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Increased oxidized methionine residues in BAL fluid proteins in acute or chronic bronchitis

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ABSTRACT: Phagocytic cells such as alveolar macrophages (AM) or polymorphonuclear neutrophils (PMN) in the bronchoalveolar tract are a potential source of the oxygen-derived free radicals which are presumed to be involved in lung tissue damage. Previous results have shown that the methionine sulphoxide (MET(O)) content of bronchoalveolar lavage fluid (BALF) protein is a reliable parameter to indicate oxidative processes in idiopathic pulmonary fibrosis (IPF).

We measured the molar ratio between MET(O) and methionine (MET) in the BALF protein from healthy nonsmokers (control group), healthy smokers and patients with acute or chronic bronchitis (AB or CB).

The MET(O)/MET ratio of the nonsmoking group (n=11) was 0.046±0.008 (mean±SEM). Healthy smokers (n=8) had similar values (0.042±0.008), even though they had strongly increased AM counts in BALF. Patients with AB (n=12) showed an increased MET(O)/MET ratio (0.191±0.031) and had high PMN but normal AM counts in BALF. Patients with CB (n=13) showed an increase in the MET(O)/MET ratio (0.086±0.010) and moderately increased PMN and markedly increased AM counts. Taking all results together, the MET(O)/MET ratio correlated positively with the relative PMN number (r=0.70; p<0.0002) and inversely with the relative AM number (r=-0.67; p<0.0002). In the group with CB, the MET(O)/MET ratio correlated inversely with forced expiratory volume in one second (FEV_1) % pred. (r=-0.77) and FEV_1/inspiratory vital capacity (IVC) % pred. (r=-0.89).

We conclude that oxidative pulmonary processes in AB and CB are reflected in an increase of the MET(O) content in the BALF protein, which is predominantly mediated by PMN.

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Oxygen-derived free radicals produced by phagocytic cells appear to be involved in the genesis of different lung diseases [1–5]. It is well-known that polymorphonuclear neutrophils (PMN) are strongly increased in the lower respiratory tract of patients with adult respiratory distress syndrome (ARDS), and it has been proposed that a H_2O_2-dependent reaction mediated by PMN contributes to acute lung injury in ARDS [6–9]. In patients with idiopathic pulmonary fibrosis (IPF), alveolar macrophages (AM) and PMN dominate the inflammatory cell population in the lower respiratory tract [10], spontaneously releasing more oxidants, such as O_2^- radicals or H_2O_2, than do the cells from healthy individuals [11–13]. In a recent study, we found significantly increased levels of oxidized methionine (MET(O)) in the bronchoalveolar lavage fluid (BALF) protein of patients with idiopathic pulmonary fibrosis, reflecting increased oxidant burden in the epithelial lining layer [14]. In patients with diffuse fibrosing alveolitis, including patients with collagen vascular disease, inverse correlations were observed between the level of MET(O) and lung function parameters, suggesting the participation of oxidants in lung tissue injury [15]. Oxidant injury of the extracellular matrix proteins also seems to be a basic mechanism in the pathogenesis of pulmonary emphysema [16].

In vitro studies with the xanthine oxidase/xanthine system have shown that soluble collagen molecules are also fragmented by reactive oxygen species [17]. Degradation of collagen has also been observed during treatment with ozone or hydroxyl radicals [18, 19]. Oxidative modification of proteins may increase their vulnerability to proteinases [18–20]. CARP *et al.* [21] reported that α_1-proteinase inhibitor (α_1-PI) in BALF from smokers has a decreased elastase inhibitory activity and contains oxidized MET residues, in contrast to the protein from nonsmokers. Further information is necessary on the role of oxidative processes mediated by phagocytes in the epithelial lining layer and the connective tissue of smokers and patients with bronchitis.

This study was designed to begin to elucidate the contribution of AM and PMN to the oxidation of protein-bound MET in the epithelial lining layer of patients with bronchitis. Additionally, *in vitro* studies were performed on the ability of N-chloramines and oxygen radicals as typical phagocytic oxidants to oxidize MET residues and to affect the inhibitory activity of α_1-PI.

