The beneficial effects of hydrogen gas inhalation during ex vivo lung perfusion on donor lungs obtained after cardiac death in pig lung transplantation model

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The beneficial effects of hydrogen gas inhalation during ex vivo lung perfusion on donor lungs obtained after cardiac death in pig lung transplantation model

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Abstract

The beneficial effects of hydrogen gas inhalation during ex vivo lung perfusion on donor lungs obtained after cardiac death in pig lung transplantation model

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INTRODUCTION: Lung transplantation (LTx) is the optimal treatment for end-stage lung diseases. However, a shortage of donor lungs is the major obstacle to LTx. To overcome this problem, the donation after cardiac death (DCD) lungs are used. The use of DCD lungs brings several disadvantages including longer warm ischemic times compared to brain death donor lung, and absence of objective methods such as arterial blood gas analysis (ABGA) and pulmonary artery pressure to evaluate DCD lung function. Ex Vivo Lung Perfusion (EVLP) is a system that circulates normothermic perfusate into procured lungs, allowing for improved lung function and lung assessment. In this study, we investigated whether ventilation with hydrogen gas during EVLP improves DCD lung function, and whether this effect persists after actual transplantation.

MATERIALS and METHODS: 10 pigs were randomly divided into control group (n = 5) and hydrogen group (n = 5). After fibrillation by electric shock, no further treatment was administered in order to induce warm ischemic injury for 1 hour. Next, the lung was procured and EVLP was applied for 4 hours. During EVLP, the control group was given room air for respiration while the hydrogen group was given 2% hydrogen gas. After EVLP, the left lung graft was orthotopically

transplanted into the recipient pig and reperfused for 3 hours. During EVLP and reperfusion, the functional parameters and ABGA were measured every hour. Superoxide dismutase (SOD), heme oxygenase (HO)-1, interleukin (IL)-6, IL-10, tumor necrosis factor-alpha (TNF-α) and NOD-like receptor protein 3 (NLRP3) were evaluated in lung tissue after reperfusion. Pathological evaluations were performed using lung injury severity (LIS) scores and the degree of apoptosis was evaluated by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Wet/dry ratio was also measured.

RESULTS: During EVLP, pulmonary vascular resistance (PVR), and peak airway pressure (PAP) were lower in the hydrogen group, but lung compliance (LC) was lower in the control group. After LTx, oxygen capacity ($p = 0.033$), LC ($p = 0.002$), and PAP ($p = 0.020$) were significantly better in the hydrogen group. The expressions of SOD ($p = 0.022$) and HO-1 ($p = 0.047$) were significantly higher in the hydrogen group. The expressions of IL-6 ($p = 0.024$) and NLRP3 ($p = 0.042$) were higher in the control group, but IL-10 ($p = 0.037$) was higher in the hydrogen group. LIS score and the number of apoptotic cells were higher, and the degree of pulmonary edema was more severe in the control group than in the hydrogen group (LIS score: 2.2 ± 0.2 vs 0.7 ± 0.2; number of apoptotic cells: 3.4 ± 1.6 vs 2.1 ± 0.4; Wet/dry ration: 2.833 ± 0.354 vs 1.766 ± 0.078, respectively).

CONCLUSION: Hydrogen gas inhalation during EVLP improved DCD lung function via reduction of inflammation and apoptosis, and this effect persisted after LTx. Hydrogen gas inhalation during EVLP may increase the utilization of DCD lungs.

Key Words: Hydrogen, Warm ischemic injury, Donation after cardiac death, Ex vivo lung perfusion, Lung transplantation, Lung preservation.

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I. INTRODUCTION

Lung transplantation (LTx) is the most effective treatment for end-stage lung diseases, but a shortage of donor lungs results in increase waitlist mortality. To overcome this problem, the donation after cardiac death (DCD) lungs started being used ever since Steen, et al. first reported a successful LTx using a DCD lung.[1]

Despite its promising potential, DCD lung brings several disadvantages compared to heart beating donor lung. First, the longer warm ischemic times of DCD lungs might deteriorate lung graft function. Moreover, donor-to-donor variations in warm ischemic times hinder the accurate prediction of peri-transplant DCD lung function.[2]

Secondly, because there is no blood supply to DCD organs, it is impossible to accurately assess DCD lung function using common diagnostic tools such as arterial blood gas analysis (ABGA). Nevertheless, several institutions have performed successful LTx procedures using DCD lungs,[3,4] and the number of cases is increasing.

Ex vivo lung perfusion (EVLP) is a system that perfuses normothermic perfusate into procured lungs and facilitates mechanical ventilation, thus mimicking an environment similar to that found in vivo. This allows for more accurate lung assessment and improved lung function. Steen, et al. reported a case of successful LTx in which EVLP was applied to evaluate the pre-transplant condition of a DCD lung.[1] However, EVLP itself can lead to organ damage because of coagulation abnormalities and inflammation.[5] Moreover, EVLP alone cannot improve the graft function pathologically.[6] Therefore, to improve graft function, various modalities have been applied in combination with the EVLP system.

