Repeated measurements of airway and parenchymal mechanics in rats by using low-frequency oscillations

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Repetitively, a thoracotomy was performed to compare the lung mechanics in the intact- and open-chest conditions. On each occasion, the rats were anesthetized, paralyzed, and intubated. Small-amplitude oscillations between 0.5 and 21 Hz were applied through a wave tube to obtain respiratory impedance (Zrs). Esophageal pressure was measured to separate Zrs into pulmonary (ZL) and chest wall (Zw) components. A model containing a frequency-independent resistance and inertance and a tissue component, including tissue damping and elastance, was fitted to Zrs, ZL, and Zw spectra. Measurements of Zrs, ZL, or Zw and the model parameters calculated from them did not differ among tests. The number of animals required to show group changes in lung mechanics was significantly lower when animals were measured noninvasively than when the group changes were calculated from open-chest measurements. In conclusion, the method reported in this study can be used to separate airway and lung tissue mechanics noninvasively over a series of tests and can detect pulmonary constrictor responses for the airways and the parenchyma separately.

Methods

The primary purpose of the present study was to develop and test a modified forced oscillatory technique that allows the partitioning of airway and parenchymal mechanics noninvasively and that would be suitable for performing long-term follow-up studies in rats. We partitioned the rats' low-frequency total respiratory impedance spectra (Zrs) into components representing the lungs (ZL) and the chest wall (Zw). Airway and parenchymal mechanics were separated from ZL by using a model containing airway and a constant-phase (5) tissue compartments. Repeating Zrs, ZL, and Zw estimates in the same animal recovered from anesthesia and paralysis, we also determined the baseline variability of the airway and lung tissue mechanical parameters in a 2-wk follow-up.

Clinical Longitudinal Studies

On the effect of aging (1, 8, 17) or to explore the long-term development of a disease process (7, 22, 24) are frequently performed to follow changes in the mechanical properties of the respiratory system. For the same purpose in animal studies, independent groups are used and long-term changes in the lung function are detected from unpaired comparisons. Nevertheless, because of the baseline variability and/or the apparent scatter in the lung responsiveness, changes occurring long term in lung mechanics may remain undetectable or require large numbers of animals in the independent groups.

Recent studies in both animals and humans have established that the pulmonary parenchyma plays an important role in determining lung function and in determining the mechanical response to various insults. Total lung resistance (RL) comprises two components, the flow resistance of the tracheobronchial airway tree against the moving air (Raw) and the resistance of the pulmonary parenchyma (Rti) manifested as a viscoelastic pressure loss across the lung tissues. Animal models have demonstrated that changes in lung function can be produced by changes occurring predominantly in the airways, in the parenchyma, or in both compartments.

The major problem limiting wider application of these findings to the general physiological assessment of fundamental research questions in animals and humans is the invasive nature of the methods used to date. Studies performed in animals have required direct measurement of alveolar pressure by using pressure capsules glued to the lung surface, usually in open-chest animals, or in vitro methods. These methods preclude repeat studies in a single animal that would be desirable for many of the studies likely to be required for the successful development of a realistic animal model of asthma. Thus the development of a noninvasive method for partitioning lung mechanics into airway and parenchymal components would represent a major advance and would allow a detailed study on lung function required to understand the pathogenesis of lung diseases such as asthma.

The primary purpose of the present study was to develop and test a modified forced oscillatory technique that allows the partitioning of airway and parenchymal mechanics noninvasively and that would be suitable for performing long-term follow-up studies in rats. We partitioned the rats' low-frequency total respiratory impedance spectra (Zrs) into components representing the lungs (ZL) and the chest wall (Zw). Airway and parenchymal mechanics were separated from ZL by using a model containing airway and a constant-phase (5) tissue compartments. Repeating Zrs, ZL, and Zw estimates in the same animal recovered from anesthesia and paralysis, we also determined the baseline variability of the airway and lung tissue mechanical parameters in a 2-wk follow-up.

