Expression of endothelial cell-specific adhesion molecules in lungs after cardiac arrest

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Abstract

Objectives: A method to compensate for donor shortages could be donation after cardiac death. In this study, we considered endothelial cell-specific molecules, claudin-5 and VE-cadherin, as possible biomarkers predicting lung injury against warm ischemia. We investigated how the expression of these molecules could change after cardiac arrest in a mouse lung, comparing other molecules presumably relating with ischemia.

Methods: At given intervals after cardiac arrest, the lungs were harvested. Quantitative analysis of mRNA expression of claudin-5, VE-cadherin, IL-1β, IL-10, HIF-α, Egr-1, VEGF, Ang-1 and Ang-2 genes in lung tissues with several periods of warm ischemia was performed.

Results: Regarding endothelial cell-specific molecules, there were significant differences in both claudin-5 and VE-cadherin mRNA expression between 0 h and 4 h after cardiac arrest. IL-1β mRNA expression 1 h, 2 h and 4 h after cardiac arrest increased significantly, compared with that at 0 h. There were no significant differences with the other genes.

Conclusions: We found that it took more time for claudin-5 and VE-cadherin mRNA expression to change significantly than IL-1β mRNA expression; therefore, endothelial cell-specific molecules, claudin-5 and VE-cadherin, might be no better candidates for clinical use than IL-1β.

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Keywords: Lung preservation; Ischemia; Lung; Non-heart-beating donor; Endothelium; Donation after cardiac death

1. Introduction

Lung transplantation (LTx) has been established as a therapeutic option for patients with end-stage lung diseases, but donor shortages remain a grave problem [1]. To resolve this problem, so-called marginal donors have begun to be utilized, but their numbers are also limited. Another method to compensate for the shortage of brain-dead donors could be the use of lungs from donation after cardiac death (DCD). In 2000, Steen et al. successfully performed LTx from an uncontrolled DCD donor [2]. In DCD donors, warm ischemia occurs inevitably during circulatory arrest. The acceptable period of warm ischemia is very short, which hampers the prevalence of LTx from DCD donors. Several experiments have been performed to inhibit lung injury due to warm ischemia is still crucial for LTx from DCD donors. Steen et al. introduced an excellent ex vivo evaluation system for DCD donors [2], and several groups performed investigated DCD donors using a similar ex vivo system [3]. This ex vivo lung assessment method not only allows us to assess the lungs for DCD donors but can also be used to assess lungs from marginal heart-beating donors. Furthermore, it might be utilized even to treat damaged donor lungs to achieve a better condition during ex vivo circulation. Although an excellent system, the ex vivo evaluation system is large and requires time and effort, so a more convenient method to assess the function of DCD donors is desired.

According to a variety of reported experiments, vascular endothelium is known to be the most vulnerable to ischemia [6–9]. Thus, as a possible marker for lung injury due to warm ischemia, specific molecules related with vascular endothelium could be considered good candidates. Claudin-5 and vascular endothelial (VE)-cadherin are the notable adhesion molecules specific to the cell–cell junction of vascular endothelial cells [10, 11]. In cellular injury by ischemia, these junctional adhesion molecules are probably damaged. For example, Koto et al. revealed that hypoxia disrupted the barrier function of neural blood vessels through changes in the expression of claudin-5 in endothelial cells [12].

In this study, we considered claudin-5 and VE-cadherin to be possible biomarkers predicting lung injury due to warm ischemia. We investigated how the expression of these molecules could change after cardiac arrest in a mouse lung, comparing other molecules presumably related with ischemia.
2. Materials and methods

2.1. Animal treatment and materials

Male ICR mice weighing approximately 30 g (Japan SLC, Ltd., Hamamatsu, Japan) were used in this study. All animals received humane care in compliance with the Principles of Laboratory Animal Care, formulated by the National Society for Medical Research, and the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health (NIH Publication No. 86-23, revised 1985). This protocol was approved by the Ethical Committee of the Faculty of Medicine at Kyoto University.

2.2. Preparation of mouse tissues with warm ischemia

Cardiac arrest was induced in mice by dislocation of the neck joint. They were then placed into a chamber in which the temperature was maintained at approximately 25 °C. At intervals (0 h, 1 h, 2 h and 4 h) after cardiac arrest, a median sternotomy was performed, and the lungs were harvested. Five mice were allocated to each harvesting time, so 20 mice were used in this experiment. For the real-time reverse transcriptase-polymerase chain reaction assay, samples were immediately snap-frozen in liquid nitrogen and stored at −80 °C until use.

2.3. RNA isolation and cDNA synthesis

Total RNA was isolated by using the RNeasy mini kit (Qiagen, Maryland, USA) following the manufacturer’s protocol. To digest contaminated DNA, extracted RNA was incubated with the DNase (Qiagen GmbH, Hilden, Germany). Reverse transcription of total RNA was performed using the Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences, Uppsala, Sweden) and random hexamer (Amersham Pharmacia Biotech, Piscataway, NJ) following the manufacturer’s protocol.

