Effect of transmural pressure on preloads and collapse of immature bronchi

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ABSTRACT: Immature airways are highly compliant compared to the adult, suggesting that trachea and bronchi from immature animals may be easily compressed. Although tracheal compression has been extensively studied, the effect of transmural pressure on occlusion of immature bronchi has been neglected.

The transmural pressure at which the lumen closed was determined from the transmittance of pressure along the lumen of isolated bronchi from late-term foetal, 1 and 4 week old pigs. Bronchi from eight cases of sudden infant death syndrome (SIDS) were also studied. In several experiments, smooth muscle tone was produced either by electrical field stimulation or carbachol challenge, and the relationship between active muscle tone and resting transmural pressure was studied.

Bronchi from foetal, 1 and 4 week old pigs were occluded by intraluminal pressures of -4, -5 and -24 cmH2O. SIDS bronchi closed at -11 cmH2O. Histological and endoscopic investigations showed that closure of the bronchi occurred along a plane and was not uniform along the bronchus. Carbachol precontraction increased the transmural pressure required to close bronchi by approximately 5 cmH2O.

The relationship between muscle tone and resting pressure was the same in all age groups, except when transmural pressure was at or below closing pressure.

Bronchi from immature animals and human infants are vulnerable to collapse by small changes in transmural pressure. Bronchial closure is partly dependent on smooth muscle tone, particularly in younger animals.

The effect of lung forces on the calibre of intrapulmonary airways is strongly influenced by the stability of the airway, which in turn is associated with the structural properties of the bronchial wall and with their compliance. Trachea and bronchi from foetal and newborn animals are more compliant than those from older animals [1–3], suggesting that they would be more easily occluded by changes in transmural pressure. For example, flow through isolated segments of foetal lamb (114–121 days gestation) trachea is stopped by an external pressure of approximately 10–15 cmH2O [4].

Preparation of bronchial segment

Lungs were obtained from freshly killed 1 and 4 week old pigs (Large White/Landrace cross). Lungs of late
term foetal pig (>100 days of the 115 day gestation) were supplied by a local abattoir. Segments of stem bronchus, approximately 25 mm long and 2 mm in internal diameter, were dissected from the lower lobes, as described previously [14]. Lumen volume was determined from the volume of Krebs solution required to fill the bronchus (coefficient of variation 8.6%) as described previously [15].

A small number of human lungs (n=8) from autopsies of infants who died of sudden infant death syndrome (SIDS) were obtained from the Western Australian State Coroner. Infant airways were lobar-segmental bronchi but exact generation was difficult to assess and the length and internal diameter varied depending on the tissue available. All bronchi dissected from human lungs were cartilaginous at the distal end.

Bronchial segments were cannulated and mounted in a modified organ bath (fig. 1). The bronchial lumen was connected to a pressure transducer (Motorola MPX2010-DP, Phoenix, AZ, USA) to measure intraluminal pressure, and to a micro-syringe to change luminal volume and pressure. When external (adventitial) pressure was altered, a pressure-tight lid was placed on the organ bath, forming a sealed chamber. This chamber was connected to a column of Krebs solution and chamber pressure was measured by manometry. During experiments involving electrical field stimulation (EFS), platinum ring electrodes were placed around the bronchi and used to deliver square wave direct current (DC) pulses (70 V, 30 Hz 1 ms pulse width) from a Grass S44 stimulator (Quincy, USA).

Airway collapse

Two different groups of bronchi were used to determine airway closure, by using either negative intraluminal pressures or adventitially applied pressures. The negative intraluminal pressure required to close bronchial segments was assessed by reducing intraluminal volume (and pressure) at the distal end in small increments (0.02 mL), while recording pressure at the proximal end (fig. 1). When the bronchial segment between the syringe (distal end) and pressure transducer (proximal end) closed, the pressure measured no longer changed with further reductions in volume. The pressure at which this occurred was taken as the internal closing pressure.

The compressive force required to close bronchi was determined manometrically by increasing the height of the Krebs column (fig. 1), while the distal end of the bronchus was open to atmospheric pressure. Until compression occluded the bronchial segment, intraluminal pressure remained at 1 atmosphere. Once the lumen closed, however, the intraluminal pressure (recorded at the proximal end) increased with adventitial pressure. The pressure at which this occurred was defined as the external closing pressure. Bronchi were then allowed to recover at 0 cmH2O transmural pressure before being contracted with 1 µM carbachol, and the external closing pressure was then redetermined with the tissue contracted.