Methods

Animal preparation. Seven male Brown Norway rats (275 ± 17 g) were studied. The animals were housed in a pathogen-free colony and allowed food and water ad libitum. Anesthesia was induced with an intraperitoneal injection of ketamine (90 mg/kg) and rompun (5 mg/kg) mixture, and an endotracheal (ET) tube (6 cm long, 2 mm ID) was inserted. The rats were then placed in the supine position and mechanically ventilated (model 683, Harvard Apparatus, South Natick, MA) with a frequency of 90 breaths/min and 9 ml/kg tidal volume. The tail vein was cannulated for intravenous (iv) drug delivery by introducing a butterfly needle through a small puncture in the skin, and pancuronium bromide (0.4 mg/kg) was administered to induce muscle paralysis. Maintenance
doses of the anesthetic mixture and the pancuronium bromide were administered iv every 40 min, or as needed. An iv bolus of atropine (50 µg/kg) and neostigmine (100 µg/kg) was used to advance recovery after the measurements were taken.

After the completed noninvasive measurement protocol, a two-step thoracotomy was performed (21) to estimate the intact-chest end-tidal transpulmonary pressure (Ptp), which was then set as a positive end-expiratory pressure (PEEP) in the open-chest measurements. Briefly, the diaphragm was exposed from the abdomen side. The trachea was then occluded, and the step change in the tracheal pressure resulting from the bilateral cut of the parietal pleura was recorded. The magnitude of this step change (1–2 cmH2O) was taken as the in situ end-expiratory pressure, and this pressure was used as the end-expiratory pressure to keep the same end-expiratory lung volume in the open-chest condition.

Measurement apparatus. The measurement setup used to collect ZL data was described in detail previously (18). Briefly, the ET tube was switched to a loudspeaker-in-box system at end expiration. The loudspeaker generated a small-amplitude pseudorandom signal containing 15 noninteger multiple components in the frequency range of 0.5–21 Hz through a 114-cm-long 2-mm-ID polyethylene wave tube. Lateral pressures were measured at the loudspeaker (Pbox) and the tracheal end (Ptr) of the tubing with two identical miniature pressure transducers (ICS model 33NA002D). Esophageal pressure (Pes) was measured with respect to atmosphere to separate Zrs into Zw and ZL via positioning of a 3-Fr single-sensor catheter-tip micromanometer (MTC 3F, Drager Medical Electronics, Best, The Netherlands) into the esophagus. The pressure drop across the chest wall was monitored during mechanical ventilation, and the catheter was positioned to obtain a smooth respiratory curve with minimal cardiac noise. The position of the catheter tip was finalized by performing a "positive-pressure" occlusion test (9). The Pbox, Ptr, and Pes signals were low-pass filtered and digitized by an analog-to-digital board of an IBM-compatible computer at a sampling rate of 128 Hz. The pressure-transfer functions Pbox/Ptr and Pes/Ptr were computed by fast Fourier transformation in the 6-s-long recordings by using a 4-s time window and 95% overlapping. Zrs was calculated as the load impedance of the wave tube (23)

\[
Z_{rs} = Z_0 \sin h (\gamma L) \left[ (P_{box}/Ptr) - \cos h (\gamma L) \right]
\]

where \(L\) is the length of the tube. The characteristic impedance \(Z_0\) and the complex propagation wave number \(\gamma\) were determined by the geometric data and the material constants of the tube and air. Because tracheal flow was not measured, Zw was calculated by assuming no flow loss to airway wall or alveolar gas compression as \(Z_w = Z_{rs}(P_{es}/P_{tr})\), and ZL was obtained by subtraction \(Z_L = Z_{rs} - Z_w\). The load impedance of the ET tube and the connecting tubing was also determined.

Study protocol. Respiratory mechanics were measured on days 0, 7, and 14. On each occasion, 6-8 data epochs were collected, and the Zrs, Zw, and ZL spectra were ensemble averaged. On day 14, after the baseline measurements were collected, aerosolized methacholine (MCh; 2 mg/ml) was administered for 60 s with a jet nebulizer (LC Plus, Pari-Werk) driven by 5 l/min air attached to the inspiratory port of the ventilator. Data collection was started 1 min after completion of MCh challenge, and successive recordings were taken every minute thereafter. Individual ZL and Zw curves were calculated, and parameter values at the peak response in ZL were reported.

