Cell Proliferation in Liver of Mmh/Ogg1-deficient Mice Enhances Mutation Frequency because of the Presence of 8-Hydroxyguanine in DNA

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ABSTRACT

The Mmh/Ogg1 gene product maintains the integrity of the genome by removing the damaged base 8-hydroxyguanine (8-OH-G), one of the major DNA lesions generated by reactive oxygen species. Using Ogg1-deficient mice, we sought to establish if cells having high amounts of 8-OH-G have the ability to proliferate and whether the mutation frequency increases after proliferation in vivo. When KBrO₃, a known renal carcinogen, at a dose of 2 grams/liter was administered to Ogg1 mutant mice for 12 weeks, the amount of 8-OH-G in liver DNA from treated Ogg1⁻/⁻ mice increased 26.1 times that of treated Ogg1⁺/⁺ mice. The accumulated 8-OH-G did not decrease 4 weeks after cessation of KBrO₃ treatment. Partial hepatectomy was performed on Ogg1⁻/⁻ and Ogg1⁺/⁺ mice after being treated with KBrO₃ for 12 weeks. The remnant liver from Ogg1⁻/⁻ mice treated with KBrO₃ regenerated to the same extent as nontreated Ogg1⁺/⁺ mice. In addition, 8-OH-G was not repaired during cell proliferation by partial hepatectomy, indicating that there is no replication coupled repair of preexisting 8-OH-G. The mutation frequency after the regeneration of liver from treated Ogg1⁻/⁻ mice showed a 3.5-fold increase compared with before regeneration. This represents a mutation frequency 6.2 times that of normal levels. The proliferation of cells having accumulated amounts of 8-OH-G caused mainly GC→TA transversions. These results showed that inactivation of the Ogg1 gene leads to a higher risk of cancer because cells with accumulated 8-OH-G still retain the ability to proliferate, leading to an increase in the mutation frequency.

INTRODUCTION

Reactive oxygen species, which are generated endogenously by cellular oxygen metabolism or exogenously by ionizing radiation, environmental mutagens, and carcinogens produce many types of DNA damage, including base and sugar modifications, DNA–protein cross-linking, and strand breaks. 8-OH-G is a major form of oxidative DNA damage and believed to contribute to mutagenesis, carcinogenesis, and aging (1). To date, the majority of studies has focused on the effect of 8-OH-G in vitro or with tissue culture cells, which have shown, by using various DNA polymerases or shuttle vectors, that 8-OH-G primarily causes GC→TA transversions as a result of mispairing with an A base (2–10).

In the mammalian cell, the Mmh/Ogg1 gene encodes for a DNA glycosylase/AP lyase that has the capacity to excise 8-OH-G from DNA (11–17). The Ogg1 protein initiates the base excision repair pathway by recognizing and excising the oxidative DNA lesion. In addition to Ogg1, the mammalian cell contains two further enzymes, MYH and MTH, that contribute to the protection from 8-OH-G damage. MYH is a monofunctional glycosylase that removes a base that has mispaired with 8-OH-G (18). MTH is an 8-OH-dGTPase that degrades 8-OH-dGTP in the nucleotide pool, thereby preventing its incorporation into DNA (19). The importance of the 8-OH-G repair systems is reflected in the fact that homologues have been identified in various species from prokaryotes to eukaryotes.

With respect to involvement of the OGG1 gene in human cancer, significant effort has been made to link the inactivation of the OGG1 gene with cancer risk in clinical tumor samples (20–24). However, the data, such as somatic mutation, loss of heterozygosity, and polymorphism, are contradictory. Moreover, it remains to be firmly established that the accumulation of 8-OH-G causes carcinogenesis by using experimental animal models. In this study, we showed that 8-OH-G accumulated in liver DNA of Ogg1⁻/⁻ mice treated with KBrO₃, which is a known carcinogen and oxidative agent for rat kidney (25–28), and persisted in the same level after cessation of KBrO₃ treatment as in the case of kidney DNA as reported previously (29). Furthermore, after partial hepatectomy, liver with such oxidative DNA lesions regenerated normally without repair of 8-OH-G, followed by a considerable increase in the mutation frequency. These results indicated that there is no major replication coupled repair of preexisting 8-OH-G. Because induction of carcinogenesis must be coupled with cell proliferation, this system would be a good animal model for long-term carcinogenesis study to elucidate the involvement of 8-OH-G.

