

Minireview

Oxidative DNA base modifications as factors in carcinogenesis[★]

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Reactive oxygen species can cause extensive DNA modifications including modified bases. Some of the DNA base damage has been found to possess premutagenic properties. Therefore, if not repaired, it can contribute to carcinogenesis. We have found elevated amounts of modified bases in cancerous and precancerous tissues as compared with normal tissues.

Most of the agents used in anticancer therapy are paradoxically responsible for induction of secondary malignancies and some of them may generate free radicals. The results of our experiments provide evidence that exposure of cancer patients to therapeutic doses of ionizing radiation and anticancer drugs causes base modifications in genomic DNA of lymphocytes. Some of these base damages could lead to mutagenesis in critical genes and ultimately to secondary cancers such as leukemias. This may point to an important role of oxidative base damage in cancer initiation. Alternatively, the increased level of the modified base products may contribute to genetic instability and metastatic potential of tumor cells.

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Abbreviations: BPH, benign prostatic hyperplasia; 5,6-diOH-Ura, 5,6-dihydroxyuracil; FapyAde, 4,6-diamino-5-formamido-pyrimidine; [•]OH, hydroxyl radical; 2-OH-Ade, 2-hydroxyadenosine; 2-OH-dATP, 2-hydroxydeoxyadenosine triphosphate; 5-OH-Cyt, 5-hydroxycytosine; 5-OH-5-MeHyd, 5-hydroxy-5-methyl hydantoin; 8-OH-Ade, 8-hydroxyadenosine; 8-oxoGua, 8-oxoguanine; ROS, reactive oxygen species; SOD, superoxide dismutase.

Reactive oxygen species (ROS) are the products of partial reduction of oxygen. These species which include superoxide anion, hydrogen peroxide and hydroxyl radical, are continuously being produced in living cells as by-products of normal metabolism [1, 2]. During mitochondrial respiration 1–5% of the oxygen undergoes single electron transfer generating the superoxide anion radical in amounts corresponding to 2 kg per year per one human being [2]. Reactive oxygen species have been postulated to play a significant role in etiology of at least 50 diseases including rheumatoid arthritis, cancer, atherosclerosis, myocardial infarction, Parkinson's disease and AIDS [3, 4]. Reperfusion of ischemic tissues and chronic inflammation also lead to generation of ROS [3, 4]. Furthermore, UV and ionizing radiation, as well as a wide variety of drugs and xenobiotics can stimulate the formation of ROS [5–9]. A variety of carcinogens, including benzene, aflatoxin and benzopyrene may generate ROS during their metabolism [5–9]. The superoxide radical is degraded by superoxide dismutase (SOD), and hydrogen peroxide by catalase. However, the reaction of hydrogen peroxide with transition metal ions leads to a highly reactive hydroxyl radical ($\cdot\text{OH}$). Interaction of this radical with cellular components may result in damage to biomolecules including DNA [1].

A considerable frequency and intensity of oxidative DNA damages in the cells of normal untreated animals or human beings is now an accepted fact. Ames & Gold [10], for example, estimated that the genome of adult rat liver cells contains about 10^6 such oxidative DNA lesions/cell and that 10^5 new or replacement lesions are added daily to this tremendous number.

Free radicals can produce different kinds of DNA lesions, among them free radical modified DNA bases [11–13]. Base damage appears to be an important class of lesions since some of them may possess mutagenic properties and may lead to carcinogenesis [14, 15].

OXIDATIVE DNA BASE DAMAGE

Hydroxyl radical attack on DNA leads to a large number of pyrimidine- and purine-derived base damage products [11–13] (Fig. 1). Some of these modified DNA bases have a considerable potential to damage the genome.

Purines can undergo oxidation of the ring atom to form different products. One of these, 8-oxoguanine (8-oxoGua) has become the species of common interest and a specific marker of oxidative DNA base damage [15]. The reaction of $\cdot\text{OH}$ radical with adenine results in formation of 8-hydroxy- and 2-hydroxyadenine [13].

Fragmented purines with ruptured imidazole ring connected to intact pyrimidine ring are also common products of $\cdot\text{OH}$ radical attack on DNA. These 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 4,6-diamino-5-formamidopyrimidine residues, usually abbreviated as Fapy, are derived from both guanine and adenine, respectively [11–13, 16, 17].

