Products of Oxidative DNA Damage and Repair as Possible Biomarkers of Susceptibility to Lung Cancer

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ABSTRACT

The broad spectrum of oxidative DNA damage biomarkers [urinary excretion of 8-hydroxy-2′-deoxyguanosine (8-OH-dGuo) and 8-hydroxyguanine (8-OH-Gua)] and the level of oxidative DNA damage and repair in leukocytes were analyzed in three groups of subjects: (a) lung cancer patients (all smokers), (b) healthy smokers with comparable smoking status (n = 26); and (c) healthy nonsmokers (n = 38). The mean level of 8-OH-Gua in urine samples of 38 healthy nonsmokers reached a value of 1.783 ± 0.785 nmol/day/kg. This level was significantly lower than that in the urine of the two smoker groups (cancer patients and healthy smokers), in whom the levels reached values of 2.319 ± 1.271 and 2.824 ± 0.892 nmol/day/kg, respectively. Urinary excretion of 8-OH-dGuo was similar in all groups of subjects. The level of 8-OH-dGuo in DNA isolated from leukocytes of cancer patients was significantly higher than that in DNA isolated from the group of healthy smokers and nonsmokers (9.44 ± 4.77 vs. 7.20 ± 2.83 and 5.88 ± 2.47 molecules/10^10 deoxyguanosine, respectively). Repair activity of 8-OH-Gua, as estimated by the nicking assay, was significantly higher in blood leukocytes of healthy volunteers (44.6 ± 20.21 and 37.54 ± 13.43 pmol/h/mg protein for smokers and nonsmokers, respectively) than in the leukocytes of lung cancer patients (24.56 ± 11.28 pmol/h/mg protein). Because oxidative DNA insult represented by urinary excretion of oxidative DNA lesions was similar in both groups of subjects with similar smoking status, it appears likely that a higher rate of generation of oxidative damage in cellular DNA of lung cancer patients is a result of deficiency of the repair mechanism(s) in this group.

INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide (1), and cigarette smoke is implicated in the development of 90% of lung tumors (2). Cigarette smoke contains many carcinogens, and the majority of works have focused on a role of polycyclic aromatic hydrocarbon adducts with DNA in lung cancer development (3). However, cigarette smoke contains also free radicals and induces oxidative damage in humans. It is suggested that these free radicals cause redox cycling that generates superoxide anion from molecular oxygen and leads to formation of hydrogen peroxide and hydroxyl radical (4). Several studies have demonstrated that cigarette smoke causes DNA damage of cultured cells (5–9). Moreover, there are some pieces of evidence implicating oxysteroidal and subsequent 8-OH-Gua\(^3\) formation in cellular DNA in human lung carcinogenesis (10). The presence of 8-OH-Gua residues in DNA leads to GC to TA transversion unless repaired before DNA replication (11). Therefore, the presence of 8-OH-Gua may lead to mutagenesis. Furthermore, many observations indicate a direct correlation between 8-OH-Gua formation and carcinogenesis in vivo (12–14). It is noteworthy that 8-OH-Gua is one of the many products of oxidative DNA damage (15).

It is generally accepted that the products of repair of 8-OH-Gua in cellular DNA are excreted into the urine without further metabolism (16–18). The level of 8-OH-Gua and 8-OH-dGuo (modified base/nucleoside) in urine may be a good indicator of oxidative DNA insult and a general index of the level of oxidative stress on the organism. Recently, using a method involving HPLC preparativization followed by gas chromatography with isotope dilution mass spectrometric detection, we found that urinary excretion of 8-OH-Gua and 8-OH-dGuo does not depend on diet in the case of humans and may reflect involvement of different repair mechanisms, namely, BER and NER (19, 20).

Although leukocytes are not the direct target of tobacco carcinogens, the level of carcinogen-DNA adducts in WBCs has been shown to correlate with tobacco carcinogen-induced damage in human lung tissues (21, 22), and cells from peripheral blood that migrate and circulate through the lung may be exposed to accumulated unmetabolized toxic compounds in this tissue (3). Moreover, leukocytes are often used as surrogate cells, which are supposed to inform about oxidative stress (measured as a certain level of 8-OH-dGuo) in other tissues (23). It is also noteworthy that leukocytes are the only available cells that may be analyzed in all subject groups (see below).

