Original

Serum type III procollagen peptide: Indicator for pulmonary fibrosis

I. A tool for early detection of pulmonary fibrosis as a complication in cancer therapy

Isao Okazaki, Koji Miura, Yumiko Kobayashi, Katsuya Maruyama,*
Hiroshi Yoshimatsu** and Toshihiko Tanaka***

Department of Preventive Medicine and Public Health, School of Medicine
Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan

* Clinical Research Unit, National Institute on Alcoholism, Kurihama
National Hospital, Kanagawa 239, Japan

** Department of Chest Surgery, School of Medicine, University Hospital
of Occupational and Environmental Health
Yahatanishi-ku, Kitakyushu City 807, Japan

*** Department of Radiation Therapy, Kanagawa Cancer Center
Yokohama 241, Japan

(Received for publication on June 6, 1986)

Abstract

To clarify whether serum type III procollagen peptide can reflect degree of pulmonary fibrosis and is useful for the early detection of pulmonary fibrosis seen as a complication of cancer therapy, 24 cancer patients were investigated before and after radiation therapy or treatment with anti-cancer drugs. The serum peptide levels in 68 healthy adults was 8.6 ± 2.4 ng/ml (mean $\pm$ SD). Before cancer therapy the serum peptide levels in 15 cancer patients without detectable liver dysfunction were within normal range. After cancer therapy thirteen cancer patients with complicating pulmonary fibrosis revealed 24.3 ± 8.0 in serum,

岡崎 勲, 三浦公嗣, 小林友美子, 丸山勝也, 吉松 博, 田中利彦

Abbreviations: P-III-P, type III procollagen peptide; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Al-Pase, alkaline phosphatase; LDH, lactic dehydrogenase; GGTP, gamma glutamyltranspeptidase; BUN, blood urea nitrogen.

while the remaining 11 cases without complication of pulmonary fibrosis showed lower values of 16.1 ± 2.5 with statistical significance ($p < 0.01$). There was no correlation between serum peptide level and degree of pulmonary fibrosis. However, the cases showing high values revealed interstitial pneumonitis followed by rapid progressive pulmonary fibrosis caused by radiation therapy or treatment with anti-cancer drugs. These results indicate that this method is useful for detecting the complication of progressive interstitial pulmonary fibrosis at an early stage.

Key words: fibrogenic marker, type III procollagen peptide, pulmonary fibrosis, drug-induced and radiation-induced pulmonary fibrosis

Introduction

Complication of pulmonary fibrosis in cancer therapy very frequently leads to a fatal prognosis, and screening tests for the early detection of pulmonary fibrosis are needed. Pulmonary fibrosis is characterized morphologically by the disorganization of normal alveolar structures, altered cellular population and disordered interstitial collagen deposition.¹⁻⁵ There is no question that collagen deposited in the lung influences the mechanical properties of the lung and causes impaired function.^{2,3,6} Recent research on collagen polymorphism in the lung has revealed that type I collagen is located in the interstitium of alveolar septa and type III collagen in the septa and the perivascular wall.⁶⁻⁹ The connective tissue reaction in wound healing or fibroproliferative process after injury is characterized first by biosynthesis of pericellular type V collagen and type IV collagen, followed by biosynthesis of type III collagen, after which type I collagen fibers are prominently deposited.¹⁰

Collagen-producing cells such as fibroblasts, endothelial cells and muscle cells can synthesize procollagen within the cells. Once procollagen is secreted extracellularly, both N-terminal and C-terminal peptides of procollagen are cleaved by endopeptidases.¹¹ These peptides can move from the tissue into the blood stream. Therefore, the released peptides of type III procollagen may reflect the degree of biosynthesis of type III collagen, and increased peptide level in serum may suggest inflammation or the beginning of fibrosis. Since Rohde *et al.*¹² reported an assay method for serum type III procollagen peptide (P-III-P), P-III-P serum marker has been applied in chronic liver diseases,^{12,13-19} pulmonary fibrosis,²⁰⁻²⁶ myocardial fibrosis,²⁷ myelofibrosis²⁸ and bone diseases.²⁹ Here we report the usefulness of this assay in the early detection of pulmonary fibrosis as a complication in cancer therapy.