Patients, materials and methods

Study population

Patients with chronic bronchitis. Thirteen patients (9 men, 4 women; mean age 49 yrs, range 24–68 yrs), all smokers (mean smoking history 32±7 pack yrs). The diagnosis was based on the presence of cough and sputum production most days for at least 3 months a year during the previous 2 yrs. Four patients in this group had irreversible obstructive lung disease defined as forced expiratory volume in one second/inspiratory vital capacity (FEV_1/IVC) <65% with no significant response to inhaled bronchodilator. The other patients had normal pulmonary function tests. Arterial blood gas measurements showed that none had hypercapnia or a Pao_2 <65 mmHg (8.7 kPa).

Patients with acute bronchitis. Twelve patients (5 men, 7 women; mean age 47 yrs, range 13–76 yrs), 6 smokers, 5 nonsmokers and 1 ex-smoker. Patients were selected for inclusion in this group when they presented with acute onset of cough and production of purulent sputum with or without fever. None had radiographic infiltrates suggestive of pneumonia. Bronchoscopic inspection prior to BAL showed erythema and oedema of the bronchial mucosa and purulent secretions. Pulmonary function tests were not performed in this group.

Normal smokers and nonsmokers. The smoker group consisted of 9 healthy smoking volunteers (5 men, 4 women; mean age 29 yrs, range 25–37 yrs) with a mean smoking history of 10±4 pack yrs. The nonsmoker group consisted of 11 healthy nonsmoking volunteers (5 men, 6 women; mean age 25 yrs, range 21–32 yrs). All volunteers were free of respiratory symptoms in the preceding 3 months, and had normal chest radiographs and pulmonary function tests.

Bronchoalveolar lavage (BAL)

Informed consent was obtained from each patient and healthy volunteer. BAL was performed by standardized washing of the right middle lobe with 5×20 ml of sterile 0.9% (w/v) saline during fibreoptic bronchoscopy as described previously [22]. The recovered fluid was filtered through gauze and centrifuged at 500×g for 10 min. The total number of cells was enumerated and differential cell counts determined from the cell pellets.

Chemicals

L-amino acid calibration mixture, *o*-phthaldialdehyde reagent solution, sample diluent buffer (1.0 M potassium borate, pH 10.4), and cyanogen bromide were obtained from Pierce (Rockford, Ill., USA); acetonitrile (high-performance liquid chromatography (HPLC) gradient grade) and tetrahydrofurane (HPLC gradient grade) from Baker (Groβ-Gerau, FRG); homoserine and α_1-PI from Sigma (Taufkirchen, FRG); and all other chemicals from Merck (Darmstadt, FRG).

Analysis of proteins for MET and MET(O)

Prior to analysis, non-oxidized MET in the BALF protein or α_1-PI was converted to homoserine and homoserine lactone by reaction with cyanogen bromide [23–25]. Portions of 100 µg protein were dialysed against water for 3 h at 4°C, lyophilized and dissolved in 840 µl 75% (v/v) formic acid (nitrogen-saturated). Conversion of MET to homoserine was performed by addition of 60 µl 3 M cyanogen bromide (dissolved in acetonitrile). The reaction mixtures were kept under N_2 saturation in the dark at room temperature. After 24 h incubation, the samples were lyophilized twice. Under these conditions, more than 99% of the non-oxidized MET is converted to homoserine and its lactone [26].

Hydrolysis and reversed-phase HPLC

After reaction with cyanogen bromide the proteins were subjected to hydrolysis with double-distilled 6 M HCl in the presence of 5 mM dithioerythritol at 110°C under vacuum. Under these conditions the residual MET(O) is recovered quantitatively as MET. The originally non-oxidized MET was determined quantitatively as homoserine by subsequent amino acid analysis with reversed-phase HPLC. Pre-column derivatization with *o*-phthaldialdehyde and separation on a 3 µm Spherisorb ODS-2 C_{18} column (4×125 mm) from LKB (Freiburg, FRG) by an acetonitrile gradient were performed as described previously [26]. Peaks were detected with a Hitachi fluorescence analyser F 1000 (excitation at 330 nm and emission at 450 nm) and evaluated with a Shimadzu integrator.

Protein determination

Protein concentrations were determined according to LOWRY *et al.* [27].

