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ORIGINAL ARTICLE

Long agonal period deteriorates cardiac death donor lung function in a rat EVLP model

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Abstract

Purpose We investigated the impact of the duration of agonal period on donor lung function after reperfusion in an ex vivo rat lung perfusion model.

Methods Three mechanical hypoventilation conditions were used for three agonal periods, which were defined as the interval between the start of hypoventilation and the time when systolic arterial blood pressure reached < 50 mmHg, i.e., < 10, 30–60, and 150–200 min for very short (VS), short (S), and long (L) groups ($n = 5$ rats/group). After flushing the lung, heart–lung blocks were reperfused ex vivo for 120 min; physiological data were obtained throughout the reperfusion process.

Results Pulmonary vascular resistance was significantly higher throughout reperfusion in group L than in the other two groups ($p < 0.05$). After reperfusion, oxygenation was worse and pulmonary edema was more severe in group L than in group S ($p < 0.05$). Potassium concentrations in the perfusates were significantly higher in group L than in group VS. Histological analysis revealed more severe injury in group L than in the other two groups.

Conclusions Long agonal periods may lead to deterioration of donor lung function; short intervals may not significantly affect donor lung function.

Keywords Lung transplantation · Controlled donation after cardiac death · Agonal period · Warm ischemic time · Animal model

Introduction

Shortage of organ donors is a chronic and serious problem worldwide and has led to the increased use of marginal donors. In 1995, Love et al. reported the first successful series of lung transplantations (LTx) using lungs from donation after cardiac death (DCD) donors in Maastricht criteria category III; this led to a continual increase in the number of this LTx type [1–7]. At institutions that perform LTx from DCD donors, the percentage of LTxs from DCD donors among all LTx cases ranged from 7 to 27% [2, 4, 5, 7, 8]. LTx from controlled DCD (cDCD) donors reportedly showed favorable outcomes compared with donation after brain death (DBD) donors [2–4, 7].

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Clinical standards for lung grafts from DCD donors vary among institutions and countries and between controlled and uncontrolled cases. The American Society of Transplant Surgeons recommended practice guidelines for cDCD organ procurement for liver, kidneys, and pancreas transplantation in 2009 [9]. However, the guidelines for procurement of cardiothoracic organs for DCD have not yet been established because of the relatively small number of cases. The published guidelines indicate that liver transplantation from cDCD donors with a total warm ischemia time (WIT) (i.e., the interval between discontinuation of mechanical ventilation and initiation of perfusion) of > 30–45 min, and kidney or pancreas transplantation from cDCD donors with a total WIT of > 45–60 min may be associated with increased complication rates. In contrast, lungs can be procured with 60 min or more of warm ischemic time.

As with other organs, the interval between withdrawal of life-sustaining therapy (WLST) and cardiac arrest, known as the agonal period, has important implications in LTx from cDCD donors. Most institutions have set an acceptable time

frame of < 90–120 min; however, the rationale behind this threshold is unclear. Potential cDCD donor characteristics often do not meet the criteria of brain death; consequently, sustained spontaneous breathing during WLST is suggested to be the major factor affecting the length of agonal period [10]. The purpose of this study was to investigate the influence of the length of agonal period on donor lung function after reperfusion in an ex vivo rat model of lung perfusion.

Materials and methods

Animal preparation

Fifteen male Lewis rats (290–330 g) were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and intubated after tracheotomy. Ventilation was initiated using a respirator (SN-480-7; Shinano, Tokyo, Japan), and followed by intravascular administration of vecuronium bromide. Rats were artificially ventilated with room air at a tidal volume of 11.7 ml/kg, a respiratory rate of 60 breaths/min, and a positive end-expiratory pressure (PEEP) of 0 cm H_2O for 5 min. Well-controlled anesthesia was maintained by intermittent administration of vecuronium bromide and continuous intraperitoneal injection of sodium pentobarbital (65 mg/kg/h). Femoral arterial blood pressure was measured continuously. Heparin was not administered.

All animals received humane care in compliance with the Principles of Laboratory Animal Care guidelines by the National Society for Medical Research and the National Institutes of Health Guide for the Care of Laboratory Animals. The ethics committee of the Graduate School of Medicine at Kyoto University, Japan, approved the study's protocol.