Methods

Healthy subjects

Serums of 68 healthy adults were obtained at the Department of Central Clinical Laboratory, University Hospital of Occupational and Environmental Health, Japan. The age distribution was 20 to 79 years, and included 31 males and 37 females. They met the following criteria: (a) no abnormal physical finding, (b) no abnormal laboratory test (urine and stool examinations, peripheral blood cell counts and blood chemistry analysis including serum total protein, total cholesterol, AST, ALT, LDH, Al-Pase, GGTP, total bilirubin, BUN and creatinine).

Patients

Twenty-four cancer patients (17 with lung cancer, 2 with breast cancer, 1 with thymoma, 1 with esophageal cancer, 1 with colon cancer, 1 with uterine cancer and 1 with malignant lymphoma) were admitted to the Department of Chest Surgery, University Hospital of Occupational and Environmental Health, Japan, and the Department of Radiation Therapy, [REDACTED]. The 24 cancer patients were investigated by chest X-ray and lung function tests, with special attention paid to the complication of pulmonary fibrosis on admission, and before and after treatment with radiation and/or several kinds of anti-cancer drugs without or after surgery. All subjects showed no abnormal liver function tests (elevated AST and/or ALT) and had no concomitant disease assumed to affect systemic collagen metabolism.

Chest X-rays were examined by [REDACTED] and [REDACTED] who did not know the serum value of P-III-P.

Type III procollagen peptide (P-III-P) assay

Serum was separated within 2 hours of venipuncture and stored at -20°C until assayed. Serum P-III-P levels were measured using a radioimmunoassay according to the method of Rohde *et al.*¹² Research kits including specific antibody against bovine type III procollagen aminopeptide, unlabeled bovine aminopeptide standard and ^{125}I -labeled aminopeptide were purchased through Hoechst (Tokyo). The serum was diluted to four different concentrations (1/5, 1/10, 1/20, 1/40), incubated with excess specific antibodies to form antigen-antibody complex and the remaining antibodies reacted with the known ^{125}I -labeled antigen. Then, both unlabeled and ^{125}I -labeled immune complexes were precipitated after the addition of separating reagent, Tachisorb®, followed by the centrifugation. The supernatant of the remaining ^{125}I -labeled antigen was discarded. The precipitate of ^{125}I -labeled immune complex was counted by γ -scintillation counter (Aloka Auto Well Gamma System ARC-600). Thus, we obtained the inhibition binding curve for each serum and calculated the peptide concentration from the standard inhibition curve using a 50% intercept method. All tests were carried out in triplicate.

Reproducibility of results obtained with different test kits was observed with an error of less than 5%. Limit of low value detecting serum P-III-P was approximately 0.6 ng/ml in this assay.

Statistical calculations are based on Student's t-test. Data are express as mean $\pm$ SD.

Results

1. Serum peptide levels in 68 healthy adults

Normal value of the serum P-III-P level was 8.6 ± 2.4 ng/ml in 68 healthy adults (9.1 ± 2.6 in 31 males and 8.2 ± 2.0 in 37 females). There was no statistical significance between males and females. The values for each age group are shown in Table 1. Those over 70 years of age showed a tendency toward higher values of serum peptide compared to levels in the other age groups, but without statistical significance.

Values exceeding the mean + 2SD (13.4 ng/ml) were taken to be abnormal.

Table 1
Serum levels of type III procollagen peptide in 68 healthy adults

Age	Number	Serum level of type III procollagen peptide (ng/ml)
20-29	6	$7.6 \pm 1.5^*$
30-39	12	8.1 ± 2.8
40-49	15	8.1 ± 2.7
50-59	14	8.6 ± 1.8
60-69	12	8.7 ± 1.7
70-79	9	10.8 ± 2.3
Total	68	8.6 ± 2.4

* Mean $\pm$ SD

There is no statistical significance between age groups.

