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Changes in levels of 8-hydroxyguanine in DNA, its repair and *OGG1* mRNA in rat lungs after intratracheal administration of diesel exhaust particles

Yosuke Tsurudome[1,4], Takeshi Hirano[1], Hiroshi Yamato[2], Isamu Tanaka[2], Masaru Sagai[5], Hideyasu Hirano[3], Naoki Nagata[4], Hideaki Itoh[4] and Hiroshi Kasai[1,6]

[1]Department of Environmental Oncology and [2]Department of Environmental Health Engineering, Institute of Industrial Ecological Sciences and [3]Department of Biochemistry and [4]Department of Surgery 1, School of Medicine, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555 and [5]Research Team for Health Effects of Air Pollutants, National Institute of Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-0053, Japan

[6]To whom correspondence should be addressed
Email: h-kasai@med.uoeh-u.ac.jp

Diesel exhaust particles (DEP), an environmental pollutant, are known to induce lung cancer in experimental animals. To clarify whether reactive oxygen species (ROS) are involved in its carcinogenic mechanism, we examined the levels of 8-hydroxyguanine (8-OH-Gua), its total repair and the repair enzyme *OGG1* mRNA in female Fischer 344 rat lungs, as markers of the response to ROS, after DEP was intratracheally instilled. The 8-OH-Gua levels in both DEP-treated groups (2 and 4 mg) were increased during the 2–8 h following exposure to DEP. The 8-OH-Gua repair activities in the DEP-treated groups decreased during the period from 2 h to 2 days following DEP exposure and then recovered to the level of the control group at 5 days after exposure. *OGG1* mRNA was induced in rats treated with 4 mg DEP for 5–7 days after administration. In conclusion, the 8-OH-Gua level in rat lung DNA increases markedly at an early phase after DEP exposure, by the generation of ROS and the inhibition of 8-OH-Gua repair activity, and induction of *OGG1* mRNA is also a good marker of cellular oxidative stress during carcinogenesis.

Introduction

Higher industrial development and increased amounts of traffic have increased the levels of environmental pollutants, such as nitrogen dioxide (NO_2) and automobile exhaust particles (1). Diesel exhaust particles (DEP) are the exhaust emission of diesel engined vehicles (particularly trucks and buses) and are known to be associated with allergic, cytotoxic, mutagenic and carcinogenic properties. DEP contain a variety of mutagenic and carcinogenic chemicals, such as benzo[*a*]pyrene (B[*a*]P) (2,3), nitropyrenes (4) and 1,6- and 1,8-dinitropyrenes (DNP) (5). In animal studies, the incidence of lung cancer was significantly increased by direct injections of lung carcinogens, such as 1,6-DNP and B[*a*]P, contained in DEP in a dose-dependent manner (6). In addition, administration of DEP to mice or rats induced pulmonary neoplasms in the lung, including lymphomas, adenomas and adenocarcinomas (7,8), and reactive oxygen species (ROS) played a crucial role in the carcinogenic process (8–10). In these studies, the levels of 8-hydroxyguanine (8-OH-Gua) were found to be increased in the mouse lung DNA after the administration of DEP.

8-OH-Gua is believed to be a major form of oxidative DNA damage (11) and a useful marker of DNA oxidation (12) and causes mainly G·C→T·A transversions in *Escherichia coli* and mammalian cells (13–16). We have measured the levels of 8-OH-Gua produced by several carcinogens and lifestyle factors, such as potassium bromate (17), ferric nitrilotriacetate (18), cigarette smoking (19) and exercise (20), which are thought to generate ROS. Repair mechanisms for 8-OH-Gua have also been reported by several researchers. Enzymatic activities for removing 8-OH-Gua have been found in both bacterial and mammalian cells (21,22) and there might be several different repair mechanisms for 8-OH-Gua (23,24).

Although several reports have indicated a relationship between oxidative DNA damage and DEP carcinogenicity, there have been no reports of a relationship between 8-OH-Gua repair activity and DEP carcinogenicity. The genes for the human and mouse glycosylase-type repair enzymes for 8-OH-Gua (*hOGG1* and *mOGG1*) were recently cloned by several researchers (25–31). Therefore, we examined the induction of *OGG1* mRNA in the lungs of rats treated with DEP by a reverse transcription–polymerase chain reaction assay (RT–PCR) to obtain more detailed information about cellular oxidative stress during DEP-induced carcinogenesis.