Hydrogen gas has anti-oxidant and anti-inflammatory effects. The therapeutic anti-oxidant effect of hydrogen have been demonstrated in ischemic reperfusion-injured organs, including the brain, the heart, the liver, and the intestines.[7] In recent reports, it was

demonstrated that hydrogen gas inhalation during EVLP could improve the function of DCD lungs in a pig model,[8] and result in better post-transplant graft function in a rat LTx model.[9]

The aim of this study was to investigate whether hydrogen gas inhalation during EVLP improves the function of donor lungs procured from a pig DCD model, and to evaluate whether this effect persists after LTx.

II. MATERIALS AND METHODS

1. Animals

Female Yorkshire pigs (XP Bio, Anseong, Korea) weighing 40 kg were used in this study. All surgical procedures and animal care were carried out in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Guidelines and Policies for Swine Survival Surgery; provided by the Institutional Animal Care and Use Committee (IACUC) of the Yonsei University Health System.

2. Donor lung procurement

Donor lung procurement and EVLP technique were performed as reported previously.[8]

Ten pigs were randomly divided into control group (n = 5) and hydrogen group (n = 5). In the control group, lung grafts were ventilated using only room air during EVLP, while in the hydrogen group, they were ventilated with 2% hydrogen.

The pigs were sedated intramuscularly with 5 mg/kg of tiletamine/zolazepam (Zoletil;Virbac, Carros, France) and 2 mg/kg of xylazine (Rompun; Bayer, Seoul, Korea). Endotracheal intubation was performed using an 8-mm diameter tube, and a Foley catheter was inserted. The pigs were then anesthetized with isoflurane (Forane; JW Pharmaceutical, Seoul, Korea). The ventilator was set in a volume control mode with a tidal volume of 10 mL/kg, a positive end-expiratory pressure (PEEP) of 5 cmH_2O, a respiratory rate of 16–18/min, and a fraction of inspired oxygen (FiO_2) of 0.5. After fibrillation was induced by a 9-V electrical shock through the vertical subxiphoid incision, the pigs were left to progress to cardiac arrest. Cardiac arrest was defined as the state when the difference between systolic and diastolic pressure was zero, and when there was an absence

of electrical activity in the electrocardiogram. After declaration of cardiac death, the pigs were left untouched at room temperature for 1 h; however, mechanical ventilation was maintained with the aforementioned settings.

One hour after declaration of cardiac death, the sternum and pericardium were opened and 15,000 U of heparin (JW Pharmaceutical, Seoul, Korea) was injected into the main pulmonary artery (MPA). Cardiac massage was performed to circulate the heparin into the lung. A Prolene 4-0 (Ethicon, Peterborough, Canada) purse string suture was placed in the MPA followed by insertion of a 20 Fr. Foley catheter. After ligating the superior and inferior vena cava, the aorta was cross-clamped, and the left atrial appendage was incised. Lung preservation solution (Perfadex; Vitrolife, Göteborg, Sweden) was flushed (60 mL/kg at 4℃) into the MPA from a height of 30 cm. After the flush, the heart was excised and a retrograde perfusion of 500 mL of Perfadex into the left atrium (LA) was performed. While maintaining airway pressure at 15 cmH_2O and a FiO_2 of 0.5, the trachea was clamped and the lungs were excised to keep the lungs inflated. A LA cuff was designed to match the size of the funnel-shaped LA cannula (Vitrolife) and was secured with a Prolene 4-0 suture. A pulmonary artery (PA)

cannula (Vitrolife) was inserted into the MPA and tied with heavy silk (Ethicon). A tracheal tube was placed in the airway to prevent collapse of the lungs.

3. Preparation of the EVLP system

The EVLP system was prepared and managed in accordance with the Toronto protocol.[10] The EVLP system consisted of a mechanical ventilator (Hamilton-C2, Hamilton Medical AG, Bonaduz, Switzerland) and a centrifugal pump (Rotaflow, Maquet Cardiopulmonary AG, Hirrlingen, Germany) to circulate perfusate through the system. Mixed gas (6% O_2, 8% CO_2, and 86% N_2) was administered while the perfusate passed through the membrane oxygenator (Quadrox PLS oxygenator, Maquet Cardiopulmonary AG); this allowed de-oxygenation before the perfusate was re-circulated into the PA. A leukofilter was placed immediately before entry to the PA and a heat exchanger (HU 35, Maquet Cardiopulmonary AG) was connected to the membrane oxygenator.