2. Serum peptide levels in 15 cancer patients before cancer therapy

The serum P-III-P levels were measured in 15 cancer patients (12 patients with lung cancer, 1 with breast cancer, 1 with colon cancer and 1 with uterine cancer) at admission, that is, before radiation therapy and/or treatment with anti-cancer drugs. The values in the 12 cases with lung cancer were 11.0 ± 4.3 ng/ml and the three other cases showed values within normal range.

3. The serum peptide levels in 24 cancer patients after cancer therapy

Twenty-four patients (17 patients with lung cancer, 2 with breast cancer, 1 with thymoma, 1 with esophageal cancer, 1 with colon cancer, 1 with uterine cancer and 1 with malignant lymphoma) were treated with radiation or anti-cancer drugs. Among 24 cancer patients after radiation therapy or treatment with anti-cancer drugs, 13 suffered from complication with pulmonary fibrosis without any involvement in other organs and the other 11 did not. The mean serum level of P-III-P in the 13 cases with complication of pulmonary fibrosis was 24.3 ± 8.0 ng/ml when abnormal findings suggesting pulmonary fibrosis appeared on chest X-rays (Table II) while that of the remaining 11 cases without the complication was significantly lower 16.1 ± 2.5 ng/ml (range of 12.4 to 19.4 ng/ml; $p < 0.01$).

However, the serum levels of the peptide were not associated with the degree of pulmonary fibrosis revealed by chest X-ray, but with the grade of progression in clinical course. That is, all three cases exceeding 30 ng/ml suffered from the acute and rapidly progressive type of interstitial pulmonary fibrosis after cancer therapy.

The serum levels of the peptide in 11 cases with the complication of pulmonary

Table 2
Serum type III procollagen peptide levels in 13 cancer patients with complication of pulmonary fibrosis

Case No.	Sex	Age	Original disease	P-III-P (ng/ml)	Degree of fibrosis	Sur-gery	Radia-tion therapy	Anti-cancer drugs
1	M	65	colon cancer	20.3	++			FU, CPA
2	M	43	ML	35.0	+			BLM, CPA, VCR
3	M	41	lung cancer	42.4	++	+		PEP, ADR, CDDP
4	M	59	lung cancer	24.4	+++		+	
5	F	32	thymoma	23.6	+++		+	
6	F	48	breast cancer	23.2	++		+	
7	F	41	breast cancer	32.5	+++	+	+	
8	F	53	lung cancer	19.7	+++		+	
9	F	59	lung cancer	13.6	++		+	
10	F	76	lung cancer	19.0	+++		+	
11	M	59	lung cancer	17.9	+++		+	
12	F	83	lung cancer	25.7	+++		+	
13	F	35	uterine cancer	18.5	++		+	

Serum P-III-P values at the appearance of abnormal findings on chest x-ray suggesting pulmonary fibrosis are listed here.

ML: malignant lymphoma.

ADR: adriamycin, BLM: bleomycin, CDDP: cis-platinum, CPA: cyclophosphamide, FU: fluorouracil, PEP: peplomycin, VCR: vincristine.

Fig. 1 Serum P-III-P levels in 11 cancer patients with complication of pulmonary fibrosis after radiation therapy and/or treatment with anti-cancer drugs. 0 = time of detection of pulmonary fibrosis by chest X-ray. $\uparrow$ indicate the value over 35 ng/ml. The shaded portion of the figure shows the normal range of serum P-III-P levels, that is, mean $\pm$ SD in 68 healthy adults.

fibrosis were examined chronologically with reference to the date of detection of pulmonary fibrosis by chest X-ray after the start of radiation therapy or treatment with anti-cancer drugs. As seen in Figure 1, the serum level of the peptide increased 2 months before the appearance of abnormal fibrous shadow on chest X-ray. The serum P-III-P levels in patients with interstitial pulmonary fibrosis diagnosed by chest X-ray showed a gradual tendency to decrease.

