Effect of Wood Combustion Smoke Inhalation on Angiotensin-1-Converting Enzyme in the Dog

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ABSTRACT

One lung of each dog was exposed to smoke from burning pine wood, while the other was subjected to acute hypoxia. Angiotensin-1-converting enzyme (ACE) activity in biopsied tissue of the smoke-exposed lung was markedly increased immediately after the injury and even higher 30 minutes later. No change in ACE activity was observed in the hypoxic contralateral lung. Serum ACE activity did not change significantly following anesthesia and before smoke inhalation. Serum aldosterone and cortisol levels increased at this juncture. Smoke inhalation caused intra-alveolar hemorrhages and edema. Pulmonary and systolic, diastolic and mean pressures, pulmonary capillary, wedge pressure, cardiac output and systemic and pulmonary arteriolar resistances remained unchanged throughout the experiment. The changes of ACE activity are presumably a direct effect of smoke inhalation. They are seen as an early response of the lung endothelial cells to many types of injury (chronic hypoxia, bleomycin or monocrotaline administration) and may represent an important step in the development of the organ’s response to the injury.
Introduction

The endothelial cells of the lungs show damage in the early phase of several types of lung injury (exposure to radiation, hypoxia, monocrotaline, bleomycin, paraquat, and thiourea). These cells are the source of several substances with vasoregulatory activity including angiotensin-1-converting enzyme (ACE), prostacyclin (PGI₂) and plasminogen activator (PLA). The secretion of these substances is altered after lung injury, and detection of changes in their levels may therefore reflect the severity of the organ damage. The same substances may also be involved in the long-term consequences of injury. It has been reported that during exposure to chronic hypoxia, the excessive production of ACE by the lung endothelial cells promotes an increased formation of the vasoconstrictor angiotensin II which might conceivably cause, as a final consequence, pulmonary hypertension, and indeed pulmonary hypertension is frequently seen in more advanced stages of the aforementioned model of injury. Furthermore, treatment with ACE inhibitors (Teprotide and Captopril) partially prevents the development of pulmonary hypertension from chronic hypoxia or monocrotaline administration. This also suggests that an excess of ACE production by lung endothelial cells is involved in the development of the pulmonary damage through the increased synthesis of angiotensin II and/or through increased inactivations of kinins.

Because preliminary results in thermally injured individuals have shown that patients with smoke inhalation have significantly higher levels of ACE activity than patients with cutaneous burns only, the present authors investigated to find out if the increase in serum ACE activity seen in humans exposed to smoke inhalation is related to the inhaled smoke, whether or not the smoke could damage the lung structure, and, if so, how. It was elected preferentially to expose to smoke inhalation one lung in dogs while the other lung was obstructed. The purpose of obstructing the contralateral lung was to rule out that other factors such as a concomitant hypoxia and/or anesthesia could also influence the lung ACE activity.

Materials and Methods

Eleven male mongrel dogs with an average weight of 25 kg were caged separately under standard conditions, with access to food and water ad libitum. Food was withdrawn 12 hours prior to surgery. On the morning of the experiment, a 22-gauge teflon catheter was inserted into the dog’s right forelimb cephalic vein and blood samples for determinations of serum A-1-CE, aldosterone, and cortisol were collected. Ringer’s lactate intravenous solution was infused at the rate of 80 ml per hour, obtaining a constant urine flow of 60 to 80 ml per hour. Through the same route, anesthesia was induced using Pentobarbital (6 percent) in the dosage of 30 mg per kg. The animal was intubated with a cuffed #8 endotracheal tube and placed on a Harvard animal respirator, model 607 (Fi O₂ 21 percent, Tidal volume 10 to 15 cc per kg, rate 12 to 16 breaths per minute). The chest, abdomen, pelvis, and inner hind legs of the animal were shaved with an Oster #80 blade clipper. The dog was then secured in the supine position to the operating table. A transurethral #10 French bladder catheter was inserted, and through this urine was allowed to drop by gravity. The left superficial musculocutaneous branch of the femoral artery was exposed for placement of a 22-gauge teflon catheter. The right internal jugular vein was used for insertion of a Swan-Ganz #6 French pulmonary out-
flow tract balloon directed catheter. Three limb electrocardiogram (EKG) electrodes were applied. Continuous monitoring of arterial pressure, pulmonary artery pressure, and EKG rhythm was then accomplished utilizing Stathan 23Db strain gauges and recorded on a Grass Model 7 polygraph. Cardiac output was performed by thermodilution technique.*