In vitro *oxidation of α_1-PI*

Oxidation with taurine monochloramine (taurine-NH-Cl) and NH_2-Cl. Taurine-NH-Cl or NH_2-Cl was obtained by reaction of hypochlorous acid with taurine or NH_3 as described previously [28, 29]. Chloramine concentrations were calculated from the reduction in absorbance during oxidation of 5-thio-2-nitro-benzoic acid [30, 31]. Oxidation of α_1-PI was performed in 20 mM potassium phosphate buffer, pH 7.0, at 25°C for 2 h.

Oxidation with the xanthine oxidase/hypoxanthine-system. Generation of superoxide anion and derived oxygen radicals was performed by the xanthine oxidase-system according to FRIDOVICH [32]. Hypoxanthine (0.3 mM), α_1-PI (0.1 mg) and xanthine oxidase from milk (0.052 U·ml^{-1}) were incubated in 0.1 M potassium phosphate buffer, pH 7.6 at 25°C in the presence or absence of 0.1 mM Fe(III) in a total volume of 21 ml. After 60 min, a further portion of 0.052 U xanthine oxidase·ml^{-1} was added. Production of superoxide anion and related oxygen radicals was estimated by uric acid formation followed at 293 nm (ε_{293}=12.0 cm^2·μmol^{-1}). At pH 7 the electron flux used for univalent reduction of oxygen to superoxide anion amounts to about 20% of the total electron flux in an air-saturated reaction mixture, as calculated from the superoxide dismutase inhibitable reduction of cytochrome c and formation of uric acid [33]. Aliquots of 4 ml were taken after 0, 30, 60 and 120 min incubation and 5 μg superoxide dismutase·ml^{-1} were added to stop the inactivation reaction.

Aliquots (1 ml) of the incubated samples were used for MET(O) analysis as described above. Elastase inhibitory activity of α_1-PI was assayed concomitantly with porcine pancreas elastase and succ-(ala)$_3$-pNA as substrate, according to BIETH *et al.* [34]. Each inactivation experiment was performed in duplicate.

Statistical analysis

Statistical analysis was performed by BMDP software from BMDP Statistical Software Inc., Los Angeles, California, USA. Data were expressed as the arithmetic mean±SEM. Data from differential cell counts, MET(O)/MET ratios and protein concentrations did not approximate a normal distribution but showed positive skewness. Thus, the Mann-Whitney test was used to test the null hypothesis. Correlation coefficients were calculated by Spearman rank correlation analysis.

Results

The results of fluid recovery and cell counts are shown in table 1, and the protein content and oxidative status of proteins in BALF in table 2.

Normal smokers and nonsmokers

The total number of BAL cells obtained from the smoker group (378×10^3 cells·ml^{-1} BALF) was increased significantly ($p<0.03$) compared to the nonsmoker group (86×10^3 cells·ml^{-1} BALF). This resulted from a big increase ($p<0.03$) in the number of AM, from 75×10^3 cells·ml^{-1} in the nonsmoker group to 352×10^3 cells·ml^{-1} in the smoker group. The PMN counts were also increased significantly ($p<0.03$) in smokers (9.4×10^3 cells·ml^{-1} BALF) compared to nonsmokers (1.1×10^3·ml^{-1}). Although the eosinophils also increased significantly ($p<0.003$) in the smoker group, the relative number (0.4% of total BAL cells) was still low. The lymphocyte counts were not significantly different in the two groups.

Table 1. – Total and differential cell counts in bronchoalveolar lavage fluids from normal nonsmokers (NS), normal smokers (S) and patients with acute bronchitis (AB) or chronic bronchitis (CB)

