Elsevier Editorial System(tm) for Transplantation Proceedings
Manuscript Draft

Manuscript Number: CLIN4858R1

Title: Comparison of ET-Kyoto Solution and Perfadex as a Preservation Solution in a Pig Ex Vivo Lung Perfusion Model: Impact of Potassium Level

Article Type: Original Works or Clinical Submission

Keywords: lung; lung transplantation; ischemia/reperfusion; organ preservation; potassium

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Abstract

Title: Comparison of ET-Kyoto Solution and Perfadex as a Preservation Solution in a Pig Ex Vivo Lung Perfusion Model: Impact of Potassium Level

Bachground. The ex vivo lung-perfusion (EVLP) system has successfully been used for assessing donor lungs. Perfadex (PX) is usually used as the flush and preservation solution in EVLP system. We have used the extracellular-type-Kyoto (ET-K) solution containing 44 mEq/L potassium in clinical lung transplantation and investigated whether the use of this solution instead of PX affects the EVLP system.

Methods. We used domestic pigs from a slaughterhouse and analyzed the EVLP system. After 20-min warm ischemia and 6-h cold ischemia, EVLP was performed for 2 h. Pig heart-lung blocks were classified into the PX (n = 5) and ET-K (n = 5) groups depending on the flush and cold preservation solution. At the beginning, we discarded the first 100 ml of effluent in the PX group and the first 200 ml in the ET-K group. Pulmonary physiological data and potassium levels were measured.

Results. In both groups, perfusion was carried out for 2 h. The 2 groups did not differ with respect to the final flow, pulmonary arterial pressure, pulmonary vascular resistance, PaO_2/FiO_2, and shunt fraction. The potassium level of the perfusate was 4.4 mEq/L in the PX group and 5.4 mEq/L in the ET-K group.

Conclusion. The pig EVLP system was not affected when ET-K was used instead of PX as the flush and preservation solution. The initial 200 ml of effluent should be discarded when using the ET-K to ensure that the potassium level does not increase.

Title:

Comparison of ET-Kyoto Solution and Perfadex as a Preservation Solution in a Pig Ex Vivo Lung Perfusion Model: Impact of Potassium Level

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This study was supported by grants 20390367 from Japan Society for the Promotion of Science.

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The number of Figures: 1

The number of Tables: 4

Text

Introduction

In 2000, Steen et al. established an ex vivo lung-perfusion (EVLP) circuit before performing lung transplantation for the evaluation of the organ from donors after cardiac death.[1] Recently, Sweden group has also reported a series of successful lung transplants of initially rejected donor organs after reconditioning with an EVLP circuit.[2] Hence, the EVLP system may be a key method for evaluating rejected organs from brain-dead donors and donors after cardiac death; this may help increase the potential donor organ supply. As a standard setting for EVLP, Perfadex with a potassium concentration of 6 mEq/L has usually been used as the flush and preservation solution along with the STEEN solution as the perfusate.[3] There are extremely few studies that have reported the use of a different preservation solution in an EVLP setting. Inci et al. recently performed a study using saline and Perfadex as preservation solutions and found that Perfadex was significantly more efficient for lung preservation.[4] In a previous study, we originally developed an extracellular-type (ET)-Kyoto solution with 44 mEq/L potassium and successfully demonstrated its use in clinical lung transplantation in Japan and South Korea.[5-7] The ET-Kyoto solution has 2 major characteristic features: (1) the use of trehalose as a saccharide and (2) a composition of extracellular ions and a potassium concentration of 44 mEq/L. Additionally, many researchers have experimentally and clinically demonstrated its effectiveness in the preservation of various organs/tissues such as the trachea, kidney, skin/muscle flap, amputated digits, liver, and pancreas.[7-9]

The aim of this study is to confirm the feasibility of using the ET-Kyoto solution as a flush and preservation solution for lungs in the EVLP setting.

Materials and methods

Preliminary experiment

A subgroup, namely, ET-K100 (n = 2), was investigated at first. The ET-Kyoto solution (Table 1) was used as the lung flush and preservation solution, and the first 100 mL of the effluent was discarded. The blocks were evaluated in EVLP for 2 h as described below.

