The Level of 8-Hydroxyguanine, a Possible Repair Product of Oxidative DNA Damage, Is Higher in Urine of Cancer Patients than in Control Subjects[1]

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Abstract

Using high-performance liquid chromatography prepurification/isotope dilution gas chromatography/mass spectrometry technique, we examined whether the amount of 8-hydroxyguanine and 8-hydroxy-2′-deoxyguanosine excreted into urine is higher in cancer patients with advanced-stage disease than in the control group. The control group consisted of 38 healthy subjects, and the patient group comprised 42 cancer patients suffering from metastasis of their primary tumors into the bones. We have found that the amount of the modified base (but not the nucleoside) excreted into urine is about 50% higher in cancer patients than in the control group. Because the presence of the modified base in urine may represent the primary repair product of oxidative DNA damage *in vivo,* our results suggest an important role of DNA glycosylases (most likely OGG1) in removal of the damage induced as a result of cancer development.

Introduction

In a normal human cell, there is a steady accumulation of DNA lesions with time (1). Substantial parts of these lesions are due to endogenous factors that damage DNA. These include ROS[3] (superoxide anion, hydrogen peroxide, and hydroxyl radical) derived from oxidative respiration. It has been shown that free radical attack on DNA generates a whole series of DNA damage, including modified bases. Hydroxyl radical (˙OH) attack on DNA leads to a large number of pyrimidine- and purine-derived base damage. Some of these modified DNA bases have considerable potential to damage the integrity of the genome (1–4).

8-OH-Gua is one of the most widely studied lesions of this type. The presence of 8-OH-Gua residues in DNA leads to GC to TA transversion unless repairs are made before DNA replication (5). Therefore, the presence of 8-hydroxyguanine may lead to mutagenesis. Furthermore, many observations indicate a direct correlation between 8-OH-Gua formation and carcinogenesis *in vivo* (3, 6).

It is generally accepted that the products of repair of 8-OH-Gua in cellular DNA are excreted into the urine without further metabolism (7, 8). There is a common belief that the presence of the modified nucleoside (8-OH-dGuo) in urine represents the primary repair product of oxidative DNA damage *in vivo*, presumably NER (9, 10). However, oxidatively damaged DNA bases are mostly repaired by the BER pathway, although the NER pathway may also play a role in the repair of some oxidized bases in DNA (11, 12). Therefore, assays that are able to determine the level of 8-OH-dGuo as well as the amount of 8-OH-Gua in urine may better reflect the oxidative damage of cellular DNA.

The analysis of 8-OH-Gua in urine presents particular difficulties (13), and until recently, there has been no reliable assay for its detection. Recently, a new methodology was developed that allowed for simultaneous determination of 8-OH-dGuo and 8-OH-Gua in the same urine sample (14). This method involved a HPLC prepurification followed by gas chromatography with isotope dilution mass spectrometric detection. Using this method, we have found that urinary excretion of 8-OH-Gua and 8-oxo-Guo does not depend on diet in the case of humans (15).

The results from different laboratories demonstrated that the background level of 8-OH-Gua in DNA of cancerous tissues is higher than that in DNA of normal tissues (16–18), and there were some data suggesting that persistent oxidative stress may be associated with cancer development (18, 19). Because the level of the modified nucleoside/base in urine may be a good indicator of oxidative DNA insult, and as a general index of oxidative stress in the present study, using the above-described method, we examined whether the amount of 8-OH-Gua and 8-OH-dGuo excreted into urine is higher in cancer patients when compared with the control group.

Materials and Methods

Subjects. The study was conducted in two groups. The control group consisted of 38 healthy volunteers. None of them had a history of smoking, diabetes, or cardiopulmonary disease. The patient group comprised 42 subjects. Patients had the following types of cancer: breast cancer (7 patients); lung cancer (30 patients); and prostate cancer (5 patients). All of the cancer patients suffered from metastasis of their primary tumors into the bones. Because smoking status may influence the excretion of modified base/nucleoside (10), the patient group was recruited from among subjects with no smoking history for at

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[3] The abbreviations used are: ROS, reactive oxygen species; 8-OH-dGuo, 8-hydroxy-2′-deoxyguanosine; 8-OH-Gua, 8-hydroxyguanine; BER, base excision repair; NER, nucleotide excision repair; HPLC, high-performance liquid chromatography.

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Table 1 General characteristics of the study groups

	Cancer group	Control group
Total no. of cases	42	38
Female	20	17
Male	22	21
Age (median, yrs)	51	50
Weight (median, kg)	68,2	70,2

Table 2 Quality control data for the analysis of 8-OH-Gua and 8-OH-dGuo in urine

	8-OH-Gua	8-OH-dGuo
n	7	7
Average (pmol/ml)	102.78	33.15
SD (pmol/ml)	3.20	2.16
% of variation	3.11	6.54
Recovery (%)	97 ± 4	94 ± 6

least 5 years. None of the patients had undergone chemotherapy at least 3 months before urine collection. There were no significant differences in diet, antioxidant use, age, sex, or weight between the control group and the group of cancer patients. Twenty-four h urine samples were collected. General characteristics of both the groups are presented in Table 1.