In the present study, to clarify the carcinogenic mechanism of DEP we investigated the dose- and time-dependent changes in 8-OH-Gua, its repair and the repair protein *OGG1* mRNA level in rat lung after DEP exposure. This is the first report of *OGG1* mRNA induction in mammalian cells.

Abbreviations: B[*a*]P, benzo[*a*]pyrene; DEP, diesel exhaust particles; DNP, dinitropyrenes; 8-OH-Gua, 8-hydroxyguanine (7,8-dihydro-8-oxoguanine); ROS, reactive oxygen species; RT–PCR, reverse transcription–polymerase chain reaction.

Materials and methods

Animals and chemicals

Female Fisher 344 rats (Seiwa Experimental Animals, Fukuoka, Japan) of an initial age of 7 weeks were used. Water and diet were available *ad libitum*. They were housed for at least 1 week in a temperature and photoperiod controlled room (24°C, 12 h/day) before the DEP instillation experiments. The DNA Extractor WB Kit was purchased from Wako Biochemicals (Osaka, Japan). Nuclease P1 and acid phosphatase (type XA, P-1435) were from Sigma Chemical Co. (St Louis, MO). The [γ-^{32}P]ATP (sp. act. >5000 Ci/mmol) was purchased from Amersham (Little Chalfont, UK). Other chemicals were of the highest purity commercially available.

Preparation of DNA substrates

The oligonucleotide (5′-GGTGGCCTGACG*CATTCCCCAA-3′, where G* represents 8-OH-Gua) used for the endonuclease nicking assay was prepared by the method of Bodepudi *et al*. (32). The 5′-end was labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase and was annealed with its complementary strand to produce the double-stranded DNA substrate.

Preparation and administration of DEP

The DEP were supplied by the Research Team for Health Effects of Air Pollutants, National Institute of Environmental Studies, where they were prepared from a light duty (2740 cc), four cylinder diesel engine as described previously (10). The DEP were suspended in buffer (sterile 50 mM phosphate buffer, pH 7.4, containing 0.9% NaCl and 0.05% Tween 80). This suspension was sonicated for 5 min in an ultrasonic disrupter (model 250/450 sonifier;

Branson Ultrasonics, Danbury, CT) at 4°C. The dispersion of the DEP was confirmed using scanning electron microscopy. The particles had a polygonal-like shape with a size range of ~0.3–3 μm. Thus, it was confirmed that the DEP had an appropriate size for transfer into the respiratory tract or alveoli.

After complete anesthesia with diethylether, the DEP suspension was administered with an intratracheal cannula to female Fisher 344 rats, as described previously (6). The rats in the control group were injected intratracheally with 0.2 ml of vehicle buffer (n = 42). The rats in the DEP group were injected intratracheally with 0.2 ml of vehicle containing either 2 (n =42) or 4 mg DEP (n = 32). Although intratracheal instillation is non-physiological as compared with the inhalation mode, 2 or 4 mg instilled instantaneously delivers what would take many days of inhalation exposure (35). Non-treated rats of the same age were also prepared (n = 11). They were killed at 2 and 8 h and 1, 2, 5 and 7 days after administration of DEP. The lungs were removed and a portion of the tissue was frozen (–80°C) until 8-OH-Gua analysis. For the nicking assay and the RT–PCR analysis, the lung tissues were freshly processed as described below.

Determination of 8-OH-Gua level

The frozen rat lung tissues were thawed and homogenized in lysis buffer and the nuclear DNA in the homogenate was extracted using the DNA Extracter WB Kit (Wako), which contains NaI, an OH radical scavenger, by the previously described method (18,33). The extracted nuclear DNA was digested to deoxynucleosides and was analyzed with a HPLC system equipped with an electrochemical detector (18,34). The level of 8-OH-Gua in the DNA was expressed as the number of residues per 10^5 guanine.