The perfusate comprised 1500 mL of Steen solution (Vitrolife) mixed with 10,000 U of heparin, 500 mg of cefazolin (Yuhan Corporation, Seoul, Korea), and 500 mg of methylprednisolone (Dong-

A Pharmaceutical, Seoul, Korea).

4. Management of the EVLP system

Lungs were placed in XVIVO chamber (Vitrolife) and both the PA and the LA cannulas were connected to this system, while ensuring that no air entered into the circulation. Circulation was initiated slowly, at a rate of 150 mL/min at 20℃, and then the perfusate temperature was gradually increased to 37℃ over a period of 30 min. After 20 min of circulation, ventilation was initiated and the perfusion flow rate was gradually increased. The ventilator settings comprised a tidal volume of 7 mL/kg, a respiratory rate of 7/min, and a PEEP of 5 cmH_2O. During EVLP, lungs in the control group were ventilated with room air (FiO_2 of 0.21), while those in the hydrogen group were ventilated with 2% hydrogen mixed with room air (FiO_2 of 0.21). Simultaneously, 0.5 L/min of mixed gas was insufflated into the membrane oxygenator and gas flow was adjusted to maintain a PCO_2 between 35 and 45 mmHg. As the perfusate temperature reached 37℃ and 40% of the expected cardiac output was attained, the perfusion flow rate was increased to 1500 mL/min.

During the entire period of EVLP, LA and PA pressure was

maintained at between 3 and 5 mmHg, and 10 and 15 mmHg, respectively, by adjusting the reservoir level. In order to keep the contents of the perfusate as constant as possible, 100 mL of Steen solution were exchanged every hour.

5. Termination of EVLP

At the end of EVLP, circulation was stopped and PA and LA cannulas were disconnected from the EVLP circuit. Next, Perfadex was flushed (60 mL/kg at 4℃) into the PA cannula from a height of 30cm. During this procedure, ventilation was maintained with a tidal volume of 7 mL/kg, a respiratory rate of 7/min, a PEEP of 5 cmH_2O, and a FiO_2 of 0.5. After infusion of Perfadex solution, the trachea was clamped to keep the lungs inflated. PA and LA cannulas were removed and the lung was kept in Perfadex solution at 4℃.

6. LTx procedure

Anesthesia of the recipient was induced as described above for the donor. A left thoracotomy was performed through the fourth intercostal space. The pulmonary hilum was dissected and the left azygos vein was isolated from the LA and ligated. The inferior

pulmonary ligament was dissected. PA and PV were ligated and divided. The left main bronchus was clamped and excised distally. The left lung was extracted from the left thoracic cavity. The donor lung was trimmed for left LTx. Bronchial anastomosis was performed with a running 3-0 Prolene suture, and PA anastomosis was performed with a continuous 5-0 Prolene suture. The LA of the donor was anastomosed to the left appendage of the recipient with a running 4-0 Prolene suture. After re-inflation of the transplanted lung, the PA clamp was removed gradually, and the lung was de-aired through the LA anastomosis.

7. Evaluation of lung function during EVLP

Lung functional parameters were measured every hour during EVLP. Ten minutes prior to each measurement, an airway recruitment maneuver was performed twice to an airway pressure of 25 mmHg, at FiO_2 of 1.0, in order to avoid atelectasis.

The measured functional parameters were oxygen capacity (OC, [LA perfusate PO_2−PA perfusate PO_2]/FiO_2) calculated using ABGA, pulmonary vascular resistance (PVR, [PA pressure−LA pressure] × 80/pulmonary artery flow), lung compliance and peak airway pressure (PAP).

8. Evaluation of lung function after LTx

At reperfusion, two 16G catheters were inserted into the ascending aorta and the MPA, respectively, and systemic and PA pressures were measured continuously. The ventilator setting was a volume control mode with a tidal volume of 10 mL/kg, a PEEP of 5 cmH_2O, a respiratory rate of 12/min, and a FiO_2 of 0.5. ABGA was measured every hour during reperfusion. Ten minutes prior to each measurement, the recruitment maneuver was performed twice to an airway pressure of 25 mmHg, at FiO_2 of 1.0. At the end of 3-hour reperfusion, sternotomy was performed and the right PA was clamped to evaluate only left lung function. FiO_2 was maintained at 1.0. After 15 minutes, ABGA was performed and the left lung was removed.

Lung specimens were excised for biological marker analysis, pathology and wet/dry ratios from the left lower lobe posterior portion. Superoxide dismutase (SOD), heme oxygenase (HO)-1, interleukin (IL)-6, IL-10, tumor necrosis factor-alpha (TNF-α) and NLRP3 (NOD-like receptor protein 3)/NALP3 (NACHT, LRR and PYD domains-containing protein 3) were measured by western blot analysis. Antibodies against SOD, HO-1, IL-6, 10, and TNF-α were manufactured by Abcam and antibodies against NLRP3/NALP3 by

R&D.

