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# Angiotensin-converting Enzyme: an Indicator of Bleomycin-induced Pulmonary Toxicity in Humans?*

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**Abstract**—*In order to evaluate bleomycin-associated lung damage in humans, lung function parameters and serum levels of the endothelial-bound angiotensin-converting enzyme (ACE) were determined by serial measurements in* 11 *patients who were treated for testicular cancer. None developed clinical or radiological evidence of pulmonary damage. While the static and dynamic lung function parameters were unchanged, carbon monoxide diffusion capacity* ($DL_{CO}$) *decreased significantly* ($P < 0.01$) *during a total of* 126 days *of pulsed regimen, indicating damage to the alveolar–endothelial membrane. S-ACE was unchanged within each treatment course but increased significantly* ($P < 0.05$) *from the initial value to the last treatment course. Two months after cessation of treatment S-ACE returned to pretreatment values. Although the changes were modest they might mirror treatment-associated endothelial damage.*

## INTRODUCTION

ADMINISTRATION of the antineoplastic drug bleomycin is associated with pulmonary inflammation, interstitial pneumonitis and fibrosis [1]. In a previous study [2] we have shown that bleomycin induces an irreversible decrease in the carbon monoxide diffusion capacity of the lungs ($DL_{CO}$), indicating a subclinical damage to the alveolar–endothelial membrane. In animal studies the first sign of bleomycin-associated pulmonary toxicity is seen in the endothelial cells [3]. Because the angiotensin-converting enzyme (ACE) is situated in the pinocytic vesicles of the pulmonary endothelial plasma membrane [4] we suggested that alterations of serum ACE (S-ACE) may occur during bleomycin treatment. Thus the aim of this study was to evaluate the changes in S-ACE activity during bleomycin treatment as a possible marker of endothelial damage.

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## MATERIAL AND METHODS

### *Patients*

Eleven patients (aged 18–29 yr) with testicular embryonal carcinoma stage II were included in the trial. None of the patients had a history, or clinical or radiological evidence of pulmonary disease. None of the patients had previously received chemotherapy or chest irradiation.

### *Regimen*

The patients received three drugs: *cis*-platinum, vinblastine and bleomycin (Fig. 1). *Cis*-platinum was given in a dosage of 20 mg/m$^2$ as a 15-min intravenous infusion for 5 consecutive days (days 1–5) every 3 weeks for six courses. Vinblastine was given in a dosage of 6 mg/m$^2$ intravenously on days 1 and 2 of each of the six *cis*-platinum courses. Bleomycin was given as an intravenous bolus in a dosage of 15 mg/m$^2$ on days 2, 9 and 16 in each of the three first *cis*-platinum courses and in a dosage of 5 mg/m$^2$ in the last three courses, to a total dose of 270 mg/m$^2$. The total treatment period was 126 days.

### *ACE assay*

S-ACE was measured prior to each treatment course on day 1 and daily before drug administra-

Fig. 1.

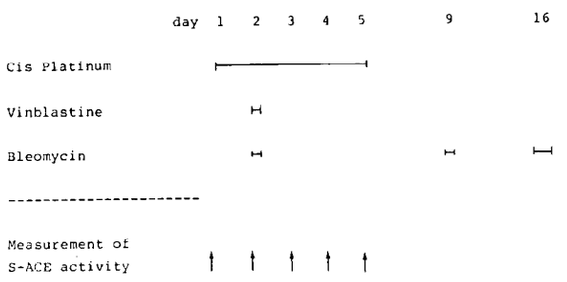


*Fig. 1. The schedule of one chemotherapeutic treatment course, with an indication of S-ACE measurements. The course was repeated five times with 3-week intervals. The total treatment period was* 126 days.

tion on days 2–5 during treatment (Fig. 1). S-ACE was also measured 2 months after cessation of treatment.

S-ACE was measured spectrophotometrically as described by Lieberman [5]. The enzyme activity was expressed in units per millilitre, one unit being nanomoles of hippuric acid liberated per minute from the substrate hippurylhistidylleucine using 60 min incubation and phosphate buffer at pH 8.3. As previously described [6], S-ACE was 24.4 ± 6.2 U/ml (mean ± S.D.) in 116 apparently healthy blood donors aged 18–65 yr. The mean ± 2 S.D. reference range was 12.0–36.8 U/ml, and values above 36.8 U/ml were considered to be abnormally elevated. The coefficient of variation for both inter- and intra-assay was less than 5% when examined on low, intermediate and high S-ACE levels. After clotting the serum was stored at -20°C until all the specimens ($N = 320$) were analysed blindly in consecutive runs.

*Pulmonary function*

Before each of the six treatment courses plain chest roentgenograms and lung function tests were performed. The lung function was assessed by spirometry, including a single-breath helium dilution manoeuvre (Hewlett-Packard computing pulmonary system). The parameters evaluated were: total lung capacity (TLC), vital capacity (VC), residual volume (RV), forced vital capacity (FVC) and forced expiratory volume in 1 sec ($FEV_1$). Carbon monoxide diffusion capacity, $DL_{CO}$, was determined by a modified method of Ogilvie *et al.* [7]. All $DL_{CO}$ values were corrected with respect to blood haemoglobin concentration by the method of Dinakara *et al.* [8].

*Statistics*

Statistical analysis of the variance of the lung function measurements was performed according to the method of Rodbard [9]. Student's $t$ test for paired samples was used to analyse the changes in lung function and S-ACE activity during treatment. As a measure of correlation we used the correlation coefficient. $P$ values $<0.05$ were considered as statistically significant.