After the MCh challenge, lung mechanics were allowed to return to the baseline. The chest was then opened, and PEEP was applied to maintain the end-expiratory lung volume as close as possible to that occurring with the chest intact (see Animal preparation). Pbox was adjusted to the PEEP level during these measurements to keep Ptp unchanged. The open-chest pulmonary impedance spectra (ZL) were determined as the load impedance of the wave tube.

One rat failed to recover after the second test, and data from six rats that completed the 2-wk follow-up form the basis of the present report.

Parameter estimation. A linear model containing a frequency-independent resistance (R) and inductance (L) and a tissue damping (G) and elastance (H) of a constant-phase tissue compartment (5) was fitted to the Zrs, Zw, ZL, and ZLo spectra by minimizing the absolute difference between the measured and the modeled impedance data

\[
Z = R + i(\omega - jH)/\omega
\]

where \(j\) is the imaginary unit, \(\omega\) is the angular frequency, and \(\alpha = 2\pi \arctan (H/G)\). Tissue parameters characterize the damping and elastic properties of each compartment. For ZL, and ZLo, the R and I represent primarily the resistance (Raw) and inductance (Lw) of the airways in the intact and open chest, respectively. In the case of Zw, R reflects the Newtonian resistance of the chest wall, while I represents the chest wall inertance. Tissue hysteresivity \(\eta\) (3) values were calculated as G/H. Impedance data coinciding with the heart rate or its harmonics had poor reproducibility and were excluded from the model fit. For sample size calculation, the resistance of the ET tube and the connecting tubing \(R_{w} = 98 \text{ cmH}_2\text{O} \cdot \text{s} \cdot \text{l}^{-1}\) was subtracted from the Raw values.

Statistics. Scatters in the parameters were expressed in SE values. Repeated measures of one-way analyses of variance with the Student-Newman-Keuls multiple-comparison procedure were used to compare the parameter values in the different states of the study. Sample size calculations were performed as follows. For the intact-chest lung data, we assumed that the changes would be detected within an individual rat. Therefore, the minimum sample sizes were determined by using the standard procedure of the paired t-test. In this case, the expected variability of the changes in the intact-chest ZL data was estimated from the 14-day follow-up of the intact-chest lung parameters. The assessment of the sample sizes for the open-chest protocol assumed two separate groups of animals. Accordingly, the SD values of the lung parameters were determined from the open-chest ZL data, and the minimum sample sizes were calculated for an intended unpaired t-test. In both cases, changes of 10, 20, and 50% were expected in Raw, G, and H, respectively. The power of the tests was 0.8, and the coefficient of variation was kept constant.

RESULTS

Noninvasive partitioning of Zrs. Representative Zrs, ZL, and Zw curves with the corresponding model fits are demonstrated in Fig. 1. All impedance curves exhibit similar frequency dependence. The low variabilities seen across all frequencies, except those coinciding with the heart rate or its harmonics, indicate highly reproducible impedance measurements. ZL contributes the majority of the high-frequency real part of Zrs, suggesting that the lung dominates the Newtonian resistive properties of the respiratory system. Zw comprises most of the increase in the real part of Zrs at low
Comparison of lung mechanics obtained in intact- and open-chest conditions. Figure 2 demonstrates that we found statistically significant differences in the airway parameters when they were determined in situ and in the open chest. Open-chest Raw was 13% lower (P < 0.005), whereas Iaw exhibited a 14% increase (P < 0.01). G showed good agreement between the in situ and open-chest condition, with a slight and not statistically significant increase of 9%. Chest opening caused a statistically significant 40% decrease in H (P < 0.05). This decrease in H with a fairly constant G resulted in a significant 34% increase in η (P < 0.001).

Sample numbers for noninvasive repeated studies and for single invasive studies. On the basis of the variability of the intact- and open-chest ZL data, we calculated the required number of rats to detect a 10, 20, and 50% change in Raw, G, and H, respectively. Tables 1 and 2 show these sample size predictions for the intact- and open-chest conditions, respectively. In all parameters, the open-chest condition required significantly greater (~2-fold) group numbers to show 10 and 20% change. This difference in the required number of rats between groups is more pronounced (5-fold) if the primary variable of interest is G.