Pyrimidines are also attacked by $\cdot\text{OH}$ radical to give a variety of products [12]. Thus, thymine glycol can exist as any of several stereoisomers but the *cis* form predominates in DNA exposed to different oxidants. Cytosine can form several products including cytosine glycol, 5,6-dihydroxycytosine and 5-hydroxycytosine [11–13, 16, 17].

Examples of oxidative DNA base damage are illustrated in Fig. 1.

MUTAGENIC AND CARCINOGENIC PROPERTIES OF OXIDIZED DNA BASES

8-Oxoguanine is one of the most critical lesions. The presence of 8-oxoGua residues in DNA leads to a GC→TA transversion unless repaired prior to DNA replication [18]. Therefore, the presence of 8-oxoGua may lead to mutations. Furthermore, many observations

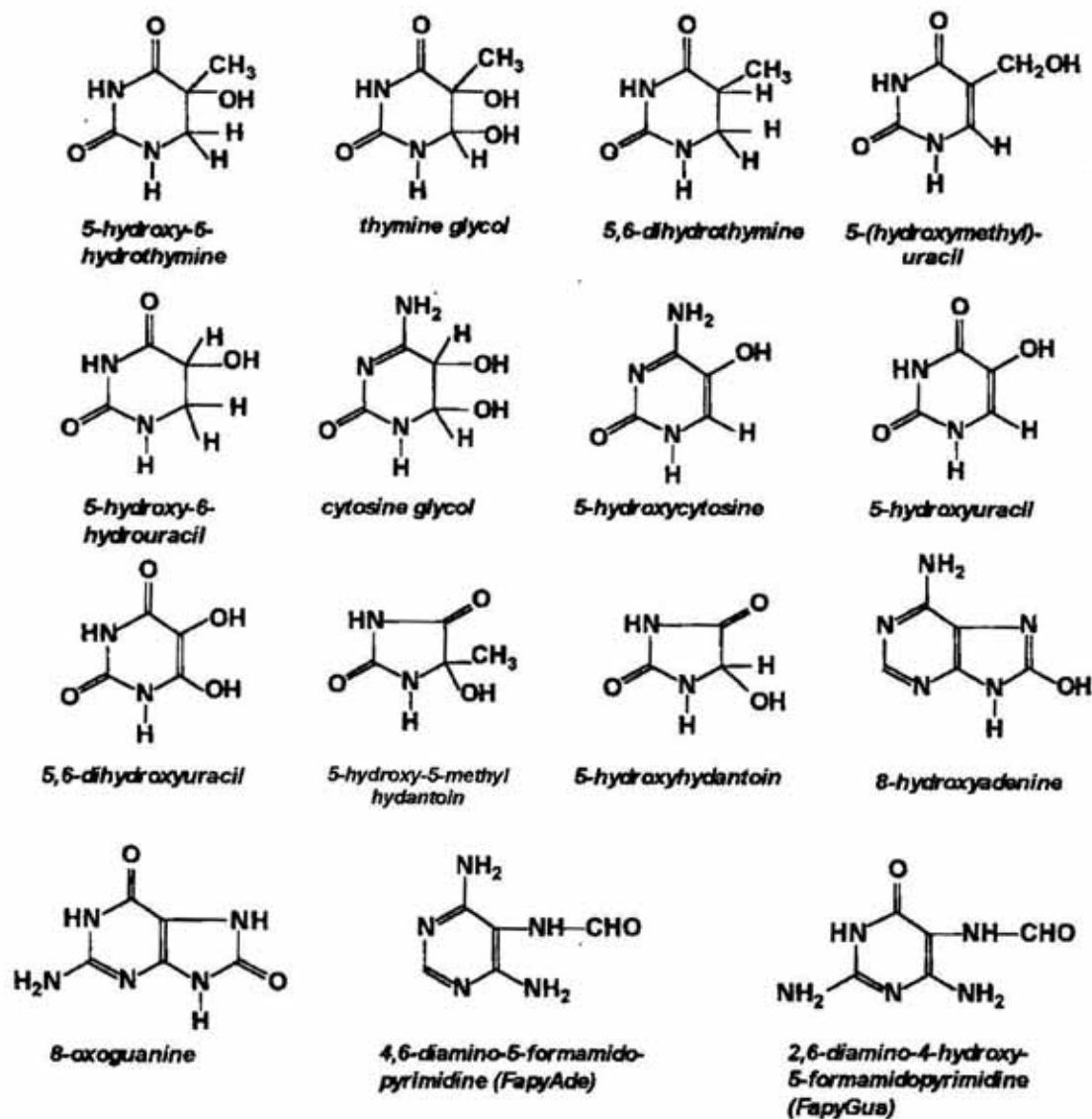


Figure 1. Structures of the modified bases.

point to a direct correlation between 8-oxoGua formation and carcinogenesis *in vivo* [14, 15]. Thus, it has been found that oxyradicals induced mutagenesis of hotspot codons of the human p53, and Ha-Ras genes [19, 20]. In agreement with this finding it was demonstrated that GC→TA transversions frequently occur in p53 gene in the case of lung carcinomas and primary liver cancer, and in *ras* protooncogene [18–20]. In this context it is noteworthy that we have demonstrated elevated levels of typical free radical-induced DNA base

modifications, including 8-oxoguanine, in human cancerous lung tissues when compared with cancer free surrounding tissues [21, 22].

Mutagenic and carcinogenic potential of any modified DNA base is reflected in its miscoding properties. Recently, it has been demonstrated that several other bases have a miscoding potential. Thus, the presence of 2-OH-Ade in DNA may induce A→C and A→T transversion [23]. It was also shown that 2-OH-dATP is a substrate for, and may be incorrectly incorporated by, DNA polymerase [24].

8-OH-Ade also has miscoding properties and induces mutations in mammalian cells [25]. 5-OH-Cyt leads to GC→AT transition and GC→CG transversion and appears to be more mutagenic than any other product of oxidative DNA damage [26, 27]. Possibly 8-oxoGua derivatives of guanine have miscoding properties [28]. On the other hand, biological consequences of other base modifications (like FapyAde, 5,6-diOH-Ura and 5-OH-5-MeHyd) have not been investigated. It is conceivable that also these lesions, could be premutagenic.

