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Biochimica et Biophysica Acta 1689 (2004) 179–181
BIOCHIMICA ET BIOPHYSICA ACTA
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Rapid report

Cytosolic carbonic anhydrase activity in chronic myeloid disorders with different clinical phenotype

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Received 30 March 2004; received in revised form 18 May 2004; accepted 18 May 2004
Available online 15 June 2004

Abstract

Carbonic anhydrase family (CAs) plays an important role in the extracellular acidification and several studies suggest a possible involvement of such enzymes in the increased tumor progression due to the acidic extracellular pH. We measured the activities of carbonic anhydrase I and II isoforms in a group of patients affected by four specific chronic haematological diseases, sharing a common origin but characterized by a different neoplastic evolution: agnogenic myeloid metaplasia (AMM), essential thrombocythemia (ET), chronic myeloid leukemia (CML) and polycythemia vera (PV) in order to understand the correlation between CAs activities and neoplastic outcome. In comparison to controls, our data demonstrate an increase of CAI and CAII activities in all our patients with a specific increase of the CAI activity in the group of the diseases with major malignancy (CML and AMM). These results suggest a possible role of such isozymes in the progression of the myeloid disorders and CAs specific inhibitors should be useful in slowing the progression of the disease.
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Keywords: Carbonic anhydrase; Myeloid; Phenotype

Acidification of extracellular milieu in malignant tumors is reported to increase the invasive behaviour of cancer cells [1]. Several studies suggest a possible role for carbonic anhydrase isozymes that catalyze the reversible hydration of CO_2 to H_2CO_3 [2]. To generate the pH gradient between the extracellular and intracellular compartments, tumor cells express ion transport proteins including vacuolar H^+-ATPase, Cl^-/HCO_3^- exchanger and Na^+/H^+ exchanger and carbonic anhydrases to catalyze the production of H^+ and HCO_3^+ [3]. At present, 13 enzymatically active CA isoenzymes were identified from mammals, namely cytosolic CAI, II III and VII, mitochondrial CA VA and VB, secretory CA VI, and membrane-associated CA IV, IX, XII and XIV. Recently a novel member of the CAs family, with high structural similarity to cytosolic isoenzymes, named CA XIII, was identified [4]. CAI is present into the erythrocytes, it is a low activity CA, and its biological role is not well understood. CAII is a high activity isozyme with the widest cytosolic distribution of the carbonic anhydrases [5]. Mutations in CAII gene result in an inherited disease [6]. The expression of CAI and CAII isozymes has been also investigated in several neoplastic and haematological diseases [7]. Immunological studies indicate that the carbonic anhydrases could have a role in tumors, including malignant brain tumors [8] and gastric and pancreatic carcinomas [9]. CAI is abundantly expressed both in the early neoplastic erythroblast in vitro and in human erythroleukemias in vivo [10], and recently has been demonstrated that there is a specific CA II increased expression in the blast cells of acute myeloid leukemia, acute lymphoblastic leukemia and chronic myelomonocytic leukemia not respective of the cell lineage [11]. Despite these studies, there are few data on the activity of CAI and CAII isoenzymes in chronic myeloid disorders (CMDs), another group of haematological diseases characterized by a slow progression, often towards a leukemic condition. We therefore decided to measure the CAI and II activities in four different myeloproliferative disorders characterized by a different progressive worst clinical phenotype, in order to evaluate a possible CAI and CAII involvement in

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doi:10.1016/j.bbadis.2004.05.003