Table 1. – Location and size of bronchial segments used to measure closing pressure

<table>
<thead>
<tr>
<th>Age</th>
<th>Length (mm)</th>
<th>Internal diameter (mm)</th>
<th>Generation</th>
<th>Carbachol contraction (cmH2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foetal (n=7)</td>
<td>25</td>
<td>1.8±0.1</td>
<td>~1–10</td>
<td>-</td>
</tr>
<tr>
<td>One (n=8)</td>
<td>25</td>
<td>2.0±0.1</td>
<td>~2–13</td>
<td>-</td>
</tr>
<tr>
<td>Four (n=8)</td>
<td>25</td>
<td>2.0±0.1</td>
<td>~8–16</td>
<td>-</td>
</tr>
</tbody>
</table>
| Bronchial segments used to measure external closing pressure
| One (n=7)    | 25          | 2.1±0.1                | ~2–12      | 21.7±5.1                      |
| Four (n=10)  | 25          | 2.0±0.1                | ~9–17      | 27.4±3.2                      |

The effect of transmural pressure on airway smooth muscle contraction was investigated by EFS of bronchial segments at different resting intraluminal pressures [13]. Passive intraluminal pressure was set by inflating or deflating the segment to the required transmural pressure (with both the microsyringe and pressure transducer connected to the proximal end of the bronchus). After equilibrating for 5 min, the segment was stimulated and active pressure measured. One set of experiments was performed at passive intraluminal pressures.
of -5, 0, 5, 10, 20 and 30 cmH\(_2\)O (foetal (n=12), 1 week (n=8), and 4 week (n=8) bronchi). A second set of experiments was performed at 5, 0, -2.5, -5, -10 and -20 cmH\(_2\)O (1 week (n=5) and 4 week (n=6) bronchi). Preliminary experiments showed no decay in active response when the tissues were stimulated every 5 min.

**Videoendoscopy**

A fibreoptic endoscope (Olympus SES 1711D, Tokyo, Japan) and CCD camera (JVC TK-1070E, Tokyo, Japan) were used to view the lumen of 1 week old pig bronchi (n=8 bronchi) as described previously [16]. Negative intraluminal pressures were produced by sealing around the endoscope (proximal end) with plasticine and reducing the pressure at the opposite (distal) end of the segment. Closure of the bronchi was observed and recorded on videotape. The effect of airway smooth muscle tone on airway patency was investigated by EFS of collapsed bronchi. In three bronchi that gave good responses to EFS, atropine (10 µM) was used to demonstrate that neurotransmission was cholinergic.

**Histology**

Bronchi were fixed at intraluminal pressures of 5, -5 or -20 cmH\(_2\)O in phosphate-buffered formalin saline (10% formalin). Frozen sections (10 µm) were cut and stained with haematoxylin (Ajax, Auburn, Australia) and chromotrope 2R (Gurr; BDH, Poole, UK).

**Solutions**

The Krebs solution used had the following composition (in mM): 121 NaCl, 5.4 KCl, 1.2 MgSO\(_4\), 1.2 NaH\(_2\)PO\(_4\), 25 NaHCO\(_3\), 11.5 glucose, 2.5 CaCl\(_2\). The phosphate-buffered saline had the following composition (in mM): 150 NaCl, 5 NaH\(_2\)PO\(_4\), 15 Na\(_2\)HPO\(_4\) and was buffered to pH 7.2. The drugs used were acetylcholine chloride, carbachol (carbamylcholine chloride) and atropine sulphate, all from Sigma (St. Louis, USA).

**Statistics**

Significance among multiple groups was tested by analysis of variance (ANOVA) with Student-Newmann-Keuls post hoc test. When only two groups were compared, Student’s t-test was used to determine significance. Paired t-tests were used to compare the external closing pressure of relaxed and contracted bronchi. All results are reported as mean±standard error of the mean. A p-value of less than 0.05 was considered to be significant.

