Matrix Metalloproteinases Activity in COPD Associated With Wood Smoke*

Martha Montaño, MSc; Carina Beccerril, MSc; Victor Ruiz, MSc; Carlos Ramos, PhD; Raul H. Sansores, MD; and Georgina González-Avila, MD, PhD

Background: Wood smoke (WS) exposure causes COPD with respiratory alterations that are similar to those described for COPD associated with tobacco smoke (TS). The aim of the present study was to analyze the effects of WS on matrix metalloproteinase (MMP) activity and expression. Design: BAL fluid and macrophages were obtained from patients exposed to WS and TS, and from control subjects. Macrophage elastolytic activity was assayed by radiolabeled elastin degradation. Gelatinolytic activity was measured by zymography in BAL fluid samples. MMP-2, MMP-9, and MMP-12 expression were analyzed by reverse transcription polymerase chain reaction in macrophages from each group.

Results: Macrophage elastolytic activity was increased significantly in WS and TS cells in comparison to control subjects with no differences between WS and TS samples. MMP-2 was identified in all groups as a 72-Kd band (proMMP-2), with the highest activity in the WS samples. MMP-9 was present in its latent and active forms with the highest gelatinolytic activity in the WS group. MMP-2 expression was increased in both groups as well as MMP-12 compared with the control. Two of three subjects studied in each COPD group had a significant increase in MMP-9 expression.

Conclusion: These findings demonstrate that WS increases MMP activity and expression that might produce lung damage similar to that observed in COPD associated with TS. (CHEST 2004; 125:466–472)

Key words: COPD; elastolytic activity; gelatinase A; gelatinase B; matrix metalloproteinases; metalloelastase; wood smoke

Abbreviations: bp = base pair; cGAPDH = competitor glyceraldehyde-3-phosphate dehydrogenase; EDTA = ethylenediaminetetraacetic acid; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; IL = interleukin; MMP = matrix metalloproteinase; PCR = polymerase chain reaction; PMSF = phenylmethylsulphonyl fluoride; RT = reverse transcription; TS = tobacco smoke; WS = wood smoke

COPD is a condition that encompasses chronic bronchitis, small airway disease, and emphysema.1 It is characterized by a progressive chronic obstruction to airflow with a persistent inflammatory process.2,3 Among the cells involved in the inflammation reaction, alveolar macrophages are the predominant cell population identified by BAL.4,5 These cells play an important role in driving the inflammatory response with the secretion of chemotactic factors such as interleukin (IL)-8, growth-related oncogene-α, and leukotriene B4.2,6,7 They also participate in the extracellular matrix degradation by synthesizing and releasing several matrix metalloproteinases (MMPs), which contribute to lung injury during COPD.

MMPs are a group of zinc-dependent and calcium-dependent endopeptidases that can degrade most of the components of the extracellular matrix.8 MMPs are divided into interstitial collagenases, stromelysins, gelatinases, and membrane-type MMPs.8,9 Alveolar macrophages are capable of synthesizing MMP-1 (interstitial collagenase 1), MMP-2 (gelatinase A), MMP-9 (gelatinase B), MMP-7 (matrilysin), membrane-type-1 MMP, and MMP-12 (macrophage metalloelastase).10 The expression of macrophage MMPs is regulated by matrix fragments and by
cytokines such as IL-10, IL-13, and tumor necrosis factor-α, which keep a balance between the synthesis of MMPs and their specific inhibitors, the tissue inhibitors of metalloproteinases. An increase in the activity and expression of macrophage MMPs has been associated with tobacco smoke (TS).

TS has been considered the major cause of COPD. Studies on COPD pathogenesis performed in humans and animals point out that prolonged exposure to cigarette smoke induces airway inflammation with an increase in macrophage MMP expression, which can lead to lung parenchyma destruction. However, TS may not be the only factor involved in COPD pathology. In developing countries, wood and other forms of biomasses have been used as domestic heating and cooking fuels. Domestic exposure to the smoke from firewood increases the prevalence of respiratory diseases such as chronic bronchitis and emphysema.

The molecular mechanisms involved in the onset of COPD associated with wood smoke (WS) are unknown. The aim of the present work was to analyze the effects of WS in the enzymatic activity and expression of MMPs.

