

Relation between exhaled carbon monoxide levels and clinical severity of asthma

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Summary

Background Carbon monoxide (CO) can be detected in exhaled air and is increased in asthmatic patients not treated with corticosteroids. However, it is uncertain whether exhaled CO is related to severity of asthma.

Objective and methods To study whether exhaled CO is related to severity of asthma in clinical courses, exhaled CO concentrations were measured on a CO monitor by vital capacity manoeuvre in 20 mild asthmatics treated with inhaled β_2 -agonists alone, 20 moderate asthmatics treated with inhaled corticosteroids, and 15 stable asthmatics treated with high dose inhaled corticosteroids and oral corticosteroids once a month over 1 years. Exhaled CO concentrations were also measured in 16 unstable severe asthmatics who visited the hospital every 7 or 14 days for treatment with high dose inhaled corticosteroids and oral corticosteroids.

Results The mean values of exhaled CO in severe asthma over 1 year were 6.7 ± 9.5 p.p.m. ($n = 31$, mean $\pm$ SD) and significantly higher than those of non-smoking control subjects (1.2 ± 0.9 p.p.m., $n = 20$, $P < 0.01$). Exhaled CO concentrations in unstable severe asthmatics were significantly higher than those in stable severe asthmatics. However, exhaled CO concentrations in mild and moderate asthmatics did not differ significantly from those in non-smoking control subjects ($P > 0.20$). There was a significant relationship between the exhaled CO concentrations and forced expiratory volume in one second in all asthmatic patients.

Conclusion These findings suggest that exhaled CO concentrations may relate to the severity of asthma and measurements of exhaled CO concentrations may be a useful means of monitoring airway inflammation in asthma.

Keywords: heme oxygenase, nitric oxide, airway inflammation, carbon monoxide, asthma

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Introduction

Carbon monoxide (CO) is produced endogenously by the class of enzymes known collectively as heme oxygenase (HO) [1]. HO-1 is induced by various stimulants including proinflammatory cytokines and nitric oxide [1]. CO is

known to be present in measurable quantities in the exhaled air of normal subjects [2,3]. Exhaled CO is increased in patients with inflammatory pulmonary diseases such as bronchial asthma, bronchiectasis, upper respiratory tract infections, and seasonal allergic rhinitis [3–7]. Treatments with inhaled and oral corticosteroids, which have been shown to reduce airway inflammation, are associated with a reduction in the exhaled levels of CO in asthma [3,8]. Furthermore, exhaled CO is increased in the exacerbations

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Table 1. Physical characteristics, type of asthma and pulmonary function test results

	Subjects	Age (n)	Sex (yr)	Type of asthma (AT/non-AT)	FVC (% pred.)	FEV ₁ (% pred.)	Brinkman's index
Control	20	64 ± 22	11M/9F	ND	105 ± 13	102 ± 13	0
Mild asthmatics	20	63 ± 18	11M/9F	6 AT/14 non-AT	98 ± 22	87 ± 9	0
Moderate asthmatics	20	63 ± 22	11M/9F	8 AT/12 non-AT	98 ± 18	85 ± 9	0
Stable severe asthmatics	15	64 ± 19	8M/7F	5 AT/10 non-AT	97 ± 23	81 ± 8	0
Unstable severe asthmatics	16	65 ± 16	8M/8F	5 AT/11 non-AT	91 ± 20	66 ± 8	0

Values are mean ± SD. AT = atopic type asthma; non-AT = non-atopic type asthma; ND = not defined.

of bronchial asthma induced by respiratory virus infections [8]. Based on these findings, it has been proposed that measurements of CO may serve as an indirect marker of airway inflammation [3–8].

Histological examinations of specimens from bronchial biopsy and autopsy in asthma patients revealed airway inflammation including increases in eosinophils, lymphocytes, mast cells, and neutrophils in airways [9,10]. Furthermore, levels of eosinophils and eosinophil cationic protein are increased in the bronchoalveolar lavage fluid from asthmatic patients and correlate with the clinical severity [11]. However, it is uncertain whether exhaled CO concentrations are related to severity of asthma.

To determine whether the level of CO is related to the severity of asthma, exhaled CO concentration was measured in asthmatic patients for 12 months when the patients attended the hospital for regular treatment or for treatment of asthma exacerbations.