**Results**

**Airway collapse**

The distal internal diameter and generation of the bronchi used is shown in table 1. Internal closing pressures for the bronchi of late term foetal (-4.47±0.96 cmH\(_2\)O) and 1 week old (-5.40±0.60 cmH\(_2\)O) pigs were similar. However, intraluminal pressures fourfold more negative (-24.90±1.92 cmH\(_2\)O) were required to close bronchi from 4 week old pigs (p<0.001) (fig. 2). External closing pressures showed a similar difference between age groups, where bronchi from 1 week old pigs closed at much lower pressures (6.86±0.77 cmH\(_2\)O) than bronchi from 4 week old pigs (19.80±1.50 cmH\(_2\)O) (p<0.001) (fig. 3). External and internal closing pressures were not measured in the same bronchi but were comparable in animals of the same age. Carbachol precontraction increased external closing pressure by approximately 5 cmH\(_2\)O in both age groups (p<0.05) (fig. 3).
Histological sections of bronchi from 1 week old pigs appeared significantly deformed when fixed at -5 cmH$_2$O (fig. 4), with a generally flattened appearance and occlusion of the lumen. The cartilage plates of 1 week old bronchi were often bent when fixed near closing pressure. In contrast, bronchi from 4 week old pigs appeared normal when fixed at an intraluminal pressure of -5 cmH$_2$O. However, when fixed at -20 cmH$_2$O, 4 week old bronchi were elliptical rather than circular in shape, and had a flattened lumen with a reduced area. Cartilage plates were seldom bent in airways from 4 week old pigs, but they were generally found to have pulled away from each other and the smooth muscle layer was often separated from the cartilage. This separation was not normally a feature of collapsed bronchi from 1 week old pigs. Serial sections showed that the bronchi from 1 week old pigs were significantly deformed when fixed at -5 cmH$_2$O, with a generally flattened appearance and occlusion of the lumen. The cartilage plates of 1 week old bronchi were often bent when fixed near closing pressure. In contrast, bronchi from 4 week old pigs appeared normal when fixed at an intraluminal pressure of -5 cmH$_2$O. However, when fixed at -20 cmH$_2$O, 4 week old bronchi were elliptical rather than circular in shape, and had a flattened lumen with a reduced area. Cartilage plates were seldom bent in airways from 4 week old pigs, but they were generally found to have pulled away from each other and the smooth muscle layer was often separated from the cartilage. This separation was not normally a feature of collapsed bronchi from 1 week old pigs. Serial sections showed that the bronchi from

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Fig. 4. – Photomicrographs of bronchi from 1 and 4 week old pigs fixed at varying intraluminal pressures. A and B) bronchi of 1 week old pigs fixed at an intraluminal pressure of +5 and -5 cmH$_2$O, respectively; C, D and E) bronchi of 4 week old pigs fixed with intraluminal pressures of +5, -5 and -20 cmH$_2$O, respectively. (Haematoxylin and chromatrope 2R stain; internal scale bar=1 mm).
each age group did not collapse uniformly, but varied in the extent of lumen occlusion along their length.

**Active-passive pressure**

Bronchi from 4 week old pigs produced more active pressure at all passive transmural pressures than bronchi from 1 week old pigs (p<0.001) (fig. 5a). Segments from all age groups produced maximum active pressure at passive intraluminal pressures of 5–10 cmH₂O. Changes in passive intraluminal pressure above 0 cmH₂O produced similar changes in active pressure production (as a percentage of maximum) in all age groups (fig. 5b). Negative pressures, in contrast, produced a more rapid decline in response to EFS in the foetal and 1 week old pigs, compared to 4 week old pigs (figs. 5 and 6). Four week old bronchi showed a steady reduction in active force generation to at least -20 cmH₂O, whereas responses from 1 week old bronchi were abolished by negative intraluminal pressures greater than -5 but less than -10 cmH₂O (fig. 6).

Four out of five bronchi from 1 week old pigs showed an unusual response to EFS at -20 cmH₂O. This response consisted of a large increase in intraluminal pressure, which was maintained even after EFS stopped.