A tracheotomy was carried out between the eighth and ninth tracheal rings with subsequent removal of the oropharyngeal endotracheal tube. The dog was then ventilated with the Harvard animal respirator via the tracheotomy. Bilateral anterolateral thoracotomies were made in the fourth intercostal space. Arterial blood was collected for determination of serum A-1-CE, aldosterone, cortisol, and blood gases. Bilateral lung biopsies were performed.

Via tracheotomy, the left main-stem bronchus was preferentially intubated with a cuffed #6 endotracheal tube. The right lung was maintained hypoxic by mechanical obstruction of the right main-stem bronchus. Smoke was generated by the ignited contents of a cylindrical chamber. This apparatus consisted of an aluminum cylinder with a diameter of 15 cm and length of 25 cm, with inflow and outflow tracts and a removable back lid. Pinewood shavings and plain tissue paper were used as combustible material. The outflow tract of the smoke generator was then coupled to the endotracheal tube entering the left main-stem bronchus while the inflow tract was coupled to a standard adult-size Ambu bag. Smoke ventilations were then given at five to seven per minute for eight minutes. Immediately after smoke inhalation, blood was collected for determinations of serum A-1-CE, aldosterone, cortisol, and blood gases, and bilateral lung biopsies were taken. Then assisted ventilation to both lungs was resumed at pre-smoke ventilatory settings. The same blood determinations as well as lung biopsies were repeated 30 minutes later, when assisted ventilation was discontinued and the animal was allowed to expire.

Each of the lung biopsies consisted of 2 x 2 cm specimen collected from the lateral margin of the lower left and lower right pulmonary lobes, each of them weighing approximately two grams. After collection, they were immediately immersed in liquid nitrogen and stored frozen at −70°C until used for chemical determinations, with the exception of a small portion which was immersed in the proper fixatives for histologic (LM and EM) studies.

When blood was collected, care was taken that heparin from the catheter would not contaminate the blood samples since any anticoagulant could interfere with ACE determinations. Activity of ACE in lung and blood serum was determined about 72 hours after collection by the spectrophotometric method of Cushman and Cheung* using the synthetic substrate hippuryl-L-histidyl-L-leucine.* Protein concentration in the lung homogenate and serum was determined by the biuret method,18 and ACE activity expressed in U per mg protein, as described previously.25 Pooled normal human blood serum was analyzed simultaneously as an interassay standard. Serum aldosterone concentration was measured by a radioimmunoassay kit.† Serum cortisol was also measured by a radioimmunoassay kit.‡

For light microscopy, the portion of the excised biopsy was formalin-fixed and sectioned at four µ thickness and stained with hematoxylin-eosin. For the electron

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* The Edwards Cardiac Output Computer Model 9520 was utilized.
† Damon Diagnostics, Needham Heights, MA.
‡ Gamma Coat Chemical Assays, Cambridge, MA.
A microscopic study, representative samples of lung tissue were fixed in five percent glutaraldehyde in Millonig's buffer, post-fixed in one percent Osmium tetroxide, dehydrated in ascending grades of alcohol and embedded in epoxy resin, and cut for thick and thin sections. Thick sections were stained in one percent toluidine blue for light microscopy. The sections were stained in uranyl acetate and lead citrate for electron microscopy. The significance of differences between groups was evaluated by student's "t" test.

