

Increased carbon monoxide levels in the nasal airways of subjects with a history of seasonal allergic rhinitis and in patients with upper respiratory tract infection

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Summary

Background Carbon monoxide (CO) has emerged as an endogenously produced gaseous mediator known to be involved in bronchial smooth muscle regulation. Increased amounts of CO have been found in exhaled air during asthma and lower airway inflammation. Recently CO has been shown to be produced in the nasal airways, but there are no reports of altered CO levels in nasal airways during inflammation.

Objective This study was designed to investigate if CO levels increase in the human nasal airways during inflammatory conditions, such as allergy and upper airway respiratory tract infection (URTI).

Methods CO was sampled separately from the upper and lower airways of 13 healthy control subjects, six patients with a history of allergic rhinitis and six patients with URTI.

Results Nasal CO levels were increased in subjects with allergic rhinitis, compared to healthy controls (2.07 ± 0.15 ppm, $n = 6$ and 1.62 ± 0.08 ppm, $n = 13$, respectively, $P < 0.01$). CO levels were also increased in patients with URTI, compared to the same controls (1.92 ± 0.09 ppm, $n = 6$, $P < 0.05$). Normal levels of CO were found in air from the lower airways among subjects with allergic rhinitis, whereas corresponding levels in the URTI patients were increased.

Conclusion The present data demonstrates that upper airway CO levels increase in parallel with different inflammatory stimuli, such as allergy and infection, suggesting a role for CO as marker or mediator of nasal inflammation.

Keywords carbon monoxide, nitric oxide, allergy, rhinitis, infection, inflammation, respiratory tract

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Introduction

Heme oxygenase (HO) is the rate-limiting enzyme responsible for endogenous carbon monoxide (CO) production [1,2]. HO activity and CO production have been demonstrated in various peripheral tissues including the human lung [3–6] and a role for CO in airway relaxation has been proposed [7]. Hypoxic challenge is reported to increase the HO expression in the lung, resulting in a CO dependent bronchodilation, further supporting the idea of CO as a mediator in airway regulation [8]. Increased amounts of CO in exhaled air have been found among patients with asthma during periods of non-steroid treatment, during asthma exacerbation and as the result of allergen challenge, suggesting a role for CO also in lower airway inflammation [9–13].

In a recent study we demonstrated HO-like immunoreactivity in the human nose, in connection with respiratory epithelium, seromucous glands and vascular smooth muscle. We also

demonstrated that the nasal cavity together with the paranasal sinuses markedly contributed to the total CO production of the human airways [14]. There are at least three reports indicating that upper airway inflammation could increase lower airway production of CO [13,15,16], but there are, to our knowledge, no published data concerning upper airway inflammation and nasal CO production. Therefore, the present study was designed to investigate if CO emanating from the nasal airways increases during different inflammatory conditions, such as seasonal allergic rhinitis and upper airway respiratory tract infection (URTI).

Materials and methods

Subjects

Twenty-five non-smoking subjects (aged 40 ± 14 years SD, 5 men) not regularly exposed to smoke passively and without current anti-inflammatory or anti-allergic medication, volunteered for the study. Before inclusion, all subjects were evaluated by an experienced ear, nose and throat consultant, and divided into three groups depending on their medical history. Individuals with a history of chronic rhinitis, nasal polyposis or recurrent sinusitis were excluded. The first group ($n = 6$)

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consisted of subjects with a history of seasonal allergic rhinitis, presenting itself with symptoms like nasal congestion, clear watery discharge, nasal itching and paroxysmal sneezing. They all exhibited a positive skin prick test for timothy pollen and some of them also responded positively when tested for other airborne allergens. The study was performed in September, two weeks after the end of the grass pollen season (according to a local pollen count at the hospital), and none of these subjects exhibited any remaining symptoms of allergy, nor did they show any symptoms of infection. The second group ($n = 6$) consisted of patients with acute URTI. They all displayed signs and symptoms of URTI, such as reddened pharynx and soft palate, nasal congestion and rhinorrhea, painful swallowing, sore throat and sneezing. The control group ($n = 13$) consisted of healthy individuals, without allergic or infectious symptoms and who all responded negatively when tested with a standard panel of 10 common airborne allergens. Approval for this study was acquired from the Local Ethics Committee of the University of Lund and an informed consent was obtained from all subjects.

Skin prick tests

Skin prick tests were performed with a standard panel of 10 common airborne allergens (ALK, Copenhagen, Denmark) including pollen (birch, timothy, artemisia), house dust mites (*D. Pteronyssinus* and *D. Farinae*), moulds (*Cladosporium* and *Alternaria*) and animal allergens (cat, dog and horse). The tests were performed on the volar side of the forearm with saline buffer as negative and histamine chloride (10 mg/mL) as positive controls. The weal reactions were measured after 20 min and designated as 4+, 3+, 2+, 1+ or 0 depending on the size in relation to histamine and saline [17]. All rhinitis patients presented 3 or 4+ reactions towards timothy. All subjects in the control group were negative when tested with the standard panel.