MATERIALS AND METHODS

Mice. The generation of Ogg1-deficient mice has been described elsewhere (30). The Ogg1⁺/⁺ mice, which were F1 hybrid of 129sv and C57BL/6J, were crossed with C57BL/6J or gpt transgenic mice of C57BL/6J background. The offspring were mated and Ogg1⁺/⁺, Ogg1⁻/⁻, gpt/Ogg1⁺/⁺, gpt/Ogg1⁻/⁻, and gpt/Ogg1⁻/⁻ mice were obtained. In the KBrO₃ treatment time course experiments, Ogg1⁺/⁺, Ogg1⁻/⁻, and Ogg1⁻/⁻ mice were used. For partial hepatectomy experiments, gpt/Ogg1⁺/⁺ and gpt/Ogg1⁻/⁻ mice were obtained by crossing gpt/Ogg1⁺/⁺ and Ogg1⁻/⁻ mice. Genotyping of the mice was performed by PCR analysis of DNA isolated from tail tips (29).

Time Course Experiment of KBrO₃ Treatment. Seven to 8-week-old Ogg1⁺/⁺, Ogg1⁻/⁻, and Ogg1⁻/⁻ mice were prepared. KBrO₃ was administered at a dose of 2 grams/liter in the drinking water to Ogg1 mutant mice. Three male and three female Ogg1 mutant mice of each genotype were killed at each time point of 1, 4, 8, and 12 weeks. Control mice were given water and killed at the same time point. A proportion of the Ogg1 mutant mice was given water 4 weeks after cessation of KBrO₃ treatment for 8 weeks and killed. The livers were excised from the sacrificed mice and stored at −80°C before measurement of the 8-OH-G levels.

Partial Hepatectomy Experiment of Mice Treated with KBrO₃. In this experiment, male gpt/Ogg1⁺/⁺ and gpt/Ogg1⁻/⁻ mice at the age of 7–8 weeks old were used. KBrO₃ was administered at a dose of 2 grams/liter to three mice of each genotype for 12 weeks. The mice were kept for 1 week after treatment with KBrO₃ before partial hepatectomy. Partial hepatectomy, involving the removal of the anterior and left lateral hepatic lobes, was performed by the procedure of Higgins and Anderson (31). At the time of partial hepatectomy, the resected liver was weighed, and this information was used to calculate the remnant liver weight for each mouse based on the assumption that the remnant liver = (the resected liver) × 3/7. All mice were killed 10 days later. On sacrifice, the remnant liver was weighed, and the proliferation ratio of the
remnant liver was calculated. The isolated liver was stored at −80°C before analysis of the 8-OH-G levels and mutation.

Measurement of the 8-OH-G Level. About 100–200 mg of liver were used for the measurement of 8-OH-G levels using an HPLC-electrochemical detector. Genomic DNA extraction, preparation of the sample, and measurement of 8-OH-G were performed as described previously (30).

**gpt Mutation Assay.** High molecular weight genomic DNA was extracted from 50–80 mg of liver using the RecoverEase DNA Isolation Kit (Stratagene). The gpt mutation assay was performed according to the procedure of Nohmi et al. (32). Briefly, extracted genomic DNA was packaged into λ phage. The phage was infected to *Escherichia coli* YG6020 and plated, and the sequence of the gpt gene from the resulting mutant colonies was obtained. To remove the possibility that mutational events might arise from clonal expansion, identical mutations occurring more than once in the same liver sample were counted as a single, independent mutation. The *gpt* mutation frequency was calculated by dividing the number of independent colonies resistant to Cm and 6-TG by the number of colonies resistant to Cm alone.

**Statistics.** The significance of differences in the measured parameters was evaluated by using the Student *t* test or ANOVA followed by Scheffe multiple comparison test.

**RESULTS**

The Ability of KBrO₃ to Produce 8-OH-G in Liver DNA. KBrO₃ is a known renal mutagen and carcinogen. It was unknown whether KBrO₃ can cause oxidative damage to DNA in other tissues, e.g., liver. The analysis of oxidative damage to liver DNA is important because liver is susceptible to oxidative stresses such as that are produced during xenobiotic metabolism. We measured the amount of 8-OH-G in liver DNA from *Ogg1*⁺/⁺, *Ogg1*⁻/⁻, and *Ogg1*⁻/+ mice to which KBrO₃ was administered at a dose of 2 grams/liter in the drinking water for 12 weeks. Three male and three female mice were analyzed at each time point. There was no observable histological difference in the livers of all groups in sections stained with H&E (data not shown).

