Available online at www.sciencedirect.com
SCIENCE DIRECT®
Mutation Research 523–524 (2003) 225–235
Fundamental and Molecular Mechanisms of Mutagenesis
www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Measurement of oxidative damage at individual gene levels by quantitative PCR using 8-hydroxyguanine glycosylase (OGG1)

Jinhee Choi [a], Dae-Yong Kim [b], Jin-Won Hyun [c], Sun-Hee Yoon [d], Eun-Mi Choi [e], Ki-Baik Hahm [f], Kwang-Ho Rhee [g], Myung-Hee Chung [d,*]

[a] *Faculty of Environmental Engineering, University of Seoul, College of Urban Science, Seoul, South Korea*
[b] *Department of Veterinary Pathology, Seoul National University, College of Veterinary Medicine, Suwon, South Korea*
[c] *Department of Biochemistry, Cheju National University, School of Medicine, Cheju, South Korea*
[d] *Department of Pharmacology, Seoul National University, College of Medicine, Seoul, South Korea*
[e] *Department of Chemistry, Incheon University, College of Natural Sciences, Incheon, South Korea*
[f] *Department of Gastroenterology, Aju University, School of Medicine, Suwon, South Korea*
[g] *Microbiology, Gyeongsang University, College of Medicine, Chinju, South Korea*

Received 15 April 2002; received in revised form 2 September 2002; accepted 9 September 2002

Abstract

In this study, an attempt was made to develop a method to estimate oxidative damage of individual genes for assessing chemopreventive potential of dietary or medicinal plants. Oxidative damage was investigated on the two genes in gastric mucosal tissue infected with *Helicobacter pylori*, which were genes of glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*), a house-keeping gene, and gene of insulin-like growth factor II receptor (*IGFIIR*), a gene known to be mutated frequently in gastric carcinoma. The oxidative damage in genomic DNA in the above tissue was confirmed by immunohistochemical study using monoclonal antibody to 8-hydroxyguanine (oh^{8}G), which showed much higher degree of staining in their nuclei. Using the method we developed, it was demonstrated that the number of oh^{8}G (indicated by 8-hydroxyguanine glycosylase (OGG1) sensitive sites) in *GAPDH* was almost not changed in *H. pylori*-infected tissue but in *IGFIIR*, it increased significantly. These results indicate that this method is valid for the estimate of oxidative damage of individual genes and also showed that the susceptibility of genomic DNA to attack of reactive oxygen species is not homogeneous but different depending upon the region of DNA. We expect to use this method in studies of carcinogenic mechanism and chemoprevention since it can provide more specific information pertaining to individual genes we are interested in.

Keywords: Gene-specific DNA damage; oh^{8}G; OGG1; *Helicobacter pylori*; *IGFIIR*; Carcinogenesis; Chemoprevention

1. Introduction

8-Hydroxyguanine (oh^{8}G; 8-oxoguanine) is easily formed in cellular DNA when organisms are exposed to reactive oxygen species (ROS). During DNA replication, oh^{8}G in DNA induces GC to TA transversion through mismatching with A instead of C [1]. Enzymes involving in repair of oh^{8}G are multiple,

Abbreviations: oh^{8}G, 8-hydroxyguanine; OGG1, 8-hydroxyguanine glycosylase; *IGFIIR*, insulin-like growth factor II receptor; *GAPDH*, glyceraldehydes-3-phosphate dehydrogenase; ROS, reactive oxygen species

* Corresponding author. Tel.: +82-2-740-8284; fax: +82-2-745-7996.
E-mail address: mhchung@snu.ac.kr (M.-H. Chung).

0027-5107/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0027-5107(02)00339-1

which are 8-hydroxyguanine DNA glycosylase to remove oh^{8}G residues from oh^{8}G:C [2], adenine DNA glycosylase to remove A from oh^{8}G:A [3] and 8-oxodGTPase to hydrolyze 8-oxodGTP formed in cytosol to 8-oxodGMP [4]. These facts imply that threat of oh^{8}G to genomic integrity is enormous and provide the background for its use as a marker for oxidative DNA damage. For this purpose, the amount of oh^{8}G residues has been measured in the genomic DNA isolated from tissues and used as an estimate of the oxidative damage of their DNA.

Mutations in cancers, however, tend to occur in gene-specific manner and mutations of this type have been frequently studied for diagnostic and prognostic purposes. Moreover, such mutations are also useful to elucidate the mechanistic basis of the carcinogenic processes [5–9]. In case of gastrointestinal cancers, particularly in gastric carcinoma, *P53* [10,11], insulin-like growth factor II receptor (*IGFIIR*) [12], transforming growth factor-β receptor type II (*TGFβRII*) [13] and *BAX* [14] are examples of genes that are frequently mutated. The gene-specific mutation suggests that genes have individual susceptibility to attack by various damaging agents. Under these circumstances, it is expected that the formation of oh^{8}G by ROS is not homogeneous throughout the genomic DNA but heterogeneous. If this is true, we should measure the amount of oh^{8}G in DNA not from total genomic DNA but from individual genes and then obtain oxidative damage pertaining to individual genes, which is much more informative and thus critically needed in many carcinogenic mechanism as well as evaluations of various chemopreventives. However, the method of this kind has not been available thus far.

In this study, an attempt was made to develop such a method, which can measure oh^{8}G content in any genes targeted. That was a quantitative PCR using the principle that the formation of PCR product is interfered with the cleavage of a DNA strand. Cleavage was induced at oh^{8}G sites by treating genomic DNA with oh^{8}G glycosylase (OGG1), which cleaves DNA at oh^{8}G residues after removing the oh^{8}G residue as a free base from the DNA strand [15–17]. The content of oh^{8}G in each gene was estimated by comparing quantitative difference between the PCR products of the targeted gene before and after treatment of genomic DNA with OGG1. This method was applied to the assessment of oxidative damage of *IGFIIR*, a gene mutated frequently in gastric carcinoma and gene of glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*), a house-keeping gene in *H. pylori*-infected gastric mucosa. The formation of oh^{8}G in genomic DNA of *H. pylori*-positive gastric mucosal tissue was confirmed by immunohistochemical staining using monoclonal antibody to oh^{8}G. However, its formation appears to be heterogeneous throughout the genome since our method demonstrates that formation of oh^{8}G was significant on *IGFIIR* but almost negligible on *GAPDH*.

