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Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

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Reports on experiments relating to long term projects

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Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
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Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Quantitative imaging of regional lung ventilation redistribution in response to IV methacholine in anesthetized and ventilated rabbit	**Experiment number**: MD213
Beamline: ID17	**Date of experiment**: from: 29/06/2006 to: 04/07/2006	**Date of report**: 20/09/2007
Shifts: 15	**Local contact(s)**: Christian Nemoz	*Received at ESRF:*

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Report:

The experiment was the continuation of the Long Term Project (LTP) for the period February 2004 to February 2006. The background and first results of the project are described in detail in an earlier report covering the period 2004-2006 (experiment series number MD76).

Background and aim of the study:

Airway narrowing is the hallmark of obstructive lung diseases such as asthma and COPD (Chronic Obstructive Pulmonary Disease), leading to regional abnormalities in lung ventilation. Histamine, an inflammatory mediator involved in the pathologic mechanism of airway narrowing, and methacholine, a cholinergic agonist with similar effects, are routinely administered as an aerosol in patients as a diagnostic test for asthma. Traditional measurements of lung function such as spirometry can at best provide overall assessments and do not give any insight into the heterogeneity of airway narrowing, nor to the determinants of this heterogeneity.

We recently introduced a novel CT imaging technique that uses synchrotron radiation to quantitatively image inhaled stable xenon gas within the airways with a high spatial resolution (1). Using this method, regional lung volume (2), ventilation (3), and airway luminal diameters down to 2 mm can be measured. The spatial resolution of this technique is the best available for regional ventilation imaging, allowing quantitative measurements of regional ventilation in small animal models such as rabbit.

We have found that histamine aerosol bronchial provocation induces patchy abnormalities of regional lung ventilation (4), the severity of this phenomenon being itself unequal depending on the anatomic location within the lungs, but highly reproducible. The local heterogeneity in lung ventilation is of major importance in the pathophysiology of asthma since it induces hypoxia through the mismatch of ventilation and perfusion, and since it is a significant challenge to get drugs administered as an aerosol to the most constricted lung

zones. However, the mechanisms of this patchy distribution remain a matter of considerable debate. One recurring hypothesis is that uneven convective transport of histamine aerosol particles can itself participate in the ventilation heterogeneity following histamine aerosol administration.

The goal of the present experiment was to address the above questions of pathophysiology and treatment of asthma by studying the changes in regional lung ventilation induced by methacholine administration through the intravenous route, therefore in the pulmonary circulation, and as an aerosol. The effect of IV methacholine is largely documented in the literature providing reference data in the rabbit model, and the effect of methacholine aerosol on lung mechanics is highly similar to that of histamine. Our working hypothesis is that aerosol distribution should have little effect on the patchy defects in regional ventilation following induced airway constriction, therefore IV and aerosol routes should both produce regional heterogeneity in ventilation. The secondary goal of this study was to determine correct dose and administration route for future experiments with sensitized animals (ongoing LTP project MD238).

Experiments

The experiment was performed in 10 anesthetized and mechanically ventilated New Zealand rabbits divided in two groups: aerosol methacholine vs. IV methacholine followed by aerosol methacholine, N = 5 in each group. Anesthesia was induced by IV sodium thiopental and maintained using inhaled isoflurane. The animals were placed in a custom-made plastic holder for immobilization, and the holder will be fixed on the translation-rotation platform usually used at ID17 for this type of experiment. Distributions of ventilation were determined using the K-edge subtraction (KES) method, where absolute density of the contrast agent can be measured. In order to facilitate comparison with earlier experiments, where histamine aerosol was used, the same protocol and imaging sequence was used. CT images were taken at 3 different lung levels and imaging was repeated at regular intervals before and after methacholine administration. IV methacholine doses varied from 5.0 to 40.0 µg/kg/min. Methacholine aerosol was produced from 125mg/ml solution, and the provocation lasted one minute. Physiological parameters, such as blood pressure, tracheal pressure, ventilation flow and tidal volume were recorded during the experiment. Full protocol was performed in following rabbits:

- 4 rabbits with IV and aerosol methacholine, excluding one rabbit that was lost before the protocol was finished. The methacholine dose varied between the rabbits.
- 5 rabbits with aerosol methacholine.

Results

Preliminary analysis of the results suggests that IV methacholine has a larger effect in the central airways.Peripheral ventilation defects were observed at higher doses.

Methacholine aerosol seems to predominantly affect peripheral airways. Images reveal peripheral ventilation defects, but the aerosol causes constriction in the central airways as well. Compared to the effect of histamine aerosol, methacholine has faster and stronger effect on the central airways, although the effects on the periphery of the lungs are similar. A more complete analysis of the results is needed, particularly to compare findings with KES-CT imaging and lung mechanics parameters. A secondary result of this experiment is the study of the circulatory effects of methacholine by systematic analysis of the calibers of several generations of pulmonary arteries.

Figure 1 shows results in one representative rabbit.