The 8-OH-G levels of liver DNA from male and female mice within the same group were similar, shown together in Fig. 1. The amount of 8-OH-G in liver DNA from both control *Ogg1*⁺/⁺ and *Ogg1*⁻/⁻ mice was constant at 2.6 ± 10⁶ dG after 12 weeks. In control *Ogg1*⁺/⁺ mice, the amount increased proportionally with time, from 8 to 15.3 ± 10⁶ dG. At 12 weeks, equal to 19–20 weeks old, the 8-OH-G level of control *Ogg1*⁻/⁻ mice was 5.9 times that of control. These results are in good agreement with those of our previous study (30).

When *Ogg1* mutant mice were treated with KBrO₃, the amount of 8-OH-G in liver DNA from *Ogg1*⁺/⁺ and *Ogg1*⁻/⁻ mice increased during the first 4 weeks but thereafter remained mainly unchanged from 4 to 12 weeks. The averages were 3.7 and 5.1 ± 10⁶ dG, respectively. The absence of OGG1 enzyme caused a high accumulation of 8-OH-G in liver DNA from *Ogg1*⁻/⁻ mice treated with KBrO₃. The amount of 8-OH-G tremendously increased in proportion to time with KBrO₃ treatment and reached 96.6 ± 10⁶ dG. This represents a 37.2-fold increase over control *Ogg1*⁺/⁺ mice and 26.1-fold increase compared with KBrO₃-treated *Ogg1*⁺/+ mice. These results show that, in mice, KBrO₃ produces 8-OH-G in liver DNA, in addition to kidney DNA (29), and that the 8-OH-G level in liver DNA of *Ogg1*⁻/⁻ accumulates because of chronic oxidative stress.

**8-OH-G Levels in Liver DNA after Discontinuing KBrO₃ Treatment.** We investigated how the levels of 8-OH-G in liver DNA from *Ogg1* mutant mice may be affected after cessation of KBrO₃ treatment. *Ogg1*⁺/⁺, *Ogg1*⁻/⁻, and *Ogg1*⁻/+ mice were given 2 grams/liter KBrO₃ solution in the drinking water for 8 weeks; the treatment was discontinuing for 4 weeks before sacrifice and analysis of the mice (Fig. 2). Three male and three female mice were killed at each point. In *Ogg1*⁺/⁺ and *Ogg1*⁻/+ mice liver DNA, the amount of 8-OH-G decreased to control levels (2.9 and 3.3 ± 10⁶ dG, respectively). By contrast, the accumulated 8-OH-G (68.3 ± 10⁶ dG) in liver DNA from *Ogg1*⁻/⁻ mice did not change (65.6 ± 10⁶ dG) after the cessation of KBrO₃ treatment. It appears that the 8-OH-G accumulated in liver by chronic oxidative stress cannot be repaired in *Ogg1*⁻/⁻ mice. These results show that the major enzyme to repair 8-OH-G in liver is the *Ogg1* gene product, which is in good agreement with our previous observations in kidneys (29).

**Regeneration of *Ogg1* Mutant Mice Liver after Partial Hepatectomy.** The ability of cells to proliferate, after accumulating high amounts of 8-OH-G, was investigated by performing partial hepatectomy after KBrO₃ treatment. In addition, the levels of 8-OH-G and mutation frequency were taken after cell proliferation. *Ogg1*⁺/⁺ mice were used as control instead of *Ogg1*⁻/⁻ mice because only a slight difference exists in the 8-OH-G levels after KBrO₃ treatment, and in addition, similar to *Ogg1*⁺/⁺ mice, the 8-OH-G level in *Ogg1*⁻/⁻ mice liver decreased to control level after cessation of treatment with KBrO₃ (Figs. 1 and 2). Only male mice were used to remove the effect of sex differences on cell proliferation after partial hepatectomy. KBrO₃ was administered at a dose of 2 grams/liter in the drinking water to *Ogg1*⁻/⁻ and *Ogg1*⁺/⁺ mice for 12 weeks. Partial hepatectomy was performed 1 week after discontinuing treatment with KBrO₃. All mice were killed 10 days after the partial hepatectomy.
We did not find any histological difference in regenerated liver sections stained with H&E. Ogg1+/− and Ogg1−/− mice 10 days after partial hepatectomy (data not shown). The weight of the remnant liver from all mice increased 2.2–2.4 times after 10 days (Fig. 3). These results indicate that liver with high amounts of 8-OH-G can proliferate normally.

