RESEARCH COMMUNICATION

Erythrocyte Catalase and Carbonic Anhydrase Activities in Acute Leukemias

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Abstract

Objective: To determine activity of catalase (CAT) as a antioxidant and carbonic anhydrase (CA) in erythrocytes from acute leukemia cases. Methods: Subjects were recruited from patients attending the outpatient clinics or hospitalised in the ward of the Hematology Department of Yuzuncu Yil University Hospital. Venous blood samples were taken from a total of 67 individuals (31 with acute leukemia and 36 healthy) included in the study. CAT enzyme activity was determined in erythrocytes using Aebi's method and CA by hydration of CO_2. Results: CAT activity was found to be significantly decreased (P<0.001) on average in acute leukemia cases as compared to the control group while erythrocyte CA activity was significantly increased (P<0.001). Conclusions: Our findings point to malfunction of the antioxidant system in acute leukemia patients. Hence we need to investigation the cause and also its possible contribution to prognosis. Furthermore, clarification of the relationship between the antoxidant system and CA inhibitors in the pathogenesis of acute leukemia appears warranted.

Key Words: Carbonic anhydrase - catalase - erythrocyte levels - acute leukemia cases

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Introduction

Acute leukemia is a rapidly progressing disease that results in the accumulation of immature, functionless cells in the marrow and blood. In acute leukemia, cancerous cells multiply quickly and replace normal cells in the bone marrow, causing bone marrow failure. The marrow often stops producing enough normal red cells, white cells and platelets. Most cases of acute leukemia have no obvious cause (Jaffe et al., 2001).

Antioxidants are subtances to hinder and delay the negative effect of free oxygen radicals on target tissues or to task in repairing of injured tissues. Antioxidants can be divided into two groups termed enzymatic and non-enzymatic. Enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). As to non-enzymatic antioxidants are vitamin E, vitamin C, vitamin A, selenium, transferin and lactoferrin. Antioxidants is frequently intracellular and sometimes may be found as extracellular forms (Halliwell and Aruoma, 1991; Evans and Cooke, 2004; Halliwell, 2007). As the result of impairment of the balance between free radicals and antioxidants, it it known that oxidative stress can be increased and that defects in DNA can lead to cancer in addition to other many diseases (Holmes et al., 1992; Halliwell, 2007). It is known that in acute leukemia, both myeloid and lymphoid tumor cells can produce excessively free radicals (Drabko and Kowalczyk, 2004).

Though there have been some studies carried out to determine the levels of enzymes such as GPx and SOD, no detailed work related with level of CAT in acute leukemia patients in literature (Drabko and Kowalczyk, 2004). Carbonic anhydrase (CA) (E.C. 4.2.1.1.) is a Zn-containing metalloenzyme that catalyzes the reversible hydration of carbondioxide. All three soluble isozymes of CA in humans (CA-I, II, and III) are monometric. CA-I is found primarily in erythrocytes. The human CA-II isozyme is widely distributed. It has been identified in erythrocytes, brain, eye, kidney, etc. Normally CA-I and CA-II each contribute about 50 percent of the total activity (Yılmaz et al., 2000). In animals and human, numerous CA isoenzymes and related proteins have been reported (Tarun et al., 2003). The maintenance of appropriate acid-base homeostasis is a prerequisite for normal cell growth. In literature, recent studies suggest that acid-base state may play an important role in tumorigenesis (Chegwidden et al., 2000; Venta, 1991). CAs has recently become a target for intensive research into carcinogenesis and tumor invasion. There are only a few studies focused on CAs in malignant hematopoietic cells. The presence of CA II in leukemia cells suggests that it may participate in the regulation of pH homeostasis in these cells. Tumor growth is generally known to involve complex interactions between cells and their microenvironment characterized at least in part by an acidic extracellular pH (Leppilampi et al., 2002).

The aim of the present study was to evaluate the catalase and the carbonic anhydrase activity in erythrocytes from acute leukemia patients.

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Materials and Methods

Biochemical Analysis

Subjects were recruited from patients attending the outpatient clinics and those hospitalised in the ward of the Hematology Department of Yuzuncu Yil University Hospital. The study included a total of 67 subjects (31 acute leukemia and 36 healthy human) and venous blood samples of acute leukemia were obtained from the antecubital veins the leukemia in accordance with the guidelines set out in the Declaration of Helsinki. The study was approved by the local ethics committee. Written informed consent was obtained from all participants.
Serum was separated by centrifugation and the samples were processed immediately. The serum samples were placed in deionised polyethylenetubes and kept at -80 oC in a deep-freeze (without thawing) until the day of study. The blood samples were centrifuged at 1500 rpm for 20 minute and the plasma and buff coat were removed. After the paced red cells were washed with NaCI (0.9%) twice, the erythrocytes were hemolyzed with cold water. The hemolysate was diluted with distilled water, and this hemolysate was used to determine the CA activity.

Biochemical analysis of erythrocyte CAT activity was performed with a method described by Aebi (Aebi, 1984) in the Biochemistry Laboratory of Chemistry Department, Faculty of Art and Science, Yuzuncu Yil University. Briefly, the supernatant (0.1 ml) was added to a quartz cuvette containing 2.95 ml of 19 mmol/l H2O2 solution prepared in potassium phosphate buffer (0.05 M, pH 7.00). The change in absorbance was monitored at 240 nm for 5 min using a spectrophotometer (Shimadzu UV-1201, Japan).

CA activity was assayed by hydration of C02. The hydration of C02 was measured by the method of Rickli and Wilbur-Anderson with bromothymol blue as indicator (Rickli et al., 1964).