**Videoendoscopy**

When bronchi underwent EFS at a transmural pressure of 0 cmH₂O, the lumen narrowed in an iris like manner, remaining circular but with numerous regularly spaced deep folds in the mucosa (fig. 7A and B). When
intraluminal pressure was reduced to closure, the lumen appeared flattened along a single plane, rather than circular, and mucosal folding was not prominent (fig. 7C). Closure of the bronchial lumen appeared to occur first at a point, with the lumen on both sides of this point narrowed but still patent. EFS of closed bronchi produced a modest opening of the lumen, visible through the endoscope (fig. 7D) in 5 of 6 segments studied, and a generally more circular appearance to the lumen. However, this dilation (of the closed lumen) was slight. Both the constrictor response to EFS at 0 cmH₂O and the dilation of closed bronchi were inhibited by atropine.

Human bronchi

The internal closing pressure, lumen volume and distal internal diameter, of the human bronchi are presented in table 2, together with details of the infants sex and age. Histological sections of closed bronchi from human infants showed a flattened lumen and bending of the membranous airway wall between cartilage plates. Neither bending of the cartilage plates nor separation of the components of the airway wall was apparent in histological sections of closed human bronchi.

Discussion

Pressure induced airway closure

Bronchi from immature animals (late term foetal and 1 week old pigs) were approximately four times more susceptible to airway closure than bronchi from more mature animals (4 week old pigs). Immature bronchi were more susceptible to collapse, regardless of whether luminal or adventitial pressure was changed. The pattern of maturational change in closing pressures was similar to the maturational changes in compliance, where bronchi from late-term foetal and 1 week old pigs are more compliant than bronchi from 4 week old pigs (unpublished data).

Bronchi from human infants were also closed by relatively small transmural pressures, suggesting that infant airways could be more vulnerable to collapse. Compression of infant airways could occur during forced expiration, such as crying and coughing, or while performing a Valsalva manoeuvre (during defaecation [17]). Increased collapsibility of bronchi can elevate closing volume and reduce maximum expiratory flow [17–20]. Both airway closure [21], and flow limitation [22] have been observed in some healthy infants during normal tidal breathing. Collapse of peripheral airways during repeated attempts to clear an obstruction [21], or prolonged expiratory apnoea [23] have been postulated as factors contributing to the SIDS. However, the transpulmonary pressure and lung volume at which airway closure occurs is dependent on the forces applied to bronchi, for example from parenchymal interdependence, as well as the mechanical properties of the bronchi themselves [18–21]. Elastic recoil pressures of immature lungs are low compared to adults [19, 21, 24], suggesting that the forces resisting bronchial closure may be less in the infant than the adult.

Because of lung growth during development, bronchi of the same size and generation cannot be compared among different ages. Changing airway diameter will alter the tension in the airway wall according to the law
of Laplace. In this study, bronchi of similar internal diameters were compared to minimize the influence of Laplace's law. This meant that bronchi from younger animals were taken from more proximal regions of the stem bronchus (table 1). However, the porcine stem bronchus has a similar composition, e.g. in proportion of cartilage, along its length (unpublished observations) and regional differences in compliance reported by others [25], cannot explain the increased collapsibility of the immature bronchi that we have reported here.

**Structural basis of airway collapse**

Bronchi from 1 and 4 week old pigs appeared collapsed when fixed at intraluminal pressures of -5 and -20 cmH\(_2\)O, respectively, which corresponds well with the measured closing pressures. Human bronchi also showed evidence of collapse when fixed at a transmural closing pressure close to their internal closing pressure. Hence, the histological appearance of the bronchi is in accord with the novel method that we have used to assess bronchial closure *in vitro*.

Collapsed bronchi from 1 week old pigs showed bending of the cartilage plates but little separation of the smooth muscle from the cartilage or between cartilage plates. In contrast, cartilage plates of collapsed bronchi from 4 week old pigs showed little bending, but the smooth muscle and underlying structures were often pulled away from the cartilage. The cartilage plates were also separated from each other in bronchi from 4 week old pigs that were fixed at -20 cmH\(_2\)O. This separation of airway wall structures seen in four week old bronchi has previously been observed in sections of adult cat bronchi fixed with a negative intraluminal pressure [6]. Closure of immature bronchi may occur at lower transmural pressures because the cartilage plates are more pliable [26], and bend easily under low pressures. In the 4 week old pigs, increased cartilage stiffness may prevent bending of the plates, and collapse may not occur until the transmural pressure is high enough to cause separation of the components of the airway wall. Human infant bronchi showed neither wall separation nor cartilage bending, with collapse appearing to occur by bending between the cartilage plates. The membranous regions, of the human bronchi, between plates of cartilage, appear to act as a hinge allowing the airway to flatten during collapse.