To assess the role of oxidative DNA damage in lung cancer development, we decided, for the first time, to analyze the broad spectrum of oxidative DNA damage biomarkers: urinary excretion of 8-OH-dGuo and 8-OH-Gua and the level of oxidative DNA damage and the rate of its repair in leukocytes. These parameters were analyzed in three groups of subjects: (a) lung cancer patients (all smokers); (b) healthy smokers with comparable smoking status; and (c) healthy nonsmokers.

MATERIALS AND METHODS

Patients. The study was conducted in three groups. The control group consisted of 38 healthy males (\(n = 30\)) and females (\(n = 8\)) with a median age of 62 years (range, 40–87 years). None had a history of smoking. The control group comprised 26 healthy smokers (20 males and 6 females) with a median age of 59 years (range, 42–79 years). The cancer patient group of 51 subjects comprised 41 males and 10 females. The median patient age was 63 years (range, 45–82 years). All of the patients were smokers and have newly recognized NSCLC.

Blood and urine were taken before surgery. Twenty-four h urine samples were collected. All of the patients had histologically proven adenocarcinomas (\(n = 6\)) and squamous cell carcinoma (\(n = 45\)) with G2 (\(n = 37\)) and G3 (\(n = 14\)) grading. No differences in the investigated analytical parameters were found between the grading groups. The patients were not treated with any drug during the time from diagnosis up to the time of surgery (up to 3 weeks). The control groups were chosen in such a way that the following criteria matched the patient group: eating habits; age; body weight; and sex. The control group and the patient group have similar smoking status.

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3 The abbreviations used are: 8-OH-Gua, 8-hydroxyguanine; 8-OH-dGuo, 8-hydroxy-2′-deoxyguanosine; BER, base excision repair; NER, nucleotide excision repair; HPLC, high-performance liquid chromatography; NSCLC, non-small cell lung cancer.
The study was approved by the medical ethics committee of The L. Rydygier Medical University, Bydgoszcz, Poland (in accordance with Good Clinical Practice, Warsaw, 1998), and all of the patients gave informed consent.

Isolation of Leukocytes from Venous Blood. Venous blood samples from the patients were collected. The blood was carefully applied on top of Histopaque 1119 solution (Sigma, St. Louis, MO), and leukocytes were isolated by centrifugation according to the procedure described by the manufacturer.

DNA Isolation and 8-OH-dGuo Determination in DNA Isolates. DNA from leukocytes was isolated using the method described by Miller et al. (24), with some modifications (25). Determination of 8-OH-dGuo by means of the HPLC/electrochemical detection technique has been described previously (26).

Urine Analysis. For urine analysis, 0.5 nmol of [15N3,13C]8-OH-Gua, 0.05 nmol of [15N]8-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 96.5%, respectively. The labeled standards were a kind gift from Dr. M. Dizdaroglu (National Institute of Standard and Technology, Gaithersburg, MD). 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, isotope-labeled internal standards of unmodified compounds (1 nmol of [13C6]guanine and 1 nmol of [15N2]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/nucleosides. Isotopic purity of the applied standards was 96.4% and 98.0%, respectively.

Urine HPLC purification of 8-OH-Gua and 8-OH-dGuo was performed according to the method described by Gackowski et al. (19) and Rosalski et al. (20). Gas chromatography/mass spectrometry analysis was performed according to the method described by M. Dizdaroglu (27), adapted for additional modifications (20). Gas chromatography/mass spectrometry analysis was performed according to the method described by M. Dizdaroglu (27), adapted for additional modifications (20).

Repair Activity Assay for 8-OH-Gua. Leukocyte pellets were homogenized with 4 volumes of buffer containing 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 10 mM DTT. Cells were disrupted by sonication (three times with 15-s pulses with 30-s intervals). The cell debris was removed by centrifugation, and the supernatant served as a source of enzymes. The protein concentration was determined by the method of Bradford (28).

A 40-mer oligodeoxynucleotide (20 pmol) containing 8-OH-Gua at position 20 in the sequence 5'-d(GTCTAGTCTACAGACCT-8-OH-Gua)-CGAC-TGTTCCAACGTCTGCAAGC-3' (Eurogentec Herstal, Belgium) was [3H]-labeled at the 5'-end by polynucleotide kinase in the excess of [γ-32P]ATP (3000 Ci/mmole). Oligomer was purified from unincorporated radioactive activity using Micro Bio-Spin P-30 columns (Bio-Rad, Hercules, CA) as described by manufacturer and annealed to complementary oligonucleotide (double molar excess) containing C opposite 8-OH-Gua. Formation of duplexes was verified by non-denaturing PAGE.