The study was approved by the medical ethics committee of The Ludwik Rydygier Medical University (Bydgoszcz, Poland), No. 141/2001 (in accordance with Good Clinical Practice, Warsaw 1998), and all of the patients gave informed consent.

Urine Sample Preparation. Aliquots of 0.5 nmol of 8-OH-[$^{15}N_3$,^{13}C]Gua and 0.05 nmol of 8-[^{18}O]OH-dGuo and 10 μl of acetic acid (HPLC grade; Sigma) were added to 2 ml of human urine. Isotopic purity of the applied standards was 97.65% and 85%, respectively. After centrifugation (2000 × *g*, 10 min), supernatant was filtered through a Millipore GV13 0.22 μm syringe filter, and 500 μl of this solution were injected onto the HPLC system. In the pilot study, isotopically labeled internal standards of unmodified compounds (1 nmol of [$^{13}C_3$]guanine and 1 nmol of 2′-[$^{15}N_5$]deoxyguanosine) were added to the urine samples to monitor fractions containing both these compounds and to avoid an overlapping of the peaks containing the modified and unmodified base/nucleoside. Isotopic purity of the applied standards was 96.4% and 98.0%, respectively.

HPLC Purification and Gas Chromatography/Mass Spectrometry Analysis. Urine HPLC purification of 8-OH-Gua and 8-OH-dGuo was performed according to the method described by Gackowski *et al.* (15).

Gas chromatography/mass spectrometry analysis was performed according to the method described by Dizdaroglu (20), adapted for additional 8-[^{18}O]OH-Gua analyses (*m/z* 442 and 457 ions were monitored).

Results

For evaluation of the accuracy and reproducibility of the analyzed compounds, the quantitative analyses of 8-OH-Gua and 8-OH-dGuo were repeated seven times on the same urine samples. To check accuracy and recovery, spiked urine samples were analyzed (10 pmol of 8-OH-dGuo and 50 pmol of 8-OH-Gua were added to 1 ml of urine sample). Quality control data

Table 3 Statistical analyses of the urinary excretion of 8-OH-Gua

	Control group	Cancer group
Mean ± SD (nmol/24 h)	138 ± 83	202 ± 102
P (Student's *t* test)	0.0015	
Mean ± SD (nmol/kg body weight)	2.03 ± 1.3	2.9 ± 1.6
P (Student's *t* test)	0.0097	

Table 4 Statistical analyses of the urinary excretion of 8-OH-dGuo

	Control group	Cancer group
Mean ± SD (nmol/24 h)	35 ± 21	36 ± 15
P (Student's *t* test)	0.4028	
Mean ± SD (nmol/kg body weight)	0.52 ± 0.29	0.51 ± 0.22
P (Student's *t* test)	0.8617	

for the analysis of 8-OH-Gua and 8-OH-dGuo are presented in Table 2.

The mean level of 8-OH-Gua in urine samples of 42 cancer patients was 202 ± 102 nmol/24 h (Table 3). It was significantly higher ($P = 0.0015$) than that in the urine of the control group, where the level reached a value of 138 ± 82 nmol/24 h. There were no differences between the patients with various types of primary cancer (data not shown). The mean levels of 8-OH-dGuo in the control group and in cancer patients were very similar and reached mean values of 35 ± 21 and 36 ± 15 nmol/24 h, respectively (Table 4). This difference was not statistically significant. Interestingly, our data concerning the control group were recently confirmed when the LC/MS/MS technique was applied to analyze urinary excretion of 8-OH-Gua and 8-OH-dGuo in human subjects (21).

Discussion

The level of the lesions in urine can depend on oxidative DNA insult and can be reflective of an involvement of different repair mechanism(s). It is rather unlikely that DNA repair mechanisms might differ in cancer patients in comparison with healthy subjects. Therefore, the higher level of 8-OH-Gua in urine of cancer patients may be explained, at least in part, by the reported oxidative stress in cancer tissues (16–18, 22, 23). However, the amount of the modified base/nucleoside excreted into urine should represent the average rate of DNA damage in whole body (9, 10). Therefore, it is rather unlikely that the elevated level of the base product in cancerous cells alone could account for the observed 50% increase of 8-OH-Gua in urine. Our results suggest rather that oxidative stress, represented by the increased amount of the compound in urine, may be characteristic not only for the diseased tissue but for some other tissues (or whole organism) of cancer patients as well. The precise mechanism(s) of the oxidative stress is still unknown.

However, some mechanisms may be suggested.

(*a*) It has recently been documented that cancer patients showed signs of extensive granulocyte activation with a release of ROS followed by a dramatic increase of 8-isoprostane, one of the biomarkers of oxidative stress. This oxidative stress exceeded that found in other diseases with documented oxidative stress (19).

