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Oxidative DNA base damage and antioxidant enzyme levels in childhood acute lymphoblastic leukemia

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Abstract We have investigated the levels of several antioxidant enzymes and the level of oxidative DNA base damage in lymphocytes of children with acute lymphoblastic leukemia (ALL) and in disease-free children. Children with ALL had just been diagnosed with the disease and had received no therapy prior to obtaining blood samples. A multitude of typical hydroxyl radical-induced base lesions in lymphocyte DNA of children were identified and quantified by gas chromatography-isotope dilution mass spectrometry. Higher levels of DNA base lesions were observed in patients with ALL than in children without the disease. The levels of the antioxidant enzymes glutathione peroxidase, catalase and superoxide dismutase in lymphocytes of ALL patients were lower than in lymphocytes of controls. These findings are in agreement with earlier observations in various types of adulthood cancer. Some of the identified DNA base lesions are known to possess premutagenic properties and may play a role in carcinogenesis. The results may indicate a possible link between decreased activities of antioxidant enzymes and increased levels of DNA base lesions due to oxidative damage, and support the notion that free radical reactions may be increased in malignant cells.

Key words: Catalase; Hydroxyl radical; Modified base; Oxidative DNA damage; Oxygen-derived species

1. Introduction

Oxygen-derived free radicals are formed in aerobic organisms by normal cellular metabolism or by endogenous sources (reviewed in [1]). These species can cause damage to biomolecules including DNA, and thus be mutagenic and carcinogenic. Of free radicals, the hydroxyl radical (•OH) is highly reactive and generates a multitude of modifications in DNA (reviewed in [2,3]). This type of damage, also called oxidative DNA damage, is implicated in mutagenesis, carcinogenesis and aging [1]. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) protect cells from the toxic effects of oxygen-derived species [1]. Of these species, superoxide radical (O_2^-) and H_2O_2 do not cause any DNA damage under physiological conditions [4–6]. The toxicity of O_2^- and H_2O_2 results from their metal-ion catalyzed reactions leading to •OH [1].

There is evidence that cancer cells produce larger amounts of oxygen-derived species than non-neoplastic cells and that the antioxidant system of cancer cells is suppressed [7]. Several carcinoma cell lines have been found to produce large amounts of H_2O_2 [8]. Low antioxidant enzyme activities are a common characteristic of cancer cells (reviewed in [9,10]). These facts indicate that there is a persistent oxidative stress in cancer [7] and cancer cells are more exposed to oxygen-derived species than their normal counterparts. Recent findings clearly showed elevated levels of typical •OH-modified DNA bases in various cancerous tissues than in their surrounding normal tissues [11–15]. Furthermore, there is evidence of an association between increased levels of modified DNA bases and decreased levels of antioxidant enzymes in human cancerous tissues [13]. Although not cancerous, human benign prostatic hyperplasia tissues have been shown to have higher oxidative DNA damage and lower antioxidant enzyme activities than surrounding normal prostate tissues [16].

Levels of oxidative DNA damage and antioxidant enzymes have not been investigated in any childhood cancer. In this work, we studied the endogenous levels of typical •OH-modified DNA bases and activities of SOD, CAT and GPx in blood lymphocytes of children with acute lymphoblastic leukemia (ALL). ALL is a malignant, clonal disorder of bone marrow lymphopoietic precursor cells and the onset of the disease is acute or subacute in previously healthy children, but rare in adults [17]. Nine children with ALL and 10 disease-free children between the ages of 3 and 12 were involved in this study. We wished to see whether this type of childhood cancer possesses characteristics similar to other types of cancers previously investigated, in terms of levels of oxidative DNA damage and antioxidant enzymes.

