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The Decrease in Prolidase Activity in Myeloproliferative Neoplasms

Zmniejszona aktywność prolidazy w nowotworach mieloproliferacyjnych

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Abstract

Backgraound. The development of bone marrow fibrosis is a severe complication in hematological diseases. The progress of bone marrow myelofibrosis is evaluated by a trephine examination and may be characterized by the biochemical markers of collagen turnover determination.

Objectives. Investigation of serum prolidase activity and biochemical markers of collagen metabolism in order to establish its role in the development of bone marrow fibrosis.

Material and Methods. The group of 37 patients with myeloproliferative neoplasms (MPN) before treatment, consisted of 16 patients with chronic myeloid leukemia (CML), 7 with primary myelofibrosis (PMF), 8 with essential thrombocythopenia (ET), and 6 with polycythemia vera (PV).

Results. It was found that the plasma activity of prolidase (Pro) was reduced to almost half together with the serum level of osteocalcin (BGL), and hydroxyproline (H-PRO) in the serum and urine of patients with MPN in comparison to the control group. In the MPN group of patients, the levels of N-terminal procollagen III peptide (PIIINP), type I procollagen (PICP) and the C-terminal telopeptide of type I collagen (ICTP) were significantly higher.

Conclusions. The alteration of collagen turnover markers in the MPN patient group (the elevation of synthesis and inhibition of collagen catabolism rate) has suggested that a diminished prolidase activity may contribute to such alteration of collagen metabolism and should be consider a biomarker of MPN progress (**Adv Clin Exp Med 2012, 21, 6, 767–771**).

Key words: collagen, markers of bone metabolism, prolidase, myeloproliferative neoplasms.

Streszczenie

Wprowadzenie. Rozwój zwłóknienia szpiku kostnego jest powikłaniem w wielu chorobach hematologicznych. Ocena histopatologiczna trepanobioptatu w chorobach mieloproliferacyjnych jest wykładnikiem stopnia zwłóknienia szpiku kostnego, a badane markery metabolizmu kolagenu mogą być biochemicznym sygnałem toczącego się procesu włóknienia.

Materiał i metody. Grupa chorych na nowotwory mieloproliferacyjne (MPN) liczyła 37 pacjentów, w tym 16 z przewlekłą białaczką szpikową (CML), 7 z pierwotnym zwłóknieniem szpiku (PMF), 8 z nadpłytkowością samoistną (ET) i 6 z czerwienicą prawdziwą (PV).

Wyniki. Stwierdzono znaczące, 2-krotne zmniejszenie aktywności prolidazy w osoczu oraz zmniejszenie stężenia osteokalcyny (BLG) w surowicy, hydroksyproliny (OH-pro) w surowicy i moczu w porównaniu z grupą kontrolną. Jednocześnie stwierdzono zwiększone stężenie N-końcowego propeptydu prokolagenu typu III (PIIINP), propeptydu prokolagenu typu I (PICP) oraz C-końcowego telopeptydu kolagenu typu I (ICTP) w grupie chorych na MPN.

Wnioski. Znaczące zmiany w stężeniu markerów metabolizmu kolagenu wskazujące na wzmożenie syntezy i zahamowanie katabolizmu tego białka sugerują, że ograniczenie aktywności prolidazy może mieć istotny wpływ na rozwój procesu włóknienia szpiku kostnego, którą należy rozważyć jako wskaźnik rozwoju choroby (**Adv Clin Exp Med 2012, 21, 6, 767–771**).

Słowa kluczowe: kolagen, markery metabolizmu kostnego, prolidaza, nowotwory mieloproliferacyjne.