Materials and Methods

Study Population

Twelve patients with COPD were examined. The diagnosis of COPD was confirmed by medical history and the results of pulmonary function tests. COPD was defined according to American Thoracic Society criteria. A history of productive daily cough for 3 consecutive months each year for the past 2 years, with an FEV1 of <80% of the predicted value, an FEV1/FVC ratio of <70%, and a reversibility in FEV1 of <10% after inhalation of 400 μg salbutamol. Subjects with a history of asthma, atopy, or allergy were excluded from the study. None of the studied subjects had emphysema detected on a CT scan.

COPD patients were divided into the following two groups: (1) the TS group, consisting of four women and two men who were current smokers for >10 years (mean ± SD smoking history, 21.5 ± 15.8 pack-years; range, 8.4 to 15.8 pack-years); and (2) the WS group, consisting of six women who not tobacco smokers, who had been exposed to domestic WS for a mean duration of 22.7 ± 9.7 years (249 ± 210 h/yr; range, 96 to 500 h/yr). These patients reported the use of traditional “three stone” stoves in their kitchens without a chimney. They also reported seeing smoke and soot on the walls in the cooking area.

Three healthy nonsmoker volunteers with normal spirometry values, no signs of infective respiratory disease during the past 3 weeks, without exposure to WS in the past 10 years, and with no history of atopy, allergy, or asthma were used as control subjects. Clinical data for each group are given in Table 1. Informed consent was obtained from each subject before BAL, and the protocol was approved by the local ethics and research committees.

BAL

Alveolar macrophages were obtained from all subjects by BAL. BAL was performed, with slight modifications, as described elsewhere. Seven 30-mL aliquots of sterile saline solution were instilled with the bronchoscope to the subsegmental bronchi of the middle lobe. The recovered fluid was strained through surgical gauze to remove debris and mucus, and was centrifuged at 400 g for 10 min. The supernatant protein content was measured by the Bradford method, and samples then were stored at −70°C until used.

Elastolytic Activity

Tritium-labeled elastin was prepared by the reductive alkylation of bovine ligamentum nuchae elastin (Elastin Products Company; Owensville, MO), using sodium boro-[3H]-hydride. Elastolytic activity was assayed by the method of Chapman and Stone. Twenty-four well plates were coated with 16 μg 3H-elastin (specific activity, 128,21 disintegrations per min [dpm]/mg). Prior to use, cultured plates were washed three times with phosphate-buffered saline solution. A total of 1 × 10^6 cells/mL added to each well and incubated in RPMI-1640 medium (Sigma; St Louis, MO) containing 10% fetal calf serum. An aliquot was counted in an hemocytometer, and cell viability was analyzed by trypan blue exclusion. Total and differential cell counts in BAL fluid are shown in Table 2. Alveolar macrophages were isolated by differential attachment to elastin-coated 24-well plates (see below).

Table 1—Clinical Characteristics of Control Subjects and COPD Patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control Group</th>
<th>TS Group</th>
<th>WS Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>51.7 ± 3.1 (49–55)</td>
<td>55.7 ± 8.9 (48–71)</td>
<td>59.8 ± 4.3 (56–68)</td>
</tr>
<tr>
<td>FEV1, % predicted</td>
<td>94.7 ± 7.5 (87–102)</td>
<td>35.7 ± 12.8 (45.9–74)</td>
<td>47.2 ± 11.4 (32.5–57.4)</td>
</tr>
<tr>
<td>FEV1/FVC, %</td>
<td>74.3 ± 3.1 (71–75)</td>
<td>38.8 ± 5.8 (33.9–47.4)</td>
<td>58.8 ± 2.8 (56–62)</td>
</tr>
<tr>
<td>FEV1 reversibility, %</td>
<td>ND</td>
<td>4.8 ± 3.6 (2–9.5)</td>
<td>6.6 ± 3.8 (1–9.6)</td>
</tr>
</tbody>
</table>

*Values given as mean ± SD (range). ND = not done.

www.chestjournal.org CHEST / 125/2 / FEBRUARY, 2004 467
Elastolytic activity related to metalloproteinase was inhibitable by EDTA but not by PMSF. The results were calculated as follows:

\[
\text{cpm macrophage sample} - \text{cpm blank} \times \frac{1}{\text{specific activity of } ^3\text{H-elastin}}
\]

where cpm is counts per minute. Results were reported as micrograms of elastin degraded per 1 × 10^6 cells in 48 h.