2. Materials and methods

2.1. Endoscopic biopsy and diagnostic tests for H. pylori

Selection of volunteers, endoscopic biopsy and various laboratory tests for *H. pylori* were performed as described in our previous study [18]. Briefly, the volunteers were recruited from young people (age of 21–23 years) that had undergone endoscopy between October 1999 and March 2000, at the Department of Internal Medicine of Gyeongsang National University Hospital, due to abdominal complaints. The same endoscopist took seven pieces of biopsy specimens of the gastric antrum and serum from each volunteer. Two specimens were used for microbial tests (culture and urease test) and two for routine histology staining and Warthin–Starry silver staining. The remaining three samples were immediately frozen and kept at −70 °C until use. Sera were used to detect anti-*H. pylori* antibody by immunoblot analysis [19]. The biopsy samples were used for immunohistochemical study to detect oh^{8}G in genomic DNA and for the quantitative PCR to measure oh^{8}G in individual genes.

2.2. Potassium bromate-treated rat kidney and liver tissues

To test the validity and applicability of the immunohistochemical staining used to detect oh^{8}G in the DNA of human gastric mucosal cells, the staining method was applied to kidney and liver tissues of rats treated with potassium bromate ($KBrO_3$, Sigma, St. Louis,

MO, USA) as below. This chemical is known to cause oxidative damage, mainly to the kidney [20]. Twelve male 5-week-old F-344 rats were reared on standard food pellets and tap water ad libitum in a specific pathogen free environment with a 12 h light/dark cycle for 2 weeks. They were grouped into three groups and each group was given a single intragastric administration of $KBrO_3$ at doses of 0, 80 or 400 mg/kg body weight in 0.9% saline as described previously [20]. At 24 h after treatment, the rats were exsanguinated under ether anesthesia. Kidneys, the target organ and livers, the controls were dissected, fixed overnight in 70% ethanol at 4 °C and embedded in paraffin.

2.3. Immunohistochemical procedure

Cryosections of frozen human gastric mucosa specimens and paraffin embedded tissue sections of rat liver and kidney (5 μm each) were placed on poly-L-lysine coated slides and fixed in 80% ethanol. Immunohistochemistry was performed according to the procedures described in our previous study [21] with some modifications. Monoclonal anti-oh^{8}G antibody was prepared in National Research Laboratory of Free Radicals (Seoul, South Korea). The sections were sequentially treated with 0.3% H_2O_2 in phosphate-buffered saline (PBS) for 30 min (to quench endogeneous peroxidase activity), 50 μg/ml RNase for 1 h at 37 °C (to exclude interference effects of oxidative RNA products), and 70 mM of NaOH in 40% ethanol for 30 s (to denature DNA), and then incubated in 10% normal goat serum in PBS for 1 h (to saturate non-specific binding sites). Sections were then incubated with diluted mouse monoclonal anti-oh^{8}G antibody (1:50) overnight at 4 °C. To visualize the antigen, the standard avidium–biotin–peroxidase complex method was used according to the manufacturer's instructions (Dako, Copenhagen, Denmark) using 3,3-diaminobenzidine (Sigma) as a chromogen. The specificity of the primary antibody was confirmed by the omission of the primary antibody, the substitution of normal mouse serum for the primary antibody and the preincubation of primary antibody with oh^{8}G (Sigma) (10 mg/ml of diluted antibody), for 24 h before being applied to tissue sections. As a result, oh^{8}G immunoreactivity disappeared completely in tissue.

2.4. OGG1 preparation and the treatment of genomic DNA with OGG1

OGG1 was prepared using OGG1 cDNA synthesized from the mRNA of mouse (R1 strain of Senescence Accelerated Mice) [22] liver by RT-PCR, as described in the previous study [23]. DNA was isolated from the human gastric mucosa specimens using a QIAamp® DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Two DNA samples (50 ng each) were prepared and one DNA sample was treated with 1 unit of OGG1 at 37 °C for 1 h in 10 μl of 50 mM KCl, 1 mM EDTA and 50 mM Tris–HCl, pH 7.5. Under this reaction condition, all sites of oh^{8}G in 50 ng DNA could be cleaved. One unit of this enzyme was defined as the activity to cleave 1 pmol of the substrate DNA in 15 min [24]. The other sample was given no OGG1 treatment. The two DNAs treated and untreated with OGG1 were then subjected to PCR amplification directed towards the two genes, *IGFIIR* and *GAPDH*.

2.5. Quantitative PCR conditions

Expand Long Template PCR System (Roche, Manheim, Germany) was used for the PCR analysis. The reaction solution (50 μl) contained either of the DNAs (50 ng), primers (300 nM), *Tac* and proofreading DNA polymerase (2.6 U), dNTP (350 μM) and $MgCl_2$ (1.75 mM). Quantitative PCR conditions were optimized after performing cycle number and DNA template tests. PCR was carried out in a TECHNE Touchgene gradient thermal cycler (Techne, Cambridge, UK). The oligonucleotides used as primers for the both genes examined, were as follows (numbers in parentheses indicate the lengths of the resulting PCR products): 5′-TCG TGA GTG CCT TCC CAG TCC ACC C-3′, 5′-GCC GCC AGG TTC CAA CTC TCA AGC C-3′ for *IGFIIR* (2867 bp) and 5′-AAC AGG AGG TCC CTA CTC CCG CCC G-3′, 5′-TCT GGG TGG CAG TGA TGG CAT GGA C-3′ for *GAPDH* (2519 bp). DNA untreated or treated with OGG1 was used for PCR, which was programmed to operate sequentially with a denaturation step of 94 °C/5 min followed by 25 (for *GAPDH*) and 30 (for *IGFIIR*) reaction cycles of 94 °C/10 s and 68 °C/3 min, plus a final extension step of 72 °C/10 min. The optimal condition for both genes was determined from the

experimental results shown in Fig. 3. After PCR, the PCR reaction mixtures were stored at 4 °C until required.