CHEMICAL DETERMINATION OF DNA BASE DAMAGE INDUCED BY REACTIVE OXYGEN SPECIES

Understanding of the biological consequences of ROS induced DNA damage depends on the chemical characterization and quantification of these lesions. There are two major techniques commonly applied to analyze them: gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC) with electrochemical detection (EC).

GC-MS is the only technique which permits simultaneous identification and quantification of various derivatives of all four bases in DNA [16, 17]. Using this technique quantification of samples is achieved by adding an appropriate internal standard to the DNA sample. Recently, custom-synthesized, stable isotope-labeled analogues of a number of the modified bases were obtained, which permitted to apply a very accurate procedure, called isotope-dilution mass spectrometry, for quantitative determination of oxidative base damage in DNA [17]. This technique was used to study the oxidative damage of DNA isolated from cancerous and precancerous human tissues [21, 22, 29].

EC detection coupled with HPLC separation is a sensitive and most commonly applied method for the measurement of 8-oxo-guanine

released from DNA by enzymatic digestion. This technique has been used to measure oxidative DNA damage in intact cells and whole organisms. A detailed comparison of these two techniques has been presented [11].

An alternative approach to measuring oxidative DNA base damage is based on the use of repair endonucleases specific for certain kinds of lesion. The enzymes create breaks at the modified sites in DNA. Measurement of these breaks by appropriate methods (most frequently by comet assay) give indirect insight into the scale of the base damage [30].

EXPERIMENTAL EVIDENCE SUGGESTING INVOLVEMENT OF OXIDIZED BASES IN CARCINOGENESIS

The role of free radical modified DNA bases in development of cancer in humans is supported by the abundant presence of oxidative base modifications in cancer tissue [21, 22, 31].

We have investigated endogenous levels of typical free radical induced DNA base modifications in chromatin of various human cancerous tissues and their cancer free surrounding tissues. In all cases the levels of modified DNA bases in cancerous tissues were found to be elevated over control levels [21, 22]. The levels of modified bases depended on the tissue type (Fig. 2).