Specimens were prepared for pathological evaluation by fixation in 10% buffered formalin and by hematoxylin and eosin staining. Pathological assessments were performed by a blinded pathologist in accordance to the lung injury severity (LIS) score.[11] This score is based on four indicators: (i) alveolar capillary congestion, (ii) hemorrhage, (iii) infiltration or aggregation of neutrophils in the air space or the vessel wall and (iv) thickness of the alveolar wall/hyaline membrane formation. Depending on the severity of the respective indicators, a score between 0 (minimal damage) and 4 (maximal damage) was assigned, and the sum of the scores was averaged to compare the results between the two groups.

Apoptotic cells were detected using the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Stained cells were counted by evaluating five fields per sample, and the mean of the five values was calculated.

The wet/dry weight ratios were calculated based on the weight difference of specimens before and after storage at 80℃ for 72 h.

9. Statistical analyses

Results were expressed as the mean ± standard error of the mean. For comparison of the lung functional parameters, such as OC, PVR, and PAP, repeated measures analysis of variance (ANOVA) was performed. A Mann–Whitney test was performed for the comparison of other variables (western blots, the wet/dry ratio, and the LIS). All statistical analyses were performed using SPSS software version 21.0 (IBM, Somers, NY, USA) and p values <0.05 were considered statistically significant.

III. RESULTS

1. Lung function during EVLP

OC, a measurement of the oxygen transfer capacity of the lung, was not different between the two groups (Fig 1A). PVR, which increases as lung function deteriorates, was higher in the control group than in the hydrogen group over the entire duration of the EVLP, although the difference was not statistically significant (Fig 1B). There was a statistically significant difference in lung compliance between the two groups (p = 0.031) (Fig 1C). PAP was not different between the

two groups over the entire duration of the EVLP, but it was significantly lower in the hydrogen group at the 4-hour time point (p = 0.027) (Fig 1D).

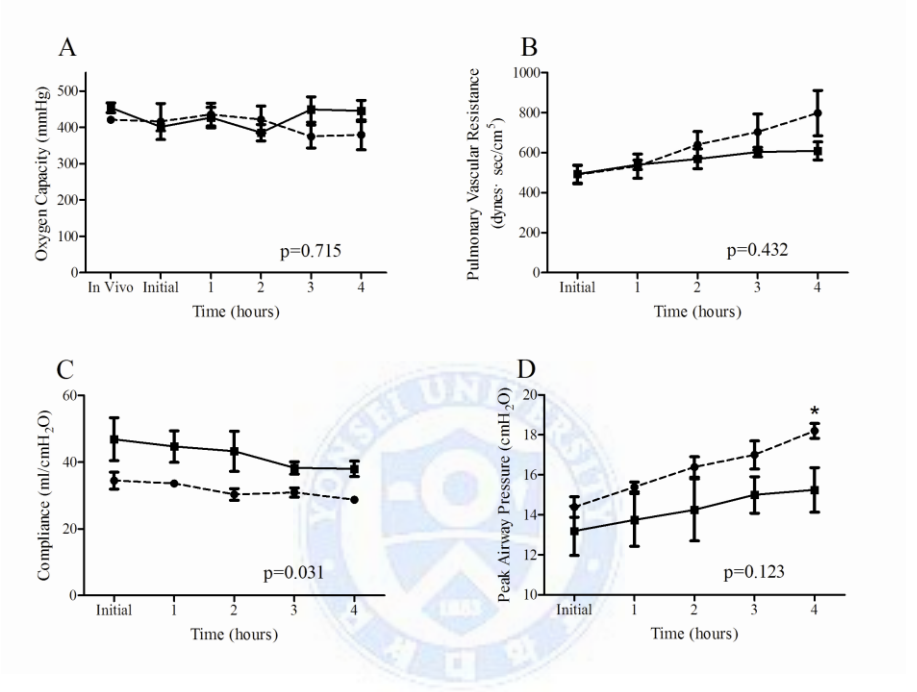


Figure 1. Functional parameters during Ex Vivo Lung Perfusion. A. Oxygen capacity (OC), B. Pulmonary vascular resistance (PVR), C. Lung compliance (LC), D. Peak airway pressure (PAP). Although OC and PVR were not different between the two groups over time, PVR tended to increase in the control group. LC was significantly higher in the hydrogen group. PAP was not different between the two groups over time, but it was lower in the hydrogen group at the 4-hour time point (*p = 0.027). Dotted line; control group, solid line; hydrogen group.

2. Lung function after LTx

(A) Functional parameters

OC was better in the hydrogen group than in the control group over the entire duration of reperfusion (p = 0.033). Especially, after right PA clamp, OC decreased suddenly in the control group (Fig 2A). Lung compliance (p = 0.002) and PAP (p = 0.020) were also significantly better in the hydrogen group than in the control group (Fig 2B, 2C).