Discussion

Complication of pulmonary fibrosis is very often seen in cancer therapy. It is well known that radiation causes radiation pneumonitis followed by interstitial pulmonary fibrosis³⁰ and that anti-cancer drugs such as bleomycin and its derivatives, cyclophosphamide and vincristine, cause interstitial pneumonitis and pulmonary fibrosis.³¹ A simple screening test for the early detection of pulmonary fibrosis is needed. Low *et al.*²⁰ demonstrated an increased concentration of P-III-P in lavage of patients with

idiopathic pulmonary fibrosis and sarcoidosis, and showed that the lung produces type III collagen. Recently Begin *et al.*²⁶ also confirmed and demonstrated that the measurement of levels of fibronectin and P-III-P in lavage fluid can assess fibrogenic activity of alveolitis. However, it is not easy to obtain samples of lavage fluid from patients, nor can it be used for occupational health surveys.

In the present study we observed significantly higher values of serum P-III-P in cancer patients with complication of pulmonary fibrosis after cancer therapy compared with patients without complication of pulmonary fibrosis. We also found that the cases showing high values of serum P-III-P suffered from interstitial pneumonitis followed by rapid progressive pulmonary fibrosis probably caused by radiation therapy or the administration of anti-cancer drugs such as bleomycin and its derivative (peplomycin), cis-platinum and vincristine. Our observations suggest that the elevated serum levels of P-III-P reflect increased synthesis of type III collagen in the lung, although no increased serum level of P-III-P was reported in patients with idiopathic pulmonary fibrosis,²⁰ sarcoidosis²⁰ or farmer's lung²⁵ compared with volunteer controls. The difference in the results between our study and others may depend either on the stage of lung fibrosis (early or advanced), or on the type of lung fibrosis (gradual or rapid progressive).

Cases of advanced or gradual progressive lung fibrosis may not show high levels of serum P-III-P as we reported in silicosis patients,²³ and cases with early and/or rapid progressive lung fibrosis may show high levels of serum P-III-P as observed in this report. The high levels of serum P-III-P in the present study may reflect marked fibrogenic activity in the lung, just as Watanabe *et al.*²⁴ observed elevated serum levels of P-III-P in cancer patients after radiation. If the above observations are correct, it is reasonable to be no correlation between the serum levels of P-III-P and the degree of pulmonary fibrosis.

The possibility that the interstitial pneumonitis seen in our cases with high levels of serum P-III-P are due to cancer cell infiltration in the lung can be eliminated, because serum P-III-P levels in lung cancer without fibrosis are not high if liver involvement is not observed.³²

Our study showed high levels of serum P-III-P in cancer patients with pulmonary fibrosis. It also demonstrated the existence of elevated serum P-III-P before chest X-rays revealed lung fibrosis, indicating that the assay is useful for detecting progressive interstitial fibrosis at an earlier stage of cancer therapy than is the present degree of fibrosis.

Acknowledgement

The authors wish to express their gratitude to Dr. Haruo Kondo and Dr. Haruhiko Sakurai (Professors, Department of Preventive Medicine and Public Health, School of Medicine, Keio University) and Dr. Kenzaburo Tsuchiya (President, University of Occupational and Environmental Health) for their warm and continued

support for this research project.

