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Protein 1 (Clara cell protein) serum levels in lung cancer patients receiving chemotherapy

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Protein 1 (Clara cell protein) serum levels in lung cancer patients receiving chemotherapy. H. Nomori, H. Horio, R. Kobayashi, S. Morinaga, Y. Hirabayashi. ©ERS Journals Ltd 1995.

ABSTRACT: As many antineoplastic drugs can cause injury to alveolar pneumocytes, Clara cells (nonciliated, nonmucous epithelial cells of the bronchioles) may also be damaged by such drugs. Protein 1 (P1) is an α-microprotein secreted by Clara cells. The effect of antineoplastic drugs on Clara cells is examined by the measurement of serum levels of P1 in patients with lung cancer receiving chemotherapy.

Serum levels of P1 were studied in 36 patients with lung cancer, before chemotherapy and 5–7, 10–12 and 14–18 days after chemotherapy. One hundred and eight healthy subjects, matched by sex and age, acted as controls.

There was no significant difference in P1 serum levels between patients with lung cancer and healthy controls. P1 serum levels decreased significantly 5–7 and 10–12 days after chemotherapy, recovering thereafter.

We conclude that P1 serum levels do not differ between lung cancer patients and healthy controls, and that antineoplastic drugs inhibit the synthesis or secretion of P1 by Clara cells in the early period after the administration of medication.

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Clara cells are nonciliated nonmucous epithelial cells of the bronchioles, having the morphological characteristics of secretory cells [1]. Although the role of Clara cells remains to be clarified, it is thought to be important in the carcinogenesis of lung cancer [2, 3], in surfactant production in terminal airways [4–6], and in the regulation of the inflammatory response in the lungs [7, 8]. However, the relationship of Clara cells to different pathological conditions of the lung remains to be studied adequately.

Protein 1 (P1), an α-microprotein with a molecular weight of 10 kDa, was initially found in the urine of patients with renal tubular dysfunction [9–11]. In 1992, BERNARD and co-workers [12] demonstrated that P1 was identical to the Clara cell 10 kDa protein (CC10), which was originally reported as a Clara cell secretory protein by SINGH *et al.* [13]. Using *in situ* hybridization, BROERS *et al.* [14] demonstrated that the CC10 gene was also expressed in nonciliated columnar epithelial cells in the large and small bronchi, as well as those in the bronchioles (Clara cells). We demonstrated, by immunohistochemical study of all types of tissue, that P1 and CC10 were most significantly distributed in Clara cells [15].

It has been reported that many antineoplastic drugs can cause injury of alveolar pneumocytes, which results in abnormal surfactant production and/or composition [16–19], and, in some patients, progresses to clinically manifested interstitial pneumonia. Although Clara cells are bronchiolar epithelial cells, they have close embryological and functional relationship with alveolar pneumocytes [4, 6, 20]. In order to examine the effect of antineoplastic drugs on Clara cells, we studied P1 serum levels in 36 patients with lung cancer who had received systemic chemotherapy. In addition, in order to evaluate the P1 serum levels in the patients with lung cancer, the prechemotherapy P1 values were compared to the P1 values in 108 healthy controls matched by sex and age.

Subjects and methods

Cases

We studied 36 consecutive patients with lung cancer (27 males and 9 females, aged 48–72 (mean 62) yrs) who underwent systemic chemotherapy between June 1993 and August 1994 at Saiseikai Central Hospital, Tokyo, Japan. Twenty four (67%) of the patients were smokers, and the remaining 12 patients were nonsmokers or ex-smokers. Histological types were adenocarcinoma (22), squamous cell carcinoma (5), large cell

carcinoma (4), and small cell carcinoma (5). Chemotherapy was administered as the first course in 22, the second in 11, and the third in 3 cases. The period from prior chemotherapy exceeded 28 days in all patients. No patients had prior thoracic radiation therapy or lung resection, nor had any suffered from clinically manifested pulmonary complications caused by antineoplastic drugs.

