

SHORT REPORT

Effects of cigarette smoking on the serum concentration of lung surfactant protein A (SP-A)

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ABSTRACT: We have tested the hypothesis that serum surfactant protein A (SP-A) production may be affected by cigarette smoke exposure, and that alterations in serum levels may reflect smoking habits.

A total of 52 subjects were divided into the following two groups: active smokers (Group A; n=23); and nonsmokers (Group B; n=29). The assessment of smoking habit was determined by self-report, carbon monoxide concentration in expired air, and cotinine concentration in urine.

Serum SP-A was compared in both groups and was found to be higher in Group A (active smokers) ($p<0.002$).

We conclude that surfactant protein A levels are increased in the serum of smokers. However, it was found that this is not a sensitive discriminating factor to separate smokers from nonsmokers.

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Surfactant is a complex substance comprising at least four specific proteins with a unique phospholipid composition [1]. The unique surfactant-associated proteins have been identified as surfactant proteins A (SP-A), B (SP-B), C (SP-C) and D (SP-D) [1]. Recently, KUROKI *et al.* [2] showed that SP-A appears in the bloodstream as a complex with immunoglobulin, and that increased concentrations are found in the serum of patients with idiopathic pulmonary fibrosis (IPF) and alveolar proteinosis. There is evidence that smoking is a potentiating factor in IPF, and biochemical studies suggest that surfactant function in smokers is impaired [3, 4].

In this study, we hypothesized that SP-A production may be affected by cigarette smoke exposure, and that alterations in serum levels may reflect smoking habit.

In this context, serum SP-A levels in healthy male cigarette smoking and nonsmoking office workers were compared.

Methods

Subjects

Sixty male office workers, both smokers and nonsmokers, aged 20–50 yrs, were selected at random. After being informed in detail of the study purpose and procedures, all subjects answered a questionnaire about their respiratory symptoms, past medical history, current medications and detailed smoking habits. Subjects with a history of wheezing, persistent cough or expectoration, present or previous cardiopulmonary disease, or those on current medication were excluded from the study.

Finally, a total of 52 subjects were entered into the study. All tests were performed on one Wednesday or Thursday, during working-time, between 14.00 and 16.00 h. Current smokers were not permitted to smoke for at least 1 h prior to testing.

A total of 52 subjects fulfilled the criteria and were included in the study. According to smoking habit, the subjects were divided into active smokers (Group A; n=23) and nonactive smokers (Group B; n=29). Twenty of those in Group B had never smoked and nine were exsmokers who had ceased smoking more than 3 yrs earlier.

Ages ranged 23–47 yrs, Group A (n=23; age (mean \pm SEM) 33 \pm 8 yrs) and Group B (n=29; 36 \pm 9 yrs).

Measurements

Pulmonary function test. Pulmonary function testing of forced expiratory volume in one second (FEV₁), FEV₁ % predicted, and FEV₁/vital capacity (VC) were performed with a spirometer (Chest HI-501, Chest Co., Tokyo, Japan). Triplicate measurements were obtained for each subject.

Urine cotinine. The first approximately 10 mL of urine expelled from each subject was used to measure cotinine. The urine was stored at -80°C until used. The concentration of cotinine was measured by high-performance liquid chromatography (HPLC) by the method of HORSTMANN *et al.* [5], with minor modification. Briefly, 1 mL of 5 N NaOH and 4 mL of chloroform were added to 5 mL of the urine sample, and the mixture was centrifuged for 10 min. The organic layer was then redissolved in 250 μ L of dichloromethane for HPLC analysis.

Chromatographic analysis was performed at 40°C at a flow rate of 1–2 mL·min⁻¹. The concentration of cotinine in the urine sample was corrected for creatinine concentration in the same sample (COT/CRE ratio). For the assay of urinary creatinine, the level in 150 µL of urine was measured using an automated clinical chemical analyser (550 Express; Ciba-Corning Instruments, New York, USA). Reproducibility and coefficients of variation in the urinary cotinine and creatinine analysis were obtained by duplicate analysis.

