn}) did not show any significant differences comparing the standard group and the modified Custodiol-N groups (**Figure 3D**). While it increased in the standard group from 12.79 ± 5.16 ml/cm H_2O to 17.91 ± 8.86 ml/cm H_2O, it remained almost constant in the CDA-group at around 17 ml/cm H_2O and slightly increased in the CD-group from 16.45 ± 4.34 ml/cm H_2O to 21.56 ± 4.6 ml/cm H_2O (n.s).

Lactate production of the lung tissue was significant lower ($P < 0.05$) in the two modified Custodiol-N perfused lungs compared to the standard protocol EVLP with STEEN Solution™ (**Figure 4A**). Mean lactate level in 4 hours of perfusion was 4.88 ± 1.56 mmol/l in SS group, 2.14 ± 0.77 mmol/l in CD group and 2.56 ± 0.88 mmol/l in CDA group.

Cell injury

As indicator of general cell injury, free LDH in perfusate was measured hourly. The activity of AP in perfusate as a marker enzyme for destroyed pneumocytes type II should additionally give an of the extent of lung cell damage. LDH activity in all groups increased during the EVLP process and did not differ significantly (**Figure 4B**; standard hour 1: 463.75 ± 137.36 U/l, hour 4: 677.97 + 196.11 U/l; CD hour 1:

