Supplemental digital content 1:

Additional experiments for selectivity of acid instillation.

Methylene blue protocol

In order to verify the selectivity of acid instillation and to exclude a contamination of the left lung, especially in higher V_T group (mice subjected to right hydrochloric acid instillation and mechanical ventilation with V_T of 25 ml/kg, HCl-VILI$_{25}$), we treated 8 animals with a bolus of 1.5 ml/kg of Methylene Blue administered in the same way used for hydrochloric acid (HCl) instillation. Four mice were ventilated with V_T of 15 ml/kg (Blue-VILI15), 4 mice with V_T of 25 ml/kg (Blue-VILI15). After 1 hour of mechanical ventilation, mice were euthanized and thorax was opened. We first clamped the left main bronchus and then removed the right lung and, at last, the left one.

The lung were separately processed: they were weighted, homogenized and centrifuged (2000 rpm for 10 min) to remove debris. The absorbance of each sample was measured at 650 nm and 740 nm. We considered the difference of the absorbances at the two wave length. Data were expressed as ratio of the adjusted absorbance of the right lung to that of the left lung.

Results

One hour after Methylene Blue instillation the excised right lungs showed a well delimited blue area (typically in the inferior lobe, see Fig 1A). Macroscopically, we did not observe blue in the left lung neither for the Blue-VILI$_{25}$ nor for the Blue-VILI$_{15}$ group.

The volume instilled resulted similar: we instilled 50±6.6ul of Methylene Blue in Blue-VILI$_{15}$ group (1.5 ml/kg; body weight: 33±4 g) and 49.5±3.9 µl in Blue-VILI$_{25}$ group (body weight: 33±2 g).

We did not observe differences between groups in adjusted absorbance in the left lung (absorbance at 650 nanometers minus absorbance at 740 nanometers) that was significantly lower than that of right lung, for both group (Fig 1B). The ratio of the adjusted absorbance of the right lung to that of the left lung resulted similar (t test: p=0.7) (Fig 1C).

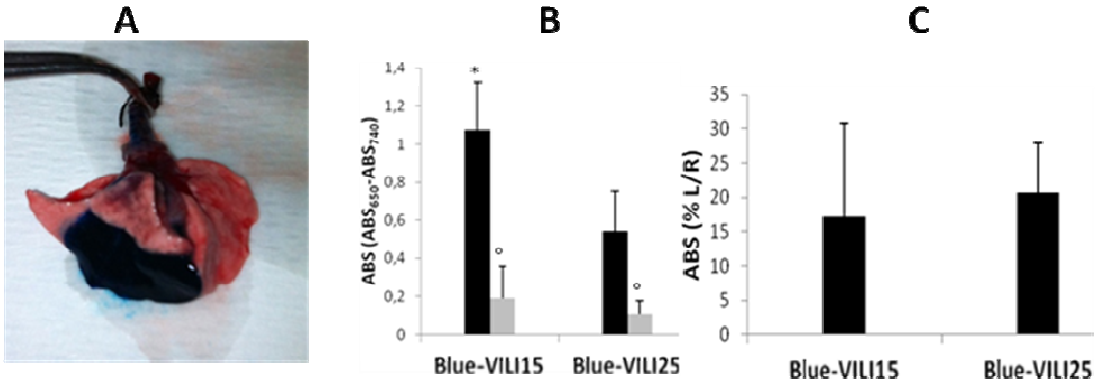

Fig 1: Panel A. Lung excised one hour after Methylene blue instillation in a Blue-VILI$_{25}$ mouse. Blue tissue was just confined in one right lobe. No macroscopically evident blue tissue was seen in left lung.

*Panel B. Absorbance (ABS) of the right lung (black bars) and the left (grey bars) lung. We considered the difference between ABS at 650 nm and ABS at 740 nm (ABS$_{650}$ -ABS$_{740}$). Data are expressed as mean±SD. * p<0.05 vs right lung of Blue-VILI$_{25}$; ° p<0.05 vs right lung of the same group. n=4/group.*

Panel C. Absorbance (ABS) expressed as ratio of the adjusted ABS of the right lung to that of the left lung. We considered the difference between ABS at 650 nm and ABS at 740 nm. n=4/group.