2. Materials and methods

2.1. Materials

Certain commercial equipment or materials are identified in this paper in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it

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Abbreviations: ALL, acute lymphoblastic leukemia; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; 5-OH-Cyt, 5-hydroxycytosine; 5-OH-Ura, 5-hydroxyuracil; 5,6-diOH-Ura, 5,6-dihydroxyuracil; 5-OH-5-MeHyd, 5-hydroxy-5-methylhydantoin; 5-OH-Hyd, 5-hydroxyhydantoin; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 8-OH-Gua, 8-hydroxyguanine; FapyAde, 4,6-diamino-5-formamidopyrimidine; 8-OH-Ade, 8-hydroxyadenine

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Table 1
Activities (U/mg of protein[a]) of antioxidant enzymes in lymphocytes of children with ALL and in lymphocytes of disease-free children

Enzyme	Control	ALL
CAT	5.06 ± 1.96	2.36 ± 0.63[b]
GPx	137.2 ± 32.4	76.2 ± 20.6[b]
SOD	86.1 ± 26.0	41.1 ± 10.4[b]

[a]Values represent the mean ± standard deviation ($n = 10$ for controls, $n = 9$ for ALL patients).
[b]Significantly different from control ($P < 0.05$ by Student's t-test).

imply that the materials or equipment identified are necessarily the best available for the purpose.

Modified DNA bases, their stable isotope-labeled analogues and other materials for gas chromatography/mass spectrometry were obtained as described [18]. Labeled 2′-deoxyguanosine (2′-deoxyguanosine-$^{15}N_5$) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA).

2.2. Blood samples, measurement of enzyme activities and isolation of DNA

Venous blood samples (15 ml) were drawn from nine children with ALL and 10 disease-free children between the ages of 3 and 12 at the Department of Pediatrics, Faculty of Medicine, University of Istanbul, Turkey. In all cases, the consent of the families for obtaining blood samples was received. Children with ALL had just been diagnosed with the disease and had not received any therapy prior to obtaining blood samples. 10 ml of blood was collected from each subject in tubes containing heparin and lymphocytes were isolated [19]. Lymphocytes were sonicated for 20 s and then centrifuged at 15000 × g for 20 min. The supernatant fractions were used to determine the activities of CAT, and GPx and the total activity of SOD (Cu,Zn-SOD and Mn-SOD) according to established methods [20–22]. Briefly, the activity of CAT was determined by measurement of H_2O_2 concentration using UV absorbance at 240 nm. The conversion of NADPH to NADP was utilized using measurement of UV absorbance at 340 nm for determination of the activity of GPx. The activity of the total SOD was determined using the inhibition by SOD of the reaction of O_2^- with nitroblue tetrazolium. Protein content was determined using a Lowry protein kit. For DNA isolation, 5 ml of blood was collected in EDTA-coated tubes. Lymphocytes were separated as described above. Subsequently, DNA was isolated from lymphocytes [23].

2.3. Analysis by gas chromatography/mass spectrometry

The concentration of DNA was estimated by the measurement of absorbance at 260 nm (absorbance of 1 = 50 μg of DNA/ml). Aliquots of stable isotope-labeled analogues of modified DNA bases were added as internal standards to 50 μg of DNA. An aliquot of 2′-deoxyguanosine-$^{15}N_5$ was also added for quantification of guanine to assess by mass spectrometry the DNA amount in each sample. Upon hydrolysis, 2′-deoxyguanosine-$^{15}N_5$ yields guanine-$^{15}N_5$, which is used as an internal standard for guanine in DNA [24]. Samples were dried under vacuum in a SpeedVac and then hydrolyzed with 0.5 ml of 60% formic acid in evacuated and sealed tubes at 140°C for 30 min. The hydrolyzates were lyophilized in vials for 18 h. Lyophilized hydrolyzates were derivatized and then analyzed by gas chromatography/isotope-dilution mass spectrometry with selected-ion monitoring as described [25].

3. Results

We investigated endogenous levels of antioxidant enzymes and oxidative DNA base damage in lymphocytes of children with ALL and disease-free children. Children with ALL were just diagnosed with the disease and had undergone no chemotherapy prior to this study. The objective of this study was to see whether this childhood cancer possesses characteristics similar to adult cancers previously investigated concerning antioxidant enzymes and potentially mutagenic DNA lesions.