## RESULTS

*Clinical course*

All the patients achieved complete or partial remission during the treatment and 24 months after the initiation of the treatment only one patient had relapsed. During the 18 weeks of antineoplastic treatment none of the patients developed clinical evidence of pulmonary disease.

*Pulmonary studies*

Neither before nor during treatment did radiological signs of interstitial pneumonitis develop. Table 1 shows that while the static and dynamic lung function parameters remained unchanged, $DL_{CO}$ decreased significantly ($P < 0.01$).

*S-ACE activity*

The pretreatment S-ACE was rather low but within normal limits in all patients (20.5 ± 1.0 U/ml). During treatment we observed no significant changes within each treatment course, while a significant increase ($P < 0.05$) occurred when initial S-ACE was compared to the S-ACE activity at the last course (25.0 ± 0.8 U/ml) (Fig. 2). Two months after cessation of treatment the S-ACE values had returned to pretreatment levels (19.1 ± 4.2 U/ml). The changes during treatment in $DL_{CO}$ and S-ACE were negatively correlated ($r = 0.93$) (Fig. 3).

## DISCUSSION

The pulmonary endothelial cells, making up 40% of the total amount of cells in the lungs [4], are the target cells of bleomycin cytotoxicity and are the main sources of ACE in the organism. Therefore it has been suggested that monitoring of the S-ACE may have clinical value in

*Table 1. Lung function before and after chemotherapeutic treatment in 11 patients with testicular carcinoma*

| | $FEV^1$ | TLC | $DL_{CO}$ |
|---|---|---|---|
| Before treatment | 98 ± 19 | 114 ± 10 | 88 ± 12 |
| After treatment | 105 ± 20 | 108 ± 20 | 59 ± 10 |
| Statistics | N.S. | N.S. | $P < 0.01$ |

The values are expressed in percentage of predicted value (mean ± S.D.).
N.S., not significant; $FEV^1$, forced expiratory volume in 1 sec; TLC, total lung capacity; $DL_{CO}$, carbon monoxide diffusing capacity.

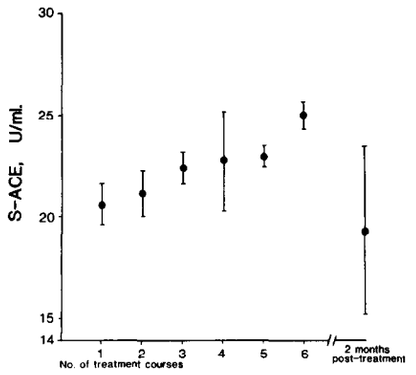


*Fig. 2. Pooled S-ACE (mean ± S.D.) during bleomycin treatment and* 2 months *after cessation of treatment.*

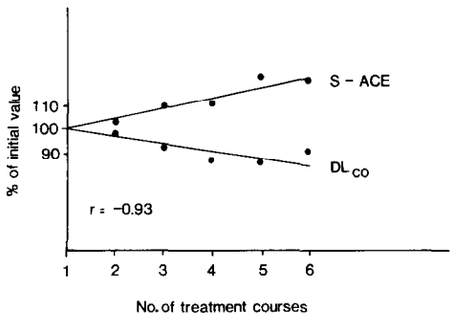


*Fig. 3. Pooled S-ACE and* $DL_{CO}$ *as a percentage of the initial value during six courses of bleomycin treatment.*

identifying impending pulmonary damage induced by bleomycin [10].

The results in animal studies are contradictory: Newman *et al.* [11] showed that a single intratracheal dose of bleomycin in rats was followed by pulmonary ACE depletion and increasing ACE activity in bronchoalveolar lavage fluid and a transient increase of S-ACE, while Catravas found no change in rabbits [12]. In contrast, Chandler and Giri [13] observed a decrease in serum ACE after intratracheal bleomycin administration in hamsters. In mice Lazo found an increase in both serum and lung ACE after subcutaneous administration of bleomycin [14], while in rabbits both serum and lung ACE decreased during subcutaneous bleomycin treatment [15]. The discrepancies may be attributed to route of administration, species variation and ability to repair damage [12]. The animal studies indicate a bleomycin-associated endothelial damage with shedding of the enzyme.

Our findings are in accordance with the results obtained by Newman *et al.* [11], although we found that S-ACE increased from course to course rather than from day to day. This may mirror a subclinical lung damage only evident by the decreased $DL_{CO}$. Although the increase in S-ACE was rather modest and all values except one were within the normal range, the inverse relationship between changes in $DL_{CO}$ and S-ACE during the treatment period was consistent with damage to the alveolar–endothelial membrane.

The evaluation of a biochemical marker of toxicity is often complicated by changes in the evaluable parameter caused by the malignant disease itself, i.e. in both leukaemia and lung cancer the activity of S-ACE is decreased when compared to healthy controls [16, 17]. This indicates one of the major problems in biochemical assessment of toxicity during antineoplastic treatment. It is difficult to tell whether an alteration is caused by the treatment or by changes of the malignant disease. However, our observation of an increasing trend of S-ACE during the whole treatment period and the decline after cessation of treatment—without evidence of relapse—suggest that the S-ACE alterations are most certainly secondary to the treatment.

Because Newman *et al.* [11] found a 30-fold increase in the bronchoalveolar fluid ACE activity after administration of a single bleomycin dose, bronchoalveolar lavage ACE measurements may be a more sensitive method for assessment of endothelial damage than the measurement of serum activity.

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