RESEARCH COMMUNICATION

Erythrocyte Catalase and Carbonic Anhydrase Activities in Lung Cancer

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Abstract

Aim: To study the relationship between the pathogenesis of lung cancer and antioxidant status and acidic media by measuring the activities of erythrocyte catalase (CAT) and carbonic anhydrase (CA). Methods: A total of 26 patients with lung cancer and 15 healthy individuals were included in the study. The CAT and CA activities of erythrocytes were defined. The catalase (CAT) activity of erythrocytes was measured using Aebi`s method. Carbonic anhydrase (CA) activity was analyzed by CO_2 hydration. Results: It was found that erythrocyte CA and CAT activities were significantly lower in patients with lung cancer compared to controls ($p<0.05$). Of the 26 patients with lung cancer, seven (26.9%) had metastasis, and the CA and CAT levels in patients with metastasis were significantly decreased (p=0,0001). Conclusions: Development of oxidative stress due to lung cancer may be related to the balance between prooxidant and antioxidant reactions. Catalase may have a preventive effect for malignant lung cancers and the gene of the antioxidant enzymes may be one of the anti-oncogenes, and inactivation of one of these genes in the process of carcinogenesis may lead to tumor development. This may be an explanation for the very low levels of antioxidant CAT in patients with lung cancer compared to healthy individuals. Carbonic anhydrase (CA) in tumor cells may be an indicator of the acid-base balance in lung cancer. Decreased levels of CA in patients with lung cancer may provide a convenient media for tumor development, growth and metastasis by creating an acidic media.

Keywords: Catalase - carbonic anhydrase - lung cancer

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Introduction

Free radicals with their structure, physicochemical properties, cellular origins, reactions and impacts- have been implicated as important mediators in many clinical disorders. The free radical oxidation of poly-unsaturated fatty acids in biological systems is known as lipid peroxidation. Lipid peroxidation initiating with the removal of a hydrogen atom from unsaturated fatty acids in the cell membrane is a typical indicator of oxidative stress (Gutteridge, 1995; Reiter, 1995).

The shift in thedelicate balance between the free radicals and antioxidant defense system towards prooxidant and oxidant substances leads to the development of oxidant stress. It has been shown that oxidative stress causes tissue damage and is effective in the development of pathological conditions such as cancer, diabetes, and atherosclerosis (Cross et al., 1987; Asayama et al., 1994).

Free radicals are continuously produced in the body as a result of various chemical events. The most effective free radicals are singlet oxygen, hydroperoxides and superoxide anions (Cross et al., 1987).

There are some defense mechanisms in the body that prevent the development of free radicals and the damage they cause. They are known as "antioxidant defense systems". Antioxidants are substances that prevent, delay or repair the damage caused by the free oxygen radicals in target tissues. Antioxidants are divided into two groups: enzymatic and non-enzymatic. The antioxidant enzymes include superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). Non-enzymatic antioxidants include vitamin E, vitamin C, vitamin A (a-carotene), selenium, transferrin and lactoferrin. Antioxidants are usually intracellular and may occasionally be extracellular (Evans and Cooke, 2004; Halliwell, 2004).

It is known that when oxidative stress increases, damage may occur in the DNA sequence leading to cancer and other diseases as a result of the deterioration in the balance between free radicals and antioxidants (Halliwell,

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2007).

Recent research indicates that the relationship between the superoxide radical and the enzymes responsible for its removal (superoxide dismutase) reflects a much more delicate balance than was first anticipated (McCord, 1993).

During the past few years, superoxide radical activity in tumor cells has received increasing attention. For this reason, many researchers have turned towards the research for SOD and catalase activity, which are the basic antioxidants in damaged tumor cells (Gonzales et al., 1984; Guner et al., 1996).

H_2O_2 (hydrogen peroxide) is a strong membrane-passing oxidant that is rapidly eliminated from the cell to prevent induction of oxidative damage to lipids, proteins and DNA. Catalase detoxifies hydrogen peroxide (Vaisberg et al., 2005) and is one of the well-known antioxidant enzymes (Roels et al., 1979; Baek et al., 2007). Catalase is a priority enzyme of the antioxidant system in defense for oxidative stress occurring in many pathological conditions including cancer, diabetes, cataract, atherosclerosis, ischemic-reperfusion damage, arthritis, neuro-degenerative disease, nutritional deficiency, and aging. Catalase deficiency may lead to accumulation of reactive oxygen metabolites and this may cause the initiation of carcinogenesis.