Lung cancer tissues removed from smokers showed the highest increases of modified bases over the control levels. Stomach, ovary, brain and colon cancerous tissues also had significantly higher number of lesions in their chromatin than their respective cancer free surrounding tissues (Fig. 2).

There may be several reasons which could explain the increased level of modified bases in cancerous tissues:

- ◆ 1. In the case of lung cancer patients who were smokers the components of cigarette smoke could be involved in the production

of DNA damage. In fact, it has been estimated that one puff of cigarette smoke contains some 10^{17} oxidant particles, among them free radicals [32].

- ◆ 2. Elevated levels of modified DNA bases in cancerous tissues could be due in part to the presence of large numbers of leukocytes in human tumors [33]. Activated leukocytes are a source of H_2O_2 , which can cross cellular and nuclear membranes and reach the nucleus to cause site specific DNA damage by producing $\cdot OH$ radical in the reaction with DNA bound metal ions. Direct proof for this suggestion came from the work of Dizdaroglu *et al.* [34]. They demonstrated that exposure to activated leukocytes in human cells caused DNA base modifications

typical of those induced by hydroxyl radical attack.

- ◆ 3. Moreover, elevated levels of modified bases in cancerous tissues could be due to the production of large amounts of hydrogen peroxide, which has been found to be characteristic of human tumor cells [35].
- ◆ 4. Furthermore, evidence exists that tumor cells have abnormal levels and activities of antioxidant enzymes when compared with their respective normal cells [36]. Low levels of antioxidant enzymes, such as superoxide dismutase or catalase in tumor cells may cause accumulation of superoxide anion and H_2O_2 with subsequent $\cdot OH$ induced damage to DNA, resulting in higher levels of