2.6. Electrophoretic quantification of PCR products

Each PCR reaction was mixed with 5 μl of the 6× loading buffer (Promega, Madison, WI, USA), loaded on 0.7% agarose gel and electrophoresed at 100 V for 30 min in a 0.5× TBE buffer. PCR products were identified using a UV detector and photographed. The densities of the product bands were determined using a densitometer (MCID, Imaging Research, Ont., Canada) and used to quantify the number of oh^{8}Gs in the genomic DNA strand.

2.7. Quantification of oxidative DNA damage in each gene

The nicks by OGG1 at oh^{8}G residues interfere with PCR and thus the difference in the amounts of PCR products of a targeted region of DNA provides as indication of the extent of oxidative damage to that DNA region. Based on this principle, oxidative DNA damage in the region of interest of each gene was quantified in terms of the frequencies of oh^{8}G in DNA by using the difference between the amounts of PCR products obtained before and after OGG1 treatment. According to Poisson equation, the average lesion frequency per genomic DNA strand was determined using $\lambda = -\ln A_D/A_O$, where A_D is the band density of the PCR product obtained from the OGG1-treated DNA, and A_O is the band density of the PCR product obtained from the untreated DNA [25,26]. Statistical analysis was performed using the unpaired Student's *t*-test.

3. Results

3.1. Bacteriological, serological and histopathological data of biopsy samples of human gastric mucosa

The gastric biopsy samples were diagnosed according to the diagnosis of volunteers and used for verifying oxidative DNA damage due to *H. pylori* infection by immunohistochemical staining of oh^{8}G in the nuclei of mucosal tissues and investigating the heterogeneous distribution of this adduct in the genome. Twenty volunteers were examined for *H. pylori* infection. A volunteer was regarded as *H. pylori*-positive when the samples taken together showed positive results on all tests of bacterial culture, urease, immunoblot analysis for anti-*H. pylori* antibody and histopathology. If a volunteer showed negative results on these tests, he/she was regarded as *H. pylori*-negative. The results are summarized in Table 1. Ten volunteers proved to be negative and others to be positive.

Table 1
Bacteriological, serological and histopathological data of *H. pylori*-positive and -negative volunteers

	H. pylori-positive group	*H. pylori*-negative group
No. of volunteer	10	10
Male/female	5/5	5/5
Median age in years (range)	21 (20–23)	22 (20–23)
Culture	+	−
Urease test	+	−
Immunoblot analysis	+	−
Histology[a] (grades 0/1/2/3)		
Lymphocytic infiltration	0/0/1/9	0/10/0/0
Neutrophilic infiltration	0/3/4/3	10/0/0/0
H. pylori density	0/1/5/4	10/0/0/0

As described in Section 2, seven biopsy samples were taken from one volunteer. Two samples were used for culture and urease test within 1 h of collection and two for routine histological staining and Warthin–Starry silver stainings. The remaining three were used for the oxidative DNA damage study. The remaining samples were described as *H. pylori*-positive if taken from a positive volunteer or *H. pylori*-negative if taken from a negative volunteer. As shown in the table, 10 volunteers were *H. pylori*-positive and 10 were *H. pylori*-negative.

[a] Grade 0, absent; grade 1, mild; grade 2, moderate; grade 3, severe (graded according to the Sydney System).

3.2. Immunohistochemical staining of oh^{8}G in H. pylori-positive gastric mucosa

The formation of oh^{8}G in genomic DNA by *H. pylori* infection was tested by comparing the immunohistochemical staining using monoclonal antibody to oh^{8}G of *H. pylori*-positive and -negative biopsy samples. Fig. 1 shows a representative immunohistochemical staining pattern of oh^{8}G in *H. pylori*-negative and

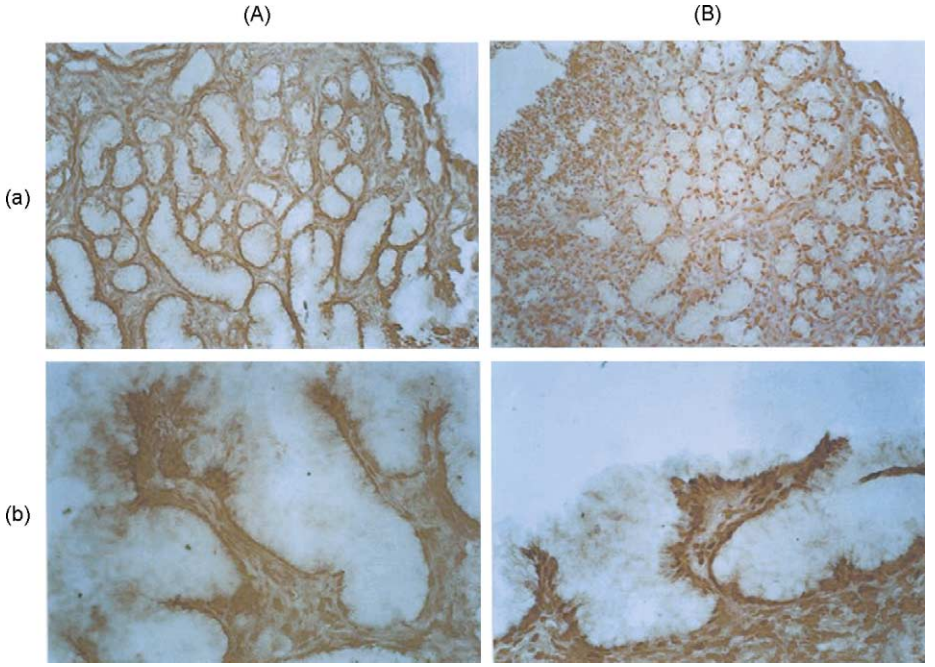

Fig. 1. Immunohistochemical staining of human gastric mucosa. Monoclonal mouse anti-oh^{8}G antibody was used. (A and B) *H. pylori*-positive and -negative specimens, respectively, and (a and b) 200× and 400× magnification, respectively.