Marrow myelofibrosis, which belongs to the histopathological picture of myeloproliferative neoplasms, occurs in that disease in various degrees. It is evaluated using the picture of marrow, obtained by trepanobiopsy as follows: MF-0 – scattered linear reticulin with no intersections (crossovers) corresponding to normal bone marrow, MF-1 – a loose network of reticulin with many intersections, especially in perivascular areas, MF-2 – a diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis, MF-3 – a diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis [1]. Bone marrow myelofibrosis with significant deposition of collagen and reticulin occurs as a result of the clonal growth of fibroblasts stimulated by PDGF and TGF-β produced by megakaryocytes existing in platelets [2]. An additional factor is the repression of PF4 by the collagenase activity secreted by granulocytes and monocytes [3]. Bone marrow fibrosis is evaluated by the amount of collagen and hyaluronan deposited in bone marrow interstitium [4]. Collagen is the main component of connective tissue. A sequence of 3 amino acids (glycine, proline and hydroxyproline) included in a polypeptide chain appears repeatedly in it. Over 20 proteins appear as collagen, and 10 more have collagen-like domains [5, 6]. Reticular fibers consist mainly of type III collagen. Type IV and V collagen occurs in basal membranes, whereas in bone marrow, collagen type I and III appears. A higher reticulin grade in the bone marrow trephine of patients with ET is associated with increased complication rates, including myelofibrosis [7].

Prolidase is a cytosolic exopeptidase which cleaves iminopeptides, possesses carboxyterminal proline or OH-pro, and plays an important role in collagen metabolism because of the high proportion of imino acids in collagen (25% of Pro and OH-Pro) [8]. Patients with a prolidase deficiency due to PEPD gene mutation manifest developmental delays and facial dysmorphism [9]. In cultured fibroblasts derived from patients with deficient prolidase, prolinase activity was higher in prolidase-deficient cells than in control cells. Prolinase activity was also higher in the plasma [10]. Prolidase activity was increased during *in vitro* stimulation of collagen synthesis in skin fibroblasts [11].

The aim of present research was to determine the collagen metabolism biomarkers – prolinase and prolidase activity in serum together with some related factors such as bone metabolism markers: the osteocalcine concentration and bone fraction of alkaline phosphatase, as well as the concentration of hydroxyproline in serum and its 24-hour excretion in urine, to apply a non-invasive method of estimating the progress of bone marrow fibrosis.

Material and Methods

A group of 37 patients, aged 24–74 years old, average age 50.5 years, 21 female and 16 male, were included in the study. The study group of patients with MPN consisted of 16 with chronic myeloid leukemia (CML); 11 in the chronic phase and 5 in blastic crisis, 7 with primary myelofibrosis (PMF), 8 with essential thrombocytemia (ET) and 6 with polycythemia vera (PV). The control group consisted of 30 healthy volunteers, aged 22–68, average age 43.7 years, 17 female and 13 male. The authors drew blood for serum, plasma, and urine (of a 24 hours excretion) after a 1-day diet without collagen, frozen to –20 degrees centigrade.

Prolidase and prolinase activity was determined according to Myara et al. [12, 13]. The serum level of the biochemical markers of collagen and bone metabolism was determined using commercially available tests except OH-pro. The N-terminal procollagen III peptide (PIIINP) with a PIIIP – RIA-gnost test (Behring, Germany), type I procollagen (PICP) and the C – terminal telopeptide of type I collagen (ICTP) – Orion Diagnostica (Finland), and total (T-ALP) and bone specific ALP (B-ALP) – the ALP MPR-3 test (Behringer, Mannheim, Germany) were used. Osteocalcin (BGL) was measured using Osteocalcin ELISA of the Dako firm. OH-pro concentration in serum and urine were determined by the method proposed by Prockop and Kivirikko [14].

The results are expressed as means ± the standard deviation (SD). Due to the small number of patients in the CML, PMF, ET and PV groups, we compared the results of the whole MPN group with the control group using the program Statistica 5.0 PL (StatSoft Polska). For the comparison of values between the two groups of subjects, the Mann-Whitney *U*-test was used. Differences were considered significant when p was less than 0.05.

Results

The biochemical laboratory test results are presented in Table 1.