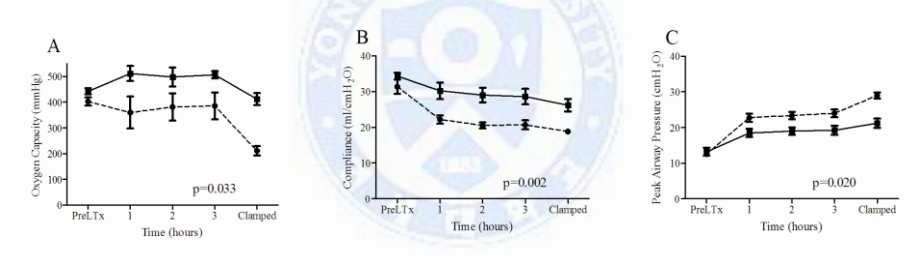


Figure 2. Functional parameters after lung transplantation. A. Oxygen capacity (OC), B. Lung compliance (LC), C. Peak airway pressure (PAP). The control group showed a decrease in OC and LC, and an increase in PAP over time. However, the hydrogen group showed relatively stable values. All parameters had statistical significance. Dotted line; control group, solid line; hydrogen group.

(B) Biological markers

The expressions of SOD (p = 0.022) and HO-1 (p = 0.047) were higher in the hydrogen group (Fig 3). The expression of IL-6 within the lung tissue was significantly lower in the hydrogen group than in the control group (p = 0.024) (Fig 4A). The expression of IL-10 was significantly higher in the hydrogen group (p = 0.037) (Fig 4B). However, there was no difference in TNF-α (Fig 4C). The expression of NLRP3 was significantly higher in the control group than the hydrogen group (p = 0.042) (Fig 4D).

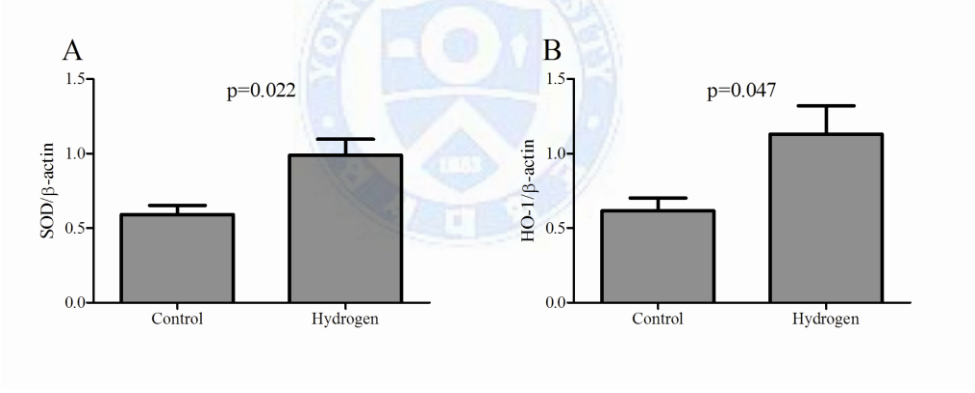


Figure 3. The effects of hydrogen on the expression of anti-oxidases in lung grafts after lung transplantation. A. The expression level of superoxide dismutase (SOD). B. The expression level of heme oxygenase (HO) -1. The expressions of SOD and HO-1 were significantly higher in the hydrogen group.