DISCUSSION

In the present study we have modified the low-frequency forced oscillation technique (4, 5, 10, 18, 20, 21) to noninvasively characterize airway and parenchymal mechanics in rats. Because the present method allows long-term investigations, in which individual rats can be used as their own control to study the development (or resolution) of a disease process, we studied the individual and group variability of lung mechanics over a 2-wk period. The results of this study demonstrated that our technique requires significantly smaller group sizes to detect small, but clinically significant (10–50%), changes in airway or parenchymal mechanics when the rats are used as their own control than that required to detect the same change by using independent groups. To assess the potential of our method to observe changes in airway and parenchymal mechanics, we determined the lung response to inhaled MCh. In agreement with previous findings we found a significant parenchymal contribution to the

**Fig. 1.** Real (R) and imaginary (X) parts of representative respiratory (Zrs; ●), pulmonary (ZL; ■), and chest wall (Zw; ▲) impedance spectra in intact-chest condition. Values are means ± SD. Open symbols represent data points corrupted by cardiac noise and are excluded from model fit. Solid lines denote corresponding model fits.

frequencies, indicating that the chest wall has a major contribution to G. In contrast, the low-frequency imaginary parts demonstrate a higher percentage of parenchymal contribution to the respiratory elastance. Negligible chest wall contribution to the inertive properties of the respiratory system is evident in the high-frequency imaginary parts of the impedance spectra. Apart from the data points corrupted by cardiac artifacts, the model fitted all impedance data well, with only a slight systematic fitting error at the low-frequency real parts.

Repeated measurements in individual animals. The results of the repeated tests are summarized in Fig. 2. Repeated measurements of respiratory, chest wall, and lung mechanics were highly reproducible, with none of the parameters showing statistically significant differences. Because repeated tests did not reveal any significant change, we used the results of the three tests to calculate the lung and chest wall contributions to the total respiratory parameters. Figure 3 demonstrates these ratios for the airway and tissue parameters. The frequency-independent resistive and inertive components of Zrs are fundamentally determined by ZL (90 ± 0.8 and 100 ± 1.6% for R and I, respectively). The lung contributed a slightly higher percentage (58 ± 1.5%) to the respiratory system H, whereas it comprised a minority of the respiratory system G (33 ± 1.0%).
Table 1. Sample size predictions from parameter values obtained in intact-chest condition

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Expected SD of Change</th>
<th>Sample No. Required for 10% Change</th>
<th>Sample No. Required for 20% Change</th>
<th>Sample No. Required for 50% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>68 ± 10 cmH₂O · s⁻¹·l⁻¹</td>
<td>12.5 cmH₂O · s⁻¹·l⁻¹</td>
<td>35</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>G</td>
<td>401 ± 72 cmH₂O/l</td>
<td>77.4 cmH₂O/l</td>
<td>38</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>H</td>
<td>2,665 ± 363 cmH₂O/l</td>
<td>578 cmH₂O/l</td>
<td>47</td>
<td>16</td>
<td>6</td>
</tr>
</tbody>
</table>

Mean and SD values were obtained from intact-chest parameters. Expected SD of change was calculated from repeated pulmonary impedance (ZL) measurements. Mean airway resistance (Raw) was corrected for resistance of endotracheal tube and connecting tubing. G and H, tissue damping and elastance parameters, respectively.
alveolar pressure, the slope of Pes/Ptr is expected to be unity. In this study the esophageal catheter was positioned on the basis of the criteria of a smooth Pes trace with minimal cardiac artifact and a good agreement between Ptr and Pes during airway occlusion. Indeed, we found Pes/Ptr of 0.97 ± 0.02, indicating an accurate estimation of the average Ppl in the rats during the control condition.

Although it is generally accepted that Pes can be used to estimate Ppl in the control state, the relevance of this conclusion during induced constriction is not evident. Suki et al. (20) measured MCh-induced responses in intact- and open-chest dogs. While they found a significantly greater lung response in the open chest, they also concluded, after a detailed analysis of the Zw spectra in control and constricted states, that Pes measurement can be used to partition Zrs to ZL and Zw even during induced constriction. In the present study, analysis of the Zw parameters revealed good agreement in the control condition and after MCh challenge, confirming that Zrs partitioning is likely to be correct via Pes measurements in rats even during MCh-induced constriction.

