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 DNA were analyzed in three groups of subjects: (a) lung cancer patients [all smokers (n = 51)]; (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 versus 7.20 ± 2.83 and 5.88 ± 2.47 molecules/10⁶ 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 oxypyridyl and subsequent 8-OH-Gua3 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 preparation 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 at 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.
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 [15N]8-OH-dGuo, 0.05 nmol of [15N]8-OH-Gua, and 10 μl of 0.2 M 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 [15C]guanine and 1 nmol of [15N]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.

Urine HPLC purification of 8-OH-Gua and 8-OH-dGuo was performed according to the method described by Gackowski et al. (19) and Roxalski et al. (20). Gas chromatography/mass spectrometry analysis was performed according to the method described by M. Dizdaroglu (27), adapted for additional [15N]8-OH-Gua analyses (m/z 442 and 457) as monitored; these ions represent the masses of characteristic ions of the base shifted in the mass spectra according to the extent of labeling.

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′-dGTACCTACTCTGAGGCTT-G(8-OH-Gua)-CGAC-TGTTCCACTGCTTGAAG-3′ (Eurogentec Herstal, Belgium) was [32P]-labeled at the 5′-end by polynucleotide kinase in the presence of [γ-32P]ATP (3000 Ci/mmol; Amersham-Pharmacia Biotech, Uppsala, Sweden), which labeled oligonucleotide was purified by a column of unincorporated radioactive material. A five-mer oligonucleotide was synthesized with 5′-biotin (Eurogentec Herstal, Belgium) by a Message Maker (Pharmacia Biotech) and was used as an internal standard. The oligonucleotide was digested with the excess of pure OGG1 enzyme (a kind gift from Dr. Serge Boiteux; Commissariat a`l Energie Atomique, Fontenay-aux-Roses, France).

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 a`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-Smirnov with Lillefors 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 10^7 deoxyguanosine respectively, Table 1). The mean levels of 8-OH-dGuo are in the range of values reported recently by others (29, 30).

<table>
<thead>
<tr>
<th>Repaired DNA base/nucleoside</th>
<th>Control group</th>
<th>Control group</th>
<th>Control group</th>
<th>P NSCLC vs. smokers</th>
<th>P NSCLC vs. nonsmokers</th>
<th>P smokers vs. nonsmokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OH-dGuo vs. DNA from leukocytes</td>
<td>9.44 ± 4.77</td>
<td>7.20 ± 2.83</td>
<td>5.88 ± 2.47</td>
<td>0.0308b</td>
<td>0.0001b</td>
<td>0.0523</td>
</tr>
<tr>
<td>Urinary 8-OH-Gua excretion (nmol/day/kg)</td>
<td>2.319 ± 1.271</td>
<td>2.824 ± 0.892</td>
<td>1.783 ± 0.785</td>
<td>0.0745</td>
<td>0.0243b</td>
<td>0.0001b</td>
</tr>
<tr>
<td>Urinary 8-OH-dGuo excretion (nmol/day/kg)</td>
<td>0.398 ± 0.167</td>
<td>0.347 ± 0.152</td>
<td>0.451 ± 0.281</td>
<td>0.1994</td>
<td>0.2677</td>
<td>0.0906</td>
</tr>
</tbody>
</table>

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 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.

8-OH-Gua repair capacity of blood leukocytes, as measured by the nicking assay, was significantly higher in the groups of healthy volunteers (44.6 ± 20.21 and 37.54 ± 13.43 pmol/h/mg protein for smokers and nonsmokers, respectively) than in the group of lung cancer patients (24.56 ± 11.28 pmol/h/mg protein; Mann-Whitney test, two-tailed P = 0.000001 and 0.000063, respectively, Fig. 1).

Table 1. General characteristics of the analytical parameters of the study groups
Gua (35–37) and that NER acts simply as a back up system in the ground level of 8-OH-dGuo in leukocyte DNA and measurement of 8-OH-dGuo 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.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1. Repair activity of 8-OH-Gua, as measured by the nicking assay, in leukocytes of lung cancer patients versus healthy volunteers. P = obtained with the Mann-Whitney test.
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