Establishment of pseudo-agonal periods

Pseudo-agonal periods were established to mimic the clinical conditions that occur in cDCD donors. During the agonal period (i.e., the interval between WLST and the declaration of death), donors develop continuous hypoventilation that leads to deterioration of the general status including a reduction in systolic arterial blood pressure. Therefore, we designed a pseudo-agonal period using several mechanical hypoventilation conditions. The pseudo-agonal period was defined as the interval between the start of mechanical hypoventilation and the time that the systolic arterial blood pressure reached < 50 mmHg. Assuming that a no-touch period is mandatory in clinical cDCD, organ procurement in the model began 5 min after reaching the target blood pressure and disconnecting the tracheal tube from the ventilator. The change in blood pressure during the pseudo-agonal period is shown schematically in Fig. 1. Three mechanical

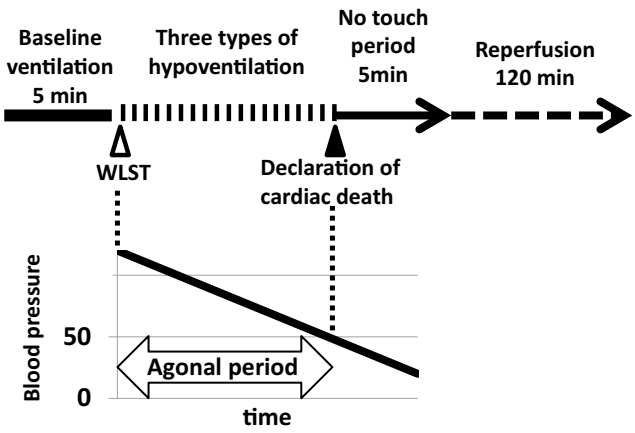

Fig. 1 Study timeline

Table 1 Pseudo-agonal periods established by hypoventilation

Pseudo-agonal period	FiO_2	Respiratory rate (/min)	Tidal volume (ml/kg)	The period from WLST to drop in blood pressure < 50 mmHg (min)
Very short	0.21	0	0	< 5
Short	0.21	40	5	30–60
Long	0.21	40	6.7	150–200

WLST withdrawal of life-sustaining therapy

hypoventilation conditions were used to create pseudo-agonal periods (Table 1). These were (1) a very short (VS) period with no ventilation and a PEEP of 0 cm H_2O, (2) a short (S) period with mechanical hypoventilation with room air, tidal volume of 5 ml/kg, respiratory rate of 40 breaths/min, and a PEEP of 0 cm H_2O, and (3) a long (L) period with mechanical hypoventilation with room air, tidal volume of 6.7 ml/kg, respiratory rate of 40 breaths/min, and PEEP of 0 cm H_2O. Systolic blood pressure decreased to < 50 mmHg within 5 min, 30–60 min, and 150–200 min, respectively. These three hypoventilation conditions were chosen based on preliminary experiments that determined the period before the systolic blood pressure decreased to < 50 mmHg.

Study design

The 15 rats were used as model cDCD lung donors, and were randomly assigned to three groups of five each for establishing the experimental pseudo-agonal conditions, as described above, before reperfusion. The tracheal tube was disconnected from the respirator when the systolic blood pressure was reduced to < 50 mmHg, and heart–lung block retrieval began after a 5-min no-touch period of hypotension in all animals. Systolic blood pressure was 0 mmHg

before retrieval commenced, with simultaneous initiation of mechanical ventilation with room air at a tidal volume of 11.7 ml/kg, a respiratory rate of 60 breaths/min, and a PEEP of 0 cm H_2O. After an antegrade flush of the lungs with 20 ml 4 °C; hypothermic Perfadex (Vitrolife, Kungsbacka, Sweden) at a pressure of 20 cm H_2O, the heart–lung blocks were partially inflated and connected to an ex vivo lung perfusion system (Model 829; IL-2 Isolated Perfused Rat or Guinea Pig Lung System; Harvard Apparatus, Holliston, MA, USA; Hugo Sachs Elektronic, Hugstetten, Germany). In the present study, this system mimicked reperfusion following lung transplantation; therefore, blood was used for the perfusate. Physiological data on lung function were collected throughout a 120-min reperfusion period.

Ex vivo lung perfusion system

Isolated rat lungs were perfused with diluted homologous blood to evaluate lung function as previously described [11, 12]. The perfusate consisted of heparinized whole blood obtained from two Lewis rat donors and saline containing 4% bovine serum albumin. The hematocrit was adjusted to 14–18%, and the pH was adjusted to 7.25–7.4 with sodium bicarbonate. No drugs, such as steroids, were added to the perfusate. The perfusate was de-oxygenated by contact with a mixture of nitrogen (92%) and carbon dioxide (8%) in a glass de-oxygenator.