The Effect of Cell Proliferation on the Levels of 8-OH-G in Liver DNA. In control and KBrO3-treated Ogg1+/− mice, the amounts of 8-OH-G in liver resected at partial hepatectomy were the same (2.4 and 2.2/106 dG, respectively; Fig. 4). After liver regeneration, there were no differences in the 8-OH-G levels in the mice as compared with before liver regeneration. In control Ogg1+/− mice, the 8-OH-G level, at 16.4/106 dG, decreased to 10.6/106 dG after liver regeneration (P < 0.05). Furthermore, in Ogg1+/− mice treated with KBrO3, the 8-OH-G level, at 113.5/106 dG, decreased to 47.1/106 dG (P < 0.01). The decreases in 8-OH-G levels observed after liver regeneration were 64.6 and 41.5% in control and KBrO3-treated Ogg1+/− mice, respectively. It appears that the decreases are related to the amount of liver proliferation. These results show that, after cell proliferation, the accumulated 8-OH-G levels in liver are diluted by a factor of two, indicating that 8-OH-G is not removed during cell proliferation.

Mutation Frequency in Liver DNA after Partial Hepatectomy. To investigate whether high amounts of 8-OH-G in tissue DNA can cause mutation, we measured the mutation frequency of the transgenic gpt gene in gpt/Ogg1+/− and gpt/Ogg1−/− mouse liver (Table 1). In control Ogg1+/− mice, the mutation frequency showed no difference before or after liver regeneration. The mutation frequency of control Ogg1+/− mice seemed higher than that of control Ogg1+/− mice, in agreement with our previous result (30), and showed a tendency to increase after liver regeneration. When Ogg1+/− and Ogg1−/− mice were treated with KBrO3, we found no large increase in the mutation frequencies compared with each control. It is likely that KBrO3 produces no mutation in mouse liver during 12 weeks. In liver DNA from Ogg1−/− mice treated with KBrO3, the mutation frequency (10.1 × 10−6) substantially increased, 3.5 times (35.4 × 10−6), after liver regeneration over 10 days (P < 0.03), This mutation frequency was 6.2-fold that of control Ogg1+/− mice (P < 0.01). The results indicate that cell proliferation plays a pivotal role in fixing the mutation in tissue with a high accumulation of 8-OH-G.

Mutation Spectrum in Liver DNA after Partial Hepatectomy. The mutation spectrum in the gpt gene of mutant colonies is shown in Table 2. We found no difference in the mutation frequency and spectrum before and after liver regeneration in Ogg1+/− mice.

**DISCUSSION**

In this study, we showed that the renal carcinogen, KBrO3, when administered chronically to mice, results in increased levels over time of 8-OH-G in liver DNA of Ogg1+/− mice. The amounts of 8-OH-G in liver DNA were approximately one-fifth as that of kidney DNA of Ogg1+/− mice treated with KBrO3 as reported previously (29). However, the increase of 8-OH-G level in liver DNA of Ogg1−/− mice treated with KBrO3 was found to be still quite significant. In addition, the accumulated DNA lesions in liver DNA did not decrease even 4 weeks after cessation of KBrO3 treatment. These results indicated that OGG1 plays a major role in repair of the bulk of 8-OH-G in mouse liver. In spleen DNA, the accumulation of 8-OH-G was dependent on KBrO3 treatment (data not shown). Thus, it is very likely that OGG1 is involved in repair of 8-OH-G ubiquitously in a variety of mouse tissues.

To our knowledge, this is the first report to clarify whether a cell having a high accumulation of 8-OH-G possesses the ability to proliferate. Studies in yeast have shown that DNA polymerase δ replicates only ~10% of DNA containing 8-OH-G when compared with normal DNA.

**Fig. 3.** Regeneration of liver in Ogg1 mutant mice after partial hepatectomy. Partial hepatectomy was performed after KBrO3 was administered at a dose of 2 grams/liter to make gpt/Ogg1+/− and gpt/Ogg1−/− mice for 12 weeks. From a measurement of liver weight, the proliferation ratio of the remnant liver during liver regeneration was calculated. Values are mean and SD.