-positive human gastric mucosa. In *H. pylori*-positive mucosa (Fig. 1B), the nuclei of the glandular epithelial cells were stained very strongly, whereas no significant staining was observed in *H. pylori*-negative nuclei (Fig. 1A). *H. pylori*-positive mucosa showed infiltrating inflammatory cells whose nuclei were also stained to almost the same degree as the epithelial cell nuclei (Panel Ba in Fig. 1). The staining observed in *H. pylori*-positive samples was abolished by the omission of the primary antibody, the substitution of normal mouse serum for the primary antibody or the preincubation of the primary antibody with oh^{8}G (data not shown). The result demonstrates the formation of oh^{8}G at genomic DNA level in *H. pylori*-infected gastric mucosa.

3.3. Test for validity of immunohistochemical staining as an indication for oh^{8}G in genomic DNA

The validity of the immunohistochemical staining observed in *H. pylori*-positive mucosal cells as an indication of oxidative DNA damage was further tested by applying this staining method to the kidney tissues of rat treated with $KBrO_3$. $KBrO_3$ has been shown to induce oxidative stress mainly to the kidney [20], thus the formation of oh^{8}G in $KBrO_3$-treated rats should be higher in the kidney than the other organs. Fig. 2 shows representative stained tissue sections of the kidneys (Fig. 2B) and livers (Fig. 2A) of $KBrO_3$-treated and -untreated rats. Kidney sections of the $KBrO_3$-treated rats (Panels Bb and Bc in Fig. 2) showed that the staining was exclusively located in the nuclei of tubular cells and was $KBrO_3$ dose-dependent. Staining in the untreated rat kidney (Panel Ba in Fig. 2) was almost negligible. In contrast to the kidney, the liver (Fig. 2A) showed a much weaker staining pattern at each $KBrO_3$ dose. Nuclear staining in the kidney was abolished by preincubating the primary antibody with oh^{8}G and substituting normal mouse serum for the primary antibody (data not shown). The results obtained from the $KBrO_3$-treated rats indicate that immunohistochemical staining is valid as a method to detect oh^{8}G in DNA in various tissues under oxidative stress including *H. pylori*-positive gastric mucosa.

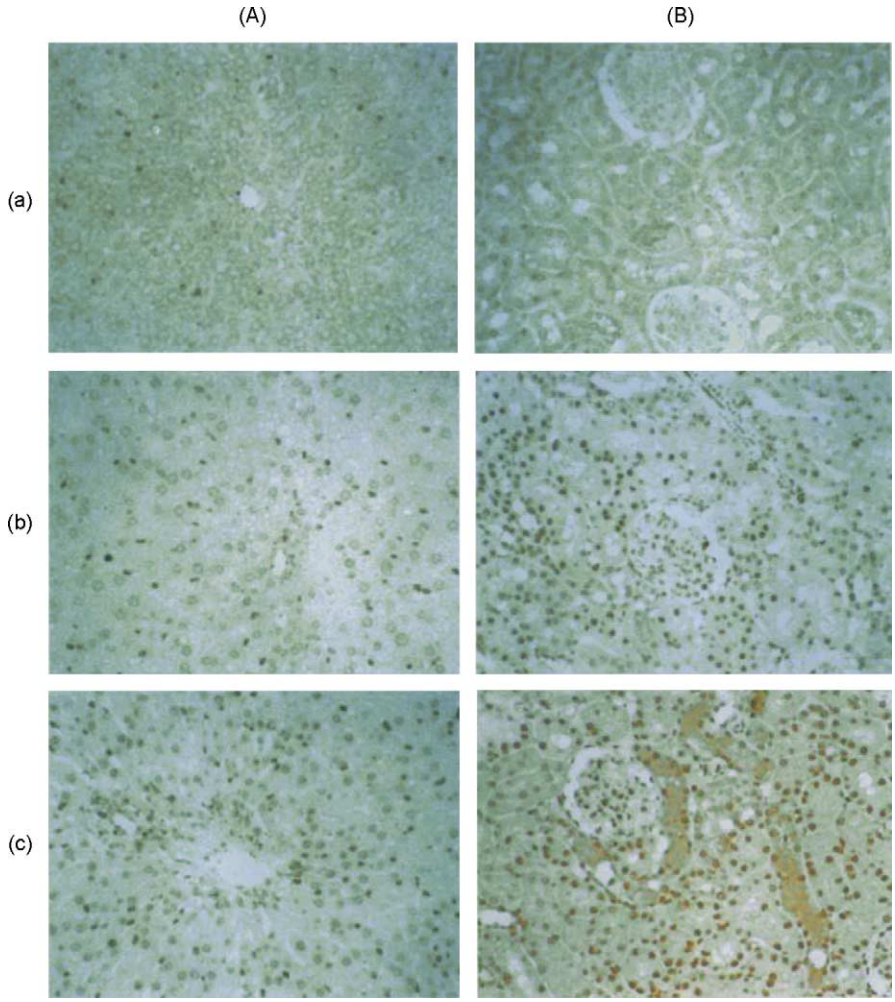

Fig. 2. Immunohistochemical staining of the kidneys and livers of rats treated with $KBrO_3$. Rats were given 0 mg/kg (a), 80 mg/kg (b) and 400 mg/kg (c) of $KBrO_3$ i.p. Livers (A) and kidneys (B) were removed 24 h after treatment and stained with monoclonal mouse anti-oh^{8}G antibody.