CO measurements

CO was measured as described before by Andersson and colleagues [14]. Briefly, CO in exhaled and sampled air was measured using an infrared analyser (Fisher Rosemount NGA 2000, provided by FLS Airloq AB, Stockholm, Sweden) with a minimum detectable concentration of 0.2 ppm CO. The analyser was calibrated with known concentrations of CO for measurements in the range of 0–10 ppm. Gaseous N₂ (Air Liquide Gas AB, Malmö, Sweden) was used between every set of measurements for baseline verification and zero calibration. All measurements were made at room temperature (22–24 °C) in a non-smoking section of the hospital. Ambient levels of CO were continuously recorded (0.72 ± 0.07 ppm) and subtracted from measured CO-values in order to compensate for alternating background levels. All subjects were seated in an upright position and all measurements were repeated three times with a resting period of 2–3 min in-between.

When CO was sampled from the nose, a nasal olive was gently introduced into the vestibulum nasi and nasal air was drawn into the CO-analyser by a vacuum pump (0.4 L/min). The contralateral nostril was left open, allowing a stream of room air to enter the nose, while the subject was constantly breathing through the mouth. This allows for a small risk of contamination of nasal air with air from the lower airways. However, we have earlier shown that nasal CO levels are higher than CO levels measured from the lower airways, and thus is of no major importance in our nasal CO measurements [14].

When CO was measured in exhaled air, the subjects were instructed to breathe normally through the mouth. After 40 s they were told to inhale maximally and then to immediately exhale, as fast as the resistance in the tubing would allow, in order to provide a stable exhalation flow rate, into a 2 L rubber bag (Silkolatex®, Willy Rüscher AG, Kernen, Germany). The tubing between the subject and the rubber bag was sufficiently narrow to create an exhalation pressure high enough to close the velum, thus preventing air from the nasal airways to contaminate air from the lower airways during these measurements [18]. During the whole procedure a nose-clamp precluded any passage of air in either direction through the nose. The CO content of the bag was instantly analysed, with the mean peak value of each set of three measurements used for calculations and statistical analysis.

Statistics

All results are expressed as means $\pm$ SEM unless stated otherwise. Statistical comparisons were made by Student's t-test and P -values < 0.05 were considered statistically significant.

Results

When CO was continuously sampled from the nose, while subjects were breathing through the mouth, stable levels of

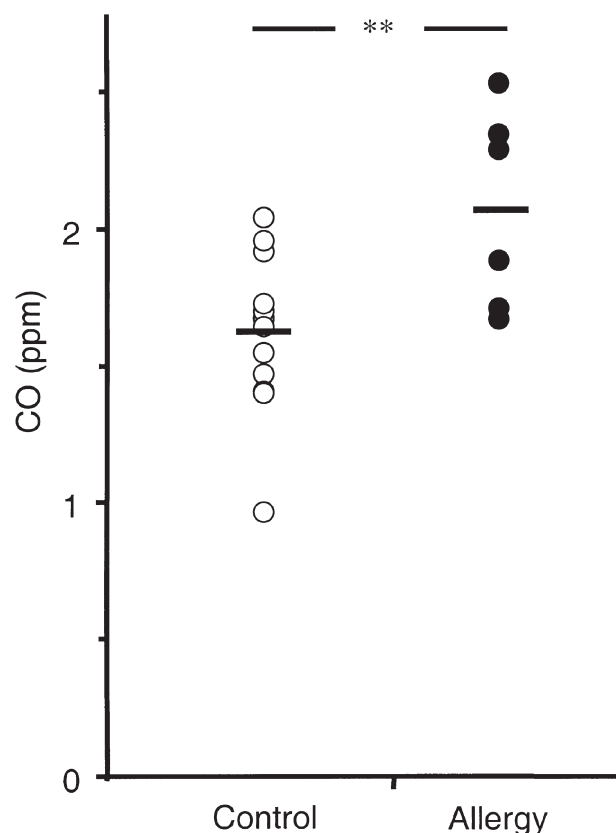


Fig. 1. CO in the nasal airways, subjects with allergic rhinitis vs. healthy controls. Nasal CO levels continuously sampled with a nasal olive in the vestibulum nasi. Six consecutive measurements were performed on each subject, alternating between the left and right nostril. Subjects with non-symptomatic allergic rhinitis (●, $n = 6$) compared to a healthy control group (○, $n = 13$). Horizontal bars represent means. ** $P < 0.01$.