Statistical Analysis

The results were expressed as the mean ± standard error (SE). One-way ANOVA was used for the comparison of mean values of the groups. Then, Student-t test was used to determine the difference between groups. In addition, Pearson's correlation analysis was carried out to determine the relationships among the variables. P value < 0.001 was considered statistically significant. Statistical analyses were carried out using the SPSS® statistical software package (SPSS for Windows version 13.0, SPSS Inc., Chicago, Illinois, USA).

Results

Acute leukemia group composed of 31 person (AML: 25;ALL: 6), eleven of them were females and the other twenty person were males. The average age of patients was 34.18±6.18. There were 36 persons in control group twelve of it females and the rest were males, and the average age of control group was 33.43±3.12. There was no difference between the two groups (p>0.001).

Parameters of haematological of acute leukemia and healthy control group are shown in (Table 1).

Table 1. Parameters of Acute Leukemia Cases and Healthy Controls

Parameter	Controls n=36	Leukemia cases n=31
WBC (109 /L)	7.2± 0.6*	78.4±24.3
Hb (g/dL)	13.4± 0.6*	6.9± 0.9
Platelets (109 /L)	228.2±17.8*	48.4±12.3
Erythrocytes (10^6/mm^3)	4.6± 0.7*	2.3± 0.5
CAT EU/(gHb)	61.4±81.9*	3.4± 1.5
CA EU/(gHb)	13.5± 2.0*	78.5±33.0

*: $p < 0.001$

Erythrocyte activity of CAT and CA of the acute leukemia and healthy human cases are also shown in Table 1. The erythrocyte CAT activity was significantly lower (P<0.001) and CA activity was significantly higher (P<0.001) in with acute leukemia than controls. The activities of CAT and CA in ALL and AML groups were measured as 3.24±1.34; 73.3±21.1 and 3.16 ±0.12; 75.2±11.9, respectively. There was no difference in the parameters of two groups (p>0.001).

Discussion

Reactive oxygen radicals (ROS), including superoxide anion (O-2), hydrogen peroxide (H2O2), hydroxyl free radical (OH) and singlet oxygen (1O2), continuously generated from mitochondrial respiratory chain, have powerfully oxidative potential. ROS is capable of attacking lipids, nuclear acids and proteins, resulting in certain degree of oxidative damages. ROS are continuously produced during normal physiologic events, and removed by antioxidant defence mechanisms (El-Habit et al., 2000; Kanter et al., 2005). In pathological conditions, ROS are over produced and result in lipid peroxidation and oxidative damage. The imbalance between ROS and antioxidant defence mechanisms leads to oxidative modification in the cellular membrane or intracellular molecules (Tunçel et al., 1998).

Cell possesses an efficient antioxidant defense system, mainly composed of the enzymes such as superoxide dismutase, glutathione peroxidase and catalase, which can scavenge the ROS excessive to cellular metabolism, and make ROS level relatively stable under physiological conditions (Gao et al., 2002; Celik and Demir, 2005;Kolusari et al., 2008). Information about the activities of antioxidant enzymes is conflicting in patients with cancer and studies on leukemia patients are rare (Devi et al., 2000). In various malignant tumor cells, catalase activity has been found to be decreased (Ono, 1966; Bozzi et al., 1976; Oberley, 1982). or in normal ranges of activity (Gonzales et al., 1984). Some studies about erythrocyte catalase activities in leukemias that we found have been made in humans (Saito et al., 1984) , (Gonzales et al., 1984) and in animals (Madej et al., 1988). In our study, CAT activity was found to be significantly decreased in red blood cells from patients with acute leukemia. CAT activity was similar to that reported in previous studies (Ono, 1966; Bozzi et al., 1976; Oberley, 1982). These findings may indicate a possible link between decreased antioxidants and increased free radical levels, supporting

the idea that there is a persistence of oxidative stress in acute leukemia.

Carbonic anhydrases (CAs) are key enzymes that regulate acid-base homeostasis in both normal and pathological conditions. At present, 13 enzymatically active CA isoenzymes were identified from mammals (Lehtonen et al., 2004). CA IX and XII, are highly expressed in some tumour cells and may be functionally related to carcinogenesis (Liao et al., 1994). Several studies suggest that these "tumour-associated" isoenzymes may be implicated in the acidification of extracellular milieu surrounding the cancer cells and thus create a microenvironment conducive to tumour growth and invasion (Kolusari et al., 2008; Ivanov et al., 1998; Ertekin et al., 2007).

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 (Hewett-Emmett and Tashian, 1996). The expression of CAI and CAII isozymes has been also investigated in several neoplastic and haematological diseases (Venta, 1991). CAI is abundantly expressed both in the early neoplastic erythroblast in vitro and in human erythroleukemias in vivo (Frankel et al., 1985). 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 (Leppilampi et al., 2002). Despite these studies, there are few data on the activity of CA in acute leukemia. Therefore, determination of CA enzyme activity is important in acute leukemia. We therefore decided to measure the CA activity. In a study, comparison to controls, data demonstrate an increase of CA-I and C-AII activities in all patients with a specific increase of the CA-I activity in the group of the diseases with major malignancy chronic myeloid leukemia and agnogenic myeloid metaplasia (Bonapace et al., 2004).

In conclusion, our findings reveal that the antioxidant system in acute leukemia patients is not well functioning. Hence there are need the investigations to protect and strength the antioxidant system, also its contribution to prognosis. Furthermore, there are need studies which verify and clarify the relationship between antoxidant system and CA inhibitors in the pathogenesis of acute leukemia patients.

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