3.4. Optimal conditions for the measurement of oxidative DNA damage at the individual gene level

We have shown that *H. pylori* infection causes oxidative DNA damage in gastric mucosal cells by immunohistochemical detection of oh^{8}G (Fig. 1). Next step was to measure the *H. pylori*-induced DNA damage at individual gene levels. Before doing this, we tried to find out two crucial conditions required for the accuracy of this method. One is the amount of OGG1, which can nick completely all oh^{8}G sites present in the DNA used for PCR. The calculation[1] indicates that 50 ng of genomic DNA used in the quantitative PCR

[1] According to a previous report, the amount of oh^{8}G in *H. pylori*-positive mucosa is $12.7 \pm 1.9/10^5$ G (normal guanine). Assuming that the level of oh^{8}G in the *H. pylori*-positive mucosa was $30/10^5$ G, which doubles the mean value, 50 ng of the DNA from *H. pylori*-positive mucosa would contain about 3.75 pg of oh^{8}G [50 ng × 1/4 (assuming that one of the four bases is G) × $30/10^5$ (assuming that molecular weights of G and oh^{8}G are equal)], which gives about 0.01 pmol of oh^{8}G (assuming that the molecular weight of oh^{8}G is about 400). Therefore, the capacity of 1 unit of OGG1 for 1 h incubation is 400 times the maximal amount (0.01 pmol) of oh^{8}G present in 50 ng of genomic DNA.

contains 0.01 pmol of oh^{8}G and that the treatment of this genomic DNA (50 ng) with 1 unit of OGG1 for 1 h can cleave all oh^{8}G site present in 50 ng of DNA. One unit of this enzyme was defined as the activity to cleave 1 pmol of the substrate DNA in 15 min and thus the capacity to cleave 4 pmol during 1 h incubation, which is in far excess over 0.01 pmol of oh^{8}G present in 50 ng of DNA. Another crucial condition is the cycle number of PCR and template DNA quantity, which can give a constant and reproducible amount of product in each PCR. To determine the optimal values for these two parameters, the kinetics of PCR product formation were studied for the candidate genes versus cycle number (Fig. 3A) and the amount of template DNA (Fig. 3B). As can be seen in Fig. 3, *IGFIIR* and *GAPDH* showed exponential kinetics when the cycle number ranged between 25 and 30. A linear relationship between the PCR yield and the quantity of template DNA used (from 20 to 200 ng) was observed for both genes. Thus, the conditions for the oxidative damage assay of both genes were fixed as follows: the amount of DNA used for PCR was 50 ng; 1 unit of OGG1 was used for 1 h; and PCR cycles was 30 for *IGFIIR* and 25 for *GAPDH*.

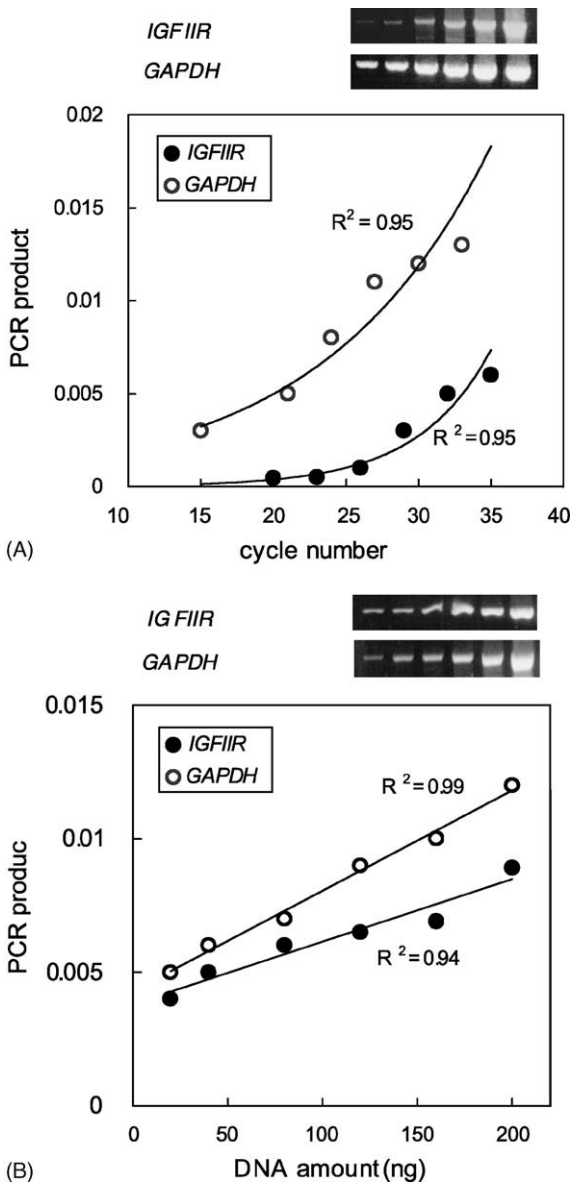

Fig. 3. Effects of cycle number (A) and the DNA template (B) on the amount of product of *GAPDH* and *IGFIIR* in the quantitative PCR. PCR product was expressed in electrophoretic band density determined by densitometry. The amounts of DNA template used varied from 20 to 200 ng and the numbers of PCR cycles varied from 15 to 33 for *GAPDH* and from 20 to 35 for *IGFIIR*.

3.5. Heterogeneous oxidative DNA damage in *H. pylori* infection

The quantitative PCR was applied to *IGFIIR* and *GAPDH* of *H. pylori*-positive gastric mucosa. Fig. 4A shows electrophoretic comparisons of the amounts of amplified PCR products obtained from *IGFIIR* and *GAPDH* before and after OGG1 treatment. *IGFIIR* showed electrophoretic band density differences in seven of the *H. pylori*-positive samples, whereas four samples proved different in *H. pylori*-negative samples. On the other hand, regardless of the states of *H. pylori* infection, little difference was found before and after OGG1 treatment on *GAPDH*. In each sample, difference in the amounts of the PCR products of each gene were quantified as OGG1 sensitive sites/kb using Poisson equation [25,26] and they were significantly different, for *IGFIIR*, between the *H. pylori*-positive and -negative groups, but not for *GAPDH* (Fig. 4B).

