Aggressive Breast Cancer Leads to Discrepant Serum Levels of the Type I Procollagen Propeptides PINP and PICP

Arja Jukkola, Riitta Tähtelä, Eric Thölìx, Klaus Vuorinen, Guillermo Blanco, Leila Risteli, and Juha Risteli

Departments of Oncology [A. J., G. B.], Obstetrics and Gynecology [K. V.], Clinical Chemistry [J. R.], and Medical Biochemistry [L. R., J. R.], University of Oulu, 90220 Oulu; United Laboratories, Ltd., Helsinki and Department of Clinical Chemistry, University Central Hospital, Helsinki [R. T.]; and Department of Oncology, Vaasa Central Hospital, Vaasa [E. T.], Finland

ABSTRACT

The propeptides PICP and PINP are derived from the synthesis of type I collagen, a major matrix protein of bone and soft tissues. The aim of this cross-sectional study was to investigate their value as indicators of the aggressiveness of breast cancer. Serum PINP, PICP, and total alkaline phosphatase were determined from 89 breast cancer patients. Forty had major bone and/or soft tissue metastases with an aggressive disease course: the progressive disease (PD) group. Forty-nine had either none or minor bone and/or soft tissue metastases with a stable clinical course: the stable disease group (SD). The mean value of PINP in the PD group was 7.2 times higher than that in the SD group (276 ± 79 μg/l versus 38 ± 3 μg/l, respectively; \( P = 0.005 \)), whereas PICP mean value was only 1.7 times higher in the PD group (174 ± 20 μg/l versus 100 ± 5 μg/l; \( P = 0.001 \)). The ratio of PICP to PINP was 1.02 ± 0.07 in the PD group and 3.07 ± 0.18 in the SD group (\( P < 0.001 \)). The correlation between PICP and PINP was linear in the SD group and nonlinear in the PD group. The results indicate that high serum PICP and PINP concentrations and a low PICP:PINP ratio are associated with a highly aggressive nature of breast cancer. Determination of PINP, in particular, may be valuable when evaluating the clinical status of a breast cancer patient.

INTRODUCTION

Different serum markers have been developed to facilitate the diagnosis of the breast cancer and its metastases. The most common of these are the carcinoembryonal antigen and the carbohydrate antigen 15-3. Although their sensitivities and specificities are not good enough (1), they are used clinically in the search for metastases (1) and in the evaluation of response to therapy.

Type I collagen is the most abundant protein in the human body, accounting for more than one-half of total collagens. Most of it is present in bone, where type I collagen forms about 90% of the organic matrix. It also occurs in soft tissues all over the body, e.g., in the skin, muscle, and internal organs (2).

The type I collagen is synthesized as a procollagen having extra domains at both ends, the N- terminal (PINP) and the COOH- terminal (PICP) propeptide. These propeptides are cleaved off before fibril formation and released into the circulation in equimolar (1:1) ratio, although there is a delay in the release of PINP. The propeptide concentrations in blood are direct indicators of ongoing type I collagen synthesis. The relative molecular masses of PICP and intact PINP are 100,000 and 35,000 daltons, respectively, and their ratio in mass terms is expected to be 3:1, which is indeed found in the serum of healthy adults (2-3:1). However, in active Paget’s disease of bone, a disease involving locally accelerated bone turnover, the circulating concentration of PINP is disproportionally increased compared with PICP (3).

Bone is a predilection site for metastases of breast carcinoma. This is why in the present study we measured serum concentrations of PICP and intact PINP in two clinically different groups of breast cancer patients. The PINP concentration was significantly elevated in aggressive disease, but surprisingly, the PICP:PINP ratio was significantly lower both in aggressive disease with bone metastases and in aggressive disease with other types of metastases. Our results suggest that aggressive breast cancer associated with poor or very poor clinical outcome causes an hitherto unknown phenomenon in type I collagen metabolism.

PATIENTS AND METHODS

Patients and Investigations. The study was approved by the ethical committees of the Medical Faculty of the University of Oulu and Vaasa Central Hospital. Eighty-nine breast cancer patients treated at the Department of Oncology, Oulu University Hospital, or the Department of Oncology, Vaasa Central Hospital, during years 1993–1996 were included in the study. Those with metastatic disease were being treated either by endocrine or chemotherapy and/or bisphosphonates. The patients were divided into two groups according to their current clinical status: the PD group of 40 patients with bone, liver, or other metastases in whom the disease was clinically evaluated as being of aggressive type; and an age-matched, disease controls, or SD group, of 49 patients with no evidence of metastases or with minor bone or other metastases and a stable clinical outcome. All of the patients had normal renal function and no metabolic bone disease.

In the PD group, 60% of the patients had bone metastases only, 5% had visceral and soft tissue metastases only, and 35% had bone, visceral and soft tissue metastases, so that the total amount of bone metastases was 95% in PD patients. In the SD group, 38% of the patients had bone metastases only, 15% had visceral and soft tissue metastases only, and 23% bone, visceral and soft tissue metastases, so that a total of 61% of the patients had metastases in bones. Twenty-four % of the PD patients had no detectable metastases.