**Zymogram Assay**

Substrate gel electrophoresis was carried out by incorporating 0.1% pig skin gelatin (Sigma) into standard 8% sodium dodecyl sulfate polyacrylamide gels, as described elsewhere. Three micrograms of protein from the BAL fluid supernatants of three COPD patients and two control subjects were added per lane under nondenaturating conditions and were run under constant current (10 mA). Prestained molecular weight markers (Sigma) were included in each gel. After electrophoresis, the gels were rinsed in 2.5% Triton X-100 and then incubated in TNC buffer (i.e., 50 mmol/L Tris-HCl, 0.15 mol/L NaCl, 20 mmol/L CaCl_2, and 0.02% sodium azide [pH 8.3]), with or without 20 mmol/L EDTA, at 37°C overnight. Each gel was stained in 0.05% Coomassie blue R-250 (Bio-Rad; Richmond, CA) and was destained in 10% methanol-10% acetic acid. Gelatinolytic activity was detected as clear bands on a blue background on the stained gel in gel/ethidium bromide. Band intensities were analyzed by scanning densitometry (Kodak Digital Science ID Image Analysis Software; Eastman Kodak; Rochester, NY) that measures the surface and intensity of lysis bands. Results were expressed as densitometry units.

**Reverse Transcription Polymerase Chain Reaction of Macrophages MMPs**

Reverse transcription (RT) polymerase chain reaction (PCR) amplification was used to analyze the gene expression of MMP-2 (gelatinase A), MMP-9 (gelatinase B), MMP-12 (macrophage metalloelastase), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), in three subjects in each group. Total RNA was isolated from adherent macrophages with Trizol (GIBCO-BRL; Rockville, MD). RNA quality was determined by resolving on denatured 1% agarose gels, and by measuring absorbance on an aliquot at 260 and 280 nm. RT-PCR was performed (ThermoScript; GIBCO-BRL) according to the manufacturer’s protocol. Briefly, to obtain complementary DNA, 1 μg RNA was added to a reverse transcription working mixture containing 15 U/μL avian ribonuclease H-reverse transcriptase, 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 5 mmol/L MgCl_2, 0.5 mmol/L deoxyinosine triphosphates, 2.5 μmol/L random hexamers, 2.5 U/μL RNase inhibitor in a 20-μL final volume.

A GADPH competitor was used to perform a semiquantitative analysis. A 155-base pair (bp) internal fragment was obtained by cutting withNeoI, a GADPH-complementary DNA cloned from an originally 1,233-bp DNA, cloned in a PBR 322 plasmid. Modified GADPH-complementary DNA was subsequently relegated. A competitor GAPDH (cGAPDH; 240 bp) sequence was obtained by PCR amplification from the modified plasmid using primers for GAPFDH. Competitive PCR for the GADPH housekeeping gene was performed with a working mixture containing 20 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2 mmol/L MgCl_2, 200 μmol/L deoxyinosine triphosphates, 1 μmol/L specific 5′ and 3′ specific primers, and 1 U/μL Taq DNA polymerase (Perkin-Elmer; Branchburg, NJ) in a final volume of 25 μL. Serial dilutions of the standard competitor (i.e., 5, 10, 15, and 20 pg) were coamplified with a constant amount of cellular complementary DNA (1 μL). Amplification was carried out (model 9600; Perkin-Elmer). Primers and cycling conditions are shown in Table 3.

PCR product aliquots of 5 μL were resolved in 1.5% agarose gel/ethidium bromide. Band intensities were analyzed by scanning densitometry using digital science electrophoresis documentation (Eastman Kodak) and by an analysis system (system 120).

### Table 2—Cell Content in Control Subjects and COPD Patients BAL Fluid*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control Group</th>
<th>TS Group</th>
<th>WS Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells/mL, 1 × 10^6 cells</td>
<td>51.4 ± 14.3</td>
<td>70.6 ± 33.3</td>
<td>105.8 ± 27.1</td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>92 ± 10.6</td>
<td>80 ± 25.4</td>
<td>75 ± 26</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>7 ± 12</td>
<td>10 ± 1.5</td>
<td>15 ± 2.8</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>0</td>
<td>6 ± 0.8</td>
<td>4 ± 0.4</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Values given as mean ± SD.