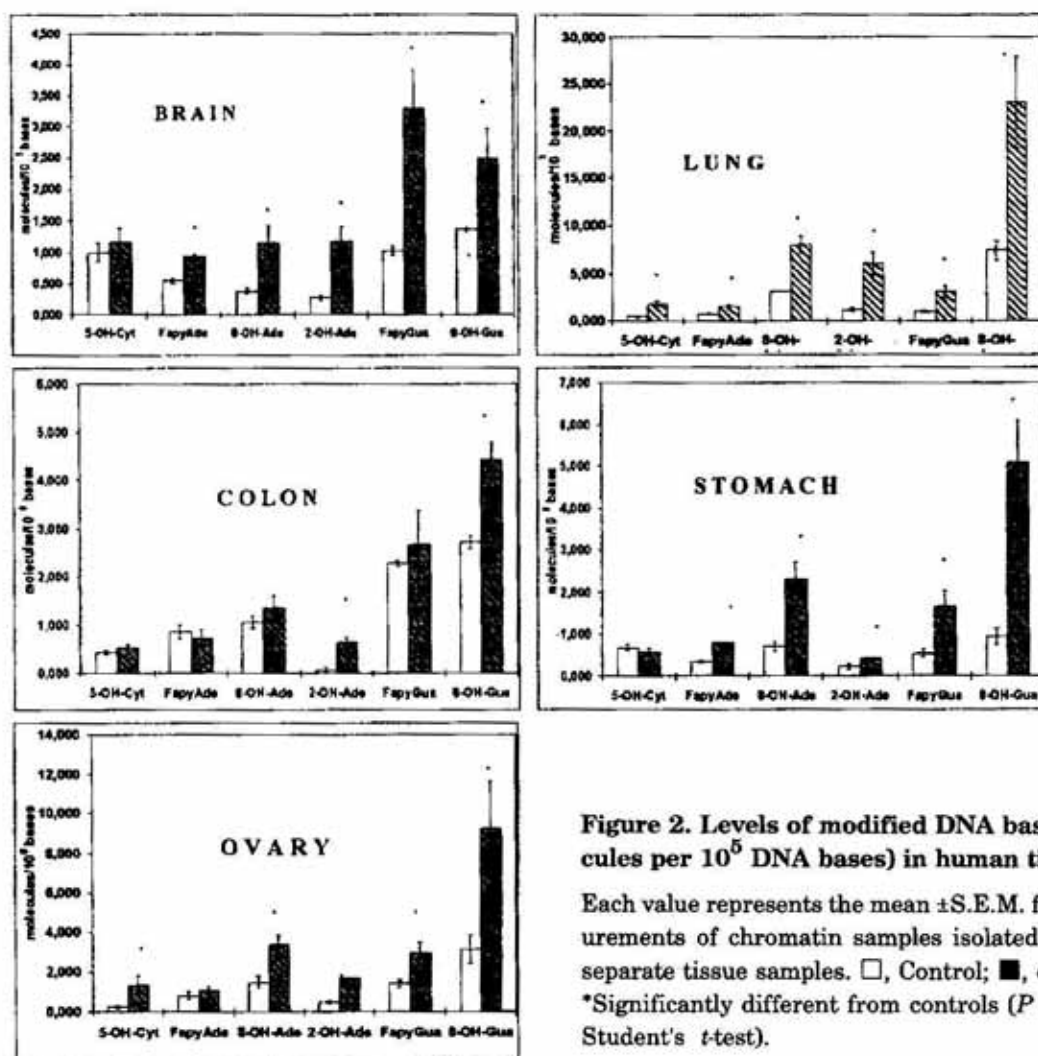


Figure 2. Levels of modified DNA bases (molecules per 10^5 DNA bases) in human tissues.

Each value represents the mean \pm S.E.M. from measurements of chromatin samples isolated from five separate tissue samples. □, Control; ■, cancerous. *Significantly different from controls ($P \leq 0.05$ by Student's *t*-test).

modified bases in tumor cells than in normal cells.

To check whether a relationship exists between oxidative DNA base modifications and antioxidant enzyme activities in cancerous tissues and in cancer free tissues we have studied the base modifications and activities of superoxide dismutase, catalase and glutathione peroxidase in normal and cancerous human lung tissues removed from five lung cancer patients. In agreement with the previous study, higher levels of DNA lesions were observed in cancerous tissues than in cancer-free surrounding tissues (Fig. 3). Most significant changes were observed in the case of 8-OH-Ade and 8-oxoGua in all five patients. Antioxidant enzyme levels were lower in cancerous tissues as compared with their surrounding cancer free tissues (Fig. 3).

The results point, for the first time, to an association between decreased activities of antioxidant enzymes and increased levels of DNA lesions in cancerous tissues [22].

It is not known whether lower levels of antioxidant enzymes in cancerous lung tissues play a causative role in carcinogenesis or are merely a result of the disease. The same is true for the pyrimidine- and purine-derived DNA lesions that were identified in cancerous tissues at higher levels than in control tissues. However, treatment of laboratory animals with carcinogenic agents which are responsible for free radical generation, causes a similar pattern of oxidative base modifications in their target organs before tumor formation occurs [37]. Nickel compounds are good examples of such agents. When injected to rats they initiated specifically renal tumors. It was found that Ni(II) treatment resulted in oxidative base damage in the rat kidneys and livers one day after the injection. However, unlike in the liver, the increased level of damage in kidneys persisted for at least 14 days. These persistent high levels of potential mutagenic lesions in rat kidney may, at least partially, explain tissue specific carcinogenic activity of nickel compounds. All these data may point to

an important role of the oxidative base damage in carcinogenesis.

On the other hand, in fully developed cancer, increased levels of modified DNA bases may contribute to the genetic instability and metastatic potential of tumor cells. Our suggestion has recently been proved. Malins *et al.* [38] showed that metastatic potential of metastasis of breast cancer tissues increased together with the increase of oxidative DNA damage.

In the next step of the study we wished to determine whether the properties of precancerous tissues, were similar to those of cancerous tissues in terms of levels of DNA base modifications and activities of antioxidant enzymes. Recent data on nuclear matrix protein patterns in both benign prostatic hyperplasia (BPH) and prostate cancer have indicated that similar phenotypic events occurs in progression of cells from normal to BPH as from normal to cancer [39]. Thus, it is possible that BPH represents a premalignant condition which may predisposed to prostate cancer. Therefore, we decided to use in our study prostate glands surgically removed from 15 BPH patients. In 1/3 of the cases changes similar to those demonstrated for lung cancer patients have been found, i.e. a drop in the activity of antioxidant enzymes and increases in the level of oxidatively modified bases in pathological tissues when compared to the control one [29] (Fig. 4.). It is likely that the patients with both decreased activity of antioxidant enzymes and increased levels of the modified bases are at a greater risk of developing prostate cancer.