Bone scans were evaluated visually by experts according to the number and the extent of pathological hot spots. An increase in their number or area was interpreted as PD, and patients with no discernible changes or showing no increase in other parameters were classified as having SD.

Visceral and soft tissue metastases were detected by ultrasound, computer tomography, or chest X-ray. AFOS activity was measured according to the Scandinavian recommendation in the Laboratories of Oulu University Hospital and Vaasa Central Hospital.

Radioimmunoassays. The PICP and intact PINP concentrations were measured by competitive RIA methods (4, 5) with commercially available kits (Orion Diagnostica, Oulunsalo, Finland). The assays are based on the use of the human antigens and polyclonal rabbit antibodies. One hundred μl of serum was used for the assay of PICP, and 50 μl were used for the PINP analysis. The intra- and interassay coefficients of variation were around 5% for both assays for the concentrations obtained in this study.

Statistical Analysis. SPSS for Windows, Origin 4.00 for Windows, and Sigmaplot for Windows were used in the statistical evaluation of the data. The results are given as mean ± SE unless otherwise indicated. Both linear and nonlinear regression were used. 95% CIs for the differences are given in the

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2 To whom requests for reprints should be addressed, at Department of Medical Biochemistry, University of Oulu, Kajaanintie 52 A, FIN-90020 Oulu, Finland. Phone: 358-8-344 233; Fax: 358-8-344 522; E-mail: Juha.Risteli@Oulu.Fi.
text. Student's t test for independent means or Mann-Whitney U test were used when appropriate. P ≤ 0.05 was considered as statistically significant when combined with CI values not crossing zero. Receiver operator characteristic curves were used to determine the accuracy of serum PINP and PICP concentrations, AFOS activity, and PICP:PINP ratio as discriminators between stable and progressive breast cancer over a range of cutoff points.

RESULTS

The values of both type I procollagen markers, PINP and PICP, were higher in the PD than in the SD group. The mean value of PINP was 7.2 times higher in the PD group (276.1 ± 79.1 µg/l versus 38.1 ± 2.9 µg/l, CI for the difference: 77.9, 398.3 µg/l, P = 0.005). However, the mean value of PICP was only 1.7 times higher (174.3 ± 19.7 µg/l versus 100.2 ± 5.0 µg/l, CI for the difference: 33.1, 115.1 µg/l, P = 0.001; Fig. 1). The somewhat better statistical significance of the latter is due to the skewness of distributions and the markedly different SDs of PICP and PINP. The statistical significances are equal (P = 0.0000) if the nonparametric Mann-Whitney U test is used.

The ratio of circulating PICP:PINP is markedly diminished in the PD patients (1.02 ± 0.07 versus 3.07 ± 0.18, CI for the difference: 1.66, 2.44, P < 0.001). This is also demonstrated in Fig. 2 in which PICP is plotted against PINP. Linear regression analysis can be applied in the SD group, whereas in the PD group, a double hyperbolic curve gives the best fit.

In Fig. 3, the PICP:PINP ratio is plotted against AFOS activity, and Log [PICP] and Log [intact PINP] are plotted against Log [AFOS]. Log [PICP] and Log [intact PINP] are linearly correlated with the Log [AFOS] values (r = 0.64, P < 0.0001 and r = 0.83, P < 0.0001, respectively). Logarithmic transformation was inevitable due to the extremely high propeptide and AFOS values of one patient, which would have added too much statistical significance if the absolute values had been used.

Using 170 µg/l as the cutoff point for PICP, a sensitivity of only 0.30 and a specificity of 0.96 were found, and using 79 µg/l as the cutoff point for PINP, the values were 0.78 and 0.96, respectively. It has been reported that in healthy people, the ratio of PICP:PINP lies between 2 and 3, and if 2 is selected as the cutoff point for the ratio, the sensitivity reaches 1.0 and the specificity is 0.84. These cutoff points are based on healthy people from northern Finland as the reference population (6, 7). The receiver operator characteristic curves for serum PINP, PICP, and AFOS concentrations and the PICP:PINP ratio are shown in Fig. 4.

The optimal sensitivity and specificity values for PINP are attained with a cutoff value 70 µg/l (0.90 and 0.90, respectively) and those for the PICP:PINP ratio with a cutoff value 1.74 (0.98 and 0.90, respectively).

Two of the three patients in the PD group that either had none or only other than bone metastasis had an elevated serum PINP concentration (86 and 111 µg/l); in all, the PICP:PINP ratio was 1.4 or lower. Only one patient of the SD group with no or only other than bone metastasis had a low PICP:PINP ratio (1.3), whereas her serum PINP concentration was normal.