Lung parameters. The Raw values obtained in the present study from the intact-chest ZL data were in all cases significantly higher than those reported previously in tracheostomized rats (4, 10–14, 18). The fact that a significant part of our Raw values can be attributable to the flow resistance of the ET tube (98 of 166 cmH2O·s·l−1) is likely to explain much of the discrepancy between our present results and previously reported Raw values. Indeed, if the contribution of the ET tube to Raw is taken into account, our results are in good agreement with those obtained by using a similar measurement technique (4, 10, 18). The systematically lower Raw values derived by Nagase et al. (11–14) with alveolar capsules during tidal breathing (ranging from 24 to 35 cmH2O·s·l−1) can be explained by the higher average lung volume present during their measurements.

Lutchen et al. (10) and Petáková et al. (18) determined G and H by modeling the low-frequency ZLo spectra in open-chest rats and found 30–50% lower values than those obtained in the present study. This can be attributable to body weight and strain differences. Our Rti values calculated from the intact-chest ZL data

Table 2. Sample size predictions from parameter values obtained in open-chest rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Sample No. Required for %Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>Raw</td>
<td>46 ± 9.3 cmH2O·s·l−1</td>
<td>83</td>
</tr>
<tr>
<td>G</td>
<td>439 ± 146 cmH2O/l</td>
<td>212</td>
</tr>
<tr>
<td>H</td>
<td>2.89 ± 465 cmH2O/l</td>
<td>50</td>
</tr>
</tbody>
</table>

Mean and SD values were obtained from open-chest parameters. Mean Raw was corrected for resistance of endotracheal tube and connecting tubing.

10–15, 18–21). Most of the previous techniques for measuring parenchymal resistance (e.g., alveolar capsule) in rats are invasive (11–15, 19). Although these techniques provide details of parenchymal mechanics, they are not suitable for repeated measurements in the same animal. Therefore, either a larger number of animals were needed or the statistics were not convincing to detect the long-term changes in lung mechanics that were present. Indeed, we statistically calculated in the present study that the baseline variability in parenchymal mechanics leads to a study by using impractically high numbers of rats (n = 64) to detect a 20% change in G. Recently, Oostveen et al. (16) developed a technique to collect Zrs data in conscious rats noninvasively by using a two-compartment whole body box. By using their method, Hessel et al. (6) examined the long-term reproducibility of Zrs data in unanesthetized mice. The frequency range used in this technique (16–208 Hz), however, is unable to provide information about the resistive properties of the respiratory tissues.

Validity of Pes measurement. A critical factor in measurement of lung mechanics in the intact chest is the ability of the esophageal catheter system to accurately represent pleural pressure (Ppl). Generally, the occlusion test is used to assess the validity of this hypothesis, in which Pes/Ptr is recorded during spontane- ous tidal breathing (ranging from 24 to 35 cmH2O·s·l−1) can be explained by the higher average lung volume present during their measurements.

Table 3. Effect of aerosolized MCh on in situ airway and parenchymal parameters

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Raw, cmH2O·s·l−1</th>
<th>Law, cmH2O·s−1</th>
<th>G, cmH2O/l</th>
<th>H, cmH2O/l</th>
<th>η</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>MCh</td>
<td>C</td>
<td>MCh</td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>176</td>
<td>348</td>
<td>0.57</td>
<td>0.35</td>
<td>437</td>
</tr>
<tr>
<td>2</td>
<td>170</td>
<td>431</td>
<td>0.68</td>
<td>−0.19</td>
<td>261</td>
</tr>
<tr>
<td>3</td>
<td>163</td>
<td>304</td>
<td>0.73</td>
<td>0.54</td>
<td>439</td>
</tr>
<tr>
<td>4</td>
<td>174</td>
<td>142</td>
<td>0.78</td>
<td>0.68</td>
<td>369</td>
</tr>
<tr>
<td>5</td>
<td>151</td>
<td>188</td>
<td>0.73</td>
<td>0.66</td>
<td>439</td>
</tr>
<tr>
<td>6</td>
<td>170</td>
<td>159</td>
<td>0.63</td>
<td>0.87</td>
<td>451</td>
</tr>
</tbody>
</table>

| Mean ± SE | 167 ± 3.7 | 262 ± 48 | 0.68 ± 0.03 | 0.51 ± 0.16 | 398 ± 30 | 1,856 ± 358* | 2,666 ± 120 | 5,544 ± 646† | 0.15 ± 0.01 | 0.32 ± 0.03‡ |