Materials and methods

Subjects

We studied 20 normal non-smoking control subjects and 71 asthmatic patients attending the Tsukidate Municipal Hospital. In order to exclude current smokers, we measured urinary cotinine concentration with high-performance liquid chromatography as described by Harlharan *et al.* [12]. We found that urinary cotinine concentrations of all control and asthmatic subjects were less than 30 ng of the cotinine/mg creatinine ratio, the level used to categorize subjects as exposed and unexposed to cigarette smoking [13]. In the 20 control subjects there was no history of respiratory and cardiovascular disease. None of these 20 control subjects were receiving long-term medication. Asthma was defined as a clinical history of intermittent wheeze, cough, chest tightness, or dyspnea, and documented reversible airflow limitation either spontaneously or with treatment during the preceding year [14].

Bronchial responsiveness to inhaled histamine was examined at the first visit to the hospital, according to the method of Yan and coworkers [15]. Testing was terminated by a fall in FEV₁ of 20% (PD20) or more. We found that PD20 of all the asthmatic patients was 7.8 µmol or less, and that all the asthmatic patients had bronchial hyperresponsiveness [15]. The type of asthma was defined from the results of a high serum IgE value and positive skin test to allergens. The patients who had a serum IgE level over 250 U/L and/or a positive skin reaction to a battery of standard antigens were classified as atopic and the others as non-atopic. Physical characteristics, the type of asthma, pulmonary function test results, and Brinkman's index (number of cigarettes/day × year) are shown in Table 1. A pulmonary function test was performed at the start of exhaled CO measurement in December, 1997. All the subjects gave informed consent for participation in the study as approved by the Tohoku University Ethics Committee.

Study protocol

A classification of asthma severity was performed according to clinical features before treatment, lung function and regular medication usually required to maintain control [14], and patients were categorized as mild (20 patients), moderate (20 patients) and severe asthma (31 patients), respectively (Table 1). Regular medication usually required to maintain control was intermittent inhaled β₂-agonists (fenoterol hydrobromide 200 µg/puff or procaterol hydrochloride 10 µg/puff) for mild asthma, daily inhaled corticosteroid (beclomethasone dipropionate 400–1200 µg/day) and daily long acting bronchodilator (procaterol hydrochloride 50–100 µg/day) for moderate asthma, or daily use of inhaled (beclomethasone dipropionate 1200–2000 µg/day) and oral (prednisolone 5–10 mg/day) corticosteroids and long acting bronchodilator (procaterol hydrochloride 50–100 µg/day) for severe asthma [14], respectively. Furthermore, severe asthmatic patients were divided into two groups: stable and unstable severe asthma.