All drugs were administered in the full dose, *i.e.* cisplatin (cis dichloro diaminoplatinum; CDDP) 80 $mg \cdot m^{-2}$ body mass intravenously (*i.v.*) on Day 1; carboplatin (CBDCA) 300 $mg \cdot m^{-2}$ *i.v.* on Day 1; vindesine (VDS) 3 $mg \cdot m^{-2}$ *i.v.* on Day 1; and etoposide (VP-16) 100 $mg \cdot m^{-2}$ *i.v.* on Days 1, 3 and 5. Of 31 cases with non-small cell carcinoma, 24 cases were treated using a combination of CBDCA and VDS, and 7 cases with CDDP and VDS. The five cases with small cell carcinoma were treated with a combination of CDDP and VP-16. No patients suffered pulmonary complications or renal toxicity due to antineoplastic drugs. Mean±SD values of serum blood urea nitrogen (BUN) were 14.1±4.2 $mg \cdot dL^{-1}$ before chemotherapy, 15.2±3.8 $mg \cdot dL^{-1}$ at 5–7, 14.6±3.9 $mg \cdot dL^{-1}$ at 10–12, and 16.1±.8 $mg \cdot dL^{-1}$ at 14–18 days after chemotherapy. Mean±SD values of serum creatinine were 0.9±0.3 $mg \cdot dL^{-1}$ before chemotherapy, 1.0±0.4 $mg \cdot dL^{-1}$ at 5–7, 0.9±0.4 $mg \cdot dL^{-1}$ at 10–12, and 1.0±0.4 $mg \cdot dL^{-1}$ at 14–18 days after chemotherapy.

Prechemotherapy P1 serum levels were measured within one week before chemotherapy. Postchemotherapy P1 serum levels were measured three times, at 5–7, 10–12, and 14–18 days after the first date of medication.

Controls

As a control for each prechemotherapy P1 serum level in the patients with malignancies, we selected three healthy subjects (total 108 cases) who underwent routine medical examination. Possible control subjects were excluded if they had any evidence of lung, heart, liver, or kidney disease, or a history of major surgery. Control cases were matched with the individual patients for sex and age (±5 yrs). Sixty two cases (57%) of the control subjects were smokers, and the remaining 46 cases were nonsmokers or ex-smokers.

Storage of samples

Serum samples were obtained in the morning before eating. When the serum samples had been examined for their original purposes, all of the remaining samples were stored at -80°C until the analysis of P1 levels.

Immunoglobulin coating of latex particles

One tenth of a millilitre of purified anti-rabbit immunoglobin G (IgG) against P1 (Dakopatts Ltd) in 0.2 mL of 0.1 mol phosphate buffered sodium (PBS), and 0.2 mL of a 10% suspension of polystyrene latex particles (0.80 μm in diameter, Baxter Ltd) were mixed well using a vortex mixer, incubated for 1 h at room temperature, and then centrifuged at 3,000 × g for 10 min. After washing in 4 mL of 0.1 mol PBS (pH 5.0), the coated particles were resuspended in 4 mL of 0.1 mol PBS containing 0.1% sodium azide whose final concentration was 0.25% (w/v). Particles were stored at 4°C.

Procedures

The P1 assay was performed using a fully automated system, *i.e.* a Behring Nephelometer Analyser (Behringwerke AG). One hundred microlitres of serum diluted 10 times with dilution buffer (Behringwerke AG), 30 μL of antiserum-coated latex particles, and 160 μL of reaction buffer (Behringwerke AG) were pipetted into a reaction cuvet and preincubated at room temperature for 6 s. The increase in light scattering energy after the mixing of the sample was measured at two defined times (6 and 726 s). The second signal measured was then subtracted from the first, and the intrinsic light scattering energy of the samples, the coating particles, and the cuvet was eliminated. Accurate sample concentrations of P1 can be determined from the standards plotted on a standard curve. The P1 assay was calibrated with purified P1 (Dakopatts). The analytical range was 3.75–3,000 $\mu g \cdot L^{-1}$.

P1 recovery test

From 20 to 200 $\mu g \cdot L^{-1}$ of the P1 standard (Dakopatts Ltd) were added to serum samples, and aliquots of the recovered P1 were assayed.