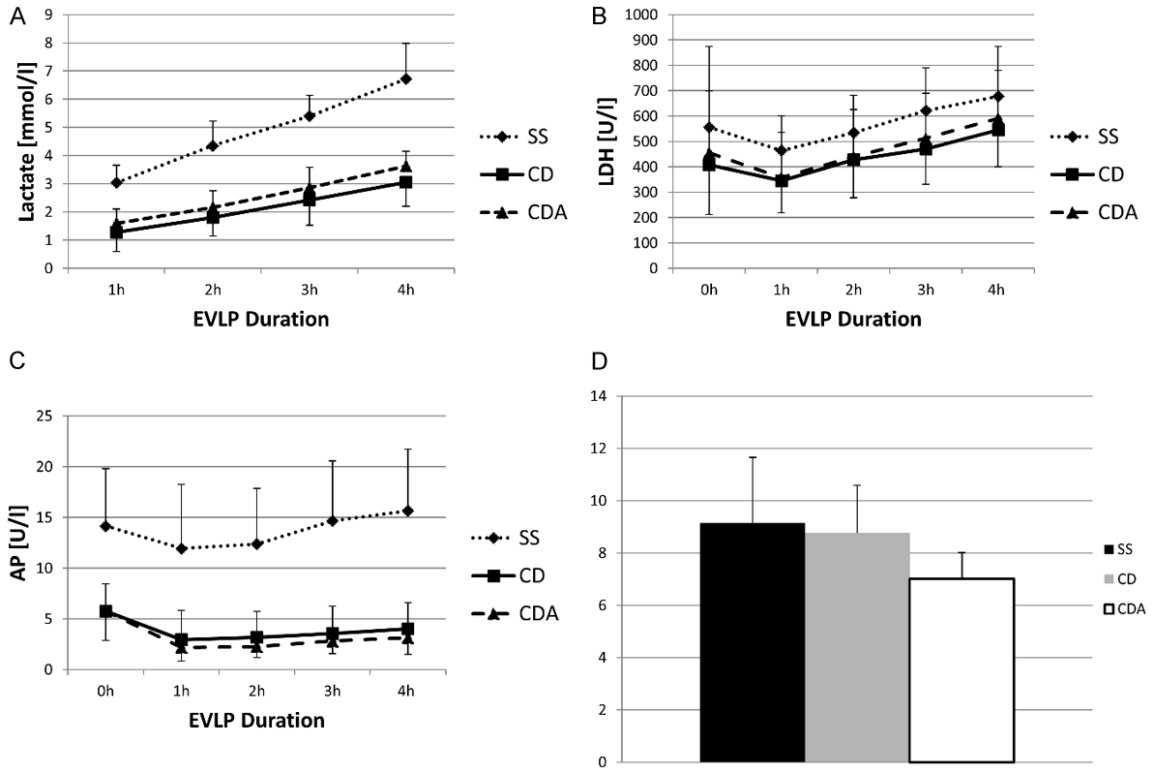

Figure 4. Lactate concentration (A) and activities of lactate dehydrogenase (LDH, B) and alkaline phosphatase (AP, C) and in the perfusate during 4 hours of EVLP. Only one side of the error bar is marked to increase the clarity of the diagram. Wet/dry-ratio as a parameter for water content in lung tissue after 4 hours of EVLP (D). Results are expressed as arithmetic mean + SD. Lactate concentration is significantly higher in the SS group than in both Custodiol-N groups ($P < .001$). The drop of LDH and AP activities in the first hour is a result of a wash-out effect due to addition of perfusion solution to the EVLP circuit. No significant differences could be found between the groups concerning the LDH activity, but the AP activity was significantly higher in the SS group ($P > 0.05$). Although there is a trend of less water content of the CD and CDA-lungs, this difference is not significant ($P > 0.05$). Comparing both Custodiol-N groups, there is a trend towards lower values in the CDA group.

345.27 ± 179.26 U/l, hour 4: 545.64 ± 189.95 U/l; CDA hour 1: 356.97 ± 126.48 U/l, hour 4: 590.22 ± 146.25 U/l). Perfusate AP activity also increased during the EVLP but with significantly lower activities in the CD and CDA group compared with the SS group ($P < 0.05$) (**Figure 4C**). The course increased in the standard group from 11.93 ± 6.3 U/l (hour 1) to 15.64 ± 6.07 U/l (hour 4), from 2.94 ± 2.9 U/l (hour 1) to 4.0 ± 2.6 U/l in the CD group and from 2.19 ± 1.33 U/l (hour 1) to 3.13 ± 1.64 U/l (hour 4) in CDA group. There were no significant differences between the CD and the CDA group in LDH or AP activity.

Water content of the lung tissue

Regarding the wet/dry-ratio, there was a trend towards lower water content levels in the CDA-group compared to the standard and the CD-group after 4 hours of EVLP (standard: 9.14 ± 2.52; CD: 8.77 ± 1.82; CDA: 7.02 ± 1.88) (**Figure 4D**). This difference is not significant.

Discussion

In a porcine model, we demonstrated for the first time that modified Custodiol-N is feasible as perfusion solution for normothermic EVLP and, moreover, that antioxidant properties and the addition of iron-chelators to the perfusion solution should be considered in the concept of normothermic EVLP because they seem to improve lung function parameters.

The intracellular formulation Custodiol-N was originally developed for static organ preservation during hypothermia and proved to be safe in preclinical lung transplantation settings [14]. Recently it has been used successfully in hypo-

thermic machine perfusion of porcine kidneys [19, 20] and as preservation solution in experimental heart transplantation in rats [18]. In the present study of porcine EVLP a modified Custodiol-N solution supplemented with dextran 40 and glucose was used as perfusion solution for normothermic EVLP. Our research group achieved promising results in adding 50 g/l dextran 40 to Custodiol-N as preservation solution for lung transplantation before [14] it was supplemented to modified Custodiol-N as perfusion solution. Moreover, 5 g/l dextran 40 is also used in STEEN Solution™ to maintain colloid pressure and to protect endothelium from subsequent excessive leucocyte interaction [23]. The addition of dextran 40 in an acellular perfusion model using an intracellular perfusion solution did not negatively influence lung function.