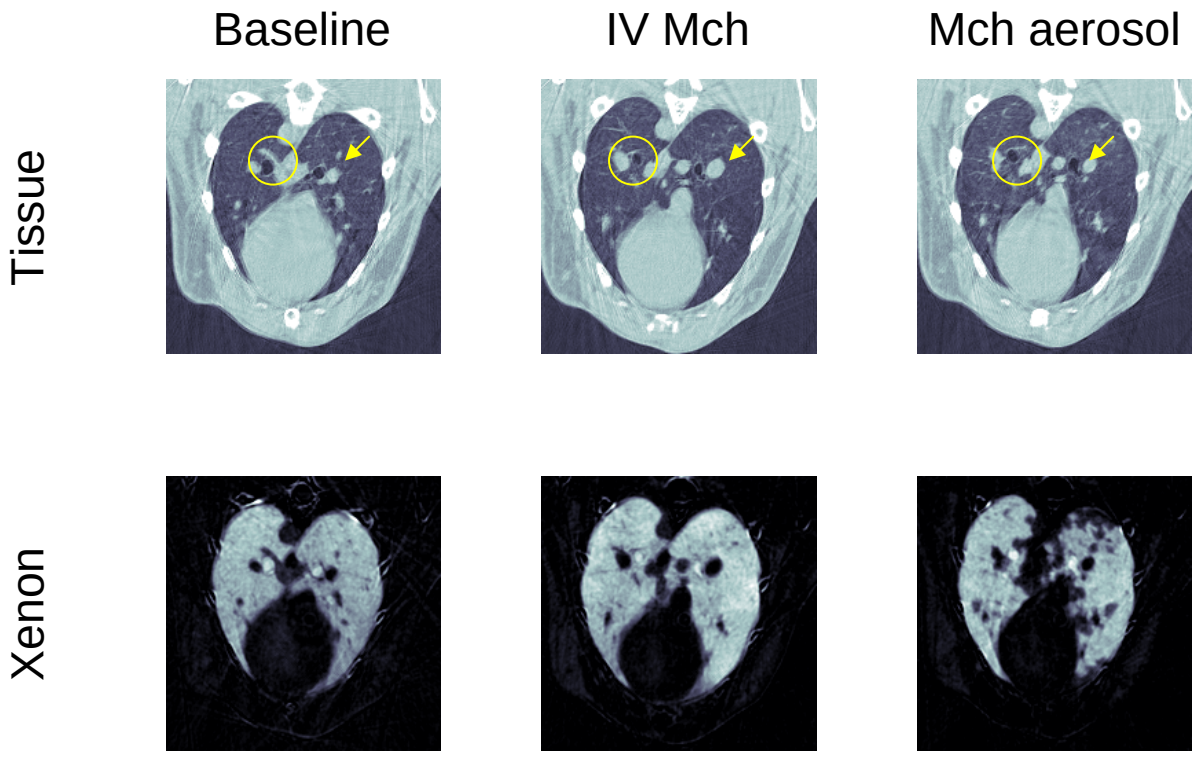

Figure 1: Transmission CT images and KES xenon images before and after methacholine provocation in one animal. Changes in the airways and blood vessels are visualized in transmission images in the upper row. After IV methacholine, large airways constrict (marked by a yellow circle) and the blood vessels dilate (marked by a yellow arrow). After methacholine aerosol, effects in the central airways are smaller. Changes in the ventilation at the periphery of the lungs are best visualized in KES images in the lower row. IV methacholine does not have large effect on the periphery of the lungs; however methacholine aerosol causes ventilation defects on the periphery of the lungs and increases the heterogeneity of ventilation.

Discussion

The results of this experiment go against our initial hypothesis, suggesting that some heterogeneity of regional ventilation is due to aerosol deposition in the lung periphery. These results were used to determine the route of methacholine challenge used in the following experiments with sensitized animals (MD238). In these experiments we decided to use IV methacholine infusion, and doses of 2.5, 5.0 and 10.0 µg/kg/min. Analysis of those results are currently underway, and will be published together with the results obtained in later experiments.

References

1. **Bayat S, Le Duc G, Fiedler S, Berruyer G, Nemoz C, Thomlinson W, Porra L, Suortti P, Grimbert F, Standertskjöld-Nordenstam CG, Sovijärvi ARA.** Quantitative functional lung imaging by synchrotron radiation using stable xenon gas as contrast agent. *Phys Med Biol*. 2001 ; 46 :3287–3299.
2. **Monfraix S, Bayat S, Porra L, Berruyer G, Nemoz C, Thomlinson W, Suortti P, Standertskjöld-Nordenstam CG, Sovijärvi ARA.** Measurement of Absolute Regional Lung Volume Using Spiral Synchrotron Radiation Computed Tomography. *Phys Med Biol.* 2005; 50 :1 - 11.
3. **Porra L, Monfraix S, Berruyer G, Nemoz C, Thomlinson W, Suortti P, Sovijärvi ARA, Bayat S.** Effect of tidal volume on ventilation distribution in rabbits; High resolution quantitative assessment with synchrotron radiation computed tomography. *J Appl Physiol.* 2004; 96: 1899–1908.
4. **Bayat S, Porra L, Suhonen H, Nemoz C, Suortti P, Sovijarvi AR.** Differences in the time course of proximal and distal airway response to inhaled histamine studied by synchrotron radiation CT. *J Appl Physiol.* 2006 Jun;100(6):1964-73.