Carbon monoxide in expired air. Each subject was asked to take a deep breath, hold it for 20 s, and then breathe out steadily and gently through the mouthpiece of the equipment described below. The concentration of end-tidal expired air carbon monoxide was measured with Mini and Micro Smokerlyzers (Bedford Technical Instruments Ltd, Manchester, UK). The equipment was calibrated daily during use with a mixture of 100 ppm carbon monoxide in air.

Surfactant protein A. The SP-A concentration in serum samples was measured according to KUROKI *et al.* [2], with minor modifications as follows. The serum samples were diluted from 50 to 200 µL in buffer A (10 mM phosphate buffered saline (PBS), pH 7.2, containing 1.5% (w/v) sodium dodecyl sulphate (SDS) and 3% (v/v) Triton X-100). Two hundred microlitres of monoclonal antibody prepared against human SP-A (provided by Y. Kuroki and T. Akino, Sapporo Medical College, Japan) and labelled with horseradish peroxidase dissolved in buffer B (10 mM PBS, pH 7.4, containing 0.25% (w/v) skimmed milk), was added and mixed thoroughly. A plastic bead coated with the monoclonal antibody to SP-A was added to each tube containing the above mixture, and the assay tubes were incubated at 37°C for 90 min.

After incubation, the beads were washed three times with distilled water and added to 400 µL of substrate solution (0.1 M phosphate-citrate buffer, pH 4.0, containing 5 mM H₂O₂ and 0.06% (w/v) tetramethylbenzidine). The mixtures were incubated at 37°C for 30 min and the reaction was stopped by the addition of 1 mL of 1 N sulphuric acid. The absorbance of each tube was measured at 450 nm. By these modifications, the assay system was able to detect SP-A in the concentration range of 2.5–250 ng·mL⁻¹, whereas the previous range of detection was 12.5–100 ng·mL⁻¹ [2]. Reproducibility and coefficients of variation were obtained by duplicate analysis.

Statistical analysis

The significance of differences between two groups was determined by unpaired Student's t-test. A p-value of less than 0.05 was regarded as significant. Results are expressed as mean±SEM.

Results

Age and spirometric data, including FEV₁, FEV₁ % pred, VC, and FEV₁/VC, were not significantly differ-

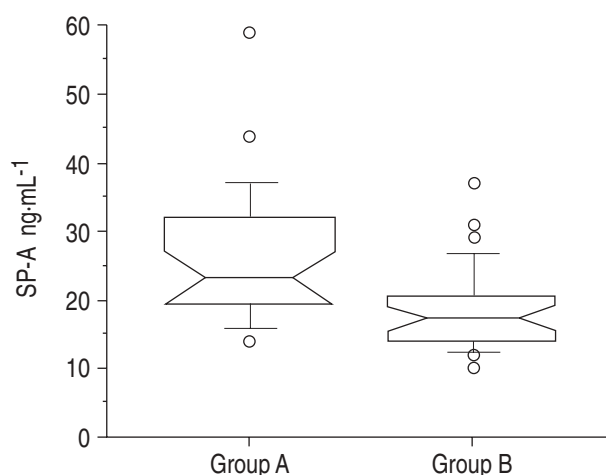


Fig. 1. – Serum surfactant protein (SP-A) concentration in smokers (Group A) and nonsmokers (Group B). Data are shown by box plot. Each plot is composed of five horizontal lines that display the 10th, 25th, 50th, 75th and 90th percentiles of a variable. Group A: smokers (n=23); Group B: nonsmokers (n=29). Group A showed higher serum SP-A concentrations than Group B ($p<0.002$).

ent between the two groups and all were within normal limits for male subjects of the same age (data not shown).

Serum SP-A levels were significantly higher in active smokers (Group A 26.22 ± 2.14 ng·mL⁻¹) than in nonsmokers (Group B; 18.35 ± 1.12 ng·mL⁻¹) ($p<0.002$), as shown by the box plot in figure 1.

The SP-A concentration increased in parallel with increased end-tidal CO ($Y=1.229 + 7.179e^{-3X}$; $r=0.449$; $p<0.01$). The SP-A concentrations were also significantly correlated with the COT/CRE ratio ($Y=1.271 + 6.971e^{-5X}$; $r=0.359$; $p<0.01$).