### Table 3—Primers and Cycling Conditions for MMPs PCR Amplification

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence</th>
<th>Amplified Product</th>
<th>Cycling Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>Sense 5′CATCCATCCCGTACCTAT3′</td>
<td>395 bp</td>
<td>95°C/10 min for 1 cycle; 95°C/30 s and 72°C/90 s for 35 cycles; and final incubation 72°C/7 min</td>
</tr>
<tr>
<td></td>
<td>Antisense 5′GCATGACTCTCAATAACG3′</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-2</td>
<td>Sense 5′GTGCTGGGCTGCTGCTTTG3′</td>
<td>180 bp</td>
<td>95°C/10 min for 1 cycle; 95°C/30 s, 60°C/30 s, and 58°C/90 s for 33 cycles; and final incubation 72°C/7 min</td>
</tr>
<tr>
<td></td>
<td>Antisense 5′GTGGGCTCACAAGTTTGGAA3′</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-9</td>
<td>Sense 5′TTCAACCGGTGTTGGAAGT3′</td>
<td>303 bp</td>
<td>95°C/10 min for 1 cycle; 95°C/30 s, 60°C/30 s, and 60°C/90 s for 35 cycles; and final incubation 72°C/7 min</td>
</tr>
<tr>
<td></td>
<td>Antisense 5′AAATGTTGGGTGTAACAGGCC3′</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-12</td>
<td>Sense 5′ATATGCTGACATCAACAT3′</td>
<td>200 bp</td>
<td>95°C/10 min for 1 cycle; 95°C/30 s, 60°C/30 s, and 72°C/90 s for 35 cycles; and final incubation 72°C/7 min</td>
</tr>
<tr>
<td></td>
<td>Antisense 5′ATAAGCCGCTTCAATGCCAG3′</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The logarithm of the GAPDH/cGAPDH ratio was plotted as a function of the logarithm of the known cGAPDH amount. The point of equivalence represented the GAPDH concentration in the unknown sample. Once the concentration of GAPDH was obtained for all samples, dilutions were performed to reach 5 pg/μL GADPH. Complementary DNA was amplified with specific primers for MMPs. The primers used for PCR reactions were custom synthesized (GIBCO-BRL). PCR amplification products were resolved in 1.5% agarose/ethidium bromide gels. Band intensities were measured by scanning densitometry. The results were expressed as densitometry units.

**Statistical Analysis**

The results were analyzed with the Mann-Whitney U test and were expressed as the mean ± SD. A p value of ≤ 0.05 was considered to be significant.

**Results**

**Elastolytic Activity**

Elastolytic activity using 3H-elastin-precoated wells was detected in all samples assayed (Fig 1). The mean WS and TS elastolytic activity (4.5 ± 1.27 and 3.0 ± 0.9 μg degraded elastin per 10^6 cells per 48 h, respectively) was significantly increased in comparison with the control group (1.42 ± 0.81 μg degraded elastin per 10^6 cells per 48 h; p < 0.05). Differences between WS and TS samples were not significant. Elastolytic activity was inhibited by EDTA but not by PMSF.

**Gelatinolytic Activity**

Zymography analysis revealed lysis bands of estimated molecular weights of 72, 85, and 92 Kd in the samples from the control group (Fig 2, top). These bands may correspond to proMMP-2 (gelatinase-A), MMP-9 (gelatinase B), and proMMP-9. The enzymatic activity of these MMPs also was observed on TS and WS samples.

ProMMP-2 (72-Kd band) densitometry analysis demonstrated a mean net intensity that was significantly higher in the WS group (8,997.7 ± 3,214.2 densitometry units) than those of the TS and control samples (1,849.1 ± 631.2 and 3,594 ± 1,742.3 densitometry units, respectively; p ≤ 0.01) [Fig 2, bottom].