Endonuclease nicking assay

After the intratracheal instillation of DEP, the rats were killed at each time point under diethylether anesthesia and the lungs were immediately excised, homogenized in buffer (50 mM Tris–HCl, pH 7.4, 50 mM KCl, 3 mM EDTA, 5 mM magnesium acetate and 3 mM β-mercaptoethanol) containing protease inhibitors (5 μg/ml each of antipain, chymostatin, leupeptin and pepstatin) and centrifuged at 12 000 *g*, as described previously (18). The supernatants (cell-free extracts) were frozen (–80°C) until the endonuclease assay. The ^{32}P-end-labeled double-stranded DNA substrate was incubated with the cell-free extract and the samples were electrophoresed on a polyacrylamide gel to analyze the cleavage of the DNA fragment at the 8-OH-Gua position. The raw repair activity was calculated as the ratio of the excised fragment activity to the total substrate (unexcised substrate activity plus excised fragment activity). In this report, the average of the raw repair activity data in the vehicle control at each time point is adjusted to 100% and each value of the repair activity in the DEP exposure groups is shown as a percentage of the control value.

Analysis of OGG1 *mRNA induction by RT–PCR*

Total RNA was prepared from fresh rat lungs and the mRNA was isolated on an oligo(dT)–cellulose column (Pharmacia Biotech AB, Uppsala, Sweden). The first strand cDNA was synthesized from mRNA primed with random hexamers using M-MLV reverse transcriptase (Gibco BRL, Grand Island, NY). Each cDNA was amplified using primers for the rat *OGG1* and *GAPDH* genes. The *GAPDH* mRNA was used as an internal standard. The primers for rat *OGG1* were designed from the consensus sequence of the human and mouse *OGG1* genes: 5′-ATCTGTTCCTCCAACAACAAC-3′ and 5′-GCCAGCATAAGGTCCCCACAG-3′. The primers for rat *GAPDH* were 5′-AACGGGAAGCTCACTGGCATG-3′ and 5′-TCCACCACCCTGTTGCTGTAG-3′. The amplification for rat *OGG1* consisted of 34 cycles at 94°C (60 s), 70°C (95 s) and 60°C (120 s). The PCR products were separated on a 5% polyacrylamide gel and were visualized with ethidium bromide staining.

Statistical analysis

All of the data are presented as the mean values of 5–11 independent measurements from different rats. The differences between each group were evaluated by applying the unpaired Student's *t*-test. All analyses were performed using the StatView-J 4.5 program (Berkeley, CA).

Results

8-OH-Gua levels in the lung DNA of DEP-administered rats

At the early phase of DEP exposure, the 8-OH-Gua levels in both DEP-treated groups increased as compared with that in the control group (Figure 1). Especially, the 8-OH-Gua levels at 2 h after exposure to 4 mg DEP were significantly higher than those in the control and 2 mg DEP-treated groups (the differences were statistically significant: $P < 0.0001$ and $P = 0.0043$, repectively). At this time point, we observed DEP phagocytosis by alveolar macrophages in the lung (data not shown). Moreover, the 8-OH-Gua levels were also significantly higher at 8 h after exposure to either 2 or 4 mg DEP than that in the control group (the differences were statistically significant: $P = 0.0218$ and $P = 0.0025$, repectively). Thereafter, the 8-OH-Gua levels in both DEP-treated groups decreased and there were almost no differences among all groups at 5 and 7 days after exposure (Figure 1).