(*b*) It has been shown that malignant cells can produce hydrogen peroxide at levels as high as those characteristic for stimulated polymorphonuclear leukocytes (24). Therefore, one

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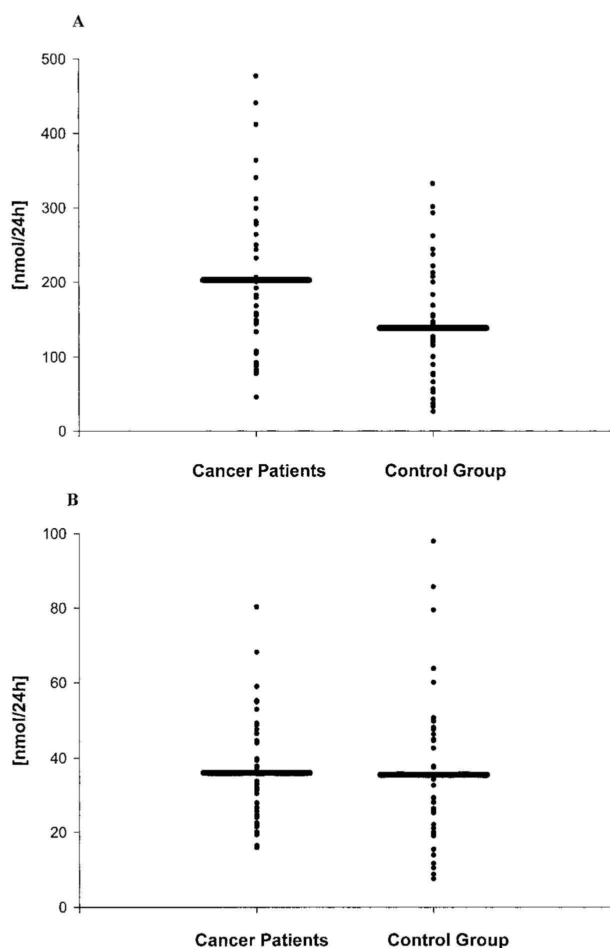

Fig. 1. The individual values for the modified base (*A*) and the nucleoside (*B*).

of the reasons for the observed oxidative stress in advanced stages of cancer may be a release of a large number of cancer cells into the blood stream (25) and their penetration into other tissues. Interestingly, it has been demonstrated that exposure to the activated leukocytes causes oxidative DNA base modifications (among them, 8-OH-Gua) in target cells (26).

(*c*) Still another reason for the observed phenomenon may be that some tumors may stimulate the defense systems of the body so that they react against the tumor to produce cytokines (27). Some cytokines can produce large amounts of ROS (28, 29). It has been shown that an elevated plasma level of tumor necrosis factor is responsible for increased oxidative DNA damage of $CD34^+$ cells (30).

In contrast to the level of modified base, the concentration of the modified nucleoside in urine samples was almost exactly the same in both groups of subjects (Fig. 1). It is possible that the levels of both the base and the nucleoside are reflective of involvement of different repair pathways responsible for the removal of 8-OH-Gua from cellular DNA, namely, the BER and NER pathways, respectively. Several glycosylases, which specifically recognize and remove 8-OH-dGuo, have been identified and cloned in mammals, including humans (31–33). Also, recent studies with OGG1 knockout mouse clearly demonstrated that the lack of glycosylase resulted in accumulation of the modified base in cellular DNA (34, 35). Therefore, it is supposed that BER plays an essential role in repair of this modification (34–36). It has been suggested that NER acts simply as a "back up" system in the repair of oxidative DNA damage (12). There have been no available data to determine what proportion of the base damage is removed by either mechanism. Our results are in good agreement with aforementioned evidence and suggest that BER in humans is several times more active in removal of the damage. Moreover, the results presented above suggest that in the case of cancer patients with advanced-stage disease, BER is mainly involved in removal of the disease-induced oxidative DNA damage.

However, we cannot exclude a possibility that other than repair processes can contribute to the 8-OH-Gua and 8-OH-dGuo level in human urine. An additional possible source of the modifications in urine may be that they derive from dead cells. Lindahl (37) suggested that 8-OH-dGuo is presumably derived from dead cells' DNA due to the action of unspecific nucleases and phosphatases. The urinary level of 8-OH-Gua may also include a contribution from oxidized RNA, particularly if mechanisms exist to maintain the integrity of RNA molecule (but such a mechanism(s) has not yet been detected). The oxidation of the cellular nucleotide pool is also a potential source of excreted 8-OH-dGuo. All these possibilities remain to be tested.

In conclusion, we have found that the amount of the modified base (but not the nucleoside) excreted into urine is almost 50% higher in cancer patients than in the control group. This suggests the important role of DNA glycosylases (most likely OGG1) in removal of oxidative DNA damage induced as a result of cancer development. The level of both compounds may be a good indicator of the involvement of two repair pathways (namely, BER and NER) that operate in human cells.

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