In a pilot study, a reduced level of glucose could be detected after one hour of EVLP, and 10 ml of 10% glucose were added. In the experimental group treated with STEEN Solution™, the ΔpO_2 averaged over four hours of EVLP was 236 mmHg and showed a decreasing course. Compared to others, our model was designed without previous lung-protective treatments ventilation of the animals or the administration of heparin ahead of medical euthanasia, which may lead to significant tissue damage reflected by decreasing oxygenation capacity lower than other models using STEEN Solution™ as perfusion solution [4, 14, 24, 25]. Probably, the model is mimicking a DCD V situation as a patient undergoing euthanasia. The model was chosen create substantial damage with a prolonged warm ischemia of the lungs.

Oxygen capacity in all 16 porcine EVLP using modified Custodiol-N were above the clinically relevant threshold of $\Delta pO_2 > 350$. These outcomes were comparable to previously reported porcine EVLP data [1-3, 7, 23-25] and significantly higher than in the standard group using STEEN Solution™. To the best of our knowledge, this is the first report of a porcine EVLP using modified Custodiol-N as perfusion solution. The median ΔpO_2, measured over four hours of EVLP was 236 in the standard group and 403 respectively 415 in the CD and CDA group. With the use of modified Custodiol-N solutions, ΔpO_2 values can be achieved comparable to porcine EVLP outcomes using STEEN Solution™ as perfusion solution [4, 25]. As shown before, Custodiol-N is suitable as preservation solution in lung transplantation [14]. Custodiol-N contains the iron-chelators LK614 and Deferoxamine to bind redox-active ions intra- and extracellularly and thereby inhibit iron-depended formation of highly reactive ROS. Dependent on the cell type and the duration of the hypothermic period, cell injury can be even more pronounced during rewarming than during cold storage itself [11, 12]. In line with these findings, a significantly higher oxygenation capacity was detectable during four hours of EVLP. It can be assumed that there is some kind of protection for the pneumocytes type 2 by the properties of modified Custodiol-N, as there was significantly lower activity of AP in perfusate of lungs which were perfused with modified Custodiol-N. Also, the trend towards lower LDH activity and lower wet/dry-ratio indicates less cell injury in lungs perfused with Custodiol-N compared to STEEN Solution™. As recently discovered, STEEN Solution™ showed antioxidant properties through downregulation of the ROS-derived NADHP-oxidase (NOX) activation in platelets, polymorphonuclear leukocytes and lymphocytes [8, 10]. Regarding the values of LDH and AP, a typical decrease after one hour of EVLP compared to the basic activity directly after the cold static preservation can be detected. This drop is caused by the dilution by filling the lungs with perfusate. Furthermore, the antioxidant effects of Custodiol-N seem to stabilize the aerobic metabolism in lung tissues noticeable in significantly lower lactate concentrations in perfusate of CD and CDA lungs which are comparable with clinical detectable values in our transplantation center.

While 70 g/l HA is used in STEEN Solution, the cellular OCS™ Solution achieves comparable values of ΔpO_2 without the addition of HA [23, 24]. Because recent studies described a positive antioxidative effect with 7 g/l HA on lung epithelial cells [8], the second Custodiol-N group used a solution supplemented with 7 g/l bovine albumin to investigate if there is a positive effect of a low albumin concentration. In hour three and four of EVLP, the CDA group achieved higher values of ΔpO_2 than the CD group (hour 3: CD 391.85 ± 27.56 mmHg, CDA 404.23 ± 69.48 mmHg; hour 4: CD 366.19 ± 95.49 mmHg, CDA 409.94 ± 58.06 mmHg)

(n.s.). The wet-dry ratio was lower in the CDA group compared with the standard and the CD group (standard: 9.14 ± 2.52; CD: 8.77 ± 1.82; CDA: 7.02 ± 1.88) (n.s.). Regarding the constant values of ΔpO_2 in the third and fourth hour and the trend of lower edema formation indicated by a lower wet/dry-ratio, we hypothesize that the addition of albumin may have a significant impact after perfusion times of more than three hours. This might be because of the higher oncotic power reached by supplementing albumin as it is described for STEEN Solution™ [21].