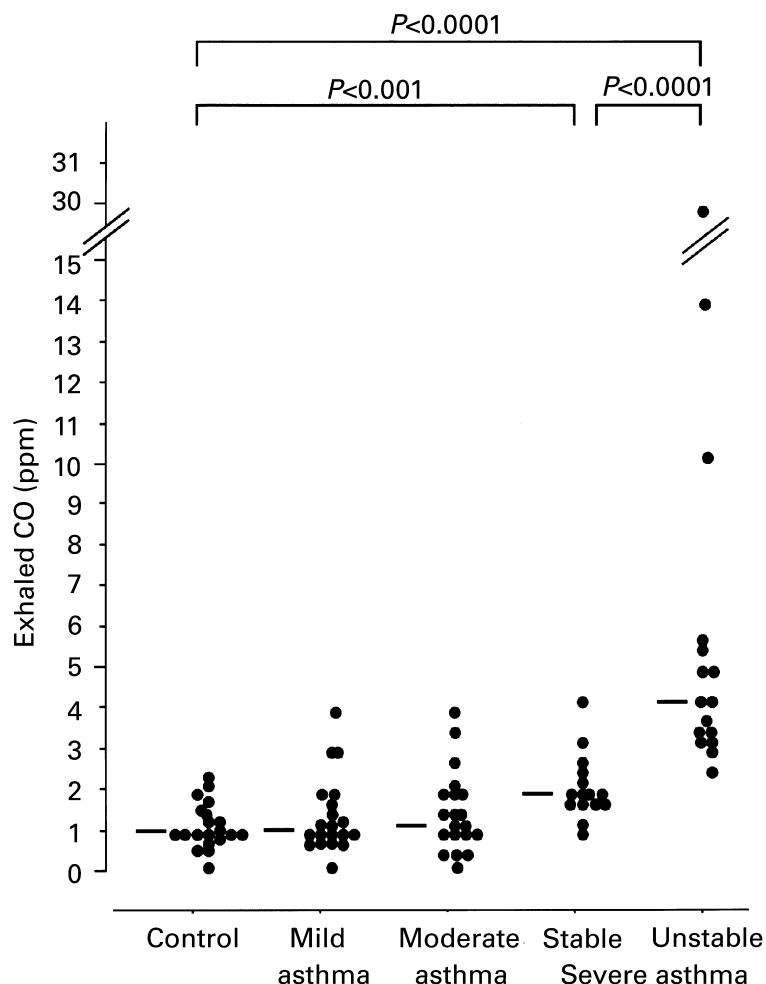


Fig. 1. Mean exhaled carbon monoxide (CO) concentrations over 12 months in individual non-smoking control subjects ($n = 20$) and individual patients with mild ($n = 20$), moderate ($n = 20$), stable severe ($n = 15$) and unstable severe ($n = 16$) asthma. Horizontal bars indicate median values for each group.

Patients with unstable severe asthma had been admitted to the hospital (1–4 times/year) or had received additional oral (prednisolone 80–560 mg/year) or intravenous (dexamethasone sodium phosphate 4–22 mg/year) corticosteroids because of asthma exacerbations.

Exhaled CO concentrations were measured for 12 months from December of 1997 to November of 1998, when the patients attended the hospital for regular treatment or for the treatment of asthma exacerbations. Exhaled CO concentrations were also measured in 16 unstable severe asthmatics who visited the hospital every 7 or 14 days for treatment with high dose inhaled corticosteroids and oral corticosteroids. The mean value of exhaled CO concentration in each season (winter December–February; spring, March–May; summer, June–August; autumn, September–November) was calculated. Mild asthmatic patients free from any symptoms for several weeks were asked to visit the hospital every month for measurements of exhaled CO. Exhaled CO concentrations during admission to the hospital with acute exacerbations of asthma were excluded from the analysis. Exhaled CO concentrations were measured 3 weeks after

treatment of acute exacerbations of asthma for the analysis in severe asthmatic patients.

Measurement of exhaled CO

Exhaled CO was measured on a portable Bedfont EC50 analyser (Bedfont Technical Instruments Ltd, Sittingbourne, UK) [2], in which subjects are asked to exhale fully, inhale deeply, and hold their breath for 20 s before exhaling rapidly into a disposable mouthpiece. This procedure was repeated three times, with 1 min of normal breathing between each repetition, and the mean value was used for analysis. Background CO values (0–1 p.p.m.) were obtained prior to the subject readings. The exhaled CO concentration was determined by subtracting the background level from the observed reading in the following experiments as reported previously [2,3]. To avoid analysis with a value of exhaled CO concentration below 1.0 p.p.m., the background level was subtracted from the average value obtained from three sequential manoeuvres in each patient. The exhaled CO concentration

Table 2. Exhaled CO concentrations in each season in non-smoking control subjects and patients with asthma

	Winter	Spring	Summer	Autumn
Control ($n = 20$)	1.2 ± 0.9	1.1 ± 0.9	1.2 ± 0.9	1.1 ± 0.5
Mild asthmatics ($n = 20$)	1.6 ± 0.9	1.5 ± 0.9	1.5 ± 1.3	1.6 ± 0.9
Moderate asthmatics ($n = 20$)	1.7 ± 1.3	1.6 ± 1.3	1.4 ± 0.9	1.5 ± 0.9
Severe asthmatics ($n = 31$)	$4.7 \pm 5.0^{**}$	$4.5 \pm 6.1^{**}$	$4.3 \pm 5.6^{**}$	$4.6 \pm 5.6^{**}$

Values are mean $\pm$ SD; $^{**}P < 0.01$, significant differences from control subjects.

values were always above 1.0 p.p.m. before subtracting the background level throughout the experiments.

Prior to the start of the study, the analyser was calibrated with a mixture of 50 p.p.m. CO in air [2,3]. Exhaled CO concentration measured by the Bedfont EC50 analyser is reported to correlate closely with blood carboxyhaemoglobin concentration over the range of values encountered in smokers and in non-smokers [2].

Statistical analysis

Statistical analysis of mean exhaled CO concentrations in each group was carried out with the statistical software package SPSS version 10.0. Since exhaled CO levels were

not normally distributed, medians between groups were compared with the Mann–Whitney *U*-test. The age, seasonal variation of exhaled CO in each group, circadian and daily variability of exhaled CO and pulmonary function test data are reported as mean $\pm$ SD. Statistical analysis of these data was performed using two-way analysis of variance (ANOVA), followed by the Newman-Keuls test. Linear regression analysis was performed using the method of least squares. Significance was accepted at $P < 0.05$.