Statistics

Conditional logistic analysis was used to determine the difference in P1 serum levels between the patients with lung cancer and their corresponding healthy controls. The differences of the paired P1 values in each patient before and after chemotherapy were analysed for significance by the two-tailed Student's t-test of paired values. Values of p less than 0.05 were accepted as significant. The relationship between the prechemotherapy and postchemotherapy P1 serum levels was analysed for significance by a two-tailed Student's t-test.

Results

The result of the P1 recovery test showed that the average recovery was 106±6%, which indicated the P1 measurement method was reliable and specific for P1 values. The storage of samples at -80°C for 1 month did not change the P1 values.

The distributions of prechemotherapy P1 serum levels in the patients and the P1 serum levels in healthy subjects are given in figure 1. P1 serum levels both in the patients and healthy subjects were widely distributed:

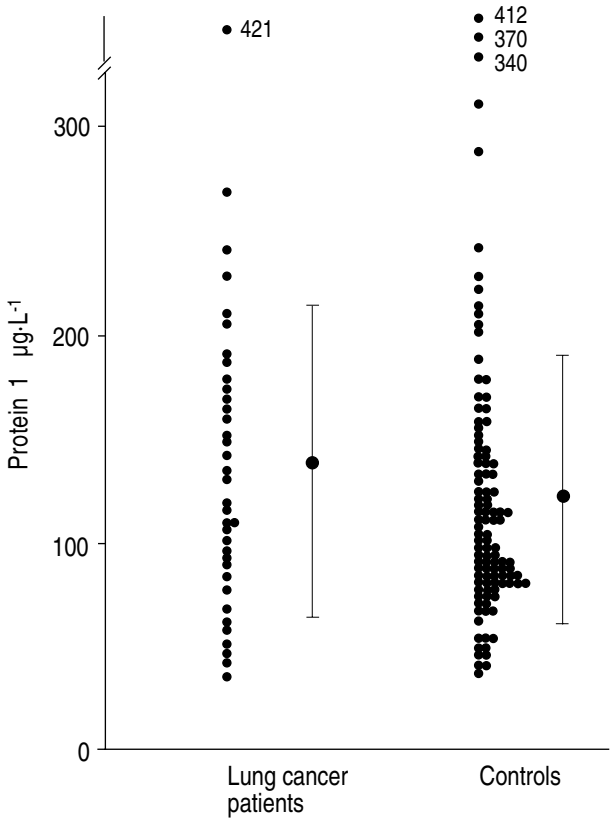

Fig. 1. – Protein 1 serum levels in patients with lung cancer and in corresponding controls. Bars represent mean±SD.

from 38–421 μg·L^{-1} in the patients with lung cancer, and from 40–412 μg·L^{-1} in the healthy subjects. Mean and SD values of the P1 serum levels were 140±76 and 122±66 μg·L^{-1} in patients with lung cancer and in the healthy subjects, respectively. Thus, there was no significant difference between them. The percentage of smokers was not significantly different between the lung cancer patients and controls.

Mean and SD values for postchemotherapy P1 serum levels in all cases were 114±62 μg·L^{-1} at 5–7 days, 131±62 μg·L^{-1} at 10–12 days, and 138±71 μg·L^{-1} at 14–18 days after chemotherapy (fig. 2). P1 serum levels 5–7 and 10–12 days after chemotherapy were significantly lower

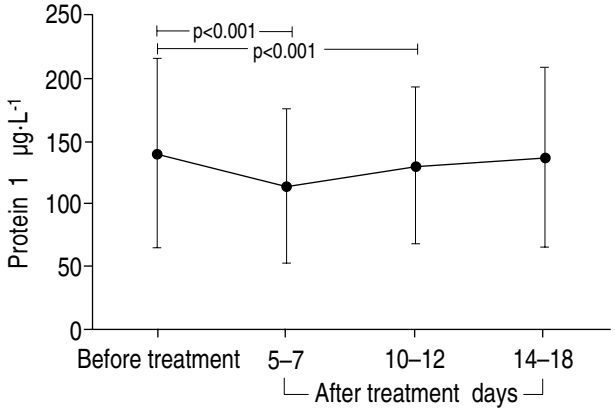

Fig. 2. – Changes in protein 1 serum levels before and after chemotherapy. Bars represent mean±SD.