After retrieval and before connection to the perfusion circuit, heart–lung blocks were inflated to an airway pressure of 20 cm H_2O to avoid atelectasis. The perfusate was pumped into the pulmonary artery, drained from the left atrium, and recirculated to the de-oxygenator. The blocks were placed in a glass chamber that mimicked the thorax, and the perfusate flow, driven by two roller pumps, was gradually increased to 10 ml/min for 12 min to stabilize the perfused lungs. The lungs were ventilated inside the artificial thorax under negative pressure at a respiratory rate of 60 cycles/min; peak inspiratory and expiratory chamber pressures of − 8 and − 4 cm H_2O, and inspiratory duration ratio of 50%. The temperature of the circuit and the chamber was maintained at 37 °C using a thermostatically controlled water bath. After stabilization of ventilation and perfusion, lung function data were collected every 10 min for a total of 120 min.

Histological assessment

At the end of reperfusion, lungs were inflated with 10% buffered formalin for fixation in all groups. Left lungs were embedded in paraffin, and sagittal sections were stained with hematoxylin–eosin for histological evaluation by light microscopy.

Statistical analysis

Statistical analysis was performed using Statview® 5.0 software (Abacus Concepts, Berkeley, CA, USA). All values were reported as means ± standard error of the mean. Data were evaluated using repeated measures analysis of variance or Scheffé's post hoc multiple comparison test. *P* values < 0.05 indicated statistical significance.

Results

Changes in arterial blood pressure during the pseudo-agonal period

Representative data on the changes in arterial blood pressure during the three hypoventilation conditions are shown in Fig. 2. At the start of hypoventilation, arterial blood

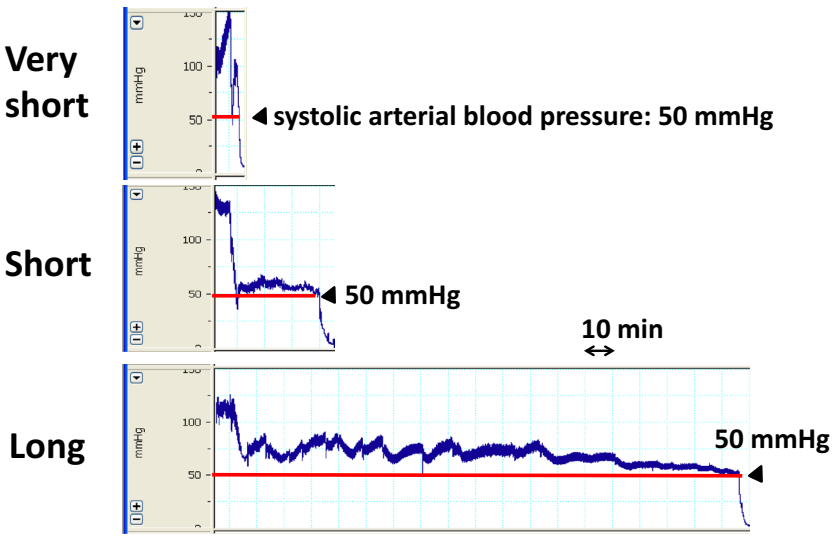

Fig. 2 Representative data of changes in arterial blood pressure under three hypoventilation conditions. Red lines indicate the agonal periods

pressure ranged between 80 and 120 mmHg in all cases. In the VS group, arterial blood pressure initially declined and then quickly increased, before declining to 0 mmHg, thus showing a bimodal curve. In groups S and L, arterial blood pressure showed a gradual decline from 60 to 70 mmHg to 0 mmHg. All the rats entered cardiac arrest within 5 min of the no-touch period after disconnection from the respirator.

Pulmonary graft function

Pulmonary vascular resistance (PVR) gradually increased throughout the reperfusion period in all groups. Throughout the reperfusion period, PVR was higher in group L ($p < 0.05$) than in the other two groups, but did not differ significantly between groups VS and S (Fig. 3a).

Dynamic lung compliance, while not significantly different among the groups, was lower in group L than in group S at 120 min after reperfusion began (0.506 ± 0.03 vs 0.556 ± 0.02, $p = 0.06$).