Cotinine concentration in the urine, corrected for creatinine concentration (COT/CRE), was significantly higher in Group A (1125.39 ± 181.79 ng·mg⁻¹ CRE) than Group B (6.74 ± 1.11 ng·mg⁻¹ CRE; $p<0.0001$). Similarly, the carbon monoxide concentration in expired-air (end-tidal CO) was higher in Group A (20.22 ± 1.73 ppm) than Group B (4.00 ± 0.22 ppm; $p<0.0001$).

Discussion

This study showed that SP-A, the major surfactant protein, is increased in serum of smokers. The molecular mass of SP-A ranges 26–36 kDa because of variable glycosylation [6]. This protein binds phosphatidylcholine and is thought to promote the formation of tubular myelin-like structures from newly secreted phospholipids [6], and to enhance the adsorption of phospholipids to the air/liquid interface in co-operation with other surfactant proteins. Thus, SP-A has a role in regulating the metabolic pathways of secretion and uptake of surfactant between alveolar type II cells and alveolar spaces [6].

In the present study, SP-A was measured by an enzyme-linked immunosorbent assay (ELISA), using monoclonal antibodies against human SP-A developed by KUROKI *et al.* [2]. The ELISA has been applied clinically to measure SP-A as a marker for surfactant in amniotic fluid [7], sputum [8], pleural effusion [9], bronchoalveolar lavage fluid [10], and serum [2].

A low yield of surfactant phospholipids from smokers was first described by FINLEY *et al.* [3] and subsequently by LOW *et al.* [4]. An abnormal surface activity of lavage fluid from smokers has been described [3, 11]. Earlier studies by FINLEY *et al.* [3] suggested no qualitative differences in the lipid analyses of surfactant from smokers and nonsmokers, but the total lipid content and, in particular, the lecithin content, was seven times less in smokers. Similarly, data obtained by LOW *et al.* [4] revealed that lavage fluid samples from smokers contain significantly lower ratios of lipid phosphorus to protein and lower albumin normalization values than those of nonsmokers. However, these earlier studies did not refer to qualitative changes in surfactant proteins, a factor that should be examined since recent data suggest independent pathways for SP-A and saturated phosphatidylcholine secretion [12]. The present data show that the serum SP-A concentration is significantly higher in healthy male current smokers than in nonsmokers ($p < 0.002$); however, it was found that serum SP-A is not a sensitive indicator for discriminating smoking habit.

It has been shown that SP-A appears in the bloodstream of patients with idiopathic pulmonary fibrosis (IPF) and pulmonary alveolar proteinosis (PAP) in a nonspecific complex with immunoglobulin G and M [2], although the precise mechanism through which SP-A and immunoglobulin complexes appear in IPF and PAP is not known. One possibility is that an influx of serum protein into alveolar airspaces occurs in IPF or PAP; therefore, it is possible that there is a corresponding efflux of SP-A from alveolar type II cells into the bloodstream, where it forms complexes. Also, it raises the possibility that some specific proteins present in the bloodstream of IPF or PAP patients bind to SP-A, as suggested by AKINO [13]. All these findings suggest that increased serum SP-A concentration is not a specific phenomenon in IPF or PAP.

It is necessary to consider whether serum SP-A concentration is superior to other known biological markers, such as cotinine or end-tidal carbon monoxide concentration, for discriminating smoking exposure. Our study suggests that this is not the case. At present, the precise factors that influence serum SP-A concentration with respect to smoking habit are unknown. A half-life value of 6.5 h for SP-A in the airspaces has been noted [12]; the decay time of serum SP-A is not known. Therefore, it is possible that the decay time is lengthened in smokers, and this leads to higher serum SP-A levels. Another possibility is that the increase in serum SP-A might arise from increased alveolar liquid permeability due to surfactant inactivation. This would be consistent with changes seen in sarcoidosis, in which the concentration of SP-A in the bronchoalveolar lavage fluid is markedly higher than in control subjects [14], and may also be supported by the observation of increased alveolar permeability in smokers as determined by technetium clearance [15].

In summary, the current study shows that serum surfactant protein A levels are increased in current smokers as compared with nonsmokers. The mechanism and implications for this observation remain unknown at present.

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