Results

ACE and Protein Determination in Lungs

Lung ACE activity in each lung is shown in figure 1 with enzyme activity values expressed as mU per 100 g of tissue and in figure 2 with activity expressed as mU per mg protein. After anesthesia, the levels of lung ACE activity were similar in both lungs, whether the values were calculated with respect to lung tissue weight or to lung protein content. Immediately after smoke inhalation, there was a significant increase in ACE activity in the left lung, while no changes were evident in the contralateral organ. Thirty minutes later, at sacrifice, values were still elevated in the left lung and unchanged in the right. The protein content in both lungs was very similar at each interval time (figure 3) varying from 1.94 ± 0.14 mg per ml* of lung homogenate to 2.05 ± 0.20 mg, which indicates that the increase in converting enzyme activity was not an artifact related to changes in lung weight owing to edema. The increase in ACE activity is more dramatically shown in figure 4 where the ratio of left versus right lung ACE activity is displayed. The ratio increases from 1:1 after anesthesia to 2:1 and then to 4:1 during the progress of the experiment.

Serum ACE Activity and Serum Aldosterone and Cortisol Levels

Levels of serum ACE activity and of serum cortisol and aldosterone are summarized in table I. No significant change in serum ACE was noted after anesthesia.

*One standard error of the mean (SEM)
or smoke inhalation, either immediately after lung exposure to smoke or 30 minutes later. An increase of serum cortisol and aldosterone levels was evident after anesthesia and persisted without alteration by exposure of the left lung to smoke.

**Histologic and Ultrastructural Studies**

The right lung, exposed to acute hypoxia, did not show, either at light or electron microscopy, any significant change of its structure but moderate atelectasia (figure 5). In the contralateral lung, exposed to smoke inhalation, scattered areas of the lung showed congestion and hemoconcentration in the perialveolar capillaries; there were numerous focal areas of intra-alveolar hemorrhage and edema (figure 6). Ultrastructural studies at this early stage of injury did not reveal definite evidence of damage to the pneumocytes or to the perialveolar capillary endothelium (figures 7 and 8). Further investigations are being carried out at longer intervals from the time of injury to determine the earliest detectable morphologic evidence of endothelial damage. [Hasleton et al. 4 have reported marked changes and, in particular, the presence of giant endothelial cells in humans lungs 24 hours following smoke inhalation in deceased burn patients.]

**Physiologic Monitoring**

No significant changes were observed in pulmonary and arterial systolic, diastolic and mean pressure, wedge pressure, cardiac output and systemic arterial and pulmonary arteriolar resistances of the dogs pre- and post-smoke inhalation. PaO₂ from an initial average value of 66.6 percent decreased to 31.8 percent post-pulmonary injury, while PaCO₂ from a value of 25.7 percent pre-pulmonary injury rose to 64.3 percent following smoke inhalation. The pH value (systemic arterial circulation) changed from 7.49 to 7.14. Overall an hypoxemia with a PaO₂ of 50 percent of the pre-injury level was produced in all animals.

**Discussion**

The present experiment demonstrated a significant increase in ACE activity in the smoke-exposed lungs of dogs in com-
TABLE I
Serum A-1-CE Activity, Aldosterone and Cortisol In 11 Dogs Exposed to Smoke Inhalation

<table>
<thead>
<tr>
<th></th>
<th>Pre Anesthesia</th>
<th>Post Anesthesia (V)</th>
<th>Post Pulmonary Injury (V)</th>
<th>Before Sacrifice (30' after PPI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum A-1-CE (mU/ml/min)</td>
<td>6.96 ±0.93*</td>
<td>5.56 ±0.66</td>
<td>5.53 ±0.70</td>
<td>5.97 ±0.87</td>
</tr>
<tr>
<td>Serum Aldosterone (pg/ml)</td>
<td>50.25 ±9.23</td>
<td>127.55† ±29.25</td>
<td>95.30† ±13.99</td>
<td>95.09† ±20.24</td>
</tr>
<tr>
<td>Serum Cortisol (ug/100 ml)</td>
<td>3.18 ±0.54</td>
<td>9.82‡ ±1.07</td>
<td>8.82‡ ±1.57</td>
<td>10.73‡ ±1.21</td>
</tr>
</tbody>
</table>

*One standard error of the mean (SEM)
†p < 0.05
‡p < 0.001

comparison to the contralateral hypoxic lungs of the same animals. The histologic findings in the lung parenchyma with red cell hemoconcentration in the perialveolar capillaries, intra-alveolar hemorrhages, and edema indicate early pulmonary vascular injury and are consistent with the chemical determinations.