Results

Exhaled CO was reproducible and there was no circadian variability. Mean exhaled CO concentrations in non-smoking

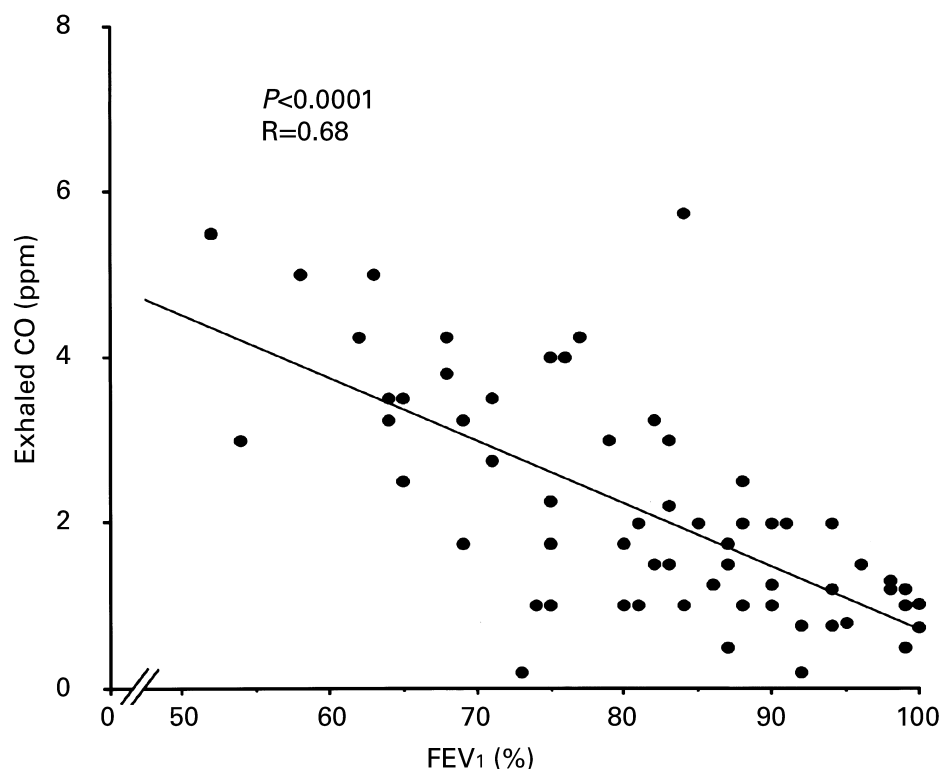


Fig. 2. Relationship between the mean CO concentration over 12 months and forced expiratory volume in one second (% pred) in individual asthmatic patients.

control subjects ($n = 10$) in the winter season were 1.2 ± 0.4 p.p.m. at 9 a.m., 1.2 ± 0.4 p.p.m. at 3 p.m. and 1.1 ± 0.4 p.p.m. at 9 p.m., respectively ($P > 0.50$). To examine the daily variability in the exhaled CO concentrations, we measured exhaled CO concentrations every day at 9 a.m. for 7 days in non-smoking control subjects ($n = 10$) and mild asthmatic patients without any symptoms of exacerbation ($n = 10$). We found that there was no daily variability in the exhaled CO concentrations in non-smoking control subjects and mild asthmatic subjects (data not shown). Furthermore, there were no seasonal changes in mean exhaled CO concentration in all non-smoking control subjects. Mean exhaled CO concentrations in non-smoking control subjects in each season were 1.2 ± 0.4 p.p.m. in the winter season, 1.2 ± 0.4 p.p.m. in the spring season, 1.2 ± 0.6 p.p.m. in the summer season and 1.1 ± 0.4 p.p.m. in the autumn season, respectively ($P > 0.50$).

At the start of treatment with corticosteroids, exhaled CO concentrations in severe asthma (7.6 ± 8.9 p.p.m.) were significantly reduced 4 weeks after treatment with corticosteroids (4.3 ± 5.7 p.p.m., $n = 31$, $P < 0.05$). However, the mean exhaled CO concentrations were significantly higher in severe asthmatic patients than those in mild and moderate asthmatic patients and non-smoking control subjects (Fig. 1). Furthermore, exhaled CO concentrations in unstable severe asthma were significantly higher than those in stable severe asthma (Fig. 1). The mean CO concentrations over the 12 months were significantly higher in three cases of mild asthma (3.3, 2.8 and 3.5 p.p.m., respectively). However, mean exhaled CO concentrations in mild asthmatic patients did not differ significantly from those in non-smoking control subjects ($P > 0.50$) (Fig. 1). Likewise, exhaled CO concentrations in moderate asthmatic patients did not differ significantly from those in non-smoking control subjects ($P > 0.50$) (Fig. 1).