Oxygenation (PO_2) at 120 min after reperfusion initiation was significantly worse in group L than in group S (96.2 ± 9.1 mmHg vs 106.8 ± 4.8 mmHg, $p < 0.05$). PO_2 was lower in group L than in group VS, but the difference was not significant. PO_2 in groups S and VS was not significantly different (Fig. 3b).

Lung weight gain at 120 min after reperfusion initiation was significantly greater in group L than in group S (92.8 ± 95.2 mg vs. -46.6 ± 36.3 mg, $p < 0.05$), indicating more severe pulmonary edema with long compared with short pseudo-agonal periods (Fig. 3c).

Detailed data are summarized in Table 2.

Potassium concentrations in the pulmonary venous effluent

Potassium concentration in the pulmonary venous effluent at the beginning of reperfusion following a 12-min stabilization period was 3.0 ± 0.6 mEq/l, 3.2 ± 0.4 mEq/l, and 4.1 ± 0.5 mEq/l in groups VS, S, and L, respectively. Potassium concentrations were significantly higher in group L than in groups VS ($p < 0.05$; Fig. 4). The data are shown in Table 2.

Histological findings after 120 min of reperfusion

Hematoxylin–eosin staining of the posterior basal segment of the left lung showed that, compared with the other two

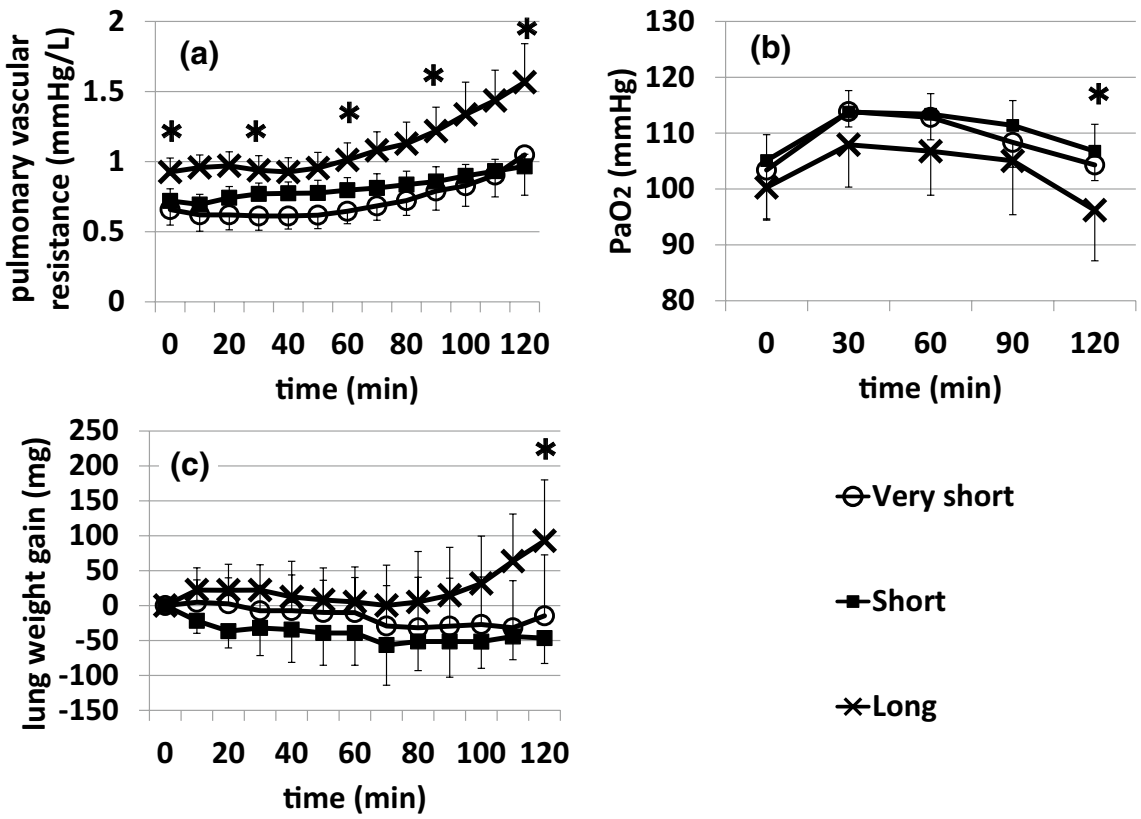

Fig. 3 Physiologic lung function during reperfusion. **a** Pulmonary vascular resistance. *$p < 0.05$, long group vs short and very short group. **b** PO_2 of lung perfusate. *$p < 0.05$, long group vs short group. **c** Lung weight gain after reperfusion. *$p < 0.05$, long group vs short group