such specific haematological conditions. Normal control samples were obtained from 30 healthy donors (HD) (18–65 years old). The patients were 14 (36–79 years) with essential thrombocythemia (ET), 8 (49–72 years) with chronic myeloid leukemia (CML), 15 (38–70 years) with agnogenic myeloid metaplasia (AMM), 7 (35–68 years) with polycythemia vera (PV). In all of patients the diagnosis was made according to the standard criteria for CMPD [12]. The patients were enrolled at the diagnosis before starting the therapy and were well matching for blast count, bone marrow cellularity, bone marrow fibrosis, disease's grade and routine haematological parameters. Only the patients with Hb values above 10 gr/dl, not transfused, were included in the study. Blood specimens were collected by venipuncture in heparinized test tubes. Leukocytes were isolated by lymphoprep density gradient centrifugation, washed twice with cold PBS and sonicated in solubilization buffer 0.1 M Tris SO_4 pH 7.5 containing 1 mM Benzamidine, 1 mM *o*-phenatroline. The pellet from lymphoprep (red cells) was collected, diluted 1:100 with bidistilled water and then stored at -20 °C until the haemoglobin and CAs were assayed. For the evaluation of Hb levels a spectrophotometric assay was used as described. Briefly, 0.2 ml of 1:100 diluted blood was added to 0.8 ml of ammonium hydroxide solution 0.006 N. The optical density at 576 nm (OD 576) was red on a Beckmann DU 60 spectrophotometer using as blank 0.2 ml of bidistilled water. The haemoglobin levels (mg/ml) were calculated by the formulae: mg Hb/ml = OD576 × 500/0.912. The total CA activity (CAI plus CAII) was evaluated according to the procedure of Maren [13] as described [14]. The CAII activity was determined in presence of sodium iodide, a CAI-specific inhibitor. The CAI activity was calculated as difference between total CA and CAII activities. The results were expressed in units of CA per milligram of Hb (Haemoglobin) for the red cells and in units of CA per milligram of total proteins for leukocytes. The protein concentration was determined by Lowry procedure using bovine serum albumin as a standard. A Kruskall–Wallis test was used to determine whether the red blood cells CAtot and CAI activities of CMPDs patients were significantly different versus normal controls. A Mann–Witney test was used to evaluate significant differences in CAII leukocytes activity of CMDs patients. Fig. 1A and B summarizes the CAtot (CAI + CAII) and CAI activities in the red blood cells from PV, ET, CML and AMM patients. All patients (44/44) showed a high activity versus normal control. The fold increase in CAtot activity varies among different groups of patients. The highest increase is for AMM patients (56 ± 21 U/mg Hb) followed by CML (41.13 ± 17 U/mg Hb), ET (36 ± 10 U/mg Hb) and PV patients (27 ± 13 U/mg Hb). Normal controls (22.3 ± 3.6 U/mg Hb). ($P < 0.0001$ Kruskall–Wallis). The same situation was observed for CAI values which are more elevated in AMM patients (32 ± 0.4 U/mg Hb), followed by CML (26 ± U/mg Hb) and ET and PT patients with 21 ± 8 and 15 ± 2 U/mg Hb, respectively; normal controls, 12.5 ± 4 U/mg Hb ($P < 0.0001$ Kruskall–Wallis). Fig. 1C summarizes the data for CAII activity in the same samples. The highest activity is present in AMM patients (24.2 ± 4 U/mg Hb) with CML (15.46 ± 6 U/mg Hb), ET (15.3 ± 3 U/mg Hb) and PV patients (12.4 ± 7 U/mg Hb); normal controls, 9.8 ± 3 U/mg Hb ($P < 0.0001$ Kruskall–Wallis). However, the fold