Experimental design

Heart-lung blocks were classified into the following 2 groups that differed in terms of the composition of the flush and preservation solution and the volume of effluent discarded at the beginning of circulation (Table 2): (1)

the Perfadex (PX) group (n = 5) in which Perfadex® (Vitrolife AB, Göteborg, Sweden) was used as the lung flush and preservation solution and the first 100 mL of the effluent was discarded and (2) the ET-Kyoto (ET-K) group ((n = 5) in which the ET-Kyoto solution was used as the lung flush and preservation solution and the first 200 mL of the effluent was discarded. After 20 min of warm ischemia and 6 h of cold ischemia, the blocks were evaluated in EVLP for 2 h as described below.

Pig heart-lung block and Ex vivo lung perfusion circuit

Heart-lung blocks of domestic pigs (average weight, 115 kg) were obtained from a local slaughterhouse as previously described.[10] Briefly, the pigs were sacrificed by exsanguination. Blood was collected and preserved at 4°C. We flushed 1 of the following 2 solutions antegradely through the pulmonary artery (PA): 2.0 L Perfadex® at 4°C that was supplemented with 10,000 IU heparin and 0.6 ml THAM set (Otsuka Pharmaceutical Factory Inc., Tokushima, Japan) or 2.0 L ET-Kyoto solution at 4°C that contained 10,000 IU heparin. They were soaked in cold Perfadex or ET-Kyoto solution and preserved for 6 h at 4°C. The EVLP circuit consisted of a hard-shell reservoir, a centrifugal pump, an artificial lung connected to a heat exchanger and gas supply, and a leukocyte/arterial filter (Fig 1), as previously reported.[10] The priming solution contained 2.0 L STEEN solution™ (Vitrolife AB, Göteborg, Sweden), concentrated autologous red blood cells, 500 mg imipenem, 10,000 IU heparin, THAM set, and 20 U insulin.

Ex vivo lung perfusion

After 6 h of cold preservation, perfusion was started at 0.1 L/min, and the first 100 or 200 mL of perfusate flushed out of the left atrium (LA) was discarded. The flow was controlled to keep the pulmonary arterial pressure less than 20 mmHg. When the temperature of LA reached 32°C, the ventilation was begun. The respirator was set as follows; tidal volume = 2 fold of the flow (L/min), respiratory rate = 15 / min, PEEP = 5 cmH_2O, FIO_2 = 1.0. The gas to the artificial lung was changed to N_2 93% and CO_2 7% to deoxygenate the perfusate. When the temperature of LA reached 37°C, the flow was increased step by step and finally set to 5.0 L/min. Within first 50 min, the flow reached to final flow. Perfusion at 5.0 L/min was continued for 2 h , as previously reported[10].

Measurement of potassium levels in the effluents and perfusates

At the start of perfusion, the effluent from the LA was collected in fractions in 50-ml Falcon tubes. Furthermore, at 1 h and 2 h after perfusion, the perfusates were obtained, and their potassium levels were measured.

Analysis of donor lungs and Statistics

The hematocrit level and blood gases of the perfusate into and out of the lung were analyzed at 10 min after the required final flow was achieved. The data obtained were used to calculate the intrapulmonary shunt fraction as previously described.[10] The Pulmonary vascular resistance was calculated as (mean pulmonary artery pressure – mean left atrial pressure) × 80/ perfusion flow (L/min). The PA pressure, LA pressure, and perfusion flow were monitored at all times. After perfusion, histological examination of the lungs was performed. The lungs were then immersed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. Statistical analyses were performed using Student's unpaired *t* test. The values are the means ± SEM. $P < 0.05$ was considered statistically significant.

Results

Preliminary experiment in the ET-K 100 subgroup

As shown in Table 3, the average potassium level in the first 50-ml aliquot of effluent and the subsequent 50-ml aliquot was 34.2 ± 2.0 and 19.9 ± 0.8 mEq/L, respectively, in the ET-K 100 subgroup. The potassium levels of the perfusate at 1 and 2 h were 6.1 ± 0.7 and 6.7 ± 0.6 mEq/L, respectively. The PA pressure was as high as 20 mm Hg and was maintained at that value for 2 h; the final flow was < 5.0 L/min (i.e., 2.5 L/min and 4.3 L/min).