Carbonic anhydrases (or carbonate dehydrates) form a family of enzymes that catalyze the rapid conversion of carbon dioxide to bicarbonate and protons, a reaction that occurs rather slowly in the absence of a catalyst (Badger and Price, 1994) The active site of most carbonic anhydrases contains a zinc ion; they are therefore classified as metalloenzymes.

The primary function of the enzyme in animals is to inter-convert carbon dioxide and bicarbonate to maintain acid-base balance in blood and other tissues, and to help transport carbon dioxide out of tissues.

Materials and Methods

Biochemical Analysis

The study cases were enrolled from patients and healthy individuals presenting to the Outpatient Clinic of the Department of Thoracic Surgery in Yuzuncu Yil University Faculty of Medicine and/or from hospitalized patients. All participants gave informed consents. The study was approved by the Local Ethics Committee.

Of the participants, 26 had lung cancer and 15 were healthy. The mean age of the patients with cancer was 56±7.29 years; eight (30.8%) were female and 18 (69.2%) were male. 50% were squamous cell carcinomas, 35% were small cell carcinomas, and 15% were adenocarcinomas. Seven patients (26.9%) had metastasis (to lung, brain, and surrenal, renal, other). None of the patients with cancer had received chemotherapy and/or radiotherapy or had undergone surgery. Of the healthy individuals, six (40%) were female and nine (60%) were male. The mean age of the controls was 49±8.16 years.

The study included a total of 41 subjects and venous blood samples from all cases were obtained from the antecubital fossa veins following the guidelines of the Declaration of Helsinki. Sera were separated by centrifugation and samples were processed immediately. The samples were placed in de-ionized polyethylene tubes and stored at -80°C in deep-freeze (without thawing) until the day of the study. The blood samples were centrifuged at 1500 rpm for 20 minutes and the plasma and buff coat were removed. After washing the paced red cells twice with NaCI (0.9%), the erythrocytes were hemolyzed with distilled water and this hemolysate was used to determine the CA activity. Biochemical analysis of the erythrocyte CAT activity was performed using a method described by Aebi in the Biochemistry Laboratory of the Chemistry Department, Faculty of Art and Science, Yuzuncu Yil University. Briefly, the supernatant (0.1 ml) was added to a quartz sink containing 2.95 ml of 19 mmol/l H_2O_2 solution prepared in potassium phosphate buffer (0.05 M, pH 7.00). The change in absorbance was monitored at 240 nm for five minutes using a spectrophotometer (Shimadzu UV-1201, Japan).

CA activity was assayed by hydration of CO_2. The hydration of CO_2 was measured by the method of Rickli and Wilbur-Anderson with bromothymol blue as the indicator (Rickli et al., 1964).

Statistical Analysis

The results were expressed as the mean±standard error (SE). One-way ANOVA was used for comparison of the mean values of the groups, and the Student-t test was used to determine the difference between the groups. Pearson's correlation analysis was performed to determine the relationships between the variables. The level of significance was set as $p<0.05$.

Descriptive statistics for the levels of catalase and carbonic anhydrase were expressed as mean, standard deviation, minimum and maximum values. The Mann-Whitney U test was used to find if there were any differences in these characteristics between the groups with and without metastasis. An ROC curve was drawn to find if catalase and carbonic anhydrase were effective in differentiating the metastasis. The level of significance was set as 5%.

Statistical analyses were performed using the SPSS® statistical software package (SPSS for Windows version 13.0, SPSS Inc., Chicago, Illinois, USA).

Results

The erythrocyte CAT and CA levels of patients with lung cancer and healthy individuals have been presented in Figure 1 and 2. Comparison of erythrocyte CAT and CA activities in patients with lung cancer and healthy individuals has been presented in Table 1. The erythrocyte CAT and CA activities were significantly lower in patients with lung cancer compared to controls ($p<0.05$).

There was metastasis in seven (26.9%) out of 26 patients with lung cancer. CAT and CA levels of patients with and without metastasis have been presented in Figure 3 and 4. Descriptive statistics for CAT and CA levels in patients with and without metastasis have been presented in Table 2. The difference in the mean values of carbonic anhydrase levels in patients with lung cancer with and without metastasis was significant ($p<0.01$), whereas it

Table 1. Erythrocyte CAT and CA Activities of Patients with Lung cancer and Healthy Individuals

PARAMETERS	HEALTHY INDIVIDUALS (MEAN±STANDARD ERROR)	PATIENTS WITH LUNG CANCER (MEAN±STANDARD ERROR)
CAT EU/(gHb)$^{-1}$	22.01± 5.25	3.75±1.11*
CA EU/(gHb)$^{-1}$	77.92± 2.96	7.11±1.61*