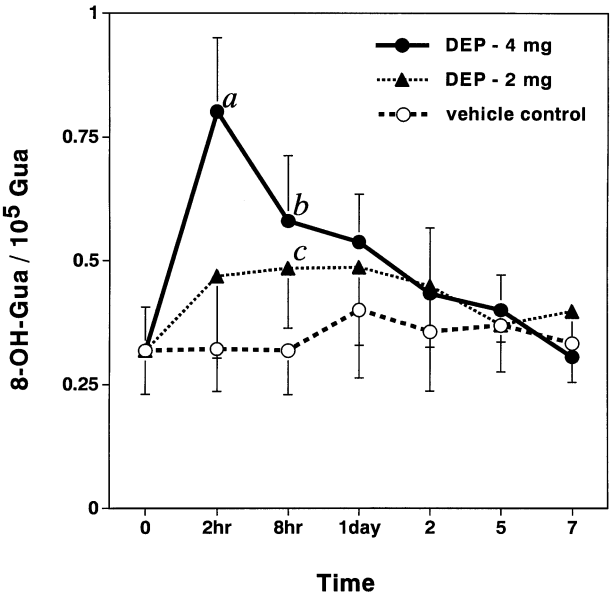

Fig. 1. Time course of 8-OH-Gua levels (mean ± SD) in rat lung DNA after DEP administration. [a]Significantly higher than vehicle control ($P < 0.0001$) and than 2 mg DEP-treated group ($P = 0.0043$). [b]Significantly higher than vehicle control ($P = 0.0025$). [c]Significantly higher than vehicle control ($P = 0.0218$).

8-OH-Gua repair activities in the lungs of DEP-treated rats

The time courses of the 8-OH-Gua repair activities are shown in Figure 2. In contrast to the 8-OH-Gua levels, the repair activities in the DEP-treated groups showed a tendency to be reduced as compared with that in the control group at an early stage (2 h), until 2 days after DEP administration. The repair activities of the groups treated with 2 and 4 mg DEP at 1 day were significantly lower than that of the control ($P = 0.0167$ and $P = 0.0169$, respectively). The repair activity of the group treated with 4 mg DEP was also significantly lower at 2 days ($P = 0.0216$). The 8-OH-Gua repair activities in both DEP-treated groups recovered by 5 days after exposure to DEP (Figure 2).

RT–PCR analysis of OGG1 *induction*

In order to determine the *OGG1* mRNA level, vehicle control, non-treated and 2 (after 5 days) and 4 mg DEP-treated (after 5 and 7 days) rats were used for RT–PCR analysis. Only small amounts of *OGG1* mRNA were detected in the vehicle control, the non-treated rats and the rat treated with 2 mg DEP, whereas it was clearly detected in the rats treated with 4 mg DEP at 5 and 7 days after administration of DEP (Figure 3A and B). Moreover, *OGG1* mRNA induction in the rats treated with 4 mg DEP was more pronounced at 7 than at 5 days after administration of DEP (Figure 3B).

Discussion

It has been reported that lung cancer in rats arises via an overload phenomenon in which chronic exposure to high levels

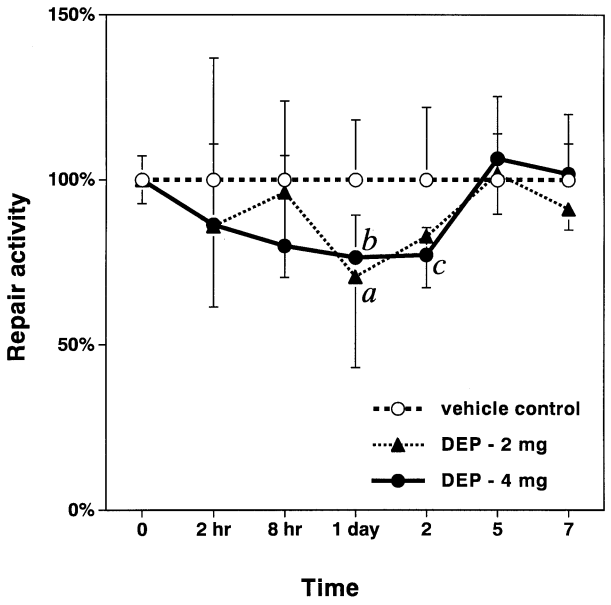

Fig. 2. Time course of repair activities for 8-OH-Gua (mean ± SD) in rat lung DNA after DEP administration. Each value of the repair activity in the DEP-exposed group is represented as a percentage of the vehicle control group at the same time point. [a]Significantly lower than vehicle control ($P = 0.0167$). [b]Significantly lower than vehicle control ($P = 0.0169$). [c]Significantly lower than vehicle control ($P = 0.0216$).