Potassium level in the first aliquot of effluent from the pulmonary vein (PV) and in the perfusates

As shown in Table 3, the average potassium level in the first 50-ml aliquot of effluent and the subsequent 50-ml aliquot was 13.9 ± 0.5 and 9.2 ± 1.1 mEq/L, respectively, in the PX group. In the ET-K group, the potassium levels in the third and fourth 50-ml aliquots were 12.7 ± 0.7 and 10.4 ± 0.5 mEq/L, respectively; these values are similar to those in the case of the first and second 50-ml aliquots in the PX group. The potassium levels of the perfusate at 1 and 2 h were 4.4 ± 0.2 and 4.2 ± 0.1 mEq/L, respectively, in the PX group, and 5.3 ± 0.1 and 5.8 ± 0.2 mEq/L, respectively, in the ET-K group.

Ex vivo evaluation of lung functions

In the 10 cases analyzed in this study, the average weight of the heart-lung blocks was 1.5 ± 0.1 kg in the PX group and 1.5 ± 0.1 kg in the ET-K group and did not significantly differ between the 2 groups ($P = 0.95$). As shown in Table 4, there was no significant difference between the PX group and the ET-K group with respect to the total flow (4.9 ± 0.1 vs. 5.0 ± 0.0 L/min, $P = 0.34$), the PA pressure (13.6 ± 1.9 vs. 14.6 ± 1.5 mm Hg, $P = 0.69$), pulmonary artery vascular resistance (198.8 ± 36.3 and 228.8 ± 20.5 dyne·sec·cm^{-5}, $P = 0.49$), PaO_2/FiO_2 ratio (473

± 11 vs. 442 ± 21 mm Hg, *P* = 0.24), and shunt fraction (21.6% ± 3.8 and 24.3% ± 3.0%, *P* = 0.60).

Histological findings in the lungs after reperfusion

In all the studied specimens, no significant findings were observed in the pulmonary architecture, including the pulmonary vessels, or in the alveolar and bronchial architecture.

Discussion

In the present study, the potassium level in the ET-K 100 subgroup was as high as 19.9 ± 0.8 mEq/L after discarding 100 mL of the effluent with a high PA pressure of 20 mm Hg. On the other hand, in the ET-K group, the potassium level was low, i.e., 10.4 ± 0.5 mEq/L, after discarding 200 ml without any change in the PA pressure; further, the potassium level was 5.3 and 5.8 mEq/L at 1 and 2 h of perfusion. It can be speculated that in the ET-K 100 subgroup, hyperkalemia (potassium levels, 6.1 and 6.7 mEq/L) of the perfusate increased the PA pressure in the EVLP circuit. Although the potassium levels (5.3 and 5.8 mEq/L) of the perfusates in the ET-K group were slightly higher than those in the PX group (4.4 and 4.2 mEq/L) at 1 and 2 h, discarding more than 200 mL of effluent may not significantly reduce the potassium level in the perfusate and may easily reduce the volume of the perfusate in the circulation. Furthermore, it must be noted that some mixtures containing high potassium levels can lead to vasoconstriction during EVLP, and that in some clinical situations, certain preservation solutions (e.g., the UW, Euro-Collins, and ET-Kyoto solutions containing 125, 115, and 44 mEq/L potassium, respectively) and concentrated red blood cells may act as sources of potassium, as was observed in the ET-K 100 subgroup.

Originally, the Steen solution may have been used in combination with Perfadex as the flush and preservation solution. The use of the ET-Kyoto solution as a flush and preservation solution in EVLP may lead to several problems other than an elevated level of potassium. The normal volume of the vascular bed in the human lung is 70 ml at rest and 200 ml during exercise.[11] Moreover, the size of the pig lungs used in this study was almost the same as that of an adult human lung. Approximately more than 50% of the ET-Kyoto solution was washed out from the pulmonary vascular beds when the first 200 ml of effluent was discarded. In the present study, we found that a mixture containing a small volume of the ET-Kyoto solution, 2 L STEEN solution, and concentrated red blood cells can be safely used in an EVLP setting. Further, the PX and ET-K groups did not differ in terms of physiological and pathological results.