Endoscopy and histology showed that closure of the lumen was focal, not uniform along the bronchus, which means that bronchi will close while the lumen is still partly filled. Focal closure of bronchi implies that the *in vitro* closing pressure of a bronchus cannot be predicted from the pressure-volume curve of that airway, as closure will occur before the pressure-volume curve reaches zero volume. The interpretation of bronchial compliance curves at negative intraluminal pressures may be complicated by focal collapse and fluid/gas trapping in bronchial segments.

**Effect of smooth muscle tone on airway stability**

The effect of smooth muscle tone on the collapsibility of immature bronchi has not been studied previously. Closing pressures were significantly increased by precontraction, although this increase was small compared to the effect of tone on tracheal collapse [4, 5, 6, 9, 10]. Structural differences between trachea and bronchi may explain why smooth muscle contraction was less effective at stabilizing bronchi than trachea. The trachealis pulls the ends of the cartilage together forming a solid cylinder [6, 7]. Bronchial smooth muscle tone appears unable to produce a similar solid ring of cartilage. This may be due to the multiple joints between cartilage plates or because bronchial smooth muscle is not directly attached to cartilage.

Although the increase in closing pressure produced by carbachol was small in absolute terms, an increase of about 5 cmH\(_2\)O represents an approximate doubling in the closing pressure of the immature bronchi. Hence, constriction of bronchial smooth muscle may be relatively more important for stabilizing immature than mature bronchi.

**Effect of bronchial stability on preload and smooth muscle operating length**

The ability of airway smooth muscle to generate tone is itself influenced by the transmural pressure and airway compliance [13], as these factors affect the preload applied to the muscle. Tracheal segments treated with papain, require higher transmural pressures to stretch the trachealis to optimum length [13]. Furthermore, after papain, the force generated by trachealis contraction changes more sharply as the resting transmural pressure is altered, implying that the smooth muscle operating length is more dependent on the resting pressure [13]. Due to their higher compliance, we expected force generation by immature airways to be more dependent on the resting transmural pressure than bronchi from older animals. However, the active *versus* passive pressure curves for all age groups were the same for positive intraluminal pressures. One possible explanation for this could be a change in the length-tension properties of bronchial smooth muscle during development that compensated for the change in compliance.

Although the active-passive pressure curves were similar at positive pressures, immature bronchi were unable to maintain contraction at negative intraluminal pressures. This impairment of force generation in the younger pigs was probably due to bronchial collapse at negative intraluminal pressures.

**Bronchial dilation produced by smooth muscle contraction**

Bronchi from 1 week old pigs showed an unusual response to EFS at an intraluminal pressure of -20 cmH\(_2\)O (see Results), where the detected pressure increased during EFS and remained elevated after stimulation was discontinued. We hypothesized that any opening of the lumen, however small, during EFS would result in pressure equalising between the two ends of the segment, producing an increase in the detected intraluminal pressure that would be sustained after stimulation ended. In response to EFS, the closed lumen became more circular producing an opening of the lumen visible with endoscopy.
Dilation of the closed bronchi was atropine sensitive, indicating that the dilation was produced by increased muscle tone. The degree of luminal dilation produced in these experiments was generally small, and probably insufficient to allow significant flow. However, this extreme case may be an example of the potential protective effect of excitatory neurotransmission to airways. This study demonstrated that closure of large conducting bronchi from immature animals and human infants can occur with a transmural pressure difference less than 10 cmH2O. The low pressure difference that can occlude immature bronchi suggests that these airways could be easily compressed during respiratory manoeuvres, such as crying or coughing. Closure of the lumen and fluid/gas trapping in bronchi, in vitro, may complicate the interpretation of bronchial pressure-volume curves. Bronchial smooth muscle tone has some influence on bronchial closure, which is relatively more important for the immature animals.

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References