Limitations: Our study is limited by its experimental character and its focus on the ex vivo lung perfusion period. The lungs were not transplanted and there are no data of the postoperative period available.

Conclusion: In a porcine DCD model of 9 hours CSP followed by four hours of EVLP, the use of modified Custodiol as perfusion solution was feasible and associated with higher oxygen capacity than standard STEEN Solution™. Addition of albumin stabilized oxygenation capacity after the third hour EVLP. This stabilizing effect of albumin addition needs to be further investigated in the future. In conclusion, use of the iron binding concept in EVLP as in Custodiol-N as perfusion solution seems favorable in regard to oxygenation capacity. Our results indicate that the concept of inhibition of free radical formation can be considered for clinical use.

Disclosure of conflict of interest

None.

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References

[1] Chambers DC, Yusen RD, Cherikh WS, Goldfarb SB, Kucheryavaya AY, Khusch K, Levvey BJ, Lund LH, Meiser B, Rossano JW, Stehlik J; International Society for Heart and Lung Transplantation. The registry of the international society for heart and lung transplantation: thirty-fourth adult lung and heart-lung transplantation report-2017; focus theme: allograft ischemic time. J Heart Lung Transplant 2017; 36: 1047-1059.

[2] Cypel M and Keshavjee S. Extracorporeal lung perfusion (ex-vivo lung perfusion). Curr Opin Organ Transplant 2016; 21: 329-335.

[3] Van Raemdonck D, Neyrinck A, Cypel M and Keshavjee S. Ex-vivo lung perfusion. Transpl Int 2015; 28: 643-656.

[4] Cypel M, Yeung JC, Hirayama S, Rubacha M, Fischer S, Anraku M, Sato M, Harwood S, Pierre A, Waddell TK, de Perrot M, Liu MY and Keshavjee S. Technique for prolonged normothermic ex vivo lung perfusion. J Heart Lung Transplant 2008; 27: 1319-25.

[5] Slama A, Schillab L, Barta M, Benedek A, Mitterbauer A, Hoetzenecker K, Taghavi S, Lang G, Matilla J, Ankersmit H, Hager H, Roth G, Klepetko W and Aigner C. Standard donor lung procurement with normothermic ex vivo lung perfusion: a prospective randomized clinical trial. J Heart Lung Transplant 2017; 36: 744-753.

[6] Aigner C, Slama A, Hotzenecker K, Scheed A, Urbanek B, Schmid W, Nierscher FJ, Lang G and Klepetko W. Clinical ex vivo lung perfusion--pushing the limits. Am J Transplant 2012; 12: 1839-1847.

[7] Yeung JC and Keshavjee S. Overview of clinical lung transplantation. Cold Spring Harb Perspect Med 2014; 4: a015628.

[8] Pagano F, Nocella C, Sciarretta S, Fianchini L, Siciliano C, Mangino G, Ibrahim M, De Falco E, Carnevale R, Chimenti I and Frati G. Cytoprotective and antioxidant effects of steen solution on human lung spheroids and human endothelial cells. Am J Transplant 2017; 17: 1885-1894.

[9] Fernandes LM, Mariani AW, Medeiros IL, Samano MN, Abdalla LG, Correia AT, Nepomuceno NA, Canzian M and Pego-Fernandes PM. Alternative solution for ex vivo lung perfusion, experimental study on donated human lungs non-accepted for transplantation. Acta Cir Bras 2015; 30: 359-365.

[10] Carnevale R, Biondi-Zoccai G, Peruzzi M, De Falco E, Chimenti I, Venuta F, Anile M, Diso D, Cavarretta E, Marullo AG, Sartini P, Pignatelli P, Violi F and Frati G. New insights into the steen solution properties: breakthrough in antioxidant effects via NOX2 downregulation. Oxid Med Cell Longev 2014; 2014: 242180.