ANTICANCER THERAPY AS A SOURCE OF OXIDATIVELY MODIFIED DNA BASES. POSSIBLE INVOLVEMENT OF THE BASE DAMAGES IN DEVELOPMENT OF SECONDARY CANCERS

Most of the agents used in anticancer therapy are paradoxically responsible for induc-

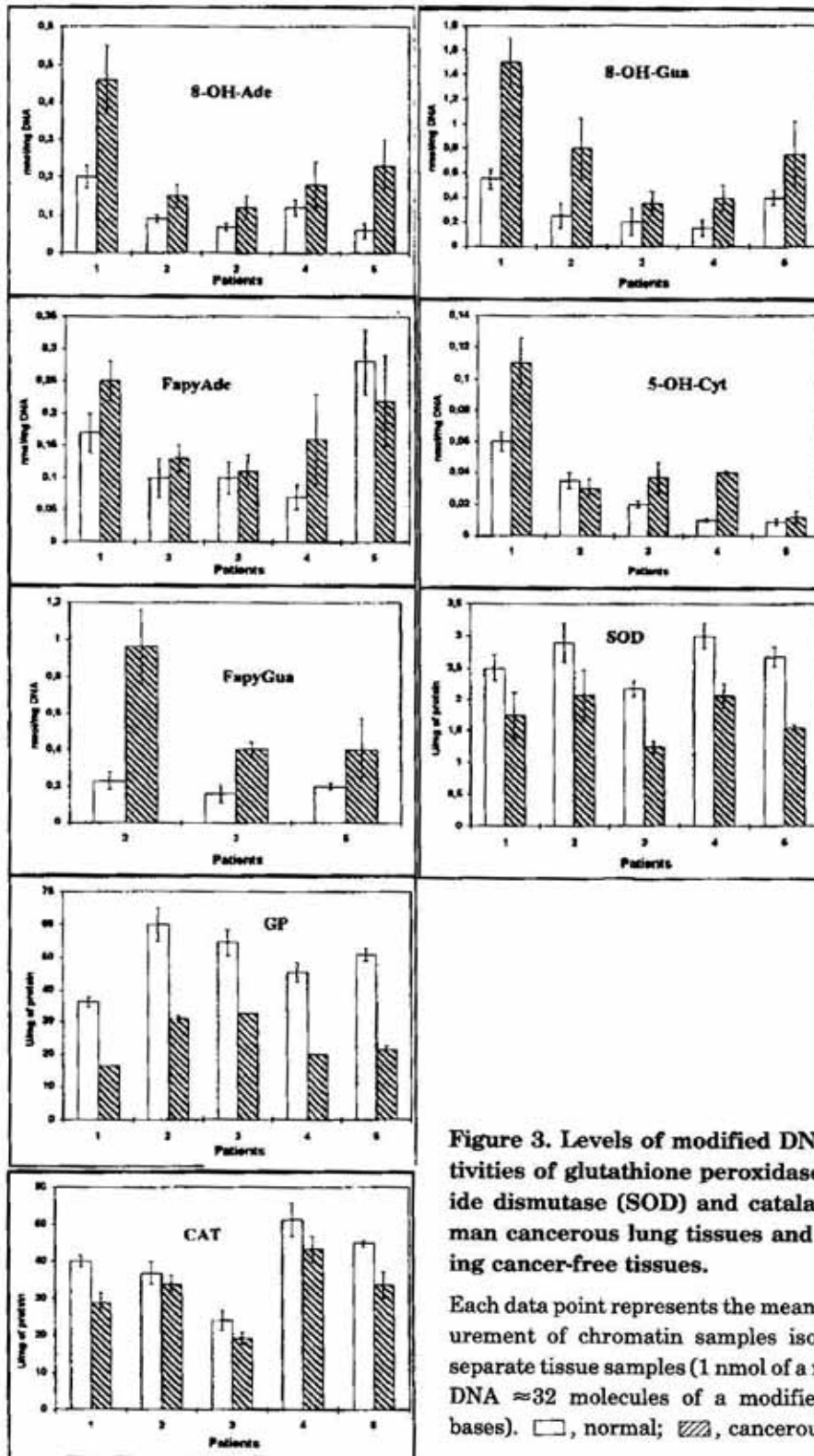


Figure 3. Levels of modified DNA bases and activities of glutathione peroxidase (GP), superoxide dismutase (SOD) and catalase (CAT) in human cancerous lung tissues and their surrounding cancer-free tissues.