Fifty % of the PD patients died during the 6-month follow-up period (one accidental death), whereas no deaths occurred in the SD group.

DISCUSSION

We demonstrate here that progressive breast cancer is associated with increased type I procollagen expression, evidenced by increased...
PINP AND PICP IN STABLE AND PROGRESSIVE BREAST CANCER

Circulating concentrations of the propeptides set free from both ends of the procollagen molecule, PICP and PINP. These markers show a positive correlation with total alkaline phosphatase. A high sensitivity and specificity were obtained for both PINP and the PICP:PINP ratio in relation to the clinical nature of the disease.

When the synthesis rate of type I collagen is high, as it can be supposed to be in metastatic breast cancer, the propeptides should appear in an equimolar ratio in the serum. In mass terms, this is 2–3:1, which was indeed found in the SD subjects and was evidenced by the linear correlation between PICP and PINP (Fig. 2). Our most intriguing finding, however, was the deviation from equimolarity between the two propeptides in the progressive disease group, i.e., the excess of the NH₂-terminal propeptide PINP related to the COOH-terminal. Thus, in the PD patients, only a nonlinear regression curve could be fitted to the data (Fig. 2). A similar phenomenon has been observed earlier with respect to quiescent and active Paget’s disease of bone (3). Discrepant levels of the type I procollagen propeptides have also been found in the serum of healthy growing females under age 20 and males under age 25 (6) and in amniotic fluid.

The observed propeptide nonstoichiometry in PD could in principle be attributed to several reasons. It is possible that the clearances of these proteins are affected by pathological conditions. Intact PINP is known to be cleared by scavenger receptors (7) and PICP by mannose receptors (8), both present on the surface of liver endothelial cells and other cells of the reticulo-endothelial system. The excess in circulating PINP could be brought about either by a mechanism leading to greatly enhanced removal of PICP, e.g., by local macrophage uptake or the receptor-mediated clearance, or by the accumulation of ligands competing with PINP for the scavenger receptor. However, it has been suggested that the mannose receptor becomes saturated when the rate of type I collagen synthesis is high, but the scavenger receptor does not (9). The liver clearance system in cancer patients may also have other unknown characteristics.

Another explanation for the nonstoichiometry could be the production of biochemical variants of type I collagen. The classical type I collagen contains two identical α1(I) chains and one homologous, different gene product, the α2(I) chain, which together form a triple-helical structure. Two abnormal variants have been detected in recent years: the so-called oncofetal collagen (10), and the α1-trimeric type I collagen (11). The former has been reported to consist of an α1(I) and an α2(I) chain of type I collagen, together with an α1(III) chain of type III collagen, whereas the latter consists of three identical α1(I) chains. All of these variant procollagens obviously give rise to corresponding propeptides, which have not been isolated, with the exception of the NH₂-terminal propeptide of the α1-trimer procollagen. The assays developed for PICP and PINP could have different specificities for the propeptides derived from these variants of type I collagen, thus giving discrepant molar concentrations. The antiserum of the PINP assay used in this study was raised against the α1-trimeric NH₂-terminal propeptide (7), and the assay measures both the classical and the α1-trimeric PINP with equal sensitivity.

Serum markers of type I collagen metabolism have been investigated previously as indicators of bone metastases in breast cancer (12, 13).

![Fig. 3. Correlation between type I procollagen propeptides, their ratio, and total AFOS. Note that logarithmic transformation has been used in intact PINP versus AFOS and PICP versus AFOS. Linear regression analysis was applied to PICP versus AFOS (r = 0.64, P < 0.0001) and PINP versus AFOS (r = 0.83, P < 0.0001). □, SD group; ○, PD group.](image)

![Fig. 4. Receiver operator characteristic curves over a range of cutoff points for serum PINP and PICP concentrations, AFOS activity, and the PICP:PINP ratio in breast cancer patients. SD versus PD.](image)
prostate cancer (14), and multiple myeloma (15). Elevated values of a degradation product of type I collagen fibers, ICTP, are associated with aggressive disease in rheumatoid arthritis (16). However, neither the procollagen propeptides PINP and PICP nor ICTP are sensitive enough for diagnosing bone disease in breast cancer patients, although these may be of use in the follow-up of treatment (12). The present results show that such markers are valuable for determining the aggressivity of the disease. This is further supported by the fact that the propeptide markers also changed in patients having none or only other than bone metastases if the disease was of aggressive type. There was no such tendency in patients with stable disease and a similar metastasis status.

We cannot say at present whether the changes in the propeptides occur before, during, or after the progression of the disease, although it has been shown that there is a tendency of the values to change according to the behavior of the bone disease in breast cancer patients (12). Unfortunately, this preliminary study only included 13 subjects. However, it is possible to evaluate the status quo of breast cancer patients either by the intact PINP value alone or by calculating the PICP:PINP ratio. Prospective follow-up studies are needed to evaluate the prognostic value of these markers.

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