References

1. Hamman, L. and Rich, A. R.: Acute diffuse interstitial fibrosis of the lungs. *Bull. Johns Hopkins Hosp.* 74: 177-212, 1944
2. Crystal, R. G., Fulmer, J. D., Roberts, W. C., Moss, M. L., Line, B. R. and Reynolds, H. Y.: Idiopathic pulmonary fibrosis; clinical, histologic, radiographic, physiologic, scintigraphic, cytologic and biochemical aspects. *Ann. Intern. Med.* 85: 769-788, 1976
3. Crystal, R. G., Bitterman, P. B., Rennard, S. I., Hance, A. J. and Keogh, B. A.: Interstitial lung diseases of unknown cause. Disorders characterized by chronic inflammation of the lower respiratory tract. *New Eng. J. Med.* 310: 154-166 and 235-244, 1984
4. Gaensler, E. A., Moister, M. V. B. and Hamm, J.: Open-lung biopsy in diffuse pulmonary disease. *N. Eng. J. Med.* 270: 1319-1331, 1964
5. Liebow, A. A. and Carrington, C. B.: The interstitial pneumonias. In *Frontiers of Pulmonary Radiology*. Simon, M., Potchen, E. J. and LeMay, M. editors. Grune & Stratton, New York. 102-141, 1969
6. Madri, J. A. and Furthmayer, H.: Collagen polymorphism in the lung. *Human Path.* 11: 353-366, 1980
7. Bradley, K. H., McConnell, S. D. and Crystal, R. G.: Lung collagen composition and synthesis. Characterization and changes with age. *J. Biol. Chem.* 249: 2674-2683, 1974
8. Hance, A. J., Bradley, K. H. and Crystal, R. G.: Lung collagen heterogeneity. *J. Clin. Invest.* 57: 102-111, 1976
9. Seyer, J. M., Hutcheson, E. T. and Kang, A. H.: Collagen polymorphism in idiopathic chronic pulmonary fibrosis. *J. Clin. Invest.* 57: 1498-1507, 1976
10. Gay, S.: The immunology of collagen. In *Connective Tissue Diseases*. Wagner, B. M., Fleischmajer, R. and Kaufman, N. editors. Williams and Wilkins, Baltimore/London. 120-128, 1983
11. Fessler, J. H. and Fessler, L. I.: Biosynthesis of procollagen. *Ann. Rev. Biochem.* 47: 129-162, 1978
12. Rohde, H., Vargas, L., Hahn, E., Kalbfleisch, H., Bruguera, M. and Timpl, R.: Radioimmunoassay for type III procollagen peptide and its application to human liver disease. *Eur. J. Clin. Invest.* 9: 451-459, 1979
13. Ackermann, W., Pott, G., Voss, B., Mueller, K.-M. and Gerlach, U.: Serum concentration of procollagen-III-peptide in comparison with the serum activity of N-acetyl- β -glucosaminidase for diagnosis of the activity of liver fibrosis in patients with chronic active liver diseases. *Clin. Chim. Acta* 112: 365-369, 1981
14. Raedsch, R., Stiehl, A., Waldherr, R., Mall, G., Gmelin, K., Gotz, R., Walker, S., Czygans, P. and Kommerell, B.: Procollagen Type III peptide concentration in chronic persistent hepatitis and chronic active hepatitis and in cirrhosis of the liver and their diagnostic value. *Z. Gastroenterol.* 20: 738-743, 1982
15. Frei, A., Zimmermann, A. and Weigand, K.: Serum procollagen III aminopeptide (PP) as index of liver fibrosis new morphometric evidence. *Hepatol.* 2: 346 (Abstr.), 1983
16. Niemela, O., Risteli, L., Sotaniemi, E. A. and Risteli, J.: Aminoterminal propeptide of Type III procollagen in serum in alcoholic liver disease. *Gastroenterol.* 85: 254-259, 1983
17. Colombo, M., Annoni, G., Donato, M. F., Fargion, S., Tiribelli, C. and Dioguardi, N.: Serum marker of type III procollagen in patients with idiopathic hemochromatosis and its relationship to hepatic fibrosis. *Am. J. Clin. Pathol.* 80: 499-502, 1983
18. Maruyama, K., Okazaki, I., Kobayashi, T., Okuno, F., Kamegaya, K., Takagi, S., Oda, M., Ishii, H. and Tsuchiya, M.: Measurement of serum type III procollagen aminopeptide as a parameter of hepatic fibrosis. *Acta Hepatol. Jap.* 25: 24-32, 1984
19. Trivedi, P., Cheeseman, P., Portmann, B., Hegarty, J. and Mowat, A. P.: Variation in