[11] Rauen U, Petrat F, Li T and De Groot H. Hypothermia injury/cold-induced apoptosis-evidence of an increase in chelatable iron causing oxidative injury in spite of low O2-/H2O2 formation. FASEB J 2000; 14: 1953-1964.

[12] Rauen U and de Groot H. Mammalian cell injury induced by hypothermia- the emerging

role for reactive oxygen species. Biol Chem 2002; 383: 477-488.
[13] Pizanis N, Gillner S, Kamler M, de Groot H, Jakob H and Rauen U. Cold-induced injury to lung epithelial cells can be inhibited by iron chelators-implications for lung preservation. Eur J Cardiothorac Surg 2011; 40: 948-955.
[14] Pizanis N, Petrov A, Heckmann J, Wiswedel I, Wohlschlager J, de Groot H, Jakob H, Rauen U and Kamler M. A new preservation solution for lung transplantation: evaluation in a porcine transplantation model. J Heart Lung Transplant 2012; 31: 310-317.
[15] Weyker PD, Webb CA, Kiamanesh D and Flynn BC. Lung ischemia reperfusion injury: a bench-to-bedside review. Semin Cardiothorac Vasc Anesth 2013; 17: 28-43.
[16] Pak O, Sydykov A, Kosanovic D, Schermuly RT, Dietrich A, Schroder K, Brandes RP, Gudermann T, Sommer N and Weissmann N. Lung ischaemia-reperfusion injury: the role of reactive oxygen species. Adv Exp Med Biol 2017; 967: 195-225.
[17] Veres G, Radovits T, Merkely B, Karck M and Szabo G. Custodiol-N, the novel cardioplegic solution reduces ischemia/reperfusion injury after cardiopulmonary bypass. J Cardiothorac Surg 2015; 10: 27.
[18] Loganathan S, Radovits T, Hirschberg K, Korkmaz S, Koch A, Karck M and Szabo G. Effects of custodiol-N, a novel organ preservation solution, on ischemia/reperfusion injury. J Thorac Cardiovasc Surg 2010; 139: 1048-1056.
[19] Minor T, Paul A, Efferz P, Wohlschlaeger J, Rauen U and Gallinat A. Kidney transplantation after oxygenated machine perfusion preservation with custodiol-N solution. Transpl Int 2015; 28: 1102-1108.
[20] Gallinat A, Luer B, Swoboda S, Rauen U, Paul A and Minor T. Use of the new preservation solution custodiol-N supplemented with dextran for hypothermic machine perfusion of the kidney. Cryobiology 2013; 66: 131-135.
[21] Nowak K, Kamler M, Bock M, Motsch J, Hagl S, Jakob H and Gebhard MM. Bronchial artery revascularization affects graft recovery after lung transplantation. Am J Respir Crit Care Med 2002; 165: 216-220.
[22] Tietz NW, Burtis CA, Duncan P, Ervin K, Petitclerc CJ, Rinker AD, Shuey D and Zygowicz ER. A reference method for measurement of alkaline phosphatase activity in human serum. Clin Chem 1983; 29: 751-761.
[23] Loor G, Howard BT, Spratt JR, Mattison LM, Panoskaltsis-Mortari A, Brown RZ, Iles TL, Meyer CM, Helms HR, Price A and Iaizzo PA. Prolonged EVLP using OCS lung: cellular and acellular perfusates. Transplantation 2017; 101: 2303-2311.
[24] Luc JGY, Jackson K, Weinkauf JG, Freed DH and Nagendran J. Feasibility of lung transplantation from donation after circulatory death donors following portable ex vivo lung perfusion: a pilot study. Transplant Proc 2017; 49: 1885-1892.
[25] Mulloy DP, Stone ML, Crosby IK, Lapar DJ, Sharma AK, Webb DV, Lau CL, Laubach VE and Kron IL. Ex vivo rehabilitation of non-heart-beating donor lungs in preclinical porcine model: delayed perfusion results in superior lung function. J Thorac Cardiovasc Surg 2012; 144: 1208-1215.