BAL fluid parameters	NS (n=11)	S (n=9)	AB (n=12)	CB (n=13)
Fluid recovery %	64±3.3	58±3.9	44±4.9	42±3.2
Total cells ×10^3·ml^{-1}	86±24	378±95*	483±179*	248±47*
Macrophages				
%	90.3±2.3	93.3±0.7	23.5±6.3†	88.4±3.8
cells ×10^3·ml^{-1}	75±18	352±88**	89±38	230±44*
Neutrophils				
%	1.2±0.3	2.3±0.6	68.3±8.2†	4.8±2.3
cells ×10^3·ml^{-1}	1.1±0.4	9.4±2.8*	363±150†	6.2±2.8*
Eosinophils				
%	0.2±0.04	0.4±0.12	0.6±0.19	0.3±0.07
cells ×10^3·ml^{-1}	0.15±0.10	1.1±0.3**	2.2±1.1**	0.67±0.32*
Lymphocytes				
%	7.8±2.2	3.8±0.4	7.4±2.5	6.2±1.4
cells ×10^3·ml^{-1}	10±6	14±5	29±11	10±3

p values from Mann-Whitney test for comparison with normal nonsmokers: *: $p<0.03$; **: $p<0.003$; †: $p<0.0003$.

Table 2. – Protein content of the BALF and oxidative status of the protein expressed as the ratio MET(O)/MET (mol/mol) from normal nonsmokers (NS), normal smokers (S) and patients with acute bronchitis (AB) or chronic bronchitis (CB)

	NS	S	AB	CB
BALF protein μg·ml^{-1}				
Mean±SEM	49.5±9.4	74.1±12.4	207.3±55.3**	93.2±24.4*
Range	18–128	33–137	45–744	31–371
MET(O)/MET mol/mol				
Mean±SEM	0.046±0.008	0.042±0.008	0.191±0.031†	0.086±0.010**
Range	0.015–0.104	0.008–0.074	0.086–0.420	0.022–0.151

p-values from Mann-Whitney test for comparison with normal nonsmokers: *: p<0.03; **: p<0.006; †: p<0.0002. BALF: bronchoalveolar lavage fluid; MET: methionine; MET(O): methionine sulphoxide.

The total BALF protein was 49.5 μg·ml^{-1} in the nonsmoker group and 74.1 μg·ml^{-1} in the smoker group. This difference was not significant. The molar MET(O)/MET ratio, which reflects the oxidative status of BAL protein, was 0.046 for the nonsmoker group (table 2, fig. 1) indicating that about 4% of the total MET residues in the protein were oxidized. There was no change in the MET(O)/MET ratio in the smoker group.

Patients with acute or chronic bronchitis

AB patients showed a significant increase in the total number of BAL cells (to 483×10^3 cells·ml^{-1} compared with 86×10^3 cells·ml^{-1} in nonsmokers; p<0.03) mainly as a result of a massive increase in PMN (to 363×10^3 cells·ml^{-1}). The absolute number of AM was normal but the relative AM content decreased significantly to 23.5% (p<0.0003) as a result of the increase in PMN (to 68.3% of total cells). The eosinophil counts were also increased significantly (p<0.003) although the total number remained low, whereas the lymphocyte counts were not markedly changed (table 1). The total BALF protein was increased significantly (p<0.006) to 207 μg·ml^{-1}, and there was a significant increase in the MET(O)/MET ratio (0.191; p<0.0002 (table 2, fig. 1)).

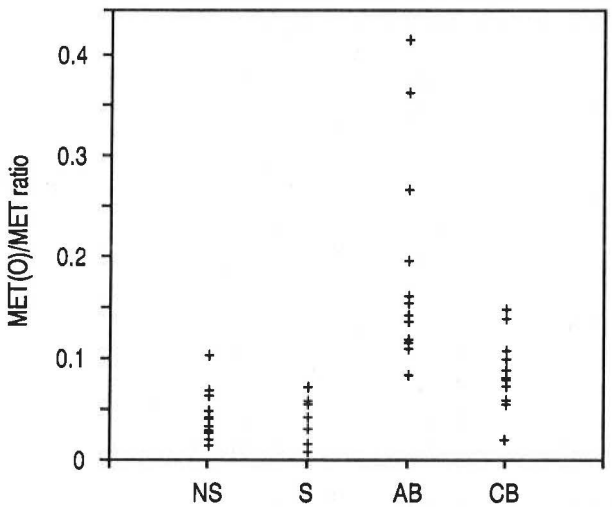

Fig. 1. – MET(O)/MET ratios in the BALF protein from healthy nonsmokers (NS), smokers (S), patients with acute bronchitis (AB) and chronic bronchitis (CB). BALF: bronchoalveolar lavage fluid; MET: methionine; MET(O): methionine sulphoxide.