**Fig. 4.** The effect of cell proliferation on the levels of 8-OH-G in liver DNA. Partial hepatectomy was performed after KBrO3 was administered at a dose of 2 grams/liter to male gpt/Ogg1+/− and gpt/Ogg1−/− mice for 12 weeks. The amount of 8-OH-G before (open bars) and after (dotted bars) liver regeneration was analyzed in samples that were taken at the time of partial hepatectomy and in liver samples taken 10 days after partial hepatectomy. Values are mean and SD. +, P < 0.05; ++, P < 0.01.
the replication of undamaged DNA (4). Asagoshi et al. (33) investigated the translesion synthesis of 8-OH-G using E. Coli DNA polymerase I Klenow fragment. The translesion synthesis of 8-OH-G was slower than that of G because of the inefficiency of nucleotide incorporation opposite 8-OH-G and extension past the lesion. When single-stranded DNA containing 8-OH-G was transfected to COS7 cells, the recovery yield of replicated plasmid was 80% when compared with DNA containing G (7). A variety of genes has been reported that 8-OH-G blocks transcription by RNA polymerase II. These reports led us to the idea that proliferation may be inhibited in cells having high amounts of 8-OH-G. Such cells, we envisaged, would experience cell cycle arrest or undergo apoptosis as a mechanism to prevent cancer development. Therefore, we investigated the effect of 8-OH-G on cell proliferation by partial hepatectomy of Ogg1−/− mice treated with KBrO3. We showed that liver with a high amount of 8-OH-G can proliferate to the same extent as normal liver. Although it could be argued that rare cells with no accumulation of 8-OH-G were responsible for the proliferated tissue, we found no significant difference in the proliferation index of all liver samples examined 48 h after partial hepatectomy (data not shown). Furthermore, in comparison with liver samples taken at partial hepatectomy, after liver regeneration, significant increases in the mutation frequency were observed. The main mutation was GC→TA transversion, believed to be caused mainly by 8-OH-G.

Table 1  Mutation frequencies in liver cells from gpt/Ogg1−/− and gpt/Ogg1−/− mice which had undergone partial hepatectomy after treatment with KBrO3 for 12 weeks

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<tr>
<th>Mouse</th>
<th>Treatment</th>
<th>Ogg1 genotype</th>
<th>Liver regeneration</th>
<th>CmR colonies (×10^5)</th>
<th>6-TG^R and CmR colonies</th>
<th>Mutation frequency (×10^−6)</th>
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* Values are mean and SD.

Table 2  Mutation spectrum of gpt gene from livers of gpt/Ogg1−/− and gpt/Ogg1−/− mice which had undergone partial hepatectomy after treatment with KBrO3 for 12 weeks

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<tr>
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The number of colonies with independent mutations are shown. The number of total colonies are in parenthesis.

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ENHANCEMENT OF MUTATION BY CELL PROLIFERATION, 8-OH-G
ENHANCEMENT OF MUTATION BY CELL PROLIFERATION, 8-OH-G

Treated with KBrO3. Our results indicate that 8-OH-G mainly causes analyzing the mutation spectrum caused by 8-OH-G. We have succeeded in investigate the mutation spectrum caused by 8-OH-G. Difficulties in experiments compounds the need for the use of an animal model to 3 and GC 3 TA, GC 3 3 TA, GC 3 GC using a similar method. Kamiya et al. (9, 10) analyzed the mutation in foci obtained on transfection of the c-Ha-ras gene containing 8-OH-G to NIH3T3 cells and found GC→TA, GC→AT, and GC→CG. The fact that discrepancies exist in the tissue culture experiments compounds the need for the use of an animal model to investigate the mutation spectrum caused by 8-OH-G. Difficulties in determining the mutation spectrum generated by 8-OH-G in animals have arisen because oxidative stress reagents often produce many kinds of DNA lesions in addition to 8-OH-G. We have succeeded in analyzing the mutation spectrum caused by 8-OH-G, which accumulated in liver DNA after partial hepatectomy of Ogg1 mutant mice treated with KBrO3. Our results indicate that 8-OH-G mainly causes GC→TA transversion in an inactive gene after cell proliferation.

The activity of the OGG1 enzyme is inhibited by nitric oxide, which is associated with chronic inflammation (38). Indeed, it was reported that the 8-OH-G level in liver with chronic hepatitis was higher than that of normal liver (39). Chronic inflammation predisposes individuals to the development of carcinoma in the esophagus, gastric mucosa, pancreas, colon, and liver (40–42). From our results, it is predicted that 8-OH-G could continue to accumulate during inflammation followed by an increase in the mutation frequency after cell proliferation. We hypothesize that inactivation of the OGG1 enzyme by chronic inflammation could contribute to the risk of cancer. It is likely that the accumulation of 8-OH-G resulting from a deficiency of the Ogg1 gene or inactivation of the OGG1 enzyme could contribute to the initiation and promotion of cancer. For induction of cancer, the cells must be proliferated. To assess this possibility, we are planning to perform a long-term carcinogenesis test using partial hepatectomized Ogg1 mutant mice.

REFERENCES

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Cell Proliferation in Liver of Mmh/Ogg1-deficient Mice Enhances Mutation Frequency because of the Presence of 8-Hydroxyguanine in DNA


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