There was no seasonal change in mean exhaled CO concentrations in asthmatic patients ($P > 0.10$) (Table 2). The CO values of severe asthma patients in the winter season appear to be higher than those in the summer season, but the differences were not significant ($P > 0.20$). There was a significant correlation between mean exhaled CO concentration over 12 months and FEV₁ in asthmatic patients ($P < 0.01$, $r = 0.55$). Even when the data of two subjects with extremely higher CO concentrations (10.3 and 14.0 p.p.m.) were excluded, there was a significant correlation between mean exhaled CO concentration over 12 months and FEV₁ in asthmatic patients ($P < 0.0001$) (Fig. 2).

Discussion

We have shown that exhaled CO concentration in severe asthmatic patients increased even after treatment with corticosteroids. In contrast, exhaled CO concentration in

mild and moderate asthmatic patients did not differ from that in non-smoking control subjects.

We observed elevated CO values in three mild asthmatic patients, but the other 17 mild asthmatic patients were free from asthma symptoms for several weeks and their exhaled CO levels remained low. It was uncertain why the CO values were elevated in three mild asthmatic patients. However, all of them had elevated serum IgE (> 250 U/L), suggesting continuous allergic airway inflammation during the treatment with intermittent inhaled β_2 -agonist. The low levels of exhaled CO in mild asthma may reflect the minimum degree of airway inflammation in the present patients. In moderate asthma, the low levels of exhaled CO could be explained by treatment with inhaled corticosteroids that decreases exhaled CO concentrations in asthmatic patients [3,8]. It is likely that corticosteroids reduce exhaled CO by inhibiting the induction of HO-1 in epithelial and inflammatory cells in the airways [16,17]. This may be by a direct effect on the HO promoter [18], or inhibition of the synthesis of pro-inflammatory cytokines [18,19] and nitric oxide [20] that can induce HO-1 in asthma. However, patients with severe asthma experienced dyspnea and wheeze even during treatment with corticosteroids, suggesting that the inhibition of airway inflammation by corticosteroids treatment was not complete, thus the higher concentrations of exhaled CO in those patients. Alternately, the higher levels of exhaled CO in severe asthma may reflect more severe airway inflammation than that in mild and moderate asthma [9–11].

There were no seasonal changes in CO concentration in asthma patients. Corticosteroids treatment may mask airway inflammation caused by inhaled antigens or mild asthma attack after airway viral infections.

Exhaled CO appears to be derived from an endogenous source, as none of the asthmatic patients were smokers, ex-smokers or passive smokers, and background CO values were subtracted from the measured values. Although the source of the CO in exhaled air is uncertain, HO-1, the inducible form of HO, is likely to be expressed in airway epithelial cells [16], endothelial cells [21] and alveolar macrophages [4,17].

In the present study, there was a significant correlation between mean exhaled CO concentration over 12 months and FEV₁ in asthmatic patients. Structural changes occur in asthma in both the large and small airways, including subepithelial collagen deposition, smooth muscle growth and thickening of the basement membrane [9,22,23]. Therefore, the persistent airway inflammation detected by increases in exhaled CO concentrations may cause structural changes in airways, thereby leading to the decreases in FEV₁ in severe asthma, although we did not perform histological examinations of the lung in the present study.

Recent reports demonstrated increases in exhaled CO concentrations in cystic fibrosis, critical illness and diabetes mellitus, suggesting increased exhaled CO concentrations in a variety of diseases other than bronchial asthma [24–26]. Therefore, the present study only suggests that exhaled CO increases with the severity of asthma, but a causal significance cannot be ascertained from only the exhaled CO concentrations. Because many control and asthmatic subjects (59%, 54 of 91) in the present study were 60 years old or more, the present study may not encompass the whole adults asthma age range. Thus, the present study suggests that exhaled CO may be a useful means of monitoring the airway inflammation in senile patients with asthma and the response to anti-inflammatory treatments with inhaled and oral glucocorticoids in individual senile asthmatic patients. However, further studies are required to substantiate the claim that exhaled CO may have a role in monitoring response to anti-inflammatory therapy in asthma generally.

