Circulating and Exhaled Markers of Nitric Oxide and Antioxidant Activity After Smoking

To the Editor:

We read with interest the article by Tsuchiya et al showing that smoking a single cigarette results in a significant acute reduction in plasma nitrite/nitrate (NOx) and antioxidant defenses. The pathway by which cigarette smoke inhalation causes systemic oxidant stress presumably begins within the lungs, but until recently, simple methods of detecting inflammatory markers from the respiratory tract were not available. Techniques such as bronchoalveolar lavage and induced sputum are invasive and unsuitable for investigating the effect of an acute stimulus. Measurement of exhaled NO concentrations have yielded contradictory results. Exhaled breath condensate (EBC) collected by cooling exhaled air is, however, noninvasive and does not influence airway inflammation, making it ideally suited to this purpose.

We have recently investigated the effects of smoking on systemic and EBC markers of NO synthesis and antioxidant status. In 12 otherwise healthy smokers (5 men, age 22.3 ± 3.8 years), NOx and glutathione were determined in both serum and EBC immediately before and 45 and 225 minutes after smoking 2 cigarettes. The same markers were measured in 12 healthy non-smokers (6 men, 21.0 ± 0.7 years) to assess the effects of habitual cigarette smoking.

At baseline, smokers had an elevated glutathione concentration in serum and in EBC compared with non-smokers (serum: 5.6 ± 0.9 μmol/L versus 3.2 ± 0.3 μmol/L, P < 0.05; EBC: 40.0 ± 0.8 μmol/L versus 12.5 ± 0.8 μmol/L, P < 0.05). Forty-five minutes after smoking, glutathione was significantly reduced in both serum and EBC (serum: 2.2 ± 0.7 μmol/L, P < 0.01; EBC: 20.0 ± 0.5 μmol/L, P < 0.05). Serum NOx was similarly reduced after smoking (39.0 ± 0.7 μmol/L versus 32.5 ± 0.7 μmol/L, P < 0.01), whereas EBC NOx remained unchanged. Serum and EBC glutathione and serum NOx returned to near baseline concentration by 225 minutes.

Our findings suggest that the acute oxidant effects of smoking are followed by depletion of local as well as systemic antioxidants, and that long-term smoking may lead to upregulation of the important antioxidant compound glutathione.

Although we did not demonstrate a change in EBC NOx at 45 or 225 minutes after smoking, the concentration of exhaled nitric oxide metabolites is difficult to interpret. Exhaled NOx is predominantly derived from airway epithelial and inflammatory cells rather than the pulmonary circulation.

We propose that EBC is a useful tool to investigate the mechanisms by which pulmonary insults contribute to cardiovascular morbidity and mortality. The systemic oxidative stress caused by smoking seems to occur in association with pulmonary antioxidant consumption. The pulmonary contribution to systemic inflammation and oxidant stress after smoking deserves further investigation.

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Response

In reviewing our article,1 Dr Nuttall et al have addressed an important issue concerning oxidative stress and resultant systemic nitric oxide (NO) reduction induced by cigarette smoke (CS). As they mentioned, CS induces these effects through its inhalation into the lungs. Theoretically, lung cells are initially attacked by the reactive oxygen species contained in CS.2 Thus, investigation of oxidative stress in lung cells may be essential for further understanding the mechanism of CS-induced systemic effects. This non-invasive method that enables the collection and analysis of exhaled breath condensate (EBC), which Dr Nuttall et al have introduced, might enable such research. Their preliminary results using this method, of which the portion obtained in serum is in good agreement with our data, seem to effectively demonstrate the increase in oxidative stress in lung coupled with smoking. However, there are two concerns with regard to their study.

The first is that NO is a gaseous molecule, whereas EBC mainly consists of nonvolatile molecules present in exhaled breath.3 Thus, the direct measurement of gaseous NO in breathing air could well reflect the NO amount generated in the lung rather than indirectly measuring NOx (nitrate and nitrite) dissolved in the EBC, although exhaled NO could be somewhat influenced by airway inflammation. In addition, exogenous NO contained in CS may possibly affect the measured value with EBC. Thus, the measurement of NOx in EBC might have limited significance as an index of the amount of NO in the lung, as shown by Dr Nuttall et al.

The second concern is whether or not changes in glutathione concentration in EBC are a reliable marker of oxidative stress in the lung. Because intracellular glutathione levels are extremely high (~10 mmol/L) as previously reported,4 it is assumed that intracellular glutathione functions as an essential antioxidant against intracellular oxidative stress. However, the lower concentrations of extracellular glutathione (~400 μmol/L) and the many other antioxidants in the extracellular space indicate that extracellular glutathione may only have a small functional contribution as one of many. Thus, the extracellular glutathione concentration, even in EBC, is not a sensitive marker of oxidative stress. In this context, the increase in glutathione concentration in EBC in chronic smokers might simply reflect the leakage of intracellular glutathione because of the CS-induced continuous destruction of lung cells. Therefore, as far as these limitations of EBC study are kept in mind, we totally agree with the proposal that EBC could be a useful tool to further investigate pulmonary events induced by CS.

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