Patients with CB had significantly increased AM counts (230×10^3 cells·ml^{-1} BALF; p<0.03) and PMN counts (6.2×10^3 cells·ml^{-1} BALF; p<0.03) compared to the nonsmokers (table 1) but the values were not markedly different from those in healthy smokers.

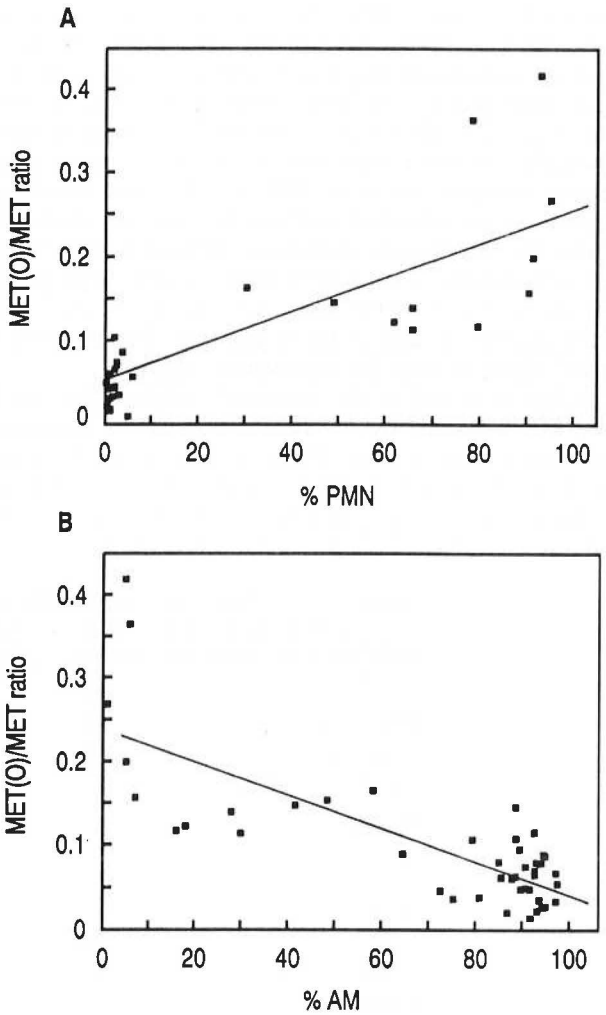

Fig. 2. – Correlation of MET(O)/MET ratio with BAL cells from healthy nonsmokers and smokers and patients with acute and chronic bronchitis. A) positive correlation of relative PMN counts with the MET(O)/MET ratios. r=0.70; p<0.0002; n=44. B) inverse correlation of relative AM counts with the MET(O)/MET ratios. r=-0.67; p<0.0002; n=44. PMN: polymorphonuclear neutrophils; AM: alveolar macrophages. For further abbreviations see legend to figure 1.

The protein content in BALF (93.2 $\mu g \cdot ml^{-1}$) was significantly increased in comparison with nonsmokers, but not with smokers, and there was a significant increase in the MET(O)/MET ratio (0.086; $p<0.006$) compared to both healthy groups (table 2, fig. 1). The increases in total protein and MET (O)/MET ratio were not as high in CB as in AB patients.

When the results of all subjects were pooled, the MET(O)/MET ratios were found to correlate positively with both the relative (fig. 2A; $r=0.70$; $p<0.0002$; $n=44$) and the absolute PMN counts ($r=0.67$; $p<0.0002$; $n=43$). There was an inverse correlation between the MET(O)/MET ratios and the relative AM counts (fig. 2B; $r=-0.67$; $p<0.0002$; $n=44$). The MET(O)/MET ratios did not significantly correlate with the smoking history expressed as pack yrs.

In the group with CB, there was a significant inverse correlation between the MET(O)/MET ratios and pulmonary function tests such as FEV_1 % pred. ($r=-0.77$; $p<0.01$), FEV_1/IVC ratio ($r=-0.82$; $p<0.05$) and FEV_1/IVC % pred. ($r=-0.89$; $p<0.001$).