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References

- Mines MD. The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 1997; 37:517–54.
- Jarvis MJ, Russell MAH, Saloojee Y. Expired air carbon monoxide: a simple breath test of tobacco smoke intake. *Br Med J* 1980; 281:484–5.
- Zayasu K, Sekizawa K, Okinaga S et al. Increased carbon monoxide in exhaled air of asthmatic patients. *Am J Respir Crit Care Med* 1997; 156:1140–3.
- Horváth I, Donnelly LE, Kiss A et al. Raised levels of exhaled carbon monoxide are associated with an increased expression of heme oxygenase-1 in airway macrophages in asthma: a new marker of oxidative stress. *Thorax* 1998; 53:668–72.
- Horváth I, Loukides S, Wodehouse T et al. Increased levels of exhaled carbon monoxide in bronchiectasis: a new marker of oxidative stress. *Thorax* 1998; 53:867–70.
- Yamaya M, Sekizawa K, Ishizuka S et al. Increased carbon monoxide in exhaled air of subjects with upper respiratory tract infections. *Am J Respir Crit Care Med* 1998; 158:311–4.
- Monma M, Yamaya M, Sekizawa K et al. Increased carbon monoxide in exhaled air of patients with seasonal allergic rhinitis. *Clin Exp Allergy* 1999; 29:1537–41.
- Yamaya M, Sekizawa K, Ishizuka S et al. Exhaled carbon monoxide levels during treatment of acute asthma. *Eur Respir J* 1999; 13:757–60.
- O'Byrne PM, Postma DS. The many faces of airway inflammation. Asthma and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 159:S41–S66.
- Djukanovic R, Roche WR, Wilson JW et al. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990; 142:434–57.
- Bousquet J, Chanez P, Lacoste JY et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323:1033–9.
- Hariharan M, VanNoord T, Greden JF. A high-performance liquid-chromatographic method for routine simultaneous determination of nicotine and cotinine in plasma. *Clin Chem* 1988; 34:724–9.
- Henderson FW, Reid HF, Morris R et al. Home air nicotine levels and urinary cotinine excretion in preschool children. *Am Rev Respir Dis* 1989; 140:197–201.
- Sheffer AL. International consensus report on the diagnosis and management of asthma. *Clin Exp Allergy* 1992; 22 (Suppl. 1):1–72.
- Yan K, Salome C, Woolcock AJ. Rapid method for measurement of bronchial responsiveness. *Thorax* 1983; 38:760–5.
- Yamada N, Yamaya M, Okinaga S et al. Protective effects of heme oxygenase-1 against oxidant-induced injury in the cultured human tracheal epithelium. *Am J Respir Cell Mol Biol* 1999; 21:428–35.
- Fukushima T, Okinaga S, Sekizawa K et al. The role of carbon monoxide in lucigenin-dependent chemiluminescence of rat alveolar macrophages. *Eur J Pharmacol* 1995; 289:103–7.
- Cantoni L, Rossi C, Rizzardini M et al. Interleukin-1 and tumour necrosis factor induce hepatic haem oxygenase: feedback regulation by glucocorticoids. *Biochem J* 1991; 279:891–4.
- Lavrovsky Y, Drummond GS, Abraham NG. Downregulation of the heme oxygenase gene by glucocorticoids and identification of 56b regulatory elements. *Biochem Biophys Res Commun* 1996; 218:759–65.
- Kim YM, Bergonia HA, Müller C et al. Loss and degradation of enzyme-bound heme induced by cellular nitric oxide synthesis. *J Biol Chem* 1995; 270:5710–3.
- Otterbein L, Sylvester SL, Choi AMK. Hemoglobin provides protection against lethal endotoxemia in rats: the role of heme oxygenase-1. *Am J Respir Cell Mol Biol* 1995; 13:595–601.
- Pare PD, Bai TR. The consequences of chronic allergic inflammation. *Thorax* 1995; 50:328–32.
- Carroll N, Elliot J, Morton A et al. The structure of large and small airways in nonfatal and fatal asthma. *Am Rev Respir Dis* 1993; 147:405–10.
- Paredi P, Biernacki W, Invernizzi G et al. Exhaled carbon monoxide levels elevated in diabetes and correlated with glucose concentration in blood: a new test for monitoring the disease? *Chest* 1999; 116:1007–11.
- Paredi P, Shah PL, Montuschi P et al. Increased carbon monoxide in exhaled air of patients with cystic fibrosis. *Thorax* 1999; 54:917–20.
- Scharte M, Bone HG, Van Aken H et al. Increased carbon monoxide in exhaled air of critically ill patients. *Biochem Biophys Res Commun* 2000; 267:423–6.