Each data point represents the mean \pm S.D. from measurement of chromatin samples isolated from three separate tissue samples (1 nmol of a modified base/mg DNA \approx 32 molecules of a modified base/ 10^5 DNA bases). \square , normal; hatched , cancerous.

tion of secondary malignancies and some of them may generate free radicals. Since free radical induced DNA damage may possess premutagenic properties and may play some

role in carcinogenesis, we wished to check whether the modalities used in anticancer therapy were responsible for production of typical free radical induced base modifica-

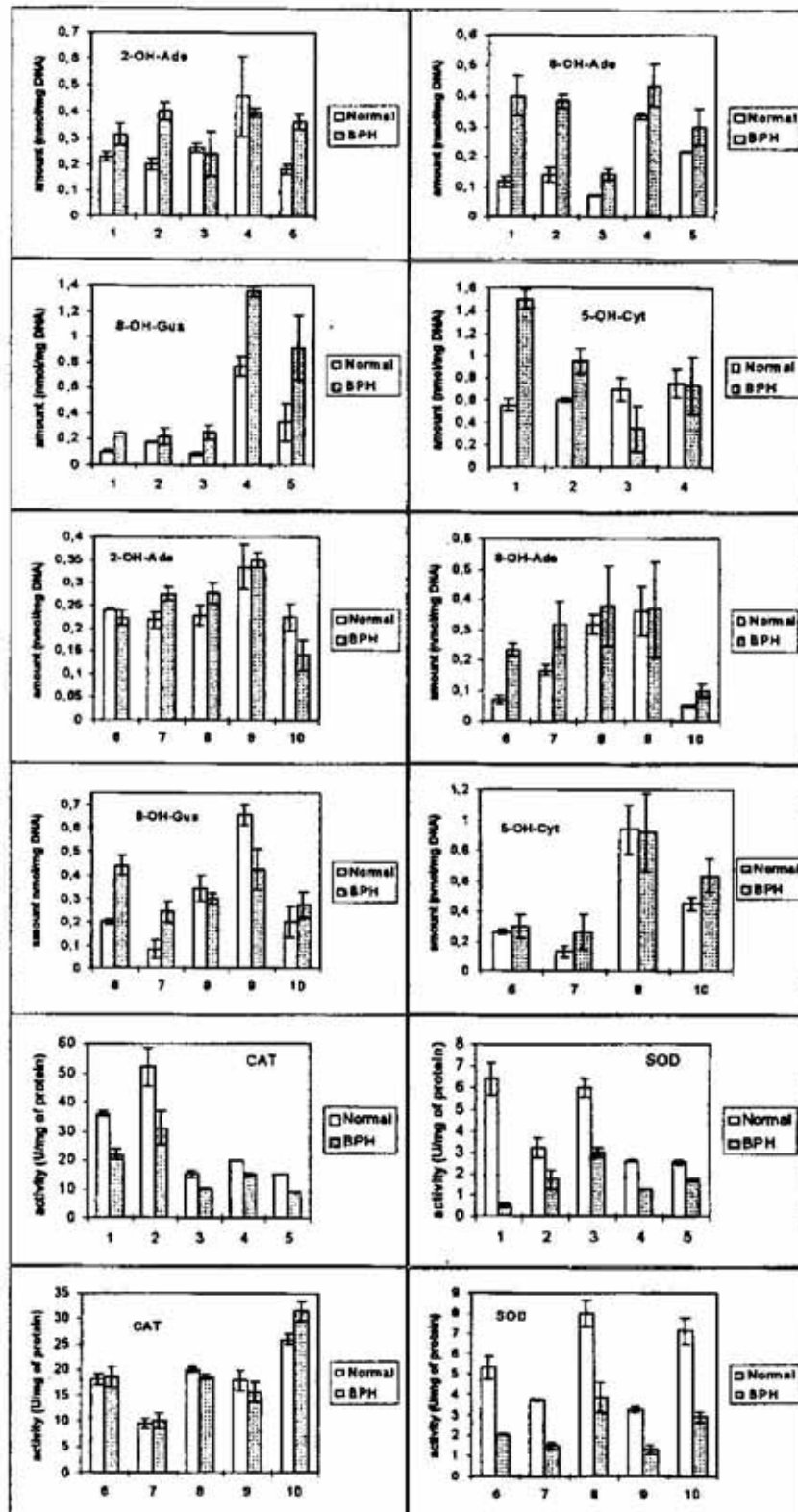


Figure 4. The levels of modified DNA bases and activities of catalase (CAT) and superoxide dismutase (SOD) in benign prostatic hyperplasia and surrounding disease-free tissues of Group I of the patients.

Each value represents the mean \pm standard error from three independent measurements (1 nmol of modified base/mg DNA \approx 32 molecules of modified bases/ 10^5 bases).

tions in nuclear DNA of lymphocytes of cancer patients who were undergoing anticancer therapy.

Ionizing radiation is one of the most commonly used therapeutic agents for cancer. In general, approximately half of cancer patients receive radiation therapy as their disease management [40]. The result of our experiments provided evidence that exposure of cancer patients to therapeutic doses of ionizing radiation caused base modifications in genomic DNA of their lymphocytes [41].

Anthracycline derivatives have been widely used in the treatment of several types of human malignancies. Cytotoxicity of these drugs has been attributed to inhibition of topoisomerase II as well as intracellular production of free radicals.

Recently, using the GC-MS method, Akman *et al.* [42] have shown that reactive oxygen reduction by the redox cycling of the doxorubicin quinone moiety is responsible for DNA base modification in isolated human chromatin. In our recent work using epirubicin (the analog of doxorubicin with a different configuration of the OH group in the C-4 position of the amino-sugar moiety) we observed similar base modifications in chromatin isolated from lymphocytes of cancer patients undergoing chemotherapy [43]. The pattern of these modifications also suggests the involvement of \cdot OH radical in their formation.