The repair activity of leukocytes was measured by nicking of the oligodeoxynucleotide at the site of the lesion. [3H]-labeled duplex (1 pmol) was incubated at 37°C for 1 h with increasing amounts of tissue extract (1–100 μg protein/sample) in a total volume of 20 μl containing 25 mM Tris-HCl (pH 7.8), 50 mM NaCl (pH 8.0), 5 mM β-mercaptoethanol, and 1 mM EDTA. Reactions were stopped, and the interfering proteins were removed by digestion with proteinase K (1 μg/ml reaction mixture, 1 h, 37°C). Because the apurinic/apyrimidinic-endonuclease activity of OGG1 is lower than that of the glycosylase function, uncleaved abasic sites were further disrupted by a 30-min incubation in 0.2 M NaOH at 70°C. The products were separated by denaturing 20% PAGE and quantified in a PhosphorImager using ImageQuant Software.

The enzyme activity was calculated from the initial velocity. All estimations were performed at least in triplicate. In each experiment, two control samples were set: (a) a negative control in which nontreated oligonucleotide was subjected to denaturing PAGE and showed any possible degradation of oligonucleotide that could appear during the procedure; and (b) a positive control in which labeled oligonucleotide was digested with the excess of pure OGG1 enzyme (a kind gift from Dr. Serge Boiteux; Commissariat à l’Energie Atomique, Fontenay-aux-Roses, France).

Statistical Analysis. All results are expressed as means ± SD. STATISTICA (version 5.1) computer software (StatSoft, Inc., Tulsa, OK) was used for statistical analysis. Values were checked to fulfill the criteria of homogeneity according to F and Leven tests. For normal distribution, variables were analyzed by the tests of Schapiro-Wilk and Kolmogorov-Smirnow with Lilliefors correction. ANOVA and Student’s t test were carried out to compare the means of variables with normal distribution. For those with non-normal distribution, the Mann-Whitney U test was used. *P < 0.05 was considered statistically significant.

RESULTS

The level of 8-OH-dGuo in DNA isolated from leukocytes of cancer patients was significantly higher than that in DNA isolated from the two other groups. (9.44 versus 7.20 and 5.88 per 10⁶ deoxyguanosine respectively, Table 1). The mean levels of 8-OH-dGuo are in the range of values reported recently by others (29, 30). Interestingly, 8-OH-dGuo level in lymphocyte DNA may vary significantly according to country. In Ireland, the level is very close to that reported in our study (31). The mean level of 8-OH-Gua in urine samples of 38 healthy nonsmokers reached a value of 1.783 nmol/day/kg (Table 1). This level was significantly lower than that in the urine of the two groups of smokers (cancer patients and healthy smokers), in whom the levels reached values of 2.319 and 2.824 nmol/day/kg, respectively (Table 1). The concentration of the modified nucleoside in urine samples was similar in all groups of subjects (Table 1). We did not find a correlation between any of these parameters and the age of the studied subjects.

DISCUSSION

Cigarette smoke can generate reactive oxygen species that can cause DNA damage, leading to mutations and cancer formation. 8-OH-Gua, one of the oxidatively modified DNA bases, is a ubiquitous biomarker of oxidative stress (32). The mean level of 8-OH-Gua in urine samples of 38 healthy nonsmokers was significantly lower than that in the urine of the two groups of smokers. Excretion of this modified base/nucleoside into urine represents the average rate of oxidative DNA damage in the total body. Therefore, it is rather unlikely that the expected increase in the level of base/nucleoside in
the lung of smokers may contribute to elevated excretion rate of the modified base. Our results suggest rather that oxidative stress, represented by the increased amount of the compound in urine, may be characteristic not only for the lung tissue but for some other tissues (or whole organism) of smokers as well. In contrast to the level of modified base, the excretion rate of the modified nucleoside in urine samples was similar in all groups of subjects (Table 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 (16, 20, 33, 34).