Table 1. – Correlation between prechemotherapy and postchemotherapy protein 1 (P1) serum levels

Post chemotherapy days	Cases n	Coefficient of correlation with P1	p-values
5–7	36	0.980	<0.001
10–12	36	0.987	<0.001
14–18	36	0.991	<0.001

Table 2. – Combination chemotherapy and changes in protein 1 (P1) serum levels before and after treatment

Combination	Cases n	P1 serum levels μg·L^{-1}			
		Before treatment	Post treatment (days)		
			5–7	10–12	14–18
CBDCA+VDS	24	133±87	107±70***	126±81**	131±82
CDDP+VDS	7	155±39	129±32**	144±29	157±28
CDDP+VP-16	5	153±57	101±48*	127±41	129±43

Values are presented as mean±SD. CBDCA: carboplatin; VDS: vindesine; CDDP: cisplatin; VP-16: etoposide. ***: p<0.001; **: p<0.01; *: p<0.05 (p-values in the difference of P1 serum levels before and after treatment).

than their paired values before chemotherapy (p<0.001), and recovered 14–18 days after treatment. P1 serum levels at 5–7, 10–12, and 14–18 days after chemotherapy showed a significant positive correlation with those of the prechemotherapy values (p<0.001) (table 1).

All types of combination chemotherapy also showed a decrease in P1 serum levels 5–8 and 10–12 days after the administration of medication (table 2). The 24 patients who received the combination of CBDCA and VDS showed a significant decrease of P1 serum levels 5–7 and 10–12 days after treatment (p<0.001 and p<0.01). The seven patients treated with CDDP and VDS, and five patients treated with CDDP and VP-16 showed a significant decrease of the values 5–7 days after treatment (p<0.01 and p<0.05, respectively).

Discussion

A large number of Clara cells are present in the bronchioles of normal human lungs. P1 secreted into the airways from Clara cells is absorbed into the blood through the bronchial epithelium [15, 21]. BERNARD and co-workers [21] demonstrated that P1 values in bronchoalveolar lavage (BAL) correlated significantly with those in serum, and that P1 values in serum could be an indicator of the total amount of protein synthesized by Clara cells. P1 values in serum would also be more reliable than those in BAL, since the latter are affected by variable dilutions of the sample.

BERNARD and co-workers [21] reported that P1 values in BAL and serum were significantly lower in eight patients with lung cancer than in 25 healthy subjects. However, the present study, using controls matched by sex and age, demonstrated that P1 serum levels were not affected by the association with lung cancer.

It has been reported that many antineoplastic drugs can cause chemically induced and/or immune-mediated pneumonitis, resulting in necrosis of type I pneumocytes, abnormal proliferation of type II pneumocytes, changes in pulmonary surfactant composition, and, in some patients, progressive advance to clinically manifested interstitial pneumonia [16–19]. The present study showed that the P1 serum levels had decreased significantly 5–7 and 10–12 days after chemotherapy, and had recovered almost to the prechemotherapy levels 14–18 days after chemotherapy. Although the antineoplastic drugs used in the present study are not known to affect the lung clinically, these drugs might cause subclinical damage of bronchiolar epithelium, such as the inhibition of P1 synthesis or secretion from Clara cells.

Several reports have suggested that Clara cell protein could have anti-inflammatory activity, *i.e.* inhibition of phospholipase A2 [7] and production and biological activity of interferon-γ [8]. Therefore, we assume that P1 secreted from Clara cells, through its anti-inflammatory activity, may play an important role in recovery from subclinical drug-induced lung damage.

Our data have demonstrated that the P1 serum levels were widely distributed both in lung cancer patients and healthy subjects. Although we cannot state the minimum P1 serum levels for normal function of Clara cells, it can be stated that the monitoring of postchemotherapy P1 values in each patient could provide important information on the recovery from lung damage due to chemotherapy.

In conclusion, we found that P1 serum levels decreased after chemotherapy. The functions and metabolism of P1 remain to be elucidated, however, and its physical and biological properties and its role in relation to lung disease i*n vivo* require much more study.

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