Anticancer therapy caused a significant increase in the level of modifications of all four DNA bases over control levels one hour after injection of the drug, in most of the patients. However, the extent of these modifications differed among patients. This interindividual variability may reflect individual differences in exposure, metabolism and repair capacity and may have, at least in part, a genetic background [44]. In the majority of patients, 24 h after infusion of the drug the base product returned to the control value. Likewise, in DNA of lymphocytes of the patients who were undergoing radiotherapy, in many cases, the modified base levels decreased after a tran-

sient increased upon irradiation, even reaching the control level [41]. Thus, the decrease in base products may well be an indication of removal of these lesions by cellular repair processes. In the case of some patients and some of the modifications the level of damage stayed high despite a considerable period of time after their induction.

There is a risk that the patients might develop secondary cancers after chemotherapy and radiotherapy [45, 46]. Long-lived B and T lymphocytes may serve as a target cells for carcinogens including some anticancer drugs and ionizing radiation [47]. Some of the base modifications which escaped repair in lymphocytes DNA could lead to mutagenesis in critical genes and ultimately to secondary cancers such as leukemias.

CONCLUSIONS

All these observations indicate that free radical induced DNA base modifications may significantly contribute to carcinogenesis. They can be responsible for mutations in genes critical for early stages of cancer development like oncogenes and tumor suppressor genes. Alternatively, in fully developed cancer, increased levels of modified DNA bases may cause further destabilization of the genome and increased metastatic potential.

The part played by oxidative DNA damage in human carcinogenesis can also be illustrated by the study of the factors involved in the total cancer burden of the U.S.A. population. It was demonstrated that approximately one third of the human cancer burden was associated with cigarette smoking, one third with diet and nutrition, and one third with all other causes such as occupational exposures to carcinogens, carcinogenic drug related compounds, environmental exposures, oncogenic viruses and radiation [48]. Since cigarette smoking, excess of calories and considerable part of carcinogens can generate ROS, it can be con-

cluded that oxidative DNA damage is an important carcinogenic factor.

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