Determination of optimal drying period in wet to dry weight ratio measurement

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Gravimetric methods are simple and reliable for evaluating the lung edema in ischemia reperfusion (IR) injury models (1). Wet to dry weight ratio (WDR) is the most frequently used gravimetric method in the literature. To determine WDR, the whole lung, lobes, or segments of peripheral lung are weighed after initial removal and dried in an oven at a constant temperature for a period (1). This drying period is not clear and ranges from 24 hours to two weeks in the literature (1-3). Our purpose was to determine the optimal drying period for this method in IR induced rat lung edema model.

Six rats were control and other six underwent hind limb IR injury (one hour ischemia and two hour reperfusion was applied to the right hind limb by tourniquet method) for constitution lung edema. After the lung removal, the right lungs were weighed wet, and then dried in an oven at 65°C, and weighed at 2, 4, 6, 12, 24th hours to 7th days. WDR was calculated using following formula; WDR = (wet-dry weight) x 100/dry weight. Mann-Whitney U test was used for analysing the difference between two groups.

Rat weights were not different in between control and IR groups (295 ± 4 vs. 297 ± 4 g, p= 0.545, respectively). In both groups, lungs lost 80% of their wet weight up to six hours and after that point, no weight loss was seen up to seven days (Table 1). Lung weights were significantly heavier in IR group than control at all drying periods (p< 0.05).

The simplest way to evaluate edema formation in the lung is to use a gravimetric method. There are four measures commonly applied: lung wet weight, WDR, lung body weight index, and extravascular lung water. Yoshikawa reported that, WDR had an excellent correlation with bronchoalveolar lavage fluid albumin and total protein during graded injury, high-airway pressure lung injury in mice (4).

Currently, we showed that, the lungs in both groups lost 80 percent of their total weight in the first six hours, and this weight loss ceased after that point up to the 7th day. Therefore, in contrast to classical application, six hours drying period is good enough to measure wet to dry weight ratio, and no need for the longer. Edematous lung weights were heavier than those of controls in all drying periods. The source of this difference resulted from the water and solute substance associated with water, such as protein, and its derivatives, accumulating in the lung tissue. During drying period, the water evaporates, but solute substances remain in the alveolar space. Here, the point that sho-
uld be emphasized is, whereas up to 6th hour, water is
the major determinant of lung weight, as the drying
period lengthened, after 6th hour, alveolar solute ma-
terials replaced with water as the major determinant of
the lung weight. Therefore, measurements after six
hours drying period shows the accumulation of solute
substance in tissues.

In this study, we used a hind limb IR model to induce
lung edema. This model is more practical than lung IR
model. We used right lungs, because total lung block
contains tissues other than lung, such as, trachea and
mediastinal fat tissue, and the right lung constitutes
2/3 of total lung weight. We perfused the lungs with
20-25 cmH2O pressure, and perfusion was continued
2-3 minutes.

In conclusion, six hours drying period is good enogh
to calculate WDR. No need for longer drying.

### Table 1. Mann-Whitney U test was used for statistical comparison of control and IR groups.

<table>
<thead>
<tr>
<th>Time</th>
<th>Lung weight (mg)#</th>
<th>Lung weight loss%</th>
<th>Wet to dry ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>IR</td>
<td>p</td>
</tr>
<tr>
<td>Wet</td>
<td>776 ± 78</td>
<td>941 ± 90</td>
<td>0.009*</td>
</tr>
<tr>
<td>2nd h</td>
<td>296 ± 62</td>
<td>508 ± 35</td>
<td>0.002*</td>
</tr>
<tr>
<td>4th h</td>
<td>157 ± 21</td>
<td>227 ± 49</td>
<td>0.041*</td>
</tr>
<tr>
<td>6th h</td>
<td>146 ± 20</td>
<td>183 ± 18</td>
<td>0.009*</td>
</tr>
<tr>
<td>12th h</td>
<td>146 ± 20</td>
<td>183 ± 18</td>
<td>0.009*</td>
</tr>
<tr>
<td>24th h</td>
<td>146 ± 20</td>
<td>183 ± 18</td>
<td>0.009*</td>
</tr>
<tr>
<td>2nd d</td>
<td>146 ± 20</td>
<td>183 ± 18</td>
<td>0.009*</td>
</tr>
<tr>
<td>3rd d</td>
<td>146 ± 20</td>
<td>183 ± 18</td>
<td>0.015*</td>
</tr>
<tr>
<td>4th d</td>
<td>146 ± 20</td>
<td>183 ± 18</td>
<td>0.015*</td>
</tr>
<tr>
<td>5th d</td>
<td>146 ± 20</td>
<td>183 ± 18</td>
<td>0.015*</td>
</tr>
<tr>
<td>7th d</td>
<td>146 ± 20</td>
<td>183 ± 18</td>
<td>0.015*</td>
</tr>
</tbody>
</table>

* Statistically significant.
# Mean ± Standard deviation.

### REFERENCES


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