CO were obtained within 40 s in all subjects. Repeated measurements indicated only minor variations in the obtained values. The nasal CO levels in allergic rhinitis patients were higher than corresponding CO levels in the healthy, non-allergic control group (2.07 ± 0.15 ppm, $n=6$ and 1.62 ± 0.08 ppm, $n=13$, respectively, $P<0.01$; Fig. 1). In patients with URTI, nasal CO levels were higher than in the same control group (1.92 ± 0.09 ppm, $n=6$ and 1.62 ± 0.08 ppm, $n=13$, respectively, $P<0.05$; Fig. 2).

In exhaled air emanating from the lower airways, there was no difference in CO levels for the patients with seasonal allergic rhinitis when compared to healthy controls (2.15 ± 0.14 ppm, $n=5$ and 2.09 ± 0.11 ppm, $n=9$, respectively, Fig. 3). This was in clear contrast to patients with URTI where increased CO levels, compared to the same controls, were found (2.44 ± 0.14 ppm, $n=6$ and 2.09 ± 0.11 ppm, $n=9$, respectively, $P<0.05$; Fig. 4).

Discussion

The presented data confirms previous findings of a steady, continuous production of CO within the nasal airways [14]. Furthermore, we now demonstrate that these CO levels are

increased postseasonally in patients with allergic rhinitis and during URTI.

Increased CO production in the nasal airways has not previously been demonstrated, neither as the result of allergic inflammation, nor during airway infections. However, increased CO levels, in air emanating from the lower airways, have been reported in patients with symptomatic seasonal allergic rhinitis and in patients with URTI [15,16]. Furthermore, increased levels of CO in the exhaled air have been reported among patients with asthma during periods of non-steroid treatment, during asthma exacerbation and after allergen challenge [9–13]. Altogether, these findings support a role for CO in airway inflammation.

The present investigation was performed two weeks after the end of the pollen season (according to a local pollen count) and none of our rhinitis patients exhibited any persisting signs or symptoms of allergy. The increased CO levels found in the nasal airways might therefore represent a persistent inflammation in the nasal mucosa. Evidence for persistent eosinophil activation and constant up-regulation of proinflammatory cytokines in the nasal mucosa after the ending of the pollen season has previously been reported in patients with seasonal allergic rhinitis [19]. We found no increase of lower airway CO levels in the rhinitis group. This might reflect the absence of inflammation in the lower airways postseasonally, indicating that the nasal airways are the primary focus of inflammation as well as CO production in allergic rhinitis.

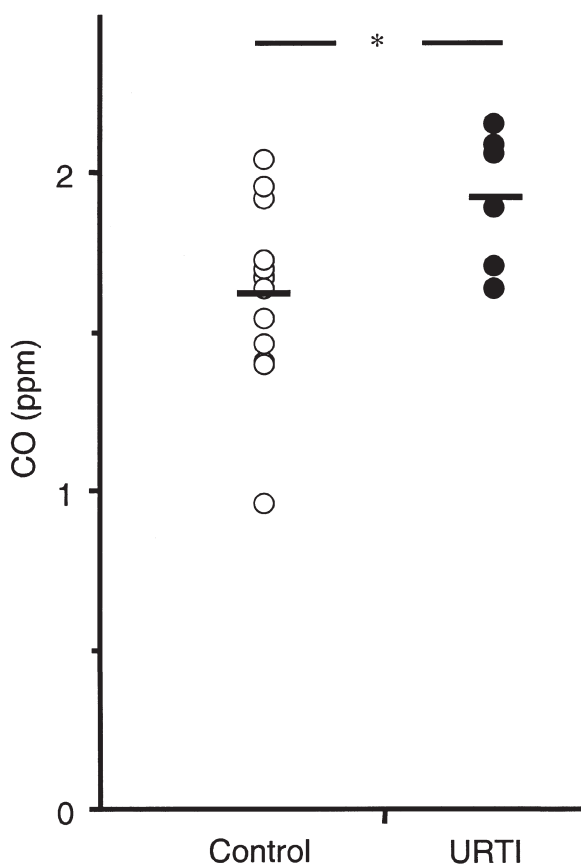


Fig. 2. CO in the nasal airways, patients with URTI vs. healthy controls. Nasal CO levels continuously sampled with a nasal olive in the vestibulum nasi. Six consecutive measurements were performed on each subject, alternating between the left and right nostril. Patients with URTI (●, $n=6$) compared to a healthy control group (○, $n=13$). Horizontal bars represent means. * $P<0.05$.