It is supposed that BER plays an essential role in repair of 8-OH-Gua (35–37) and that NER acts simply as a “back up” system in the repair of oxidative DNA damage (38). Our results also show a several times higher level of 8-OH-Gua than 8-OH-dGuo in urine, which could support this hypothesis.

We cannot entirely exclude the possibility that pathways other than repair processes can contribute to 8-OH-Gua and 8-OH-dGuo level in human urine, e.g., 8-OH-dGuo may derive from dead cells (33). Alternatively, 8-OH-dGuo in urine could derive from sanitation of cellular nucleotide pool by MutT-directed pathway (39), and the excretion of 8-OH-Gua may also include a contribution from oxidized RNA.

The background levels of 8-OH-dGuo in cellular DNA represent a dynamic equilibrium between rates of oxidative DNA damage and rates of repair of the damage. Therefore, the excretion rate can be combined with measurements of 8-OH-dGuo in cellular DNA to study the question of rates of repair versus rates of damage. In this study, for the first time, we have measured all of the parameters, which may represent oxidative DNA base damage. Besides urinary excretion of the modified base and nucleosides, the level of 8-OH-dGuo in leukocyte DNA was also analyzed. The level of 8-OH-dGuo in DNA isolated from leukocytes of cancer patients was significantly higher than that in DNA isolated from the two control groups. Because oxidative DNA insult represented by urinary excretion of oxidative DNA lesions was similar in cancer patients and the control group with similar smoking status, it appears likely that a higher rate of generation of oxidative damage in cellular DNA of lung cancer patients is a result of a deficiency of repair mechanism(s) (most likely BER pathway) in this group.

This suggestion was confirmed with the measurement of 8-OH-Gua repair activity in the leukocytes of both smoker groups. In the smokers, this activity was significantly lower than that seen in healthy smokers. The nicking assay that was used in this study reflects the activity of BER pathway, although it does not discriminate between the OGG1 and formamidopyrimidine glycosylase type of 8-OH-Gua-DNA-glycosylases or between the nuclear and mitochondrial form of enzymes. It is also noteworthy that human OGG1 protein also removes the 2,6-diamino-4-hydroxy-5-formamidopyrimidine from DNA with kinetics similar to that of the removal of 8-OH-Gua (40).

It has recently been demonstrated that many types of DNA repair pathways are reduced in lung cancer patients (41). The precise mechanism(s) of the reduced repair capacity in cancer patients is still unknown. However, some mechanisms may be suggested, as shown below.

(a) It has been documented recently that lung cancer patients showed loss of heterozygosity at the hOGG1 gene locus (42, 43). The patients exhibiting loss of heterozygosity contained a higher level of 8-OH-dGuo adducts (43).

(b) It has been suggested that polymorphism in DNA repair genes may be associated with differences in the repair efficiency of DNA damage (44). Moreover, some studies suggest involvement of hOGG1 polymorphism in lung cancer development (45).

We cannot exclude the possibility that the elevated level of 8-OH-dGuo in the cellular DNA of cancer patients is an additive effect of oxidative stress that originates not only from cigarette smoke but also from development of the disease itself. However, it is less likely because (a) we did not observe an increase in urinary excretion of 8-OH-Gua in the cancer patient group in comparison with healthy smokers (Table 1), and (b) oxidative DNA damage did not depend on the grading of the disease development (see “Materials and Methods”).

In conclusion, the analysis of urinary excretion of the oxidatively modified base/nucleoside combined with the determination of background level of 8-OH-dGuo in leukocyte DNA and measurement of 8-OH-Gua repair activity appears to predict an individual’s susceptibility to tobacco-related lung cancer. It is possible that the above-mentioned parameters can distinguish “high responders” from “low responders.” Assuming that both groups have similar oxidative insult (see above), high responders may have decreased DNA repair capacity, shared by leukocytes and lung, which may be genetically determined. This conclusion is reinforced by a recent study published in Cancer Research (46) that clearly pointed out that OGG1 knockout mice are predisposed to develop lung carcinoma and that 8-OH-Gua was found to accumulate in their DNA. The fact that only 10–15% of cigarette smokers develop lung cancer and that there is an elevated frequency of individuals with reduced repair capacity among cancer patients and their relatives (47, 48) is a further suggestion that repair capacity of DNA damage is genetically determined and contributes to the pathogenesis of lung cancer.

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REFERENCES

DNA DAMAGE AND REPAIR IN LUNG CANCER PATIENTS


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