4. Discussion

In this study, we developed a method, which can estimate oxidative damage of individual genes by measuring the formation of oh^{8}G at any genes targeted. This can be quantified using the difference in the

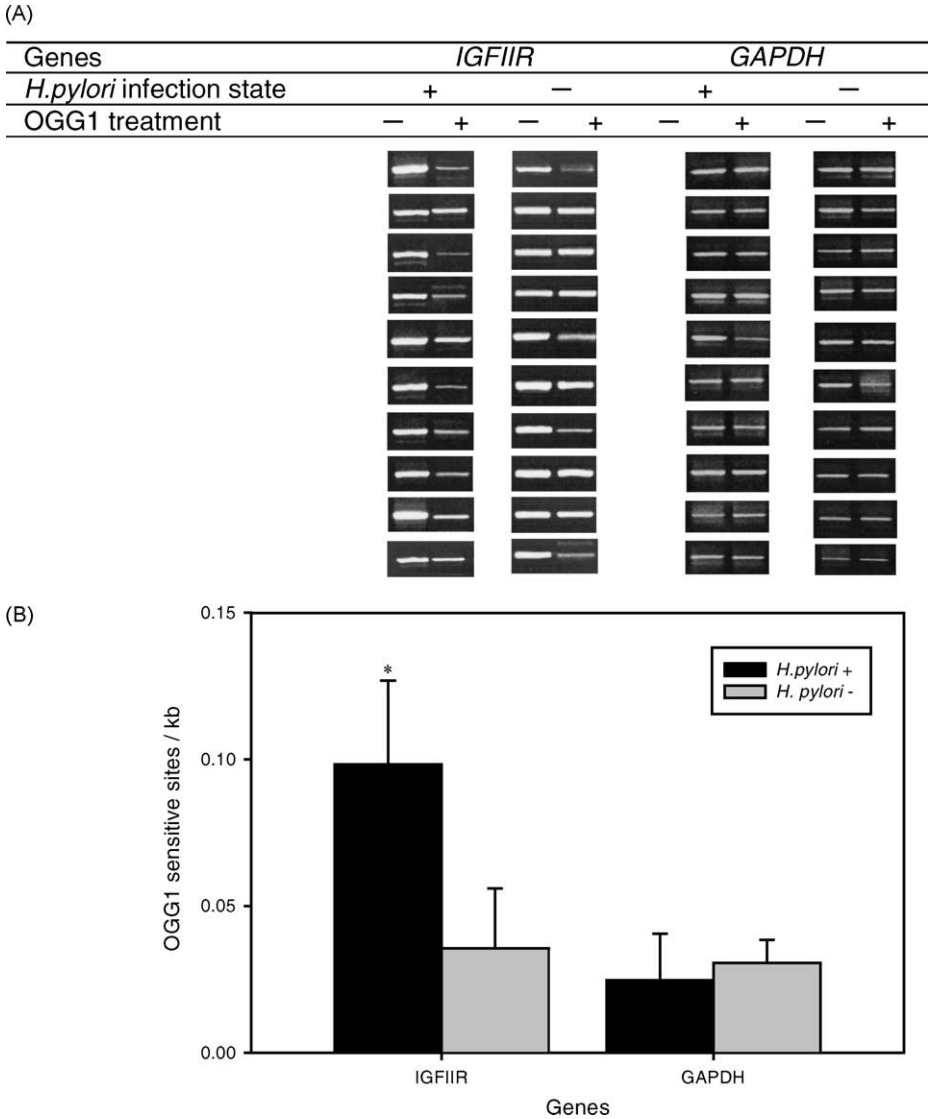

Fig. 4. Electrophoretic detection of the PCR products of each gene before and after the DNA isolated from individual biopsy samples was treated with OGG1. Oxidative DNA damage to *IGFIIR* and *GAPDH* was measured, using the paired band densities of biopsy sample: (A) electrophoresis of PCR products; (B) lesion frequency in *IGFIIR* and in *GAPDH*. (∗) Significantly different from the *H. pylori*-negative group ($P < 0.05$).

amount of PCR products of the targeted region of DNA obtained before and after the treatment of genomic DNA with OGG1. Using this method, it was found that *GAPDH*, a house-keeping gene was not attacked by ROS as indicated by no increase in the formation of oh^{8}G in *H. pylori*-positive gastric mucosa (Fig. 4), whereas *IGFIIR*, a gene mutated frequently in gastric carcinoma was attacked by ROS, as shown by the increase in formation of this adduct in the same gastric tissue (Fig. 4). These results indicate that oxidative DNA damage does not occur homogeneously but heterogeneously, since the immunohistochemical staining

using the monoclonal antibody to oh^{8}G showed that genomic DNA in gastric tissue was attacked by ROS generated by *H. pylori* infection (Fig. 1). Now, it is possible to assess the oxidative DNA damage at individual gene levels and thus, to obtain the information that may be crucial in the field of carcinogenesis and chemoprevention.

The frequent mutation observed in *IGFIIR* was found to be due to microsatellite (oligonucleotide repeat sequence) instability, which is considered a mutator phenotype that results from insufficient mismatch repair [11–14,27]. In the present study, oxidative attack to *IGFIIR* was observed in regions not containing repeat sequences, because the PCR was designed to avoid these repeat sequences. Therefore, in addition to microsatellite instability, frequent oxidative damage may also contribute to frequent mutation in this gene. However, the reasons for this frequent oxidative attack on *IGFIIR* are unclear. The extent of damage and repair in certain regions of DNA appears to be influenced by several factors, for example, the nucleotide sequence, the transcriptional or replicative state, the nature of the damaging agents and the type of lesion [28–33]. *IGFIIR* is known to function as a cell growth suppressor by activating transforming growth factor-β1 (TGFβ1), a potent growth inhibitor, or by degrading insulin-like growth factor II (IGFII), a potent growth stimulant [34–37]. ROS attack cellular macromolecules and impair various cell functions. In stressful conditions, cells are generally in the state of being non-proliferating rather than proliferating as indicated by the finding that cells are arrested at a certain phase of cell cycle, for example, G1, when subject to noxious stimuli such as irradiation [38] and at the same time, suppressor genes are activated for preparedness of this state [39]. *IGFIIR* as a suppressor gene may be a gene to be recruited for this purpose and thus rendered susceptible to ROS attack. The frequent exposure of this gene to ROS attack eventually links to its frequent mutation, which is expected to increase the likelihood of uncontrolled cell growth and contribute to the carcinogenesis of *H. pylori*-infected gastric mucosa.