*p<0.0

Table 2. Descriptive Statistics and Comparison Results of the Groups with and Without Metastasis

		N	MEAN	STANDARD DEVIATION	MINIMUM	MAXIMUM	P
Carbonic anhydrase	No metastasis	19	7.9615	0.65583	6.61	9.07	0.000
	Metastasis	7	4.1729	0.71712	3.21	5.21	
	Total	26	6.9415	1.83580	3.21	9.07	
Catalase	No metastasis	19	4.1279	1.05606	2.51	5.73	0.376
	Metastasis	7	3.6671	1.40807	2.06	5.28	
	Total	26	4.0038	1.14990	2.06	5.73	

Table 3. Test Result Variable(s): Carbonic Anhydrase (Coordinates of the Curve)

Positive if ≤(a)	Sensitivity	1 - Specificity
2.2100	0.000	0.000
3.3950	0.143	0.000
3.5900	0.286	0.000
4.0500	0.429	0.000
4.5050	0.571	0.000
4.5550	0.714	0.000
4.9050	0.857	0.000
5.9100	1.000	0.000
7.0800	1.000	0.053
7.5550	1.000	0.158
7.5700	1.000	0.211
7.5950	1.000	0.263
7.6195	1.000	0.526
7.6395	1.000	0.579
8.0900	1.000	0.632
8.5800	1.000	0.737
8.8500	1.000	0.895
10.0700	1.000	1.000

Table 4. Test Result Variable(s): Catalase (Coordinates of the Curve)

Positive if Greater Than or Equal To(a)	Sensitivity	1 - Specificity
1.0600	0.000	0.000
2.2800	0.143	0.000
2.5050	0.286	0.000
2.5150	0.286	0.053
2.8650	0.429	0.211
3.5500	0.571	0.263
4.0100	0.571	0.316
4.2450	0.571	0.526
4.5900	0.571	0.684
4.9350	0.571	0.737
5.1650	0.857	0.842
5.5050	1.000	0.895
6.7300	1.000	1.000

was not significant for catalase.

ROC analysis was performed to find if carbonic anhydrase and catalase levels were determinants of differentiating groups with and without metastasis. The area under the curve was 100% and significant for carbonic anhydrase (p<0.01) (Figure 5). However, it was 59.8% and insignificant for catalase (Figure 6). Thus, with a cut-off value of 5.91 for carbonic anhydrase, the sensitivity

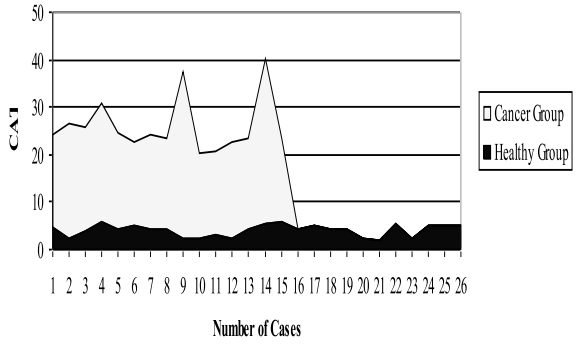

Figure 1. Erythrocyte CAT Levels in Patients with Lung Cancer and Healthy Indivduals

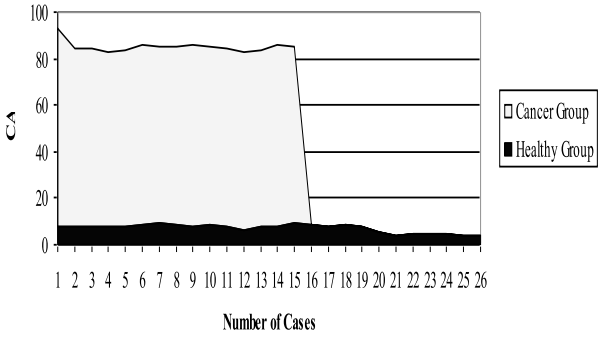

Figure 2. Erythrocyte CA Levels in Patients with Lung Cancer and healthy Indivduals