A significant increase in the mean net intensity (ie, gelatinolytic activity) of the 85-Kd band was observed (MMP-9) in the TS (4,119.3 ± 1,762 densitometry units) and WS samples (11,092 ± 3,040 densitometry units) in comparison with the control (469 ± 309.7 desitometry units; p ≤ 0.01 for all values) [Fig 2, bottom].

The mean net intensity of the 92-Kd band (proMMP-9) also was significantly increased in TS subjects (1,167.9 ± 548.2 densitometry units) and WS subjects (2,502.8 ± 265.4 densitometry units) in comparison with the control subjects (181.4 ± 31.9 densitometry units; p ≤ 0.01 for all values) [Fig 2, bottom].

**Statistical Analysis**

The results were analyzed with the Mann-Whitney U test and were expressed as the mean ± SD. A p value of ≤ 0.05 was considered to be significant.

**Results**

**Elastolytic Activity**

Elastolytic activity using 3H-elastin-precoated wells was detected in all samples assayed (Fig 1). The mean WS and TS elastolytic activity (4.5 ± 1.27 and 3.0 ± 0.9 μg degraded elastin per 10^6 cells per 48 h, respectively) was significantly increased in comparison with the control group (1.42 ± 0.81 μg degraded elastin per 10^6 cells per 48 h; p < 0.05). Differences between WS and TS samples were not significant. Elastolytic activity was inhibited by EDTA but not by PMSF.

**Gelatinolytic Activity**

Zymography analysis revealed lysis bands of estimated molecular weights of 72, 85, and 92 Kd in the samples from the control group (Fig 2, top). These bands may correspond to proMMP-2 (gelatinase-A), MMP-9 (gelatinase B), and proMMP-9. The enzymatic activity of these MMPs also was observed on TS and WS samples.

ProMMP-2 (72-Kd band) densitometry analysis demonstrated a mean net intensity that was significantly higher in the WS group (8,997.7 ± 3,214.2 densitometry units) than those of the TS and control samples (1,849.1 ± 631.2 and 3,594 ± 1,742.3 densitometry units, respectively; p ≤ 0.01) [Fig 2, bottom].

A significant increase in the mean net intensity (ie, gelatinolytic activity) of the 85-Kd band was observed (MMP-9) in the TS (4,119.3 ± 1,762 densitometry units) and WS samples (11,092 ± 3,040 densitometry units) in comparison with the control (469 ± 309.7 desitometry units; p ≤ 0.01 for all values) [Fig 2, bottom].

The mean net intensity of the 92-Kd band (proMMP-9) also was significantly increased in TS subjects (1,167.9 ± 548.2 densitometry units) and WS subjects (2,502.8 ± 265.4 densitometry units) in comparison with the control subjects (181.4 ± 31.9 densitometry units; p ≤ 0.01 for all values) [Fig 2, bottom].

**Statistical Analysis**

The results were analyzed with the Mann-Whitney U test and were expressed as the mean ± SD. A p value of ≤ 0.05 was considered to be significant.

**Results**

**Elastolytic Activity**

Elastolytic activity using 3H-elastin-precoated wells was detected in all samples assayed (Fig 1). The mean WS and TS elastolytic activity (4.5 ± 1.27 and 3.0 ± 0.9 μg degraded elastin per 10^6 cells per 48 h, respectively) was significantly increased in comparison with the control group (1.42 ± 0.81 μg degraded elastin per 10^6 cells per 48 h; p < 0.05). Differences between WS and TS samples were not significant. Elastolytic activity was inhibited by EDTA but not by PMSF.

**Gelatinolytic Activity**

Zymography analysis revealed lysis bands of estimated molecular weights of 72, 85, and 92 Kd in the samples from the control group (Fig 2, top). These bands may correspond to proMMP-2 (gelatinase-A), MMP-9 (gelatinase B), and proMMP-9. The enzymatic activity of these MMPs also was observed on TS and WS samples.

ProMMP-2 (72-Kd band) densitometry analysis demonstrated a mean net intensity that was significantly higher in the WS group (8,997.7 ± 3,214.2 densitometry units) than those of the TS and control samples (1,849.1 ± 631.2 and 3,594 ± 1,742.3 densitometry units, respectively; p ≤ 0.01) [Fig 2, bottom].