- serum Type III procollagen peptide with age in healthy subjects and its comparative value in the assessment of disease activity in children and adults with chronic active hepatitis. *Eur. J. Clin. Invest.* 15: 69-74, 1985
20. Low, R. B., Cutroneo, K. R., Davis, G. S. and Giancola, M. S.: Lavage type III procollagen N-terminal peptide in human pulmonary fibrosis and sarcoidosis. *Lab. Invest.* 48: 755-759, 1983
 21. Lohmann, J., Pott, G., Bertram, H. P. and Gerlach, V.: Procollagen-III-peptide in progressive respiratory failure after paraquat intoxication. *Intens. Care Med.* 9: 220 (Abstr.), 1983
 22. Kirk, J. M. E., Laurent, G. J., Bateman, E. D., Haslam, P. L. and Turner-Warwick, M.: Assessment of the clinical value of the measurement of serum type III procollagen peptide levels in cryptogenic fibrosing alveolitis. *Thorax* 38: 712-713, 1983
 23. Okazaki, I., Maruyama, K., Okuno, F., Suzuki, H., Aoyagi, K. and Sera, Y.: Serum type III procollagen peptide in patients with pneumoconiosis. *J. UOEH* 5: 461-467, 1983
 24. Watanabe, Y., Yamaki, K., Yamakawa, I., Takagi, K. and Satake, T.: Type III procollagen N-terminal peptides in experimental pulmonary fibrosis and human respiratory diseases. *Eur. J. Respir. Dis.* 67: 10-16, 1985
 25. Anttinen, H., Terho, E. O., Myllyla, R. and Savolainen, E.-R.: Two serum markers of collagen biosynthesis as possible indicators of irreversible pulmonary impairment in Farmer's lung. *Am. Rev. Respir. Dis.* 133: 88-93, 1986
 26. Begin, R., Martel, M., Desmarais, Y., Drapeau, G., Boileau, R., Rola-Pleszczynski, M. and Masse, S.: Fibronectin and procollagen 3 levels in bronchoalveolar lavage of asbestos-exposed human subjects and sheep. *Chest* 89: 237-243, 1986
 27. Bolarin, D. M. and Andy, J. J.: Enzymes of collagen synthesis and type III procollagen amino-propeptide in serum from Nigerians with chronic endomyocardial fibrosis. *Ann. Clin. Res.* 15: 123-127, 1983
 28. Vellenga, E., Mulder, N. H., van Zanten, A. K., Nieweg, H. O. and Woldring, M. G.: The significance of the aminoterminal propeptide of type III procollagen in paroxysmal nocturnal haemoglobinuria and myelofibrosis. *Eur. J. Nucl. Med.* 8: 499-501, 1983
 29. Simon, L. S., Krane, S. M., Wortman, P. D., Krane, I. M. and Kovitz, K. L.: Serum levels of type I and III procollagen fragments in Paget's disease of bone. *J. Clin. Endocrinol. Metab.* 58: 110-120, 1984
 30. Libshitz, H. I. and Southard, M. E.: Complication of radiation therapy: The thorax. *Semin. Roentgenol.* 9: 41-49, 1974
 31. Weiss, R. B. and Muggia, F. M.: Cytotoxic drug-induced pulmonary disease: Update 1980. *Am. J. Med.* 68: 259-266, 1980
 32. Bolarin, D. M., Savolainen, E.-R. and Kivirikko, K.: Enzymes of collagen synthesis and type III procollagen amino-peptide in serum from Nigerians with hepatocellular carcinoma and other malignant diseases. *Int. J. Cancer* 29: 401-405, 1982