The activities of SOD, CAT and GPx in lymphocytes of children with ALL and disease-free children are given in Table

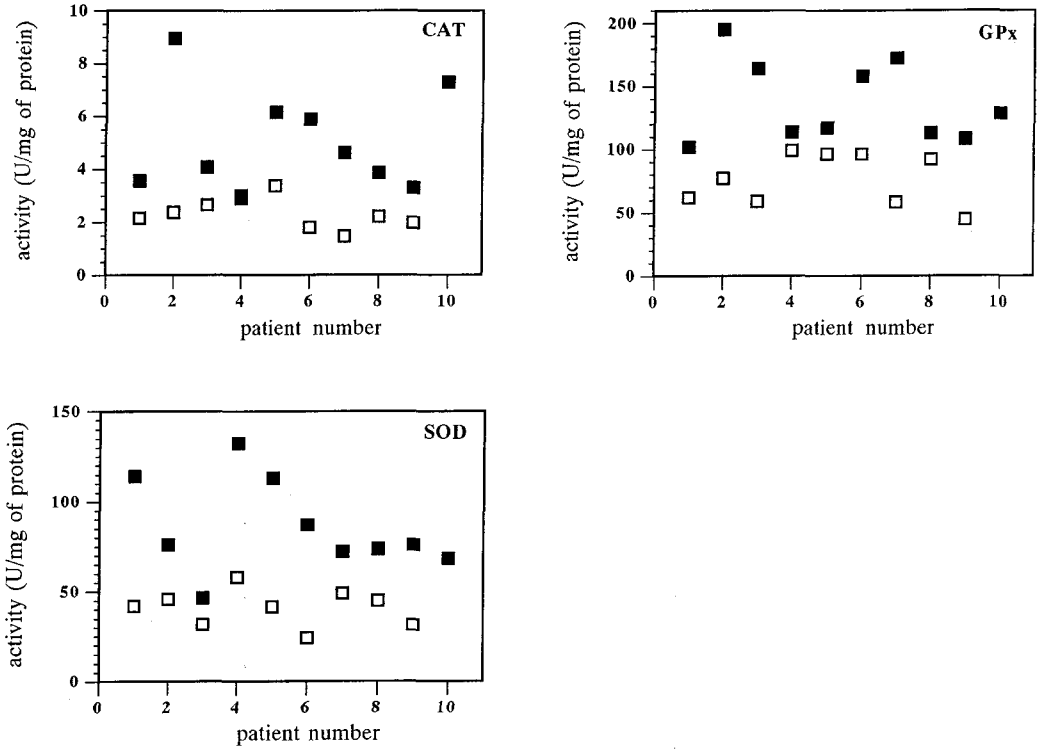

Fig. 1. Levels of activities of CAT, GPx and SOD in lymphocytes of individual ALL patients and disease-free children. Each data point corresponds to one individual. The same individual has the same number in each plot and the numbers were given arbitrarily. ■, controls; □, patients.