Table 2 Potassium concentration in the pulmonary venous effluent at the beginning of reperfusion following a 12-min stabilization period and pulmonary graft function at 120 min after reperfusion initiation

Pseudo-agonal period	Very short	Short	Long
Potassium concentration (mEq/l)	3.02 ± 0.69	3.17 ± 0.47	4.14 ± 0.46
PVR (mmHg/l)	1.05 ± 0.29	0.97 ± 0.09	1.57 ± 0.28
PO_2 (mmHg)	104.30 ± 2.79	106.76 ± 4.83	96.20 ± 9.06
Lung weight gain (mg)	− 14.6 ± 87.2	− 46.6 ± 36.3	92.8 ± 95.2
Dynamic lung compliance (ml/cm H_2O)	0.53 ± 0.03	0.56 ± 0.02	0.51 ± 0.03

Values are mean ± standard deviation

PVR pulmonary vascular resistance

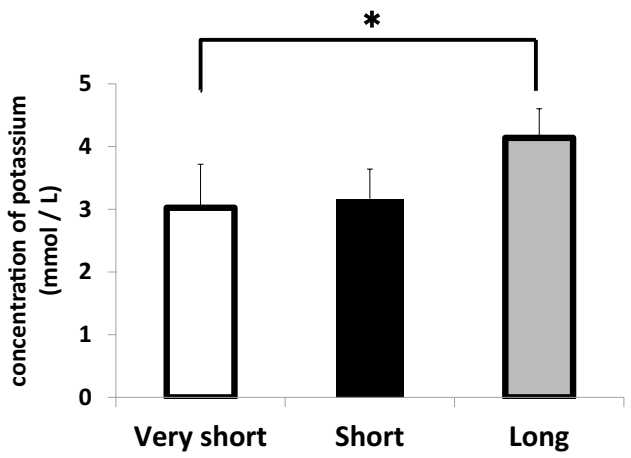

Fig. 4 Potassium concentration in pulmonary venous reperfusion effluent following a 12-min stabilization. *$p < 0.05$, long group vs very short group

groups, group L rats had more severe edema, hemorrhage, and inflammatory cell infiltration (Fig. 5). In group L, the alveolar wall was covered with a thin hyaline membrane (arrow) and contained intramural neutrophils with congestion. Conversely, the lungs in groups VS and S were almost normal.

Discussion

We confirmed that long agonal periods negatively affected donor lung function more severely than short and very short agonal periods. We also found that very short and short agonal periods had very similar effects on donor lung function, implying that there might be a critical threshold agonal period after which donor lung function following reperfusion might be adversely affected.

This ex vivo model of agonal period is unique in that the agonal period leading to sustained circulatory instability was achieved only by hypoventilation and because the agonal period mimicked true WIT. Ho et al. suggested that the duration of severe hypotension was a better predictor of delayed graft dysfunction than time from extubation to asystole in cDCD for liver and kidney transplantation [13]. In addition, the American Society of Transplant Surgeons indicated that a true WIT, i.e., the interval between significant ischemic insult, such as a drop in mean arterial pressure below 60 mmHg and initiation of perfusion, longer than 20–30 min had an unfavorable effect on cDCD liver transplantation outcomes [9].

Unified WIT criteria are recognized for both living donor and DBD donor transplantation. However, WIT is not consistently defined for cDCD because standards for the declaration of death vary. Without a clear definition for the declaration of death, it is difficult to define WIT for cDCD

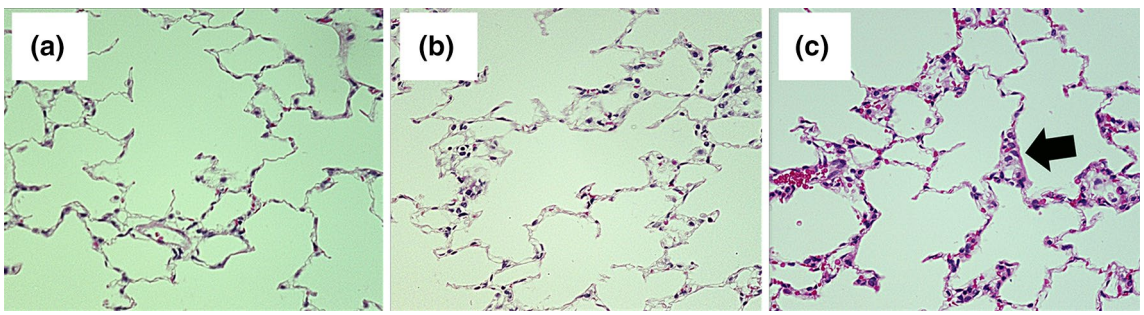

Fig. 5 Hematoxylin–eosin staining of the posterior basal segment of the left lung 120 min after reperfusion. **a** Very short group. **b** Short group. **c** Long group. Original magnification **a**–**c**: × 200