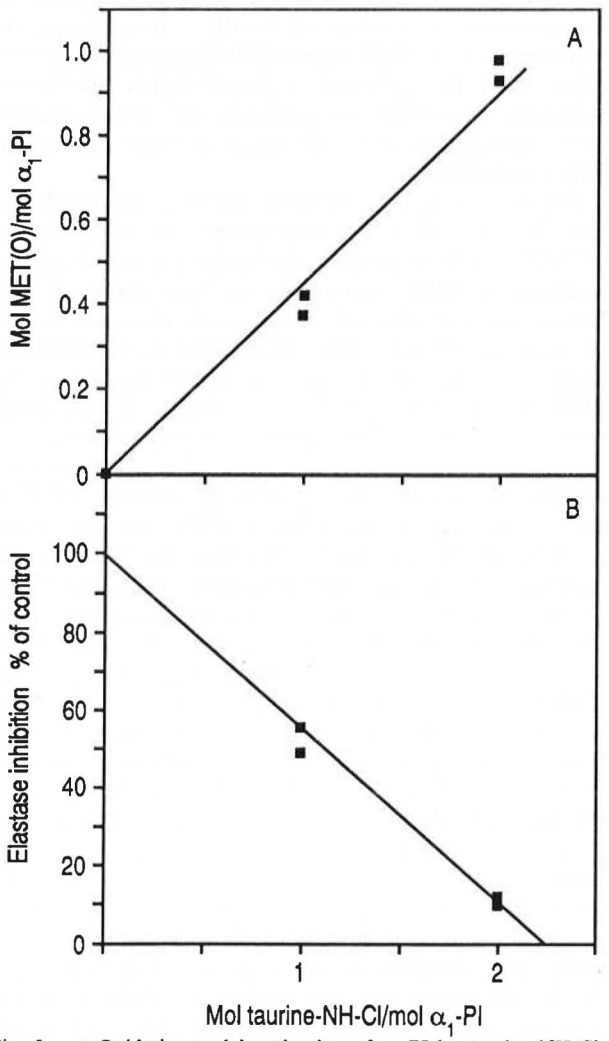

Fig. 3. – Oxidation and inactivation of α_1-PI by taurine-NH-Cl. A) formation of MET(O) residues. B) effect on the inhibitory activity against pancreas elastase. Untreated α_1-PI (control) is assumed to exhibit 100% inhibitory activity. α_1-PI: alpha$_1$-proteinase inhibitor; MET(O): methionine sulphoxide.

In vitro *oxidation of MET in α_1-PI by taurine-NH-Cl, NH_2-Cl and the xanthine oxidase/hypoxanthine-system*

Studies with these oxidants were performed to estimate their ability to oxidize MET residues in α_1-PI. The effect of taurine-NH-Cl, a myeloperoxidase-derived oxidant, on α_1-PI is shown in figure 3. Oxidation of one MET residue was achieved with a 2.15 fold molar excess of taurine-NH-Cl (fig. 3A), indicating a high susceptibility of MET to this oxidant. Complete loss of the inhibitory capacity of α_1-PI against pancreas elastase was obtained with a 2.2 fold molar excess of taurine-NH-Cl (fig. 3B). Identical results were obtained with regard to oxidation of MET residues and inactivation of α_1-PI with NH_2-Cl as oxidant (data not shown). These findings indicate that the oxidation of a specific MET residue in α_1-PI is responsible for its inactivation.

In contrast, the superoxide anion and related oxidants such as the hydroxyl radical generated by the xanthine oxidase/hypoxanthine-system are less efficient at oxidizing MET residues. A 43 fold molar excess of the superoxide anion was needed in the absence of Fe(III), and a 35 fold molar excess in the presence of 0.1 mM Fe(III), for the oxidation of one MET residue in α_1-PI (fig. 4).