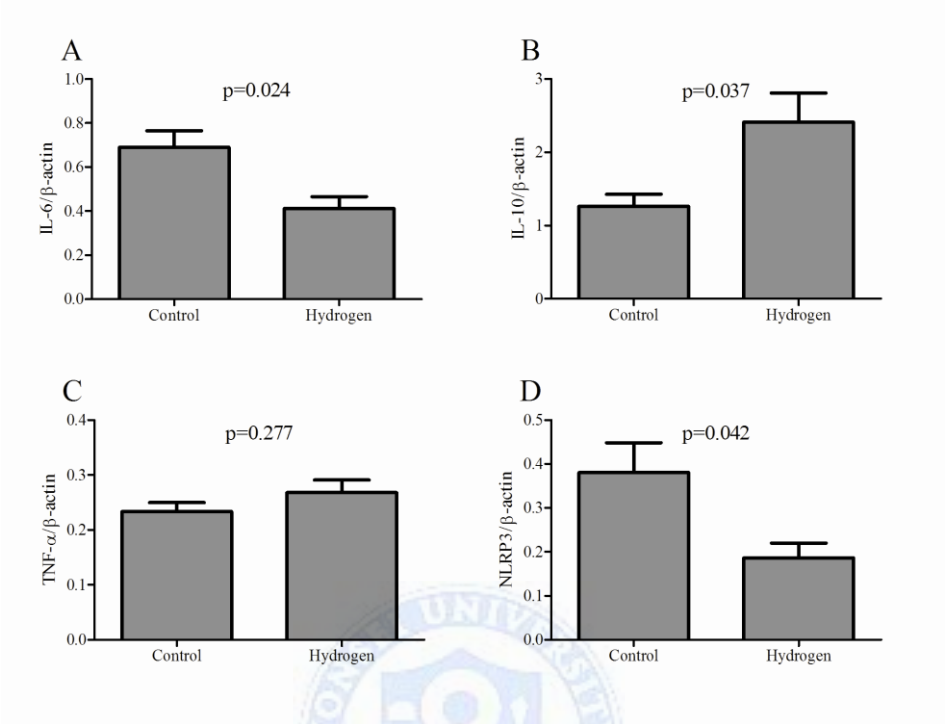


Figure 4. The effects of hydrogen on inflammation of lung grafts after lung transplantation. A. The expression level of interleukin (IL)-6, B. IL-10, C. Tumor necrosis factor (TNF)-α, D. NOD-like receptor protein 3 (NLRP3). The expression level of proinflammatory cytokine (IL-6) was higher in the control group, but the expression level of anti-inflammatory cytokine (IL-10) was higher in the hydrogen group. The expression of NLRP3, a regulator of inflammation and apoptosis was lower in the hydrogen group.

3. Lung injury severity score

The LIS scores were 2.2 ± 0.2 in the control group and 0.7 ± 0.2 in the hydrogen group, indicating that the degree of lung injury was the lower in hydrogen group (Fig 5).

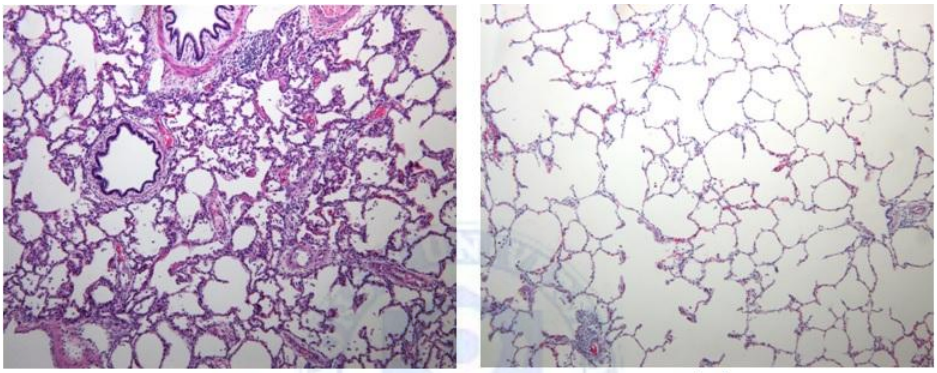
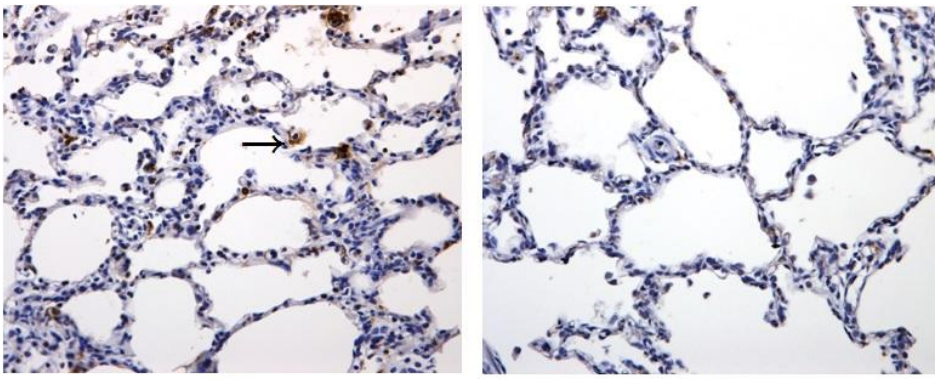


Figure 5. Pathologic findings in lung grafts (H&E, x100). A. Control group. Severe pulmonary edema was found, and many inflammatory cells were accumulated in the lung grafts of the control group. B. Hydrogen group. Alveolar structures were maintained relatively well and no pulmonary edema was found in the hydrogen group.

4. TUNEL assay

The numbers of apoptotic cells per field as assessed by TUNEL were 3.4 ± 1.6 in the control group and 2.1 ± 0.4 in the hydrogen group (Fig 6).



(A) (B)

Figure 6. TUNEL assay in lung grafts (x400). A. Control group. B. Hydrogen group. Lung grafts of the control group had more apoptotic cells than those of the hydrogen group. There were more inflammatory cells and edema in lung grafts of the control group. Black arrow; apoptotic cell.