First limitation of our study was that the harvest procedure was a little different from the standard method,

because the warm ischemic time was only 20 min and no retrograde flush was used. Therefore, the warm ischemic time should be increased, i.e., up to 1 or 2 h in addition to a retrograde flush, for simulating the donor lung after cardiac death for further investigation. Secondly, this study may have uneven comparison between Perfadex group and ET-K group because 100 ml was discarded in Perfadex, while 200 ml in ET-K group. However, it was an essential procedure for reducing potassium level in ET-K group and the volume of possible toxic substances in the discarded efferents can be minute due to its short warm ischemia time and its difference can be ignored in this study.

In conclusion, we have demonstrated that the use of the ET-Kyoto solution containing 44 mEq/L potassium as a flush and preservation solution in the pig EVLP system was as effective as the use of the Perfadex solution. However, it is important to discard the initial 200 ml of effluent in order to prevent an increase in the potassium level in the EVLP setting.

Acknowledgments

We thank Kenji Kita, Kouzo Nishimura, and Hajime Yoshida for providing technical assistance in the use of the EVLP circuit, and Kazuo Umihira for arranging pig organs from the slaughterhouse.

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Tables

Table 1. Composition of the lung preservation solutions

	Perfadex	ET-Kyoto
Na^+, mmol/l	**138**	**100**
K^+, mmol/l	**6**	**44**
Mg^{2+}, mmol/l	**0.8**	**-**
Cl^-, mmol/l	**142**	**-**
Sulfate, mmol/l	**0.8**	**-**
Phosphate, mmol/l	**0.8**	**25**
Gluconate, mmol/l	**-**	**100**
Glucose, g/l	**0.91**	**-**
Trehalose, g/l	**-**	**120**
Dextran 40, g/l	**50**	**-**
HES, g/l	**-**	**30**

Table 2. Experimental design

Groups	Flush	Preservation	EVLP
Perfadex	**Perfadex**	**Perfadex**	**First 100 ml discarded STEEN solution**
ET-K	**ET-K**	**ET-K**	**First 200 ml discarded STEEN solution**
ET-K 100	**ET-K**	**ET-K**	**First 100 ml discarded STEEN solution**

Table 3. The potassium level in each 50-ml aliquot of flushed effluent and perfusate at 1 h and 2 h

	50 ml	100 ml	150 ml	200 ml	1 hr	2 hr
Perfadex	**13.9 ± 0.5**	**9.2 ± 1.1**			**4.4 ± 0.2**	**4.2 ± 0.1**
ET-K 100	**34.2 ± 2.0**	**19.9 ± 0.8**			**6.1 ± 0.7**	**6.7 ± 0.6**
ET-K	**37.1 ± 1.5**	**18.1 ± 0.7**	**12.7 ± 0.7**	**10.4 ± 0.5**	**5.3 ± 0.1**	**5.8 ± 0.2**

In Perfadex and ET-K 100 groups, first 100 ml of flushed fluid was discarded, while first 200ml was disposed in ET-K group.