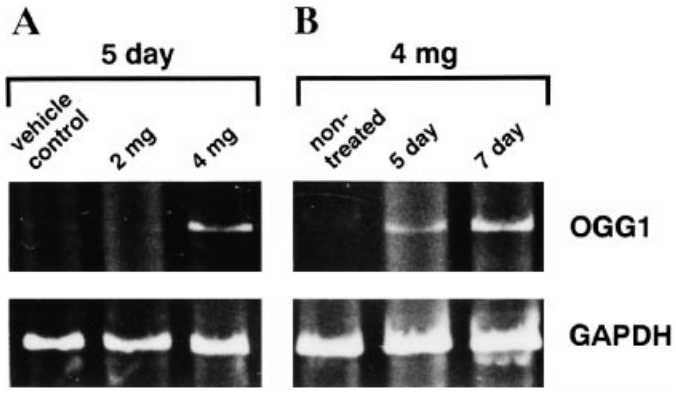

Fig. 3. *OGG1* mRNA levels in rat lung DNA after intratracheal instillation of DEP. (**A**) *OGG1* mRNA levels at 5 days after the instillation of vehicle control and DEP (2 and 4 mg). (**B**) *OGG1* mRNA levels of non-treated rats and at 5 and 7 days after DEP (4 mg) instillation.

of DEP overwhelm the normal clearance mechanisms (35) resulting in an influx of inflammatoy cells (36), structural alterations in the lung (37,38), release of cytokines (39,40), mutagenic changes (40) and development of lung cancer (41). Chronic exposure of rats to carbon black also produces lung cancer, suggesting that the carbonaceous particles (either carbon black or diesel soot) induce the cancer and that polyaromatic hydrocarbons in the diesel soot are not essential (38,40,42). Involvement of ROS in the pathogenesis of lung cancer by DEP and carbon black exposure in rats has been suggested (43). However, caution is needed in interpreting the findings in DEP-exposed rats with regard to human risk from DEP exposure (42,43).

In this paper we have examined the dose- and time-dependent changes in the levels of 8-OH-Gua, its repair and the induction of *OGG1* mRNA after an intratracheal instillation of DEP. The levels of 8-OH-Gua were significantly increased from 2 to 8 h and decreased to that of the control level by 5–7 days after DEP exposure (Figure 1). These results support the previous report that hydroxyl radicals produced by phagocytosis of DEP contribute to an increase in the 8-OH-Gua level (8,9). Indeed, the intake of a radical scavenger, β-carotene, reduced the level of 8-OH-Gua (8,9). In addition to analyzing 8-OH-Gua, it was also of interest to measure its repair, since both the 8-OH-Gua level and its repair are known to be induced in rat kidney and human leukocytes by oxidative stress (18,19). No previous reports, however, have described any changes in 8-OH-Gua repair activity associated with DEP carcinogenesis thus far.

In the present study we found that the repair activities for 8-OH-Gua decreased from 2 h to 2 days after exposure to DEP, as compared with that in the control group, and recovered to the level of the control group at 5 and 7 days after exposure (Figure 2). In addition, *OGG1* mRNA levels in the DEP-treated rats were enhanced as compared with those of the control or non-treated rats at 5–7 days after administration of DEP (Figure 3).

These results suggest that the inhibition of the 8-OH-Gua repair activity is, in part, responsible for the increase in 8-OH-Gua level in the early phase of DEP exposure. However, the mechanism of inhibition in this early phase is not clear. Since DEP contain several components, including mutagens and carcinogens, some of them might react with the OGG1 protein directly and may have affected repair activity. The other possibility is that some chemical agents in DEP inhibit the repair action. It is also known that exposure to DEP causes marked infiltration of inflammatory cells (44) and results in allergic rhinitis and allergic asthma by enhanced IgE production (45,46). The chemical mediators, such as histamines and prostaglandins, that are involved in these processes might affect the repair activity. On the other hand, we found that the repair enzyme *OGG1* mRNA was induced from 5 to 7 days after administration of DEP and a concomitant recovery of repair activity was observed during this period. However, the exact role of OGG1 in total 8-OH-Gua repair activity is still unclear, because the entire pathway of 8-OH-Gua repair is not yet understood completely.

In conclusion, we found that the 8-OH-Gua level in rat lung DNA increases after administration of DEP, partly by the generation of ROS and partly by the inhibition of repair activity for 8-OH-Gua. We also demonstrated that *OGG1* mRNA is a useful marker of cellular oxidative stress during carcinogenesis, in addition to the analyses of 8-OH-Gua levels in DNA and its repair activity.

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