5. Wet/dry ratio

The wet/dry ratio was 2.833 ± 0.354 in the control group and 1.766 ± 0.078 in the hydrogen group, indicating that the extent of pulmonary edema was the lower in the hydrogen group.

IV. DISCUSSION

DCD lung is a good solution to manage the shortage of brain death donor lungs in the LTx. Although the safety of LTx using DCD lungs has been repeatedly reported,[1,12] the relatively longer warm ischemic times of DCD lungs might deteriorate lung graft function. In this study, we demonstrated that hydrogen gas inhalation during EVLP in pig DCD lung model improved DCD lung function by down-regulating inflammatory and apoptotic markers, and this effect persisted after LTx.

EVLP maintains lung function by providing oxygen and appropriate nutrients necessary in graft survival to the procured lung, enabling an objective evaluation of the lung condition before transplantation.[10] In addition, various modified techniques can be applied to improve the lung function even if the lung function is marginal.[13,14]

However, there is still controversy on whether EVLP itself can improve lung function or change the pathology as it can induce lung damage associated with the mechanical ventilator or pump.[6] Therefore, in order to improve lung function further, additional procedures such as nitric oxide (NO) ventilation, surfactant injections, or gene therapy

have been performed simultaneously during EVLP.[15-17]

Recently, there have been animal study results reporting that hydrogen gas inhalation before lung procurement for lung transplant decreases ischemic reperfusion injury and primary graft dysfunction after transplantation.[18,19] Another study reported that hydrogen gas inhalation decreases EVLP-related adverse effects in a rat model, thereby improving the graft function after transplantation.[9] We also demonstrated previously that hydrogen gas inhalation during EVLP improved the lung function in a pig DCD lung model.[8] The aim of this study was to investigate whether such protective effects of hydrogen gas during EVLP can continue after LTx and to elucidate the mechanisms of the positive effects of hydrogen inhalation.

In the evaluation of lung function during EVLP, OC was not significantly different, which was in agreement with our previous result.[8] The hydrogen group exhibited significantly better lung compliance than the control group during the whole period of the EVLP, although PAP was statistically lower only at the 4-hour time point. Such a superior function of lung graft during EVLP could be maintained after LTx. All of OC, lung compliance and PAP reflected the better lung function statistically.

In the pathologic evaluation, the degree of LIS was more severe in the control group than in the hydrogen group. The lower wet/dry ratio in the hydrogen group demonstrated that hydrogen gas inhalation may also play a role in reducing pulmonary edema.

Generally, the mechanisms underlying the beneficial effects of hydrogen involve the action of anti-oxidases[20] as well as anti-inflammation[21] and anti-apoptosis.[22] Firstly, to demonstrate the anti-oxidant effect of hydrogen, we evaluated the expression levels of SOD and HO-1 in lung tissue after LTx. Zhai, et al. reported that hydrogen could activate NF-E2-related factor 2 (Nrf2), the protein that regulates the expression of anti-oxidant proteins that protect against oxidative damage. Activated Nrf2 initiates the anti-oxidative pathway via production of anti-oxidants such as SOD.[23] Meanwhile, HO-1, a critical downstream molecule of Nrf2, is induced by oxidative stresses such as hyperoxia and hypoxia. HO-1 can reduce ischemic reperfusion injury (IRI) after organ transplantation.[24,25] In accordance with previous reports, the present study showed the elevated expression levels of SOD and HO-1.

Secondly, to demonstrate the anti-inflammatory effect of hydrogen, we measured the expression levels of pro- and anti-

inflammatory cytokines (including IL-6, IL-10 and TNF-α) and NALP3 in the transplanted lung tissues. These results showed that IL-6, a pro-inflammatory cytokine, was over-expressed in the control group. On the contrary, the expression level of IL-10, an anti-inflammatory cytokine was higher in the hydrogen group. NALP3, a component of inflammasome, was over-expressed in the hydrogen group. Therefore, we could confirm that hydrogen inhalation might exert an anti-inflammatory effect by inducing anti-inflammatory cytokines and inhibiting pro-inflammatory cytokines.

Thirdly, we evaluated the effect of hydrogen on apoptosis using TUNEL assay. The number of apoptotic cells was higher in the control group. Various stressful conditions induce apoptosis, among which the accumulation of reactive oxygen species is a major cause of the apoptotic process.[26] Because hydrogen gas can selectively reduce the most toxic reactive oxygen species such as hydroxyl radicals and peroxinitrate anions,[27] we think that hydrogen gas exerts an anti-apoptotic effect in ischemia reperfusion injured lung grafts.

Although this study showed that hydrogen inhalation during EVLP in DCD lungs could improve lung function after LTx, reperfusion time was relatively short to evaluate the effect of hydrogen

on primary graft dysfunction and graft rejection.