There are a number of shortcomings in the present study, which should be mentioned. First, the stomach lesions were multi-focal in nature and each biopsy specimen was taken from a different site. For this reason, the biopsy specimens used for the oh^{8}G examinations and those for the diagnostic laboratory tests were unavoidably different, which suggests that there is a possibility that *H. pylori*-negative samples might exist in the *H. pylori*-positive group and vice versa. In Fig. 4A, four *H. pylori*-negative samples showing different band densities or three *H. pylori*-positive samples showing negligible differences may provide examples of this limitation. Another problem encountered concerned the size determination of the PCR products. Naturally, the longer the piece of DNA within the gene of interest amplified, the more correct the estimation of the damage on the gene of interest. However, the longer strands tend to be associated with fluctuations in the amounts of PCR products obtained. We examined various PCR product sizes up to several thousand bp and found that a size of about 2000 bp was satisfactory in terms of producing reproducible amounts of PCR products and oxidative damage detection sensitivity. Lastly, oh^{8}G formation, expressed as OGG1 sensitive sites, might be over-estimated by additional DNA damage other than that caused by oh^{8}G, or to the apoptotic degradation of DNA in infected tissues [40], which could block the progression of thermostable polymerase during the PCR. However, even with such limitations, this method was found capable of detecting susceptible genes since significant differences were found in oxidative DNA damage of *IGFIIR*, whereas no significant differences were found in *GAPDH*.

In conclusion, we developed a quantitative PCR, which can measure the oxidative damage at individual gene levels as OGG1 sensitive sites, i.e. oh^{8}G residues. Using this method, we demonstrate that the oxidative damage in genomic DNA is not homogeneous but heterogeneous. Therefore, this method will be useful in studies of carcinogenic mechanism and in the evaluation of chemopreventive potential of various dietary or medicinal plants, since it can provide more specific information to pertaining to individual carcinogens and anticarcinogens.

Acknowledgements

This work was supported by a grant from the Ministry of Science and Technology of Korea, through the National Research Laboratory Program for Free Radicals.

References

[1] S. Shibutani, M. Takeshita, A.P. Grollman, Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG, Nature 349 (1991) 431–434.

[2] J. Tchou, H. Kasai, S. Shibutani, M.H. Chung, J. Laval, A.P. Grollman, S. Nishimura, 8-Oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 4690–4694.

[3] M.L. Michaels, J. Tchou, A.P. Grollman, J.H. Miller, A repair system for 8-oxo-7,8-dihydrodeoxyguanine, Biochemistry 31 (1992) 10964–10968.

[4] H. Maki, M. Sekiguchi, MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis, Nature 355 (1992) 273–275.

[5] B. Vogelstein, K.W. Kinzler, The multistep nature of cancer, Trends Genet. 9 (1993) 138–141.

[6] L.A. Loeb, Cancer cells exhibit a mutator phenotype, Adv. Cancer Res. 72 (1998) 25–56.

[7] K.R. Loeb, L.A. Loeb, Significance of multiple mutations in cancer, Carcinogenesis 21 (2000) 379–385.

[8] Y. Dai, Y. Morishita, K. Mase, N. Sato, E. Akaogi, T. Mitsui, M. Noguchi, Application of the p53 and K-ras gene mutation patterns for cytologic diagnosis of recurrent lung carcinomas, Cancer 90 (2000) 258–263.

[9] S.W. Kim, K.H. Her, J.Y. Jang, W.H. Kim, Y.T. Kim, Y.H. Park, K-ras oncogene mutation in cancer and precancerous lesions of the gallbladder, J. Surg. Oncol. 75 (2000) 246–251.

[10] T. Matozaki, C. Sakamoto, K. Matsuda, T. Suzuki, Y. Konda, O. Nakano, K. Wada, T. Uchida, H. Nishisaki, M. Nagao, M. Kasuga, Missense mutations and a deletion of the p53 gene in human gastric cancer, Biochem. Biophys. Res. Commun. 182 (1992) 215–223.

[11] M.G. Rhui, W.S. Park, Y.J. Jung, S.W. Choi, J. Meltzer, Allelic detections of MCC/APC and p53 are frequent late events in human gastric carcinogenesis, Gastroenterology 106 (1994) 1584–1588.

[12] R.F. Souza, R. Appel, J. Yin, S. Wang, K.N. Smolinski, J.M. Abraham, T.T. Zou, Y.Q. Shi, J. Lei, J. Cottrell, Microsatellite instability in the insulin-like growth factor II receptor gene in gastrointestinal tumors, Nat. Genet. 14 (1996) 255–257.

[13] Y.J. Chung, J.M. Song, J.Y. Lee, Y.T. Jung, E.J. Seo, S.W. Choi, M.G. Rhyu, Microsatellite instability-associated mutations associates frequently with the intestinal type of primary gastric carcinomas in high-risk population, Cancer Res. 56 (1996) 4662–4665.

[14] N. Rampino, H. Yamamoto, Y. Ionov, Y. Li, H. Sawai, J.C. Reed, M. Perucho, Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype, Science 275 (1997) 967–969.

[15] M.H. Chung, H. Kasai, D.S. Jones, H. Inoue, H. Ishikawa, E. Ohtsuka, S. Nishimura, An endonuclease activity of *Escherichia coli* that specifically removes 8-hydroxyguanine residues from DNA, Mutat. Res. 254 (1991) 1–12.

[16] M.H. Chung, H.S. Kim, E. Ohtsuka, H. Kasai, F. Yamamoto, S. Nishimura, An endonuclease activity in human polymorphonuclear neutrophiles that removes 8-hydroxyguanines from DNA, Biochem. Biophys. Res. Commun. 178 (1991) 1472–1478.

[17] J. Tchou, H. Kasai, S. Shibutani, M.H. Chung, J. Laval, A.P. Girollman, S. Nishimura, 8-Oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 4690–4694.

[18] S.C. Baik, H.S. Youn, M.H. Chung, W.K. Lee, M.J. Cho, G.H. Ko, C.K. Park, H. Kasai, K.H. Rhee, Increased oxidative DNA damage in *Helicobacter pylori*-infected human gastric mucosa, Cancer Res. 56 (1996) 1279–1282.