2.4. Quantification of mRNA expression by real-time polymerase chain reaction

To quantify gene expression, a real-time polymerase chain reaction (PCR) assay was performed with the LightCycler thermal cycler system (Roche Diagnostics Japan, Tokyo, Japan) following the manufacturer’s protocol. The sense and antisense primers used for the quantitative amplification of each gene are shown in Table 2. A 20 µl reaction mixture containing 3 mmol/l MgCl₂, 0.5 µmol/l sense and antisense primers and 0.1 µg of cDNA in FastStart DNA SYBR Green I mix (Roche Diagnostics Japan) was prepared. PCR amplification was carried out with an initial 10-min pre-incubation at 95 °C to activate the FastStart Taq DNA Polymerase, followed by 40 cycles of the following profile: denaturation at 94 °C for 15 s and annealing at 55 °C for 15 s. After completion of PCR amplification, melting analyses were conducted by increasing the temperature from 65 to 95 °C with a temperature transition rate of 0.1 °C/s. The identities of PCR products were confirmed by DNA sequencing. Quantification data were analyzed with LightCycler software (ver. 3.5). Expression level of each gene was normalized by using β-actin as an internal control and is therefore presented as the ratio of each gene mRNA value to β-actin mRNA.

2.5. Statistical analysis

Statistical analyses were performed using Stat View 5.0 software (Abacus Concepts, Inc., Berkeley, CA) on an AT-compatible computer and values are the mean±standard error of the mean (S.E.M.). Data were evaluated by one-way analysis of variance (ANOVA), Fisher’s PLSD post-hoc multiple comparison test and Student’s paired t-test. P<0.05 was considered significant.

3. Results

Relative quantitative analysis of mRNA expression of claudin-5, VE-cadherin, IL-1β, IL-10, HIF-α, Egr-1, VEGF, Ang-1 and Ang-2 genes in lung tissues with several periods of warm ischemia was performed using real-time PCR (n=5).

For endothelial cell-specific molecules, mRNA expressions of claudin-5 in lung tissues 0 h and 4 h after cardiac arrest were 0.085±0.003 and 0.104±0.009, respectively (Table 1). VE-cadherin mRNA expression 0 h and 4 h after cardiac arrest were 0.107±0.005 and 0.133±0.012, respectively (Table 1). There were significant differences in both claudin-5 and VE-cadherin mRNA expressions between 0 h and 4 h after cardiac arrest (P=0.010 and 0.023, respectively).

Regarding inflammatory cytokines, mRNA expression of IL-1β of 0 h, 1 h, 2 h and 4 h after cardiac arrest were 3.18±1.25, 13.62±3.12, 11.20±3.71 and 13.51±3.37,

Table 1

<table>
<thead>
<tr>
<th>mRNA expression of several genes</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
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<tbody>
<tr>
<td>Claudin-5</td>
<td>0.085±0.003</td>
<td>0.093±0.007</td>
<td>0.088±0.006</td>
<td>0.104±0.009*</td>
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<tr>
<td>VE-cadherin</td>
<td>0.107±0.005</td>
<td>0.107±0.005</td>
<td>0.099±0.013</td>
<td>0.133±0.012*</td>
</tr>
<tr>
<td>IL-1β</td>
<td>3.18±1.25</td>
<td>13.62±3.12**</td>
<td>11.20±3.71**</td>
<td>13.51±3.37**</td>
</tr>
<tr>
<td>IL-10</td>
<td>12.58±5.65</td>
<td>19.86±7.05</td>
<td>23.36±10.40</td>
<td>29.10±11.17</td>
</tr>
<tr>
<td>HIF-α</td>
<td>0.007±0.0004</td>
<td>0.007±0.0001</td>
<td>0.005±0.0002</td>
<td>0.005±0.0001</td>
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<tr>
<td>Egr-1</td>
<td>0.003±0.0005</td>
<td>0.003±0.0010</td>
<td>0.003±0.0004</td>
<td>0.003±0.0010</td>
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<tr>
<td>VEGF</td>
<td>0.069±0.003</td>
<td>0.062±0.008</td>
<td>0.062±0.006</td>
<td>0.068±0.007</td>
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<tr>
<td>Ang-1</td>
<td>0.001±0.0002</td>
<td>0.001±0.001</td>
<td>0.001±0.0004</td>
<td>0.001±0.0003</td>
</tr>
<tr>
<td>Ang-2</td>
<td>0.003±0.0002</td>
<td>0.003±0.0004</td>
<td>0.004±0.001</td>
<td>0.004±0.001</td>
</tr>
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</table>