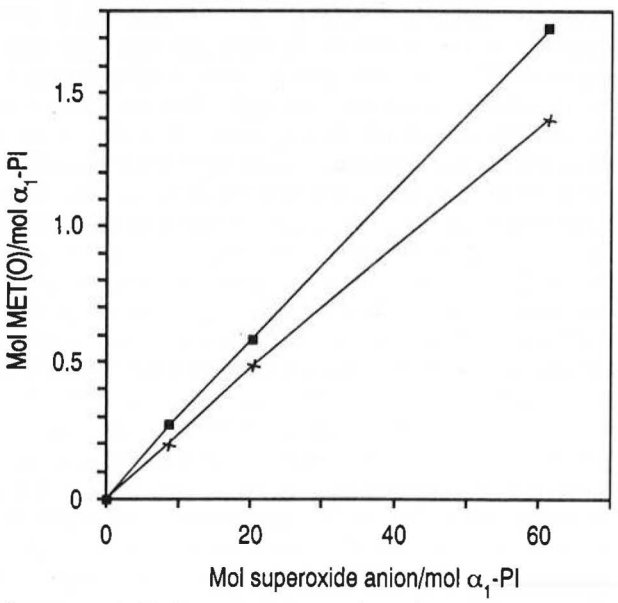

Fig. 4. – Oxidation of MET(O) residues in α_1-PI by xanthine oxidase/ hypoxanthine-derived reactive oxygen species. ×——× : in the absence of Fe(III); ■——■ : in the presence of 0.1 mM Fe(III). For abbreviations see legend to figure 3.

Discussion

The results show that the level of MET(O) was significantly increased in BALF proteins from patients with acute or chronic bronchitis as compared to the control groups. The molar MET(O)/MET ratio found in the BALF proteins from healthy nonsmokers (ratio=0.046) indicates that about 4% of the protein-bound MET in the normal epithelial lining fluid is oxidized. BALF proteins from smokers showed the

same oxidative status compared to the nonsmokers, even though the AM counts were increased fivefold and the PMN counts were increased ninefold. NAKASHIMA *et al.* [35] recently reported that AM from smokers release more than double the amount of superoxide anion, in a resting state as well as in a state of stimulation, in comparison to AM from nonsmokers. The question is why the higher oxidant burden provided by alveolar macrophages and neutrophils in the lungs of smokers is not reflected in an increased MET(O)/MET ratio of the BALF protein. One reason might be that the epithelial lining fluid from healthy smokers contains about twofold higher levels of the antioxidant glutathione compared to that from healthy nonsmokers [36]. Glutathione is known to be an efficient scavenger for numerous oxidants. A further explanation could be the low efficiency of the superoxide anion and the hydroxyl radical in oxidizing MET residues.

CARP *et al.* [21] observed that α_1-PI isolated from BALF from healthy smokers (mean age 28 yrs; 18.1±3.5 pack yrs) contained oxidized MET residues and that the total elastase inhibitory capacity of α_1-PI in this group was decreased by 40% as compared to the nonsmoker group. The authors estimated that four MET residues were oxidized in the inactive α_1-PI from smokers' BALF, whereas no oxidized MET was detected in the active α_1-PI obtained from the nonsmokers [21]. At first glance, these results appear to be in conflict with our findings. However, there is an explanation of the discrepancy. The inactivation of α_1-PI by the myeloperoxidase/H_2O_2/chloride-system from PMN has been studied extensively and it is well-established that oxidation of MET is responsible for the loss of elastase inhibitory activity of α_1-PI [37–42]. One of the nine MET residues in α_1-PI has recently been shown to be highly susceptible to myeloperoxidase-derived oxidants, and is presumed to be identical with the reactive-site MET [29] located in position 358 of the polypeptide chain of α_1-PI [40]. Oxidation of α_1-PI by taurine-NH-Cl is also accompanied by loss of the inhibitory capacity against pancreas elastase, suggesting that the critical MET residue is easily accessible to N-chloramines. We therefore believe that this specific MET residue may compete particularly efficiently with the natural scavengers in the epithelial lining layer for the myeloperoxidase-related oxidants. In contrast most MET residues in native proteins, for example in lysozyme or ribonuclease, appear to be much less sensitive to oxidants [24]. Antigenic α_1-PI, however, accounts for only 2–3% of total BALF protein, as estimated from the data given by CARP *et al.* [21]. If conditions were such that the α_1-PI MET residues were oxidized, but MET residues in other native proteins were not, then there would only be a very small increase in total levels of MET(O). This could explain why CARP *et al.* [21] detected oxidized met residues in isolated α_1-PI from smokers, whereas we did not observe increased MET(O) levels in the total BALF protein from the smoker group.

