Metabonomics in the evaluation of lung fibrosis

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Introduction
- Pulmonary fibrosis corresponds to the deposition of cicatrictial tissue in the lung. Several causes may lead to this condition, including medication, interstitial pneumonia or asbestos exposure. This transformation is accompanied by a loss of lung function which can be fatal.
- Metabonomics can be used to find small changes in the metabolism that appear under pathological condition. The detection of biomarkers characteristic of such troubles is the primary goal of this new technology.
- There is no data in the literature about metabonomics in pulmonary fibrosis.
- This is well known that collagen synthesis is associated with a production of proline and glycine.
- The main goal of the present study is to isolate a biomarker of pulmonary fibrosis in Broncho Alveolar Lavage Fluid (BALF). To do so, metabonomics profile of BALF from bleomycin-induced fibrotic rats were done.

Material and Methods
- In rats, 3IU of bleomycin was injected intratracheally and animals were sacrificed after different periods of time (3, 7, 14 and 21days). A control group was injected intratracheally with a saline solution.
- Physiological saline (8x 5cc) was instilled in the lungs and the bronchoalveolar fluid recovered was centrifugated (250g for 10 min, 4°C) and the supernatant was stored at -80°C.
- Nuclear Magnetic Resonance (NMR) spectral analysis of BALF was performed at 300MHz on a Bruker instrument.
- 7mL of the BALF was lyophilised and prepared for the analysis with addition of 800μL of D₂O and 50μL of TSP to the lyophilisat. D₂O were used to avoid the water peak.
- Residual water peak was suppressed by using a saturation pulse. 64 scans were done by acquisition.

Results

Proton NMR spectra of the aliphatic region of BAL fluid. Time before rat sacrifice is noted close to the spectra. TSP is at 0ppm.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>frequency (ppm)</th>
<th>multiplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-hydroxybutyrate</td>
<td>1.15/d, 2.34/m</td>
<td>2.42/m</td>
</tr>
<tr>
<td>lactate</td>
<td>1.33/d, 4.12/q</td>
<td>1.48/d, 3.78/m</td>
</tr>
<tr>
<td>alanine</td>
<td>1.95/s</td>
<td></td>
</tr>
<tr>
<td>proline</td>
<td>2.07/m, 2.35/t,</td>
<td>3.33/m</td>
</tr>
<tr>
<td>creatine</td>
<td>3.04/s</td>
<td></td>
</tr>
<tr>
<td>choline</td>
<td>3.21/s, 3.52/m</td>
<td></td>
</tr>
<tr>
<td>betaine</td>
<td>3.25/s, 3.89/s</td>
<td></td>
</tr>
<tr>
<td>glycine</td>
<td>3.58/s</td>
<td></td>
</tr>
<tr>
<td>β-glucose</td>
<td>3.25/m, 3.40/t,</td>
<td>3.45/m, 3.70/m</td>
</tr>
<tr>
<td></td>
<td>3.90/m</td>
<td></td>
</tr>
</tbody>
</table>

A transient increase in some peaks corresponding to a change in β-hydroxybutyrate, lactate, alanine, proline, choline, betaine and glycine is observed.

Conclusion
- Our results obtained in the BALF underlined a transient increase in proline, glycine and its precursors (choline and betaine) which is supposed to reflect the production of collagen.
- Complementary analyses are necessary to confirm the identification and the concentration of these metabolites. The correlated variation will be studied with multivariated analyses that allow the recognition of the biochemical pathways involved in the present process.

Perspectives
- Labelled amino acid precursors could be administrated with bleomycin to confirm the proposed interpretation. Histological observation of the lung can then confirm the presence of these compounds in the lung and if the glycine come from the metabolism of choline.

References
- Gerhard M, Biochemical Pathways, an atlas of biochemistry and molecular biology. Wiley, 1999