Of the markers of collagen degradation, prolidase activity was reduced from 813.5 U/ml to 453.1 U/L in the MPN group but prolinase activity was almost unchanged 14.96 U/l in the control group and 14.90 in the MPN group of patients. The level of OH-proline in serum and urine was

Table 1. Biochemical markers of bone turnover and connective tissue in patients with myeloproliferative neoplasms (MPN) and the control group

Tabela 1. Biochemiczne wskaźniki obrotu kostnego i tkanki łącznej u chorych na nowotwory mieloproliferacyjne (MPN) i grupy kontrolnej

Group (Grupa)	Prolidase [U/L]	Prolinase [U/L]	OH-pro [μg/ml]	OH-pro in urine [mg/ 24h/ m^2]	ICTP [μg/L]	PIIINP [U/ml]	PICP [μg/L]	BGL [μg/L]	T-ALP [U/L]	B-ALP [U/L]
N CML X SD	16 433.0 ± 218.0	16 18.74 ± 8.75	14 8.64 ± 1.75	16 11.32 ± 9.61	16 7.68 ± 3.4	16 0.92 ± 0.17	16 168.5 ± 60.0	16 4.67 ± 2.4	15 197.6 ± 99.5	14 52.9 ± 33.3
N PMF X SD	7 408.3 ± 185.7	7 12.11 ± 5.4	7 8.77 ± 1.84	7 9.73 ± 8.01	7 6.54 ± 1,8	7 0.846 ± 0.11	7 214.1 ± 58.5	7 4.18 ±1.5	7 304.4 ± 205.1	7 88.9 ± 96.8
N ET X SD	8 463.0 ± 225.3	8 7.93 ± 3.15	7 8.76 ± 3.37	8 8.24 ± 3.45	7 6.21 ± 2.9	6 0.84 ± 0.13	7 156.9 ± 35.5	7 4.25 ± 2.6	7 143.7 ± 59.6	7 34.2 ± 16.8
N PV X SD	6 607.3 ± 190.2	6 11.34 ± 7.5	6 10.24 ±1.68	6 7.93 ± 2.78	6 6.1 ± 1.65	6 0.998 ± 0.14	5 139.8 ± 18.4	4 3.82 ± 1.6	6 181.3 ± 48.9	6 52.4 ± 28.4
N MPN X SD	37 453.1 ± 171.3	36 14.9 ± 12.0	34 8.90 ± 2.04	37 10.15 ± 7.8	36 6.82 ± 2.71	35 0.88 ± 0.15	35 172.53 ± 56.7	34 4.54 ± 1.9	35 194.45 ± 48.9	34 52.9 ± 34.8
Control N X SD	 20 813.5 ± 183.0	 15 14.96 ± 4.45	 20 12.9 ± 1.8	 20 13.8 ± 2.1	 20 4.49 ± 1.12	 20 0.53 ± 0.08	 25 133.1 ± 56.7	 20 9.20 ± 2.3	 30 142.7 ± 35.1	 30 60.6 ± 20.3
p value	0.0001	ns.	0.0001	0.0001	0.0001	0.0001	0.02	0.0001	0.001	ns.

reduced from12.9 μg/ml to 8.90 μg/ml and from 13.8 mg/24 h/m^2 to 10.15 mg/24 h/m^2 in the MPN group, respectively. The marker of bone collagen degradation, ICTP, was elevated from 4.49 μg/l to 6.82 μg/l in the MPN group. Both markers of collagen synthesis, PIIIP and PICP were elevated from 0.53 U/l to 0.88 U/l and from 133.1 μg/l to 172.5 μg/l in the MPN group, respectively. The marker of bone synthesis, BGL, was reduced from 9.20 μg/l to 4.54 μg/l in the MPN group. Total ALP activity was significantly elevated but bone-specific ALP was slightly reduced from 60.6 U/l to 52.9 U/l.

Discussion

In the process of collagen degradation, prolidase and prolinase are involved. Collagen is degraded by prolinase (hydrolyzing dipeptides of type Pro-X) and prolidase (hydrolyzing dipeptides of type X-Pro) [15].