V. CONCLUSION

This study confirmed hydrogen gas ventilation during EVLP of DCD lungs improves lung function through the action of anti-oxidases, anti-inflammation, and anti-apoptosis, and such beneficial effects persist after actual transplantation. Therefore, the application of hydrogen gas inhalation during EVLP before DCD lung transplantation is expected to help solve the shortage of brain death donor lungs.

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APPENDICES

LTx = lung transplantation; DCD = donation after cardiac death; ABGA = arterial blood gas analysis; EVLP = Ex Vivo Lung Perfusion; IL = interleukin; TNF-α = tumor necrosis factor-alpha; NLRP3 = NOD-like receptor protein 3; LIS = lung injury severity; PVR = pulmonary vascular resistance; PAP = peak airway pressure; LC = lung compliance; OC = oxygen capacity

Abstract

돼지의 심장사후 기증 폐 모델에서 체외폐관류장치 적용 시 수소기체의 흡입이 이식 후 폐 기능에 미치는 영향

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서론: 폐 이식은 말기 폐질환에 효과적인 치료법이지만 공여 폐의 부족으로 인해 시행에 제한을 받고 있으며, 이러한 문제를 극복하기 위해 최근 심장사후 기증(Donation after cardiac death; DCD) 폐를 이용하기도 한다. 그러나, DCD 폐는 몇 가지 단점을 가지고 있는데, 뇌사 기증 폐에 비해 필연적으로 온열허혈손상 시간이 길다는 점과 동맥혈 검사나 폐혈관압과 같은 폐 기능을 평가할 수 있는 객관적인 지표가 없다는 점이다. 한편, 체외폐순환장치(Ex Vivo Lung Perfusion; EVLP)는 체외로 적출된 폐에 정상체온의 관류액을 순환시켜 폐 기능의 회복을 돕고 평가할 수 있게 하는 장치다. 본 연구에서는 EVLP 를 적용하면서 동시에 수소 기체를 호흡시키는 것이 폐 기능을 향상 시킬 수 있는지 알아보고, 그 효과가 이식 후에도 지속되는지 살펴보고자 하였다.
재료 및 방법: 10 마리의 돼지를 5 마리씩 각각 대조군과 실험군으로 나누고, 전기자극으로 심실세동을 유발시켜 심장을 정지시킨 후, 1 시간 동안 실온에 방치함으로써 온열허혈손상을 받도록 하였다. 이후 폐를 적출하여 4 시간 동안 EVLP 를 적용시켰으며 이때 대조군은 공기를 이용하여, 실험군은 2%의 수소 기체를 이용하여 인공 호흡하였다. EVLP 적용 후 좌측 폐를 적출하여 수혜돼지에 정위이식하고 3 시간 동안 재관류 되도록 하였다. EVLP 와 재관류 시 매시간 기능적 지표들과 동맥혈 검사를 시행하였다. 이후 좌측 폐를 적출하여 조직 내 Superoxide

dismutase (SOD), heme oxygenase (HO)-1, interleukin (IL)-6, IL-10, tumor necrosis factor-alpha (TNF-α) and NOD-like receptor protein 3 (NLRP3) 의 발현 정도를 측정하였다. Lung injury severity (LIS) scores 를 계산하여 조직학적 손상 정도를 측정하였고 TUNEL assay 를 시행하여 세포자멸사정도를 평가하였다. 또한, wet/dry 비를 측정하여 폐부종 정도를 평가하였다. **결과**: EVLP 시행 중 폐혈관저항과 기도내압, 폐순응도는 실험군에서 낮았으며, 이식 후에도 산소교환능 (p = 0.033), 폐순응도 (p = 0.002), 기도내압(p = 0.020)이 통계적으로 유의하게 실험군에서 양호하였다. 조직 내 SOD (p = 0.022) 와 HO-1 (p = 0.047)의 발현 정도는 실험군에서 통계적으로 유의하게 높았고, IL-6 (p = 0.024)와 NLRP3 (p = 0.042) 는 대조군에서 높았으나, IL-10 (p = 0.037)은 실험군에서 높았다. LIS score (대조군: 실험군 = 2.2±0.2: 0.7±0.2)와 자멸사 세포수 (대조군: 실험군 = 3.4±1.6:2.1±0.4)는 대조군에서 높게 나타났다. 또한, 폐부종의 정도(Wet/dry 비; 대조군: 실험군 = 2.833±0.354: 1.766±0.078)도 대조군에서 더욱 심하였다. **결론**: EVLP 적용 중 수소 기체의 흡입은 염증반응과 세포자멸사를 감소시킴으로써 DCD 폐의 기능을 향상시켰으며, 그 효과는 이식 후에도 지속되었다. 따라서, 이러한 치료 방법은 DCD 폐의 활용을 더욱 늘릴 수 있을 것으로 기대된다.

핵심되는 말: 수소, 온열 허혈 손상, 심장사후 기증, 체외폐순환술, 폐이식, 폐보존

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