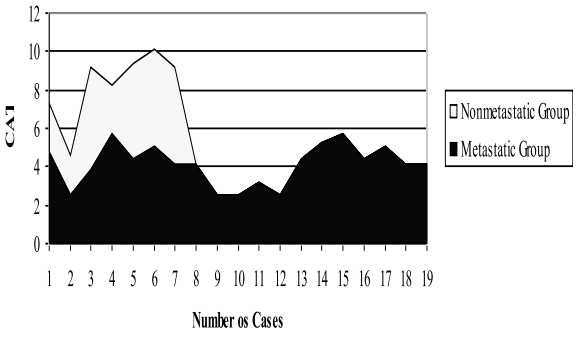

Figure 3. CAT Levels in Patients with Lung Cancer with and without metastasis

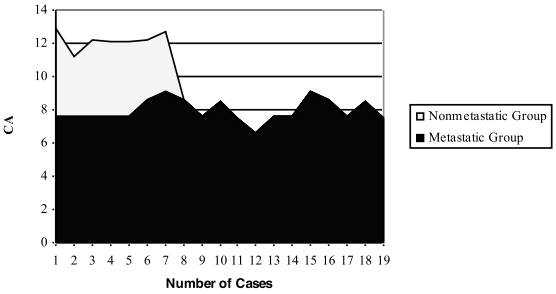

Figure 4. CA Levels in Patients with Lung Cancer with and without metastasis

and specificity were found as 100%. We can suggest that a value of 5.91 for carbonic anhydrase is effective in differentiating the presence and absence of metastasis in patients with lung cancer (Table 3). With a cut-off value of 3.55 for catalase, the sensitivity was 57.1% and the specificity was 73.7% (Table 4). However, the area under curve was not significant.

Discussion

Free oxygen radicals are found in the circulation under physiological conditions and are controlled by cellular redox systems and antioxidants. However, increased oxyradicals in the circulation and poor cellular redox homeostasis cause oxidative stress and oxidative stress causes tumorigenesis (Feeney and Berman, 1976; Goldstein and Witz, 1990; Cerutti, 1994; Halliwell, 1994). Free radicals are found to be involved in the initiation and promotion of multi-stage carcinogenesis. These highly reactive compounds can act as initiators and/or promoters, they cause lipid peroxidation and DNA damage, activate pro-carcinogens, and alter the cellular antioxidant defense system if free radicals are over-produced or antioxidant defense is inadequate (Sun, 1990).

The most important antioxidant enzyme systems against the toxic effects of free radicals are as follows: superoxidodismutase (SOD), catalase (CAT), glutathione-peroxidase (GP-x), glutathione-reductase (GR) and sulfhydryl compounds (Corrocher et al., 1986; Jaruga et al., 1994). Hydrogen peroxide produced directly or by dismutation of superoxide radicals is detoxified by converting into water by GPx and CAT enzymes. GPx, which is a selenoenzyme has essential function in the detoxification of hydrogen peroxide produced in the cell under normal conditions. It is accepted that CAT has an important efficiency in conditions of increased production of hydrogen peroxide.

Antioxidant enzymes are vital in defense to oxidative stress. The activities of antioxidant enzymes in various cancer tissues and cells have been measured and found to be low (Corrocher et al., 1986; Tang, 1991; Jaruga et al., 1994). Jaruga et al., (1994) observed higher levels of DNA lesions in cancerous tissues. The levels of antioxidant enzymes such as SOD and catalase were found to be lower in cancerous tissues. They reported that free radical reactions may be increased in malignant tumor cells. In a study, the results of bronchoalveolar lavage (BAL) in 11 patients with lung cancer were compared to those of 21 normal individuals, and the SOD and CA activities were found to be significantly lower in patients with lung cancer than that in patients without lung cancer (Tang, 1991). It was reported in another study that catalase activity was significantly reduced in malignant lung tumors (Korotkina et al., 2002). Similarly, the data in the literature are as follows: 1.42±0.24 U/mg protein (Mean±SEM) of superoxide dismutase in lung cancer and 3.13±0.51 U/mg protein in normal lung tissue; and 33.53±6.09 U/mg protein of catalase in lung cancer and 71.33±14.38 in normal lung tissue, and these differences were significant at the level of $p<0.01$ for both enzymes. The reduced activity of the catalase enzyme in lung cancer erythrocytes may lead to elevated levels of reactive oxygen metabolites, resulting in damage to key subcellular structures such as DNA, cell membranes and other vital cellular components (Guner et al., 1996).

We found that the erythrocyte catalase activity was significantly lower in all patients with lung cancer as a whole compared to controls (Table 1).

CA is a metalloenzyme consisting of zinc and providing a reversible hydration of C02. The essential function of this enzyme is to produce bicarbonate for metabolism and to maintain ion, water, and pH balance in the body (Maren, 1967). Numerous studies in the last decade have demonstrated fundamental roles of carbonic anhydrase (CA) in tumor progression and shown a negative correlation in CA levels in cancer cases (Venta, 1991; Gramlich et al., 1990; Yokoyama et al., 1997; Pastorekova et al., 2008).

