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CIGARETTE SMOKING INDUCES FORMATION OF 8-HYDROXYDEOXYGUANOSINE, ONE OF THE OXIDATIVE DNA DAMAGES IN HUMAN PERIPHERAL LEUKOCYTES

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Active oxygen species (AOS) such as O_2^- and H_2O_2 have been shown to be generated from both gas and tar phases of cigarette smoke and it has been suggested that they are involved in carcinogenesis due to cigarette smoking. Therefore, we investigated the effect of cigarette smoking on oxidative DNA damages in human peripheral blood cells using 8-hydroxydeoxy-guanosine (8-OH-dG) as a marker.

From ten healthy male volunteers aged 20–22 years, 5 ml of blood was taken before and 10 minutes after smoking 2 cigarettes in 10 minutes. After lysis of blood cell membranes leukocyte DNA was isolated using a DNA extractor and 8-OH-dG levels were determined using high performance liquid chromatography (HPLC) with electrochemical detection.

The mean levels of 8-OH-dG increased significantly ($P < 0.05$) from $3.3 \pm 0.8/10^6$ dG (mean $\pm$ SD) to 5.1 ± 2.5 after smoking.

These results indicate that cigarette smoking induces oxidative DNA damage in peripheral blood cells in a relatively short time.

KEY WORDS: Cigarette smoking, active oxygen species, 8-hydroxydeoxyguanosine, oxidative DNA damage, human leukocyte

INTRODUCTION

Although the mechanism of carcinogenesis by cigarette smoking is not clear, epidemiological studies have provided evidence that smoking is one of the major causes of human cancers, especially in the case of lung cancer and overall 30% to 40% of all cancer mortality is estimated to be related to smoking.[1]

Cigarette smoke is a complex mixture consisting of thousands of compounds, many of which are known to be carcinogens.[2] In addition, AOS such as H_2O_2 and O_2^- have been shown to be generated from both gas and tar phases in cigarette smoke[3]. Recently free radicals or AOS have been suggested to be involved not only in initiation but also in promotion of carcinogenesis.[4] These radicals are known to attack DNA molecules and produce a number of lesions in DNA such as single strand breaks or modified bases in DNA. Kasai and Nishimura have previously demonstrated that the C-8 position of deoxyguanosine residues in DNA is hydroxylated to produce

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8-OH-dG by various oxygen radical-producing agents.[5] It was also found that this hydroxylated residue is misread at the modified base and adjacent base residues in an *in vitro* DNA polymerase reaction,[6] which may offer an explanation for the carcinogenic or mutagenic properties of oxygen radical forming compounds.

We previously reported for the first time that 8-OH-dG could be quantitated in human lymphocyte DNA according to Floyd's method[7] and suggested that the assay for 8-OH-dG is useful as a biomarker for oxidative stress to humans.[8] In the present study we investigated whether or not 8-OH-dG formation is induced in human peripheral blood cell DNA after cigarette smoking.

METHODS

Ten healthy male volunteers aged 20–22 years who smoke cigarettes daily were recruited and asked not to smoke on the morning of the day when blood samples were taken. Five ml of the blood samples were taken in standard EDTA-contianing tubes before and 10 minutes after they smoked 2 cigarettes of their choice in 10 minutes.

Nine volumes of lysis buffer (0.32 M sucrose, 10 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, 1% Triton X-100) were added to the fresh whole blood. They were mixed gently to lyse all cell membranes, kept at 4°C for 5 minutes, and centrifuged at 3000 rpm for 15 minutes. The pellets of blood cells were rinsed with 5 ml of phosphate buffer saline (PBS, pH 7.4), and recentrifuged at 3000 rpm at 4°C for 10 minutes. These samples were stored at −80°C until DNA extraction. The pellets resuspended in 400 μl PBS were injected into Nucleic Acid Extractor Model 340A (Applied Biosystem, USA) to extract leukocyte DNA. 8-OH-dG levels were determined on the same day after DNA hydrolysis using HPLC coupled to an electrochemical detector as reported previously.[9]

The values shown here represent mean ± SD, and the statistical significance was determined by a matched pair t test (two-tailed).