In this aspect, smoke inhalation is reminiscent of the changes of A-1-CE described in other lung injuries such as bleomycin or monocrotaline in which the enzyme activity is significantly increased at a time when histological evidence of injury is slight. In these latter two models, a time interval from several hours to a few days is necessary to produce the same enzymatic modification that occurs within minutes following smoke inhalation.

The effect of hypoxia on lung ACE activity varies in relationship to the exposure time of the organ to injury. Chronic hypoxia is associated with an increase in lung ACE activity. The reports on the effects of acute hypoxia are controversial. Some authors reported a decrease of ACE activity; others reported no changes.

Our experiment adds further support to the concepts that the changes of ACE seen in the left lungs of the dogs are related to smoke inhalation. Although the lungs exposed to smoke inhalation have some interstitial and intra-alveolar...
Figure 6. Photomicrograph of a subpleural area of the contralateral dog lung (smoke inhalation). There is massive intra-alveolar hemorrhage, capillary congestion and emphysema. (× 125).

Figure 7. Electron microscopy of right hypoxic lung illustrated in figure 5. Alveolar septa, type 1 (P1) and 2 (P2) pneumocytes and endothelial cells (E) are unremarkable. (× 4000).

hemorrhage and some areas of intra-alveolar edema, their protein content did not significantly differ from that of the contralateral organ exposed to acute hypoxia. This finding is also supportive of our suggestion that the increased A-1-CE activity, which was expressed in relation to the lung content of protein, is a real one and not related to edema of the organ which could have affected its weight.

Serum ACE did not change in our experiment although ACE activity in the smoke-exposed lungs increased to four times that of the control lungs (figure 4). In bilateral lung injury owing to bleo-
mycin, a 60 percent increase of lung ACE over baseline was associated with 25 percent increase in serum ACE;\textsuperscript{10,24} while, in monocrotaline toxicity, a 150 percent increase in lung activity for approximately three days was not associated with serum change.\textsuperscript{8,11} In another injury model in which only one lung is injured, radiation caused a drop in ACE activity\textsuperscript{25} of the injured lung to 14 percent of the control lung without change in serum ACE.

From these experiments it appears that changes of ACE activity in the lungs are not always reflected by parallel changes of the serum enzyme. Perhaps only a sustained lung injury with lung ACE increases for a long time will eventually be reflected by serum increase.

The changes in serum cortisol and aldosterone were evident following anesthesia. The values of these hormones remained elevated throughout the experiment; most likely they were related to stress rather than lung injury.

It is not known whether or not the increase in ACE activity after smoke inhalation will lead to increased secretion of angiotensin II, vasoconstriction and pulmonary hypertension as was seen in chronic hypoxia or administration of monocrotaline.\textsuperscript{27,11} The possibility, however, that ACE changes may influence the systemic blood pressure through a modulation of the renin-angiotensin-aldosterone system is supported by the extensive use of A-1-CE inhibitors (Teprotide, Captopril) as anti-hypertensives.

Our data here in the early phase of injury in dogs indicate that further studies are necessary with the animals kept alive for a longer period and with comparison of the effect of smoke in one lung versus the exposure of both organs. In addition, A-1-CE activity during the early stage of burns should also be evaluated in humans.

From these studies it should eventually be known whether or not changes in lung and serum A-1-CE may become an indication of the extension and severity
of the pulmonary damage as it has been recognized in other conditions of pulmonary injury. It will also be possible to ascertain whether or not lung changes of ACE activity may locally and diffusely influence the RAA system and the patient’s general homeostasis.

References