Table 4. Physiological data of the 2 groups during EVLP

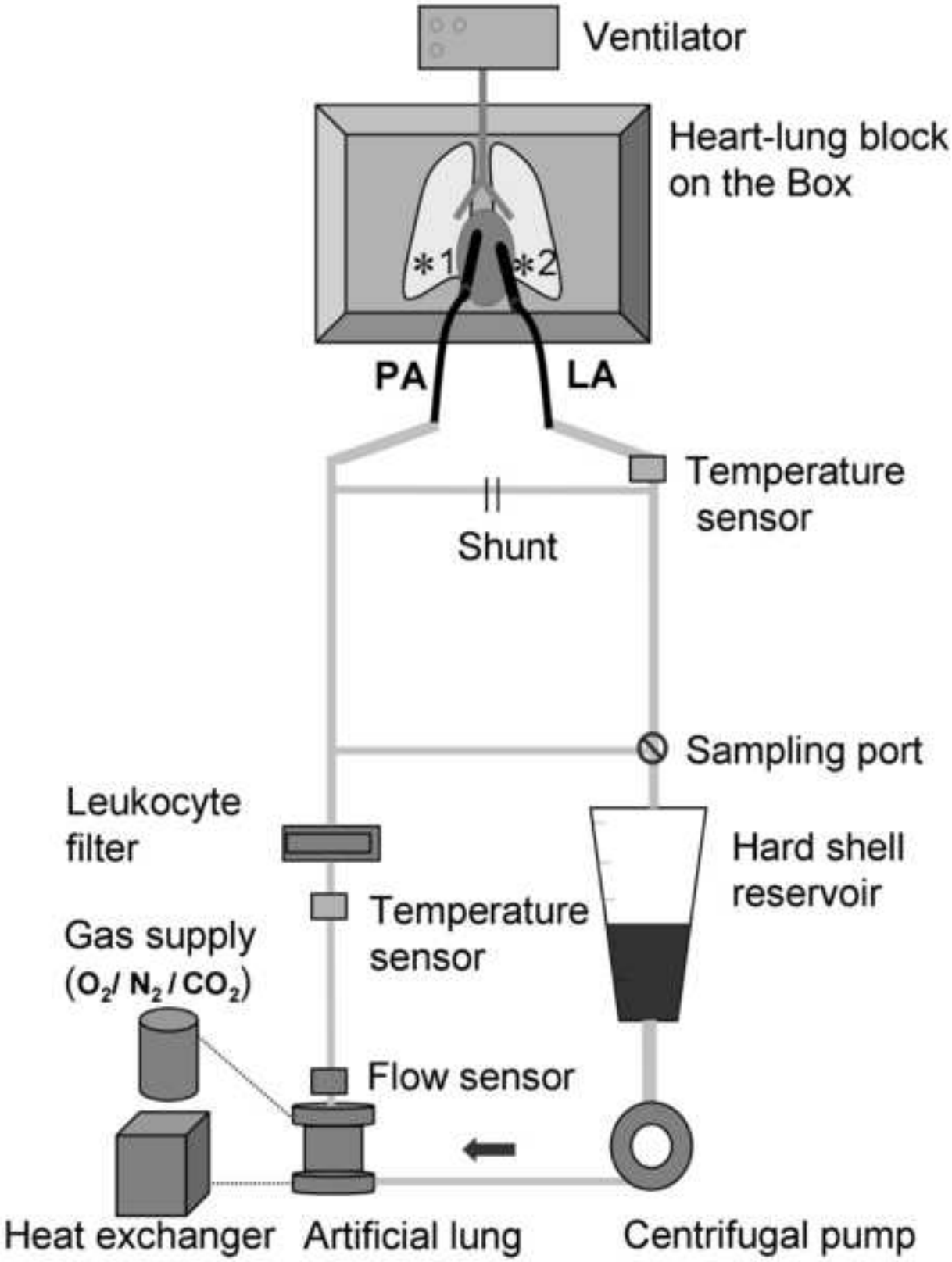
	Perfadex	**ET-K**	***P***
Flow, l/min	**4.9 ± 0.1**	**5.0 ± 0.0**	**0.34**
PA 1hr, mmHg	**16.0 ± 1.3**	**13.2 ± 1.0**	**0.13**
PA 2hr, mmHg	**13.6 ± 1.9**	**14.6 ± 1.5**	**0.69**
PVR	**198.8 ± 36.3**	**228.8 ± 20.5**	**0.49**
PaO_2/FiO_2	**473.6 ± 11.1**	**442.8 ± 21.2**	**0.23**
Shunt, %	**21.6 ± 3.7**	**24.3 ± 3.0**	**0.59**

PVR: pulmonary vascular resistance (dyne·sec·cm^{-5})

Figure legend

Fig 1. Ex vivo lung perfusion circuit. The circuit consists of a hard-shelled reservoir, a centrifugal pump, an artificial lung, a leukocyte/arterial filter, and a heart-lung block on the box. Sensors for temperature are set on the pulmonary artery (PA) and the left atrium (LA) tubes. The tracheal tube is connected to the ventilator. CO_2, carbon dioxide; N_2, nitrogen; O_2, oxygen.*1: Pressure sensor of PA, *2: Pressure sensor of LA.

Figure


Ventilator
Heart-lung block on the Box
*1
*2
PA
LA
Temperature sensor
Shunt
Sampling port
Leukocyte filter
Hard shell reservoir
Temperature sensor
Gas supply
(O_2/ N_2 / CO_2)
Flow sensor
Heat exchanger
Artificial lung
Centrifugal pump