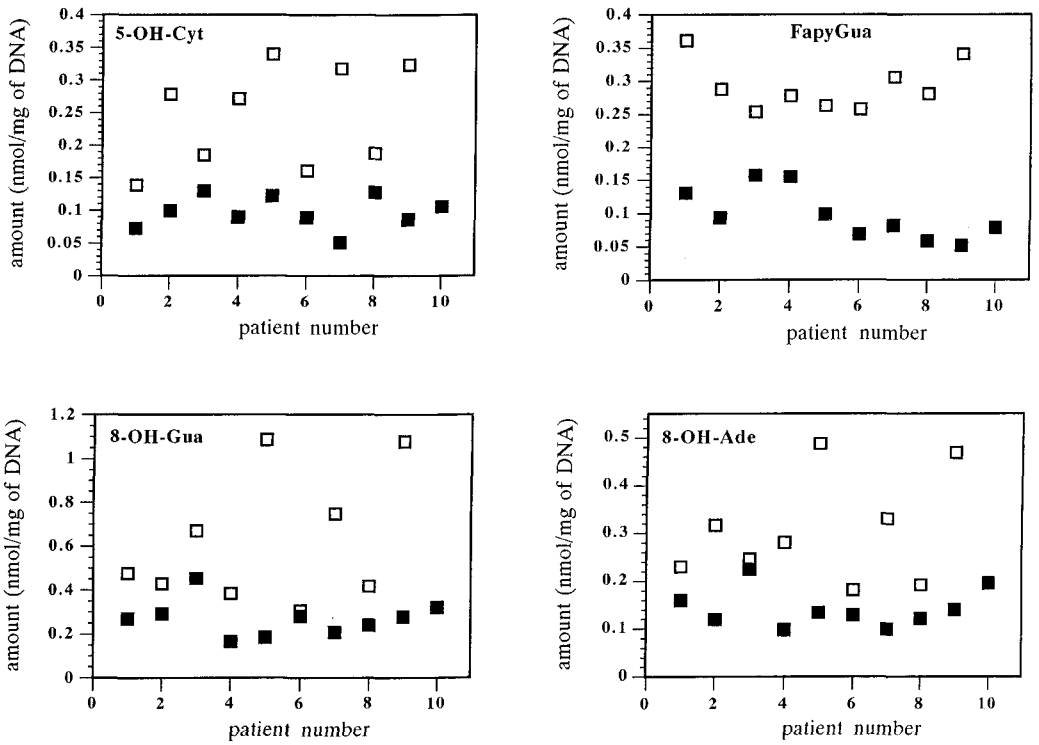

Fig. 2. Levels of four modified DNA bases in lymphocytes of individual ALL patients and disease-free children. Each data point corresponds to one individual. The same individual has the same number in each plot and the numbers were given arbitrarily. ■, controls; □, patients. 1 nmol/mg of DNA corresponds to approximately 32 lesions/10^5 DNA bases.

1. The levels of all three enzymes in ALL patients were found to be significantly decreased compared to the levels in disease-free children. Fig. 1 illustrates the enzyme levels in lymphocytes of individual patients and controls. These plots clearly show the differences between patients and controls and, as expected, the individual variations among patients and among normal children. It should be pointed out that the identification numbers of individuals were arbitrarily chosen, and each individual has the same number on each plot.

Nine modified DNA bases were identified and quantified in lymphocyte DNA samples of children with ALL and disease-free children. These were 5-hydroxycytosine (5-OH-Cyt), 5-hydroxyuracil (5-OH-Ura), 5,6-dihydroxyuracil (5,6-diOH-Ura), 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd), 5-hydroxyhydantoin (5-OH-Hyd), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), 8-hydroxyguanine (8-OH-Gua), 4,6-diamino-5-formamidopyrimidine (FapyAde) and 8-hydroxyadenine (8-OH-Ade). The structures of these modified bases can be found elsewhere [18]. The levels of nine modified DNA bases are given in Table 2. The mean values of the levels of seven of the modified bases in DNA samples from ALL patients were significantly higher than those from disease-free children. In the case of FapyGua, 5-OH-5-MeHyd and 5-OH-Hyd, the increases were more than 3-fold (Table 2). No significant differences for the mean values of 5-OH-Ura and 5,6-diOH-Ura between the patients and controls were observed. As examples, Fig. 2 illustrates the levels of four modified DNA bases in lymphocytes of individual patients and controls. These plots clearly demonstrate the differences between the patients and disease-free children. Substantial individual variations among the patients were noted whereas the levels of modified bases in controls were somewhat similar to one another.

Table 2
Levels of modified DNA bases (nmol/mg of DNA[a]) in lymphocytes of children with ALL and in lymphocytes of disease-free children

Modified base	Control	ALL
FapyGua	0.094 ± 0.032	0.292 ± 0.035[b]
8-OH-Gua	0.269 ± 0.077	0.621 ± 0.278[b]
FapyAde	0.043 ± 0.014	0.085 ± 0.012[b]
8-OH-Ade	0.142 ± 0.039	0.304 ± 0.105[b]
5-OH-Cyt	0.097 ± 0.024	0.245 ± 0.073[b]
5-OH-Ura	0.053 ± 0.030	0.064 ± 0.033
5-OH-5-MeHyd	0.089 ± 0.043	0.286 ± 0.065[b]
5-OH-Hyd	0.084 ± 0.03	0.269 ± 0.057[b]
5,6-diOH-Ura	0.011 ± 0.006	0.013 ± 0.003

[a]Values represent the mean ± standard deviation ($n = 10$ for controls, $n = 9$ for ALL patients). 1 nmol/mg of DNA corresponds to approximately 32 lesions/10^5 DNA bases.
[b]Significantly different from control ($P < 0.05$ by Student's t-test).