MCh, methacholine; Law airway inertance; η, tissue hysteresivity parameter; C, control. Statistically significant differences from baseline: *P < 0.005; †P < 0.0001; ‡P < 0.0005.
at the ventilation frequency (ranging from 32 to 70 cmH₂O·s⁻¹⁻¹) are comparable to those reported in the literature previously by Nagase et al. (11–14) (38–43 cmH₂O·s⁻¹⁻¹) and Navajas et al. (15) (35 cmH₂O·s⁻¹⁻¹). Calculating lung elastance from the imaginary intact-chest lung reactance data at 1.5 Hz revealed significantly higher values (2,400 to 4,057 cmH₂O/l) than those in previous studies performed in open-chest rats (1,233 to 1,530 cmH₂O/l) (10–14). Besides body weight and strain differences, this discrepancy can be attributable to methodological differences because, in the latter studies, lung elastance was determined in thoracotomized rats during tidal ventilation.

Lung and chest wall contributions. Hantos et al. (4) measured low-frequency Zrs and ZL spectra in intact- and open-chest rats and found a minor chest wall contribution to the Newtonian resistive properties of the respiratory system, whereas the chest wall conferred the majority of the frequency dependence of RL (analogous to G). These findings are in qualitative agreement with the results of the present study. Previous studies examining lung and chest wall components of the total respiratory elastance in rats reported a relatively low (32–35%) chest wall contribution. The results of the present study, with chest wall elastance contributing 42% to respiratory elastance, are consistent with these previous findings.

Comparison of in situ and open-chest conditions. Although we kept the lung configuration in the open chest as close as possible to that of the intact chest, significant differences were found in the pulmonary mechanics. Although no change was observed in G, significant differences were seen in Raw, Iaw, H, and T between the intact- and open-chest conditions. Suki et al. (21) investigated the differences in airway and lung tissue mechanical properties in dogs when they measured in situ and open-chest conditions and found small but significantly different airway and parenchymal properties. In their study no change was found in G, whereas Raw and T tended to increase after chest opening. They also found a statistically significantly higher Iaw and lower H in the open-chest condition. The present study demonstrated a similar pattern with the only exception being Raw, which decreased. The matching of lung mechanics between the intact- and open-chest conditions is critically dependent on matching the respective lung volumes. If the slope were underestimated, resulting in an increased lung volume in the open-chest condition, a decreased Raw may be expected. The fact that our PEEP levels were relatively low (1–2 cmH₂O) makes this argument unlikely; however, species differences may play a role: relatively small absolute errors in the PEEP would lead to a larger relative error in rats than in dogs because the slope in the latter is higher. Alternatively, the differences seen could be due to alterations in the ventilation distribution. With the chest wall intact, the increase in lung volume will occur in a more or less even fashion. However, without the restraining influence of the chest wall, most of the increase in volume may occur anteriorly, with little increase in the volume of the posterior lung units. The resulting distortion of the lungs could have variable and unpredictable effects on lung mechanics.

Summary and implications. In conclusion, we have adopted a method to characterize airway and parenchymal mechanics in rats noninvasively. We demonstrated that this measurement technique is suitable for studying the progression of disease or long-term treatment effects with relatively small group size. The chest wall has been shown to contribute significantly to the overall tissue mechanics of the respiratory system, whereas it has a negligible effect on airway mechanics. Therefore, the present technique can be used to estimate long-term changes in airway mechanics without PEs measurement, whereas estimation of Ptp (either in an open-chest animal or Pes) is advocated to study long-term responses in Rti.

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