Expression level of each gene was normalized by representing the ratio of each gene mRNA value to β-actin mRNA. Values are expressed as the mean±S.E.M. *P<0.05 between 0 h and 4 h after cardiac arrest in claudin-5 and VE-cadherin. **P<0.05 between 0 h and 1, 2 and 4 h after cardiac arrest in IL-1β.
Claudin-5
VE-cadherin
HIF-α
Egr-1
Ang-1
Ang-2
β-Actin

<table>
<thead>
<tr>
<th>Table 2</th>
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<tbody>
<tr>
<td><strong>Nucleotide sequences of the polymerase chain reaction primer pairs</strong></td>
</tr>
<tr>
<td><strong>Sequence</strong></td>
</tr>
<tr>
<td>5'-3'</td>
</tr>
<tr>
<td>Claudin-5</td>
</tr>
<tr>
<td>VE-cadherin</td>
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<tr>
<td>HIF-α</td>
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<td>Ang-1</td>
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<td>Ang-2</td>
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<td>β-Actin</td>
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respectively (Table 1). IL-1β mRNA expressions 1 h, 2 h, and 4 h after cardiac arrest increased significantly, compared with 0 h (P = 0.005, 0.021 and 0.005, respectively); however, there were no significant differences in mRNA expression among 1 h, 2 h and 4 h after cardiac arrest. For IL-10, there was no difference among the different timings (Table 1).

Expression of HIF-α and EGR-1 mRNA did not show any significant differences in proportion to the time course. As for VEGF, Ang-1 and Ang-2, there were no differences in their mRNA expression among the different timings (Table 1).

4. Discussion

The levels of biological materials such as protein and mRNA in a dead body are useful to estimate the post-mortem interval as well as the cause of death in forensic medicine [13, 14]. It was reported more recently that no substantial loss of mRNA was observed in tissue specimens from dead rabbits stored at room temperature until 96 h post-mortem [15]. Therefore, it is most likely that the stability of mRNA was adequate for analysis in our settings. If specific mRNA levels relevant to various donor lung conditions can be accurately assessed from tissue samples of a dead body by RT-PCR, we can obtain valuable information to estimate how the donor lung might function after transplantation.

One of the important functions of endothelial cells is to determine and regulate vascular permeability, which is important not only in normal physiology to maintain the tissue environment but also in pathological conditions such as pulmonary edema and lung injury due to ischemia. In organ preservation, the integrity of the vascular endothelium is a crucial factor. In view of the assessment with transmission electron microscopy, we considered that protection of the vascular endothelial cells was critical to preserve pulmonary function after LTx [7–9]. Tight junctions in endothelial cells are thought to determine vascular permeability. Recently, claudin-1 to -15 were identified as major components of tight junction strands [11, 16, 17]. Among these, claudin-5 has been considered a notable endothelial cell-specific component of tight junction strands [11]. Among cadherin isoforms, VE-cadherin is an endothelial cell-specific component of adherence junction strands [10]. In this way, claudin-5 and VE-cadherin could be considered good candidates for possible markers of lung injury due to warm ischemia.

LTx from DCD donors is performed all over the world, but in low numbers. Compared to LTx from cadaveric donors, the evaluation of DCD donors before LTx is very difficult and remains controversial. Currently there is no reliable biologic marker for the assessment of donor lung grafts prior to LTx. Furthermore, there are no reports on the study of biologic markers for DCD donors. Some researchers have reported the usefulness of multi-cytokine analysis of the cadaveric donor lung graft with quantitative real-time PCR [18, 19]. In our study, there was a significant increase in the mRNA expression of claudin-5 and VE-cadherin 4 h after cardiac arrest, compared to 0 h after cardiac arrest. In contrast, IL-1β mRNA expression was higher as early as 1 h after cardiac arrest, compared with 0 h after cardiac arrest. IL-1β is said to be a potential risk factor and therefore this result was compatible with former studies [18]. As for the other genes studied, there were no differences in mRNA expression in comparison to the time course. We tried to identify notable and reliable biologic markers for the assessment of DCD donors, but it took more time for claudin-5 and VE-cadherin mRNA expression to change significantly than IL-1β mRNA expression; therefore, endothelial cell-specific molecules, claudin-5 and VE-cadherin, proved to be no better candidates than IL-1β, because IL-1β is an inflammatory cytokine, whose expression usually changes more dramatically in reaction to stimuli. Injury due to ischemia affected several proteins forming tight junctions, resulting in the decreased expression of claudin-5 and VE-cadherin [12, 20]. Indeed, adhesion molecules specific to the cell–cell junction of vascular endothelial cells such as claudin-5 and VE-cadherin might probably target molecules for hypoxia or ischemia; however, in our study, significant changes in the mRNA expression of claudin-5 and VE-cadherin required as long as four hours.

We used mice for this study and therefore we need to consider the differences between mice and other animals, including humans. In addition, we evaluated the lung only after ischemia, not after ischemia and reperfusion. As a next step, with lung transplantation from DCD donors, it is necessary to confirm the result of this study using a large animal model and ischemia-reperfusion model.

References