A significant increase in the mean net intensity (ie, gelatinolytic activity) of the 85-Kd band was observed (MMP-9) in the TS (4,119.3 ± 1,762 densitometry units) and WS samples (11,092 ± 3,040 densitometry units) in comparison with the control (469 ± 309.7 desitometry units; p ≤ 0.01 for all values) [Fig 2, bottom].

The mean net intensity of the 92-Kd band (proMMP-9) also was significantly increased in TS subjects (1,167.9 ± 548.2 densitometry units) and WS subjects (2,502.8 ± 265.4 densitometry units) in comparison with the control subjects (181.4 ± 31.9 densitometry units; p ≤ 0.01 for all values) [Fig 2, bottom].

**Statistical Analysis**

The results were analyzed with the Mann-Whitney U test and were expressed as the mean ± SD. A p value of ≤ 0.05 was considered to be significant.

**Results**

**Elastolytic Activity**

Elastolytic activity using 3H-elastin-precoated wells was detected in all samples assayed (Fig 1). The mean WS and TS elastolytic activity (4.5 ± 1.27 and 3.0 ± 0.9 μg degraded elastin per 10^6 cells per 48 h, respectively) was significantly increased in comparison with the control group (1.42 ± 0.81 μg degraded elastin per 10^6 cells per 48 h; p < 0.05). Differences between WS and TS samples were not significant. Elastolytic activity was inhibited by EDTA but not by PMSF.

**Gelatinolytic Activity**

Zymography analysis revealed lysis bands of estimated molecular weights of 72, 85, and 92 Kd in the samples from the control group (Fig 2, top). These bands may correspond to proMMP-2 (gelatinase-A), MMP-9 (gelatinase B), and proMMP-9. The enzymatic activity of these MMPs also was observed on TS and WS samples.

ProMMP-2 (72-Kd band) densitometry analysis demonstrated a mean net intensity that was significantly higher in the WS group (8,997.7 ± 3,214.2 densitometry units) than those of the TS and control samples (1,849.1 ± 631.2 and 3,594 ± 1,742.3 densitometry units, respectively; p ≤ 0.01) [Fig 2, bottom].

A significant increase in the mean net intensity (ie, gelatinolytic activity) of the 85-Kd band was observed (MMP-9) in the TS (4,119.3 ± 1,762 densitometry units) and WS samples (11,092 ± 3,040 densitometry units) in comparison with the control (469 ± 309.7 desitometry units; p ≤ 0.01 for all values) [Fig 2, bottom].

The mean net intensity of the 92-Kd band (proMMP-9) also was significantly increased in TS subjects (1,167.9 ± 548.2 densitometry units) and WS subjects (2,502.8 ± 265.4 densitometry units) in comparison with the control subjects (181.4 ± 31.9 densitometry units; p ≤ 0.01 for all values) [Fig 2, bottom].

**Statistical Analysis**

The results were analyzed with the Mann-Whitney U test and were expressed as the mean ± SD. A p value of ≤ 0.05 was considered to be significant.
MMP Expression

Macrophage MMP expression was examined by semiquantitative RT-PCR (Fig 3, top). Complementary DNA samples were adjusted to equal the quantified housekeeping gene GADPH before amplification. Densitometry analysis showed that there were no significant differences among the net intensities from the GADPH bands obtained from the three groups (Fig 3, bottom), demonstrating that the DNA used for MMP amplification was similar for all groups. RT-PCR showed that the TS group samples had the highest MMP-2 expression (21,847 ± 6,501 densitometry units; p < 0.01) when compared to those of the control group (3,565.1 ± 345.5 densitometry units) and the WS group (7,324.9 ± 3,215.8 densitometry units) (Fig 3, bottom). WS group MMP-2 expression was significantly increased in comparison with that of the control group (p = 0.028).

MMP-9 expression appeared to be elevated in four of six COPD subjects, although there were no significant group differences among the TS, WS, and control groups (61,812.9 ± 39,861.1, 67,946.2 ± 52,679.9, and 16,719.6 ± 1,842.7 densitometry units, respectively). One subject in the TS group and one subject in the WS group had MMP-9 expression similar to that of the control group (15,807.5 and 14,084.2 densitometry units, respectively).