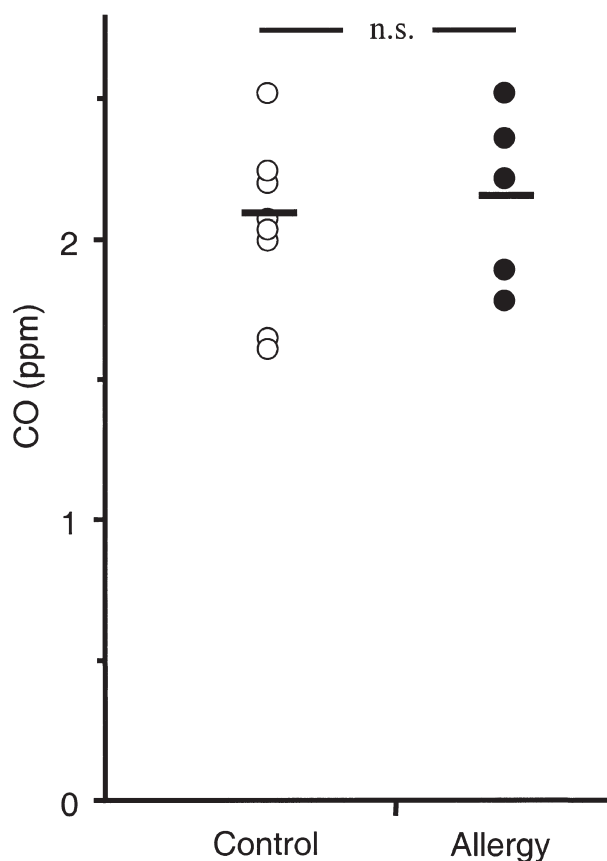


Fig. 3. CO in the lower respiratory tract, patients with allergic rhinitis vs. healthy controls. CO levels measured in exhaled air, emanating from the lower airways. Subjects with non-symptomatic allergic rhinitis (●, $n=5$) compared to a healthy control group (○, $n=9$). Horizontal bars represent means.

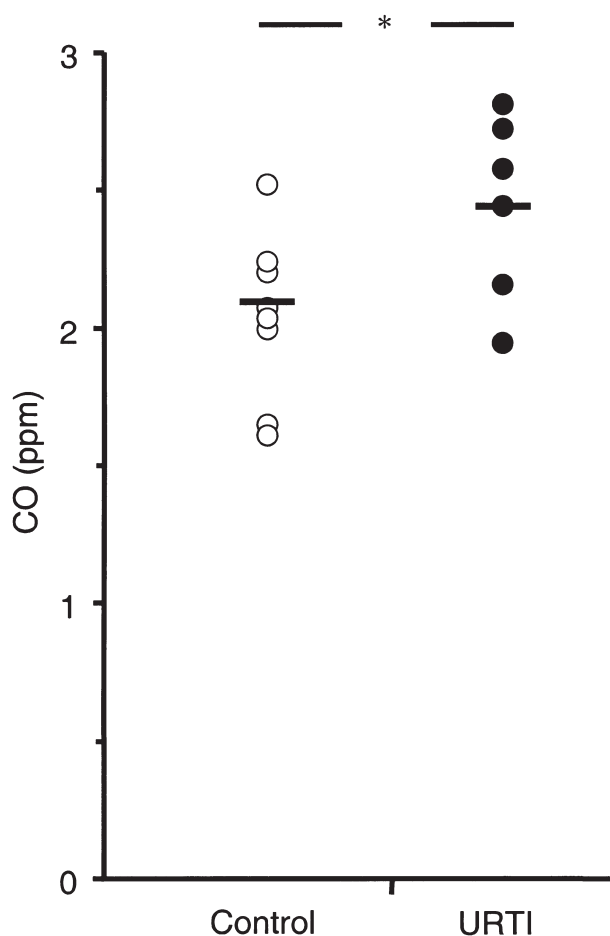


Fig. 4. CO in the lower respiratory tract, patients with URTI vs. healthy controls. CO levels measured in exhaled air, emanating from the lower airways. Patients with URTI (●, $n = 6$) compared to a healthy control group (○, $n = 9$). Horizontal bars represent means. * $P < 0.05$.

In the present study, patients with URTI displayed an increase of CO levels in air sampled both from the upper and lower airways. The URTI patients exhibited infectious symptoms mainly from their upper airways, but our finding of increased CO levels emanating from the lung, might reflect the involvement of the lower airways in these infections. Such lower airway activity has previously been demonstrated in URTI [16]. However, the focus of the inflammation must, as the name implies and our results indicate, be located in the upper airways.

A number of studies have put forward CO as a new inflammatory marker in the lower airways, mainly since it shares a number of properties with nitric oxide, a well established inflammatory marker in both the upper and lower airways. The ability of the nasal airways to increase CO production during allergic rhinitis and URTI strongly suggests a role for CO also as a marker or mediator of nasal inflammation. Thus, several studies need to be undertaken in order to comprehend in full the role of CO in human airways.

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