and to compare findings of WIT evaluations in multiple centers. However, cDCD kidney and liver transplantation is increasingly commonplace; consequently, several reports are available, and an acceptable WIT has been defined following the published guidelines. In an analysis of 1567 patients with liver transplantation from cDCD donors, an interval of > 35 min between WLST and organ flushing indicated a poor prognosis [14]. The relationship between the interval and graft failure is not linear. Graft failure rates with intervals < 35 min were not significantly different from those with intervals < 5 min. Our results are consistent with those observations, suggesting the presence of an acceptable agonal period duration.

In this study, potassium concentrations in the pulmonary venous effluent during perfusion were significantly higher in group L than in group VS after a 12-min stabilization period. High potassium concentrations reflect the severity of cell injury. A long agonal period might lead to severe graft injury [15], as reflected by our data indicating that graft function with agonal periods of 150–200 min was inferior to that with agonal periods < 60 min.

Several reports have suggested that catecholamine concentrations were elevated during the agonal period because of stress effects on the graft [16, 17]. Van De Wauwer et al. reported that catecholamine concentrations were higher with asphyxiation than with exsanguination in a swine model [18]. The extent of injury of DBD lung grafts is greatly influenced by the catecholamine storm, and the duration of exposure to catecholamine storm may greatly affect lung graft outcome. In a rat model of DBD, serum cytokine concentrations increased with longer exposure to conditions of brain death [19]. We believe that the stress associated with hypoventilation leading to severe hypotension strongly affects the graft.

In this study, arterial blood pressure was continuously monitored during the agonal period. In the VS group, the change in blood pressure was large, whereas the decline in blood pressure was gradual in groups S and L. Snell et al. reported blood pressure changes observed in 11 potential cDCD donors: eight of the donors went into cardiopulmonary arrest within 90 min, with a linear reduction in blood pressure, and the interval between WLST and blood pressure < 50 mmHg was < 30 min in those donors [6]. Changes in blood pressure observed by Snell et al. might be similar to those observed in groups S and L in the present study. However, the change in blood pressure in group VS was extreme and might have been influenced by the catecholamine storm. The influence of catecholamine concentrations might depend on the degree of hypoxia. Additionally, we showed that erratic fluctuations in blood pressure in the VS group did not deteriorate the lung graft. In this study, we could not collect blood during the agonal period because blood loss led to hypotension. Notably, all the rats entered asystole within 5 min of the no-touch period, as indicated in Fig. 2. The asystole occurred as a result of apnea, and this pseudoagonal period appeared to worsen the rats' general conditions, similar to the clinical situation.

Our model has several limitations. First, physiological respiration after WLST differs from mechanical ventilation. Agonal gasping is caused by extremely low oxygen concentrations [20] and contributes to the maintenance of life by increasing venous perfusion, raising arterial blood pressure, coronary perfusion pressure, and cerebral perfusion pressure [21]. During a true agonal period, gasping may cause a number of confounding factors. Further, in the clinical case, morphine and/or other analgesics may be given to minimize distress in a dying patient according to accepted end-of-life protocols, even if this might hasten death as an unintended consequence [9]. In addition, mechanical ventilation itself is an invasive procedure, and the pulmonary flow in this experiment was much lower than that of normal cardiac output. Ex vivo lungs perform better at lower flows, so the flow volume in the recent study was as usual in the isolated perfused lung system [22, 23]. Finally, we could not determine the mechanism underlying our findings in this study.

In summary, a long agonal period with circulatory instability led to more severe deterioration of donor lung function than short or very short agonal periods. Very short and short agonal periods had nearly identical effects on donor lung functions. Based on our findings the appropriate cutoff time for an acceptable agonal period is between 60 and 150 min. The results warrant future studies investigating the effects of the agonal period on graft function.

Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

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