MMP-12 gene expression was significantly increased in both COPD groups. TS samples exhibited the highest mean intensity (28,082.4 ± 10,065.5 densitometry units) in comparison with the control group (11,897.4 ± 5,438.2 densitometry units; p = 0.01). WS group MMP-12 expression also was significantly higher than that of the control group (21,393.7 ± 5,684.3 densitometry units; p = 0.03). The differences between the TS and WS samples were not significant.

Discussion

The use of wood and other biomasses for cooking and heating is a very frequent practice worldwide, especially in developing countries, but respiratory alterations due to the exposure to WS scarcely have been investigated.\textsuperscript{19–21} The clinical respiratory alterations associated with long-term WS exposure are the same as those for cigarette smoking. Moreover, chronic bronchitis and emphysema have been observed in non-tobacco smokers exposed to WS.\textsuperscript{29} The effects of TS on the inflammatory process and the molecular mechanisms involved in lung damage have been amply studied. In this context, a large amount of elastolytic activity, due to an increase in the expression of neutrophil elastase and/or MMP-12 from macrophages associated with TS, has been observed.\textsuperscript{17,30} In this study, we tried to determine whether WS has any effect in macrophage metalloelastase activity. We found that WS, as well as TS, increased the elastolytic activity, and that this enzymatic activity corresponds to MMP-12 since PMSF has no effect on it. This finding was confirmed by RT-PCR analysis, which revealed an increase in MMP-12 expression in subjects exposed to WS.

Other MMPs explored in this work were MMP-2 and MMP-9. These enzymes have the capacity to degrade type IV collagen, the main protein of basement membranes damaged in COPD. Increased gelatinolytic activity associated with MMP-2 (gelatinase A) and MMP-9 (gelatinase B) has been identified in BAL fluid from COPD patients.\textsuperscript{18} In the
In the present work, we found gelatinolytic activity corresponding to both gelatinases. MMP-2 activity in WS group samples was elevated compared to that in samples from the TS and control groups. In contrast, MMP-9 activity was higher than that in control subjects in both COPD groups, with the largest activity in WS group samples. There is some evidence that MMP-9 is one of the main proteolytic enzymes involved in emphysema pathology. This enzyme has been identified as a 92-Kd (proenzyme) and an 86-Kd band (active form), but also as high-molecular-weight bands (130 Kd) that correspond to lipocalin-progelatinase-B complex in BAL fluid samples. This complex (neutrophil gelatinase-associated lipocalin) is characteristic of neutrophils. In this work, we were not able to detect these molecular complexes, probably because the neutrophil count in the BAL fluid samples was very low.

According to our MMP enzymatic activity results, macrophage RNA was analyzed to see whether WS and TS had any effect on MMP-2 and MMP-9 expression that could explain in part the increase in gelatinolytic activity. MMP-2 expression was significantly increased in both groups. The increase in MMP-2 expression correlates with the MMP-2 activity observed in the WS group. However, the increase in MMP-2 expression in TS samples did not correspond to the low MMP-2 gelatinolytic activity found in BAL fluids from subjects in this group. It is possible that the low enzymatic activity observed in the TS group was due to a low translation rate and/or to low enzyme activation.

In contrast, the results obtained in MMP-9 expression analysis were not homogenous, and this could be due to the presence of polymorphism in the promoter region of MMP-9 (−1,562 C/T [cytosine/thymine]). It has been reported that the T allele has a higher promoter activity than the C allele because there is preferential binding of a repressor to the C allelic promoter. Moreover, subjects with an increase in MMP-9 expression (ie, the T allele) had a high MMP-9 gelatinolytic activity. It is possible that in our study the subjects with a high MMP-9 expression had the T allele that corresponds to an increase in gelatinolytic activity.

In conclusion, although the analyzed population was small, this study demonstrates that chronic exposure to WS has similar effects to that of TS in MMPs enzymatic activity and expression. The presence of these enzymes in the respiratory tract might degrade the interstitial extracellular matrix and basement membrane components, and cause lung damage similar to that observed in COPD associated with tobacco smoking.

References
30 Snider GL. Collagen vs elastin in pathogenesis of emphysema: cellular origin of elastases; bronchiolitis vs emphysema as a cause of airflow obstruction. Chest 2000; 117:244S–246S