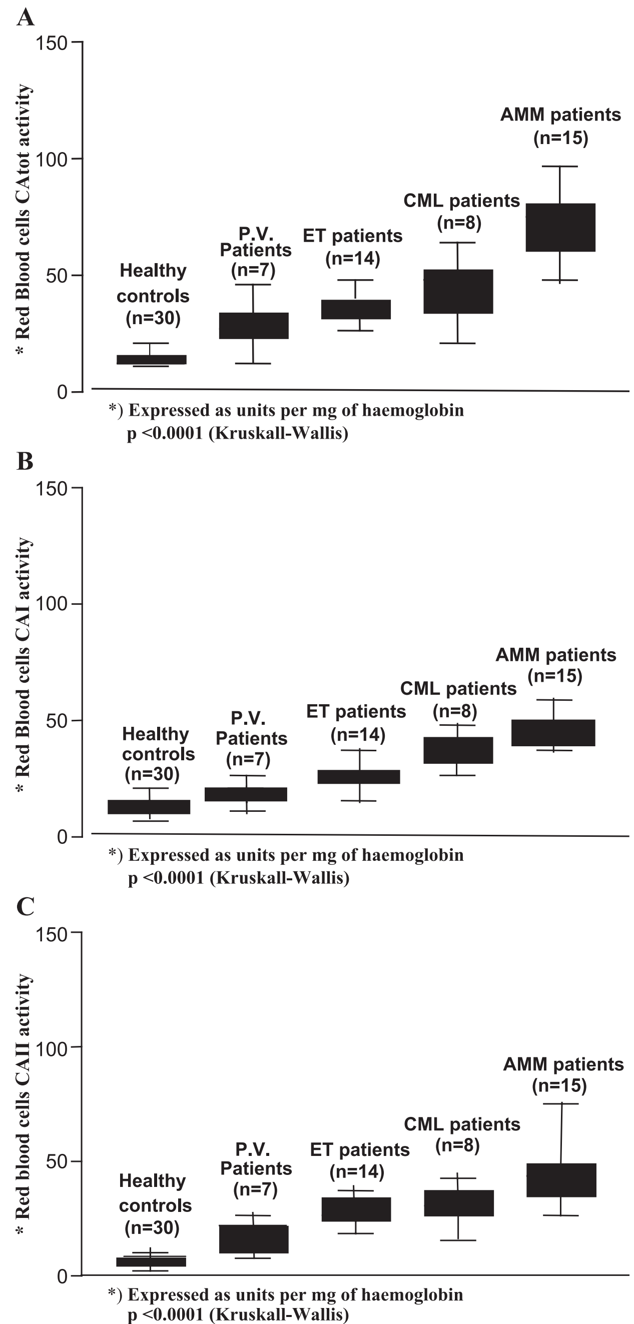

Fig. 1. Red blood cell total carbonic anhydrase (CAI + CAII) (A), specific CAI (B) and CAII activities (C) in patients affected by polycythemia vera (PV), essential thrombocythemia (ET), chronic myeloid leukemia (CML), and agnogenic myeloid metaplasia (AMM). Total CA activities, CAII and CAI activities were significantly elevated in all of four groups of chronic myeloid patients.

elevation was less than CAI. The CAII activities measured in separated leukocytes were only significantly increased in CML and AMM patients in comparison to normal control; CML (20 ± 3 U/mg of total proteins), AMM (15.2 ± 1.3 U/mg of total protein), normal control (9.5 ± 0.8 U/mg of total protein) ($P < 0.001$ Mann–Witney). No CAI activity was present in the leukocytes lysate. The chronic myeloproliferative neoplasias are clonal stem cell disorders caused by the transformation of an undifferentiated hematopoietic stem cell. Typically such disorders are classified in four different groups that recognize a common etiology but with different clinical outcome. The worst outcome is displayed by CML that frequently tends to evolve in acute leukemia and by AMM where the clonal myeloproliferation is characteristically accompanied by reactive myelofibrosis and extramedullary hematopoiesis. The median survival is estimated to be between 3 and 6 years and the causes of death include leukemic evolution. The other two groups include ET and PV that have a milder outcome and with a median survival higher than 10 years [12]. According with the leukemic evolution of the two worst outcome CMDs, and with the well-documented overexpression of CAs in course of several acute leukemias, we evaluated the possible contribution of the CAI and CAII isoforms in course of myeloproliferative disorders with different clinical phenotype. Our data, show: (i) a significant increase of total CA (CAI plus CAII), CAI and CAII specific activities in the red blood cells of all of four types of chronic myeloproliferative disorders versus normal controls, with the highest increase in the group of AMM and CML patients with the more unfavourable prognosis. (ii) a fold CAII elevation lesser then CAI, suggesting a possible major contribution of CA I isoenzyme in the total red blood cell CA activity in the course of CMDs. As expected, the leukocyte CA activity was related only to the CAII isoform, according to the previous published immunohistochemical data (Leppilampi M., 2002), and was significantly increased only in the CML and AMM patients. Although a study with a larger number of patients is necessary, our observations, suggesting a possible role for CAI and CAII isozymes in CML and AMM diseases, could be relevant for the diagnosis, and follow-up, as well as for the management of the patients since, as well described for other neoplasias [15,16], also in these haematological disorders the CA inhibitors may be useful to control the progression of the disease.

Acknowledgements

This work was supported by grants 9818306-006 M.U.R. 1998 and MM06182533-006 C.O.F.I.N. 2000 to Prof Pietro Strisciuglio.

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