RESULTS AND DISCUSSION

Within-run reproducibility was checked by analyzing blood samples in aliquots from one person. The coefficient of variation by this method was 11.2% ($n = 7$) for within-run determination of 8-OH-dG levels in human peripheral leukocyte DNA. The mean 8-OH-dG levels in peripheral leukocyte DNA from 10 volunteers increased from $3.3 \pm 0.8/10^6$ dG to 5.1 ± 2.5 after smoking 2 cigarettes in 10 minutes, which was statistically significant ($P < 0.05$, Figure 1).

In this study we investigated an acute effect of smoke exposure on the formation of 8-OH-dG in human peripheral leukocyte DNA, and it was observed that 8-OH-dG levels increased in a relatively short time after cigarette smoking similar to usual smoking habits.

It is reported that DNA damage developed within 20 seconds of exposing target cells to AOS such as H_2O_2 or stimulated leukocytes and reached a maximal effect in minutes, and the repair of the DNA occurred over the succeeding hours.[10] Nakayama *et al.* reported that cigarette smoke caused DNA single strand breaks in human cancer cell DNA in vitro and such activity is mainly due to action of AOS, especially hydroxy radicals generated from cigarette smoke.[11] Cigarette smoke contains a large amount

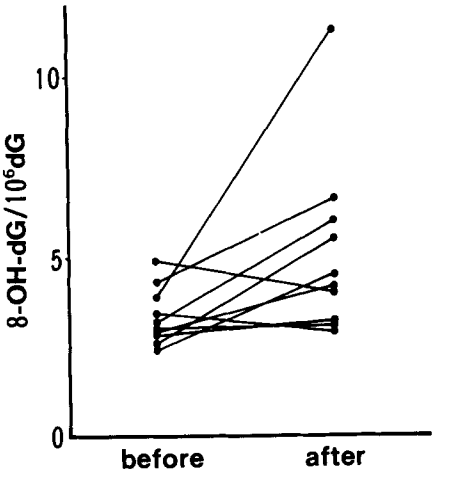

FIGURE 1 The changes of 8-OH-dG levels in human peripheral leukocyte DNA 10 minutes after smoking 2 cigarettes. The mean levels of 8-OH-dG increased significantly ($P < 0.05$) from 3.3 $\pm$ 0.8/10^6 dG to 5.1 $\pm$ 2.5.

of free radicals (about 5×10^{14} radicals are reported to be generated per one pull)[3]. H_2O_2 is very stable and has a half life long enough to penetrate target cells and generate hydroxy radicals, the most active oxygen radical in the vicinity of DNA in the cells. Cigarette tar also contains a significant amount of iron which promotes the Fenton type Haber-Weise reaction with H_2O_2 and produces hydroxy radicals. Alveolar macrophages of smokers are reported to contain increased levels of iron.[12] In addition, nitgrogen oxides from smoke gas phase are also involved in generating free radicals.[13] Moreover, cigarette smoking causes increased accumulation and metabolic activation of both polymorphonuclear leukocytes and macrophages in the lung.[14] These cells from smokers have been shown to release more O_2^- spontaneously and after stimulation by phorbol myristate acetate than that from nonsmokers.[15] These additive effects might be relevant to the higher incidence of lung cancer among smokers.

Many investigators have reported that frequencies of sister chromatid exchange or carcinogen DNA adducts levels in human peripheral lymphocytes are increased in smokers.[16,17] In contrast, some studies have failed to show such an increase.[18,19] We also previously observed that 8-OH-dG levels in smokers lymphocyte DNA were only slightly higher than nonsmokers.[20] Possible explanations for these results would be an efficient function of the DNA repair system such as removal of 8-OH-dG in the cells or organ(s) specificity. Although in the body most DNA damages caused by cigarette smoke are expected to be repaired rapidly and effectively, some would remain unrepaired and the accumulation of DNA damage over a long period of time may have more serious consequences, especially for heavy smokers.

Our results also showed that nearly two fold inter-individual variations of 8-OH-dG levels in human leukocyte DNA exist among the males who were almost the same age before smoking. At present the reasons why these variations occur are not clear, genetic factors including gene(s) involved in the repair of 8-OH-dG, the differences of dietary contents or antioxidants levels might be related. Further investigations to

clarify these points should determine whether the higher levels of 8-OH-dG in DNA is correlated to high incidence of carcinogenesis or aging.

Although it is not certain whether this DNA damage actually occurs in target organs such as lung, the results of this study are important at least as a marker to estimate the levels of oxidative DNA damage in target organs from the standpoint of public health.

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