Several studies have demonstrated the ability of human PMN to oxidize MET residues in proteins such as α_1-PI [25, 43]. However, no information exists on the function of AM in this oxidation process. The finding that the strongly increased number of AM in BALF from smokers was not associated with an increase of MET(O) in the total BALF protein, suggests that AM play only a minor role in the oxidation of MET. AM are devoid of the myeloperoxidase activity (except for those AM which have phagocytosed PMN [44]), which readily oxidizes MET to the sulphoxide. We therefore assume that AM are not essentially involved in the oxidation of MET residues in proteins. This assumption was substantiated by the *in vitro* studies on the oxidation of α_1-PI with the xanthine oxidase/hypoxanthine-system [33, 34], which provides superoxide anions. This system simulates the production of oxygen-derived free radicals by the respiratory burst oxidase [45], *i.e.* the situation with AM. Detectable oxidation of MET residues in α_1-PI was observed only at a high molar excess of superoxide anions produced by the xanthine oxidase in the presence or absence of Fe(III). In contrast, N-chloramines (*i.e.* PMN-derived oxidants [46, 47]) oxidized MET in α_1-PI with a much higher efficiency, indicating that PMN are probably the major effector cells contributing to the oxidation of MET residues in BALF proteins.

The studies of patients with AB also supported the idea that the PMN are responsible for the oxidation of MET. These patients had dramatically increased numbers of PMN, accounting for two thirds of total BAL cells, and showed significantly increased levels of oxidized MET in total BAL protein (fourfold the values in nonsmokers). A considerable fraction of the PMN in AB was of bronchial origin, as concluded from the presence of purulent secretions observed during bronchoscopic inspection. The patients with CB showed a small but significant increase in PMN, and an increase in the MET(O)/MET ratio in BALF protein, which was significant but less than in AB patients. When the MET(O)/MET ratios and PMN levels in the two disease groups are compared, then the PMN in CB seem, on a single cell basis, to be more efficient at oxidizing MET than in AB. The PMN level in AB is about fiftyfold higher than in CB, whereas the MET(O)/MET ratio is only twofold higher. This apparent difference in efficiency between PMN from patients with AB and CB might be due to the fact that PMN in AB are, in part, of bronchial origin, exhibiting a different activation state than PMN of alveolar origin. The same may be true for PMN from AB compared to PMN in IPF. BAL proteins from patients with IPF have MET(O)/MET ratios [46] comparable to those found in this study in the AB group, but the number of PMN in BALF was increased much less (18 fold) than in the AB group (350 fold), in comparison with the nonsmoker group. The reasons for this difference are unclear. The PMN may have different properties in different disease states. Equally there may be different

antioxidant concentrations in the epithelial lining fluid in AB and CB, or the reaction may reach a saturation *in vivo* at relatively low levels of oxidation.

The most prominent consequence of oxidation of MET residues in the epithelial lining fluid is the modification of α_1-PI leading to a reduction of the association constant between the inhibitor and PMN elastase by a factor of 2,000 [48]. This change in the kinetics of complex formation increases the relative affinity of free PMN-elastase to the connective tissue and, thus, favours elastase degradation, the basic mechanism of lung emphysema.

Numerous other peptides, proteins and enzymes (*e.g.* chemotactic peptides, calmodulin, cytochrome c and lysozyme) are known to be affected in their activity by oxidation of a critical methionine [49]. However, the role of these oxidations in the pathogenesis of bronchitis is still speculative.

The inverse correlation between MET(O)/MET ratio and lung function parameters of airflow obstruction in CB suggests the participation of reactive oxygen species in the genesis of chronic obstructive lung disease. Apart from a direct action of oxidants on extracellular matrix proteins, functional modification of α_1-PI by neutrophil-derived oxidants, as discussed above, should be considered as a major mechanism leading to impaired lung function in CB [50].

In conclusion, patients with AB or CB showed a marked increase of MET(O) in BALF protein. This kind of oxidation appeared to be PMN-related, also leading to the inactivation of α_1-PI. Smokers showed low levels of MET(O) in BALF protein comparable to that in nonsmokers, in spite of a massive increase in AM. This finding suggests that AM are not essentially involved in the oxidation of MET residues and this was confirmed by *in vitro* studies.

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