Clinical studies have shown increased prolidase activity in chronic liver disease [12].

Plasma prolidase activity in patients with diabetes mellitus type 2 was significantly lower than in healthy control subjects, which may be interpreted as evidence of decreased bone resorption. The authors have suggested that serum prolidase activity may be a better marker of osteoporosis in a diabetic state than deoxypyridoline Dpy [16]. No relationship between increasing bone marrow turnover markers, bone density and prolidase activity was observed in postmenopausal women [17]. The prolidase activity was significantly lower in patients with osteoarthritis than in the control. Prolidase activity was negatively correlated with the total peroxide and oxidative stress index and positively correlated with serum total antioxidant capacity. The decreased collagen metabolism may be related to oxidative stress, which has a role in the etiopathogenesis and/or in progression of the disease [18]. The lower activity of prolidase with normal activity of prolinase in MPN indicates that the process of collagen degradation is mainly the result of the activity of prolinase. The decrease of the concentration of hydroxyproline in serum and urine confirm this hypothesis. Recently, no data concerning the activity of prolidase and con-

centration of hydroproxyline in MPN-patients is available.

In the group of patients with MPN, an alteration of collagen metabolism markers is observed. The concentration of ITCP expresses the rate of degradation of type I collagen and is connected with the increased resorption of the bones and degradation of type I collagen. The rise of PINP and PICP concentration shows an increased synthesis of I type collagen, which leads to the growth of bone tissue. In bone marrow reticulin, the type III collagen is predominant. Peptide PIIINP is a sensitive marker of fibrosis and its high concentration has been found in patients with myeloproliferative syndrome [19]. Barosi et al. [20] found a lack of relationship between PIIIP and the morphometric grading of bone marrow fibrosis, megakaryocyte numbers or lymphoid infiltrations. It was suggested that the PIIIP level correlates with overall disease activity rather than with bone marrow fibrosis. The growth of the synthesis of PICP and PIIINP correlates positively with the growth of the secretion of PDGF and TGFβ [21]. In the present study, higher concentrations of PICP and PIIINP have been observed in patients with MPN, which suggests an increased synthesis of collagen of types I and III, in particular by PMF- and CML-patients.

The concentration of osteocalcin in MPN patients is essentially lower than in the control group, which seems to indicate the inhibition of the bone building process. The concentration of osteocalcin in MPN-patients is essentially lower than in the control group, which seems to indicate a worse bone building function of osteoblasts [22]. Total ALP-activity in the present patients was essentially higher then in the control group, but the activity of B-ALP is larger in PMF-patients, although its activity in the full group does not differ from that of the control group. The low concentration of osteocalcine and lower activity of the bone fraction of alkaline phosphatase (except in PMF-patients) in the serum indicate a slacking calcification process in MPN-patients.

MPN share several phenotypic similarities, mainly the mutation of JAK-2 tyrosine kinase but patients with ET of both types, JAK-2 positive and JAK-2 negative, have shown pronounced abnormalities in granulopoesis and megakariopoesis, suggesting that reticulin accumulation reflects the similar biologic mechanisms in both subtypes of ET [7]. In myelofibrosis, the JAK2V617F mutation is restricted to hematopoietic cells, and cannot explain the stromal alteration being observed in this disorder [23].

The authors concluded that statistically significant elevated concentration of PIIINP and PICP and diminished concentration of hydroxyproline shows the superiority of the synthesis of collagen over its degradation in MPN-patients may be indicative of the accumulation of collagen in marrow. Maximal concentration of PICP and ICTP was found in CML- and PMF-patients, suggesting that the parameters of transformation of type I collagen may be essential markers in the process of fibrosis.

The deficiency of prolidase activity in studied MPN-group of patients has shown the possibility of diminished collagen degradation, which may be an important reason for higher fibrosis in these disorders. The diminishing of the hydroxyproline level in serum and urine supports these conceptions.

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