

## Detection of oxidative stress from bronchoalveolar lavage fluid in animal model

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Oxidative stress results from an oxidant/antioxidant imbalance in favour of oxidants. A large number of studies have demonstrated that increased oxidative stress occurs in airways diseases, but there is now substantial evidence that it really plays an important role in the injurious and inflammatory responses in acute lung injury and in asthma. We developed a model that allows the quantification of the airway responsiveness (BHR) and the characterizations of the BAL cellular profile repeatedly within the same rat. The detection of carbonyl proteins (CP) is a wide spread method to detect oxidative stress, where the values are increased. In the present study with the help of our method we examined, whether oxidative stress measured by CP plays a role in the pathomechanism of different lung abnormalities, so acute lung injury and asthma in animal model.

We studied 28 male white, Wistar rats (weight range 350–500 g). The animals were housed in a healthy colony and allowed food and water ad libitum. Anesthesia was induced, intubations were performed, muscle relaxation was achieved and mechanical ventilation was used, BALF was collected. The BAL fluid was then centrifuged and CP was detected by spectrophotometry method. Acute lung injury was caused by intraperitoneal (ip) injection of E Coli lipopolisaccharide (LPS) and asthma was induced by the combination of ip. and inhalative ovalbumin (OVA).

The acute lung injury caused by LPS was due together with significant oxidative stress which was reflected by the increase of the CP values in BALF. OVA sensitization was connected with a less significant oxidative stress right after the procedure, but the CP values increased further until the next measurements, one month later (12,9 vs 13,9 vs 16,4).

The results confirm the role of oxidative stress in the pathomechanism of acute lung injury and asthma in rats. Our method is suitable to detect the oxidative stress in animal model and will be also suitable later to investigate the protective effects of different antioxidants substances.

## Examination of biochemical parameters of oxidative stress in childhood asthma

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Increased levels of reactive oxygen intermediates (ROI) and cellular injury have been implicated in many pulmonary diseases including COPD, cystic fibrosis and asthma bronchiale. According to earlier examinations, the production of ROI was elevated in the asthmatic airways due to the action of macrophages, eosinophils and neutrophils, which led to bronchial hyperresponsiveness and caused more severe airway inflammation. ROI also play important roles in the modification of immune response and in the structural alterations of the lung parenchyma.

The aim of our study was to analyse the alterations of several biochemical parameters such as malondialdehyde (MDA), carbonylated proteins, ratio of oxidized/reduced glutathione, vitamin E concentration, total antioxidant capacity and activities of antioxidant enzymes (catalase, glutathione reductase, glutathione peroxidase, superoxide dismutase). The blood samples were obtained from asthmatic children (n=21) and healthy controls (n=12). The clinical state of the patients was compared to the examined biochemical markers.

According to our results, elevated oxidative stress was observed in asthmatic patients even with stable clinical condition. Elevated MDA (mean. 0,86 vs. 0,58 nM/mg prot.) and carbonylated proteins (mean.  $4,711 \times 10^{-2}$  vs.  $3,859 \times 10^{-2}$   $\mu$ g/mg prot.) levels were detected from the blood samples of the patients versus controls.

Our results also proved the presence and importance of oxidative stress in asthma and the possible use of biochemical parameters in the clinical practice. Additionally we set out to examine the expression of the gp91phox subunit of NADPH oxidase enzyme and HO-1 enzyme from blood.