[19] H.S. Youn, S.C. Baik, W.K. Lee, M.J. Cho, H.H. Ryou, H.J. Choi, K.H. Rhee, Serodiagnosis of *Helicobacter pylori* infection, J. Korean Soc. Microbiol. 25 (1990) 463–474.

[20] H. Kasai, S. Nishimura, Y. Kurokawa, Y. Hayashi, Oral administration of the renal carcinogen, potassium bromate, specifically produces 8-hydroxydeoxyguanosine in rat target organ DNA, Carcinogenesis 8 (1987) 1959–1961.

[21] M.H. Won, T.C. Kang, G.S. Jeon, J.C. Lee, D.Y. Kim, E.M. Choi, K.H. Lee, C.D. Choi, M.H. Chung, S.S. Cho, Immunohistochemical detection of oxidative DNA damage induced by ischemia-reperfusion insults in gerbil hippocampus in vivo, Brain. Res. 836 (1999) 70–78.

[22] T. Takeda, M. Hosokawa, K. Higuchi, Senescence-accelerated mouse (SAM): a novel murine model of accelerated senescence, J. Am. Geriatr. Soc. 39 (1991) 911–919.

[23] J.Y. Choi, H.S. Kim, H.K. Kang, D.W. Lee, E.M. Choi, M.H. Chung, Thermolabile 8-hydroxyguanine DNA glycosylase with low activity in senescence-accelerated mice due to a single-base mutation, Free Radic. Biol. Med. 27 (1999) 848–854.

[24] H.S. Kim, Y.W. Park, H. Kasai, S. Nishimura, C.W. Park, K.H. Choi, M.H. Chung, Induction of *E. coli* oh^{8}Gua endonuclease by oxidative stress: its significance in aerobic life, Mutat. Res. 363 (1996) 115–124.

[25] F.M. Yakes, Y. Chen, B. Van Houten, PCR-based assays for the detection and quantification of DNA damage and repair, in: G.P. Pfeifer (Ed.), Technologies for Detection of DNA Damage and Mutations, Plenum Press, New York, 1996, pp. 171–184.

[26] S. Ayala-Torres, Y. Chen, T. Svoboda, J. Rosenblatt, B. Van Houten, Analysis of gene-specific DNA damage and repair using quantitative polymerase chain reaction, Methods 22 (2000) 135–147.

[27] Y.J. Chung, K.M. Kim, J.R. Choi, S.W. Choi, M.G. Rhui, Relationship between intratumor histological heterogeneity and genetic abnormalities in gastric carcinoma with microsatellite instability, Int. J. Cancer 82 (1999) 782–788.

[28] W.T. Briscoe, L.E. Cotter, The effects of neighbouring bases on *N*-methyl-*N*-nitrosurea alkylation of DNA, Chem. Biol. Interact. 52 (1984) 103–110.

[29] W.T. Briscoe, L.E. Cotter, DNA sequence has an effect on the extent and kinds of alkylation of DNA by a potent carcinogen, Chem. Biol. Interact. 56 (1985) 321–331.

[30] V.A. Bohr, D.H. Phillips, P.C. Hanawalt, Heterogenesis DNA damage and repair in the mammalian genome, Cancer Res. 47 (1987) 6426–6436.

[31] P.C. Hanawelt, Transcription-coupled repair and human disease, Science 266 (1994) 1957–1958.

[32] T.Y. Brandt, F. Ali-Osman, Detection of DNA damage in transcriptionally active gene by RT-PCR and assessment of repair of cisplatin-induced damage in the glutathi1 *S*-transferase-π gene in human glioblastoma cells, Toxicol. Appl. Pharmacol. 143 (1997) 22–29.
[33] T.H. Quan, J.C. State, Preferential DNA damage in the p53 gene by benso[a]pyrene metabolites in cytochrome P4501A1-expressing xeroderma pigmentosum group A cells, Mol. Carcinog. 16 (1996) 32–43.
[34] A.T. De Souza, G.R. Hankins, M.K. Washington, T.C. Orton, R.L. Jirtle, M6P/IGF2R gene is mutated in human hepatocellular carcinomas with loss of heterozygosity, Nat. Genet. 11 (1995) 447–449.
[35] G.R. Hankins, A.T. De Souza, R.C. Bentley, M.R. Patel, J.R. Marks, J.D. Iglehart, R.L. Jirtle, M6P/IGF2 receptor: a candidate breast tumor suppressor gene, Oncogene 12 (1996) 2003–2009.
[36] S. Wang, R.F. Souza, D. Kong, J. Yin, K.N. Smolinski, T.T. Zou, T. Frank, J. Young, K.C. Flanders, H. Sugimura, J.M. Abraham, S.J. Meltzer, Deficient transforming growth factor-beta1 activation and excessive insulin-like growth factor II (IGFII) expression in IGFII receptor-mutant tumors, Cancer Res. 57 (1997) 2543–2546.
[37] R.F. Souza, S. Wang, M. Thakar, K.N. Smolinski, J. Yin, T.T. Zou, D. Kong, J.M. Abraham, J.A. Toretsky, S.J. Meltzer, Expression of the wild-type insulin-like growth factor II receptor gene suppresses growth and causes death in colorectal carcinoma cells, Oncogene 18 (1999) 4063–4068.
[38] J.W. Hyun, G.J. Cheon, H.S. Kim, Y.S. Lee, E.Y. Choi, B.H. Yoon, J.S. Kim, M.H. Chung, Radiation sensitivity depends on OGG1 activity status in human lukemis cell lines, Free Radic. Biol. Med. 32 (2002) 212–220.
[39] S.J. Kuerbitz, B.S. Plunkett, W.V. Walsh, M.B. Kastan, Wild type p53 is a cell cycle checkpoint determination following irradiation, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 7491–7496.
[40] K.B. Hahm, K.J. Lee, S.Y. Choi, J.H. Kim, S.W. Cho, H. Yim, S.J. Park, M.H. Chung, Possibility of chemoprevention by the eradication of *Helicobacter pylori*: oxidative DNA damage and apoptosis in *H. pylori* infection, Am. J. Gastroenterol. 92 (1997) 1853–1857.