The main paradigm of body acid-base regulation is based on keeping the extracellular pH (pHe) at 7.4 (Cardone et al., 2005). This pH depends on the concentrations of CO_2 and HCO_3-which are carbonic tampon compounds-as shown by the Henderson-Hasselbalch equation. This pH is stabilized normally by a well-regulated plasma CO_2 partial pressure and HCO_3 concentration, respectively, by the lungs and the kidneys.

The major sources of acid are aerobic and anaerobic cellular respiration producing CO_2 and lactic acid, respectively. If acids accumulate in the cell, the intracellular pH (pHi) may decrease dangerously. The effect of acidic pH on cell function, growth and division may cause the induction of a clastogenic effect or even apoptosis (Roos and Boron, 1981; De Brabander et al, 1982; Isfort et al., 1993).

Nobel prize winner Warburg demonstrated in 1931 that tumors have the capacity of producing much higher levels of lactic acid than normal tissue (even in the presence of O_2) (Warburg, 1930). The acidic natures of the tumors were confirmed by electrode studies (Wike-Hooley et al., 1984; Kallinowski et al., 1989) and it was suggested for long time that both pHe and pHi were lower in tumor cells. 31P nuclear magnetic resonance (NMR) technology used in live cells demonstrated in 1980 that tumor cells were in fact alkaline (Griffiths et al., 1981).

The controversial results by these two methods showed that it was mostly pHi measured by 31 P nuclear magnetic resonance technique, whereas it was mostly pHe measured by electrode method showing that tumor

cells had low pHe and high pHi (Gillies et al., 1994). In conclusion, it was found that most tumors had a pHi value of between 7.0 and 7.4, and a pHe value between 6.9 and 7.0, similar to non-tumor cells (Vaupel et al, 1990). In fact, some in vivo tumors may acidify the patient's venous blood by their low pHe levels (Koukourakis et al, 2006). Low pHe levels are convenient for tumor growth and progression (Gatenby and Gillies, 2004). It has been shown that extracellular acidity is effective by degradation of the extracellular matrix and the basement membrane in the invasion of melanoma cells and in the metastasis of sarcoma (Martınez-Zaguilán et al., 1996). Reaction-diffusion models have shown that low pHe values have a key role in tumor invasion (Gatenby and Gillies, 2004; Martınez-Zaguilán et al., 1996; Schlappack, 1991).

The CO_2 level is important in low pHe. High partial CO_2 pressures were measured in solid tumors until the early 1960s (Gullino et al., 1965). This CO_2 has three probable origins: a) Krebs cycle producing the CO_2 molecule for each carbon atom. Although this is probably seen in the tumor stroma, it can be ignored in many tumor cells due to its contribution to glycolysis. CO_2 is also obtained by the pentose phosphate shunt mostly during nucleotide synthesis, and probably the most common way in the appealing mechanism in tumors (Helmlinger et al., 2002). Another production method of CO_2 is the titration of metabolically produced acid by HC03 independent from glycolytic or Krebs cycle enzymes. HC03 is the conjugated base of carbonic acid. CO_2/HC03 may have the function of an open tampon, particularly in high pHi conditions.

As discussed previously, cellular CO_2 may probably partially comprise some of the reasons for extracellular acidosis. Extracellular acid-trapping activity is dependent on the capacity of hydration of CO_2 to HC03 and H+ outside the cell (Svastova et al., 2004). In the deficiency of carbonic anhydrase, this tampon system will not work properly and the amount of acid in the acidic media will increase.

In this study, out of 26 patients with lung cancer, seven (26.9%) had metastasis. The difference in the mean CA levels in the two groups was significant when the CA levels of patients with and without metastasis were compared (Figure 4) ($p<0.01$). The value of 5.91 for CA was accepted as the cut-off value to differentiate the cases with and without metastasis in patients with lung cancer (Table 3).

In conclusion, our results suggested that oxidative load was increased in patients with lung cancer; however, enzymatic free radical defense mechanisms were damaged. These results suggest that reactive oxygen metabolites may produce harm to specific genes which control cellular growth and differentiation, and may induce rapid growth and malignancy. Besides, genes of the antioxidant enzymes may be anti-oncogens and inactivation of one of these genes in the process of carcinogenesis may lead to tumor development. The lower levels of antioxidant CAT in patients with lung cancer compared to healthy individuals may be explained by this. In addition, the decrease in CA levels in patients with lung cancer may provide a convenient media for tumor development, growth, and metastasis by creating an acidic media.

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