4. Discussion

The results of the present work show lower activities of three antioxidant enzymes in lymphocytes and higher endogenous levels of typical •OH-induced products of DNA bases in lymphocyte DNA of children with ALL when compared to children without the disease. The findings suggest a possible link between increased levels of oxidative DNA damage and decreased levels of antioxidant enzymes in ALL.

The levels of GPx, CAT and SOD were consistently lower in ALL patients than in control children. A statistically significant 2-fold difference between the patient group and the

control group was observed for all three enzymes. There were also differences of up to 2–3-fold between individuals in each group, reflecting possible individual variations in enzyme activities. Nine modified DNA bases were quantified in DNA of lymphocytes. The levels of seven of these lesions in ALL patients were significantly higher than those found in controls.

The results of this work are consistent with earlier observations of higher levels of typical $^{\bullet}OH$-induced DNA lesions in various human cancerous tissues than in their cancer-free surrounding tissues [11–14]. The difference between those studies and the present study is that tissues from the same individual, i.e. cancerous tissue versus cancer-free surrounding tissue, were compared in earlier studies, whereas, in this work, lymphocyte DNA samples were compared between cancer patients and disease-free children. The findings on antioxidant enzymes in this work are also in agreement with an earlier report that antioxidant enzyme activities in human lung cancerous tissues were higher that those in their surrounding cancer-free tissues [13]. Recently, similar findings were reported on DNA base damage and activities of antioxidant enzymes in human benign prostatic hyperplasia tissues and surrounding disease-free tissues [16].

In general, activities of antioxidant enzymes in tumor cells have been found to be lower than in normal cells [10]. In fact, in tumor cells, Mn-SOD activity is always low, CAT activity is almost always low and Cu,Zn-SOD activity is usually low. On the other hand, levels of GPx were found to be highly variable in some tumor cell lines and higher in others [10]. The results of this work are consistent with the general trends observed in previous studies. Decreased levels of GPx, SOD and CAT may cause the accumulation of O_2^- and H_2O_2 in tumor cells [10]. Moreover, human cancer cells have been shown to produce excess amounts of H_2O_2 [8]. Since H_2O_2 itself is not reactive, it may pass through cellular membranes and reach any cellular compartment including the nucleus and DNA [1]. This may result in greater DNA damage in tumor cells than in normal cells by production of $^{\bullet}OH$ from reactions of H_2O_2 with DNA-bound metal ions [1]. In fact, the treatment of human and other mammalian cells with H_2O_2 or with activated leukocytes has been shown to induce modification of all four bases in cellular DNA with a pattern similar to that observed in the present work [26–28]. Higher levels of oxidative DNA base damage as observed in ALL patients and earlier in other cancers support the hypothesis that free radical reactions may be increased in malignant cells concurrent with decreased levels of antioxidant enzymes [7,9].

It is not known whether an increase in the endogenous level of oxidative DNA damage concurrent with a decrease in the levels of antioxidant enzymes plays a causative role in carcinogenesis, or is merely a result of the disease. Some of the DNA lesions that were found in ALL patients in increased amounts have been shown to possess premutagenic properties. 8-OH-Gua, which is the most investigated lesion, has been found to cause GC→TA transversions [29–32]. The other prominent purine lesion, 8-OH-Ade, possesses premutagenic properties in mammalian cells [33]. 5-OH-Cyt appears to be more mutagenic than any other DNA lesion thus far investigated [34,35]. On the other hand, the mutagenic effects of the remaining DNA base lesions, which were found in ALL patients in increased levels, are not known [36]. These lesions may also contribute to the mutagenic effects of oxidative DNA base damage. The findings on the mutagenic effects of oxidative DNA lesions may indicate an important role for the lesions in carcinogenesis. Increased levels of oxidative DNA lesions may also contribute to the genetic instability and metastatic potential of cancerous cells in ALL and other cancers [15].

In conclusion, the present work provides evidence for increased levels of oxidative DNA base damage and decreased levels of antioxidant enzymes in lymphocytes of ALL patients, suggesting a possible link between these two important parameters in this cancer. The results of this work are consistent with previous observations on other human cancerous tissues. Higher levels of DNA base lesions may indicate an increase in free radical reactions in ALL cells and may contribute to their genetic instability and metastatic potential.

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