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Forum Review

Oxidative and Nitrative DNA Damage as Biomarker for Carcinogenesis with Special Reference to Inflammation

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ABSTRACT

Reactive oxygen and nitrogen species are known to participate in a wide variety of human diseases. Oxidative DNA damage is involved in chemical carcinogenesis and aging. Monocyclic chemicals induce mainly oxidative DNA damage, whereas polycyclic chemicals can induce oxidative DNA damage in addition to DNA adduct formation. Recently, chronic infection and inflammation have been recognized as important factors for carcinogenesis. Nitrative DNA damage as well as oxidative DNA damage is induced in relation to inflammation-related carcinogenesis. The authors examined the formation of 8-nitroguanine, a nitrative DNA lesion, in humans and animals under inflammatory conditions. An immunofluorescence labeling study demonstrated that 8-nitroguanine was strongly formed in gastric gland epithelial cells in gastritis patients with *H. pylori* infection, in hepatocytes in patients with hepatitis C, and in oral epithelium of patients with oral lichen planus. 8-Nitroguanine was also formed in colonic epithelial cells of model mice of inflammatory bowel diseases and patients with ulcerative colitis. Interestingly, 8-nitroguanine was formed at the sites of carcinogenesis regardless of etiology. Therefore, 8-nitroguanine could be used as a potential biomarker to evaluate the risk of inflammation-related carcinogenesis. *Antioxid. Redox Signal.* 8, 1047–1058.

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in a wide variety of human diseases, including cancer. Oxidative and nitrative stress refers to the situation of a serious imbalance of production of these reactive species and antioxidant defense system (22). ROS and RNS are capable of causing damage to various cellular constituents, such as nucleic acids, proteins, and lipids, leading to carcinogenesis, aging, and many other diseases. ROS are generated from multiple sources, including inflammatory cells, carcinogenic chemicals and their metabolites, and electron transport chain in mitochondria (Fig. 1). On the other hand, nitric oxide (NO) is generated specifically during inflammation via inducible nitric oxide synthase (iNOS) in inflammatory and epithelial cells (Fig. 1). Recent studies have provided evidence that inflammation is associated with carcinogenesis (14). Inflammation can be induced by chronic infection with various infectious agents and other physical, chemical and immunological factors (14, 77).

ROS and RNS are considered to play important roles in carcinogenesis through oxidative and nitrative DNA damage (34, 77). Guanine is most easily oxidized among the four DNA bases, because the oxidation potential of guanine is lower than the other three DNA bases, adenine, cytosine, and thymine (10, 108). ROS can induce the formation of oxidative DNA lesions, including 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) (10, 20, 46, 119). 8-OxodG is considered to be a mutagenic DNA lesion. It was reported that misincorporation of adenine occurs opposite 8-oxodG during DNA synthesis, leading to G → T transversions (8, 104). The mutational spectra induced by other oxidative DNA lesions have been investigated. 2,5-Diamino-4H-imidazol-4-one (Iz) and 2,2,4-triamino-5-(2H)-oxazolone (Oz) can be generated by oxidation of guanine and 8-oxodG (10). Similarly to 8-oxodG, Oz induced G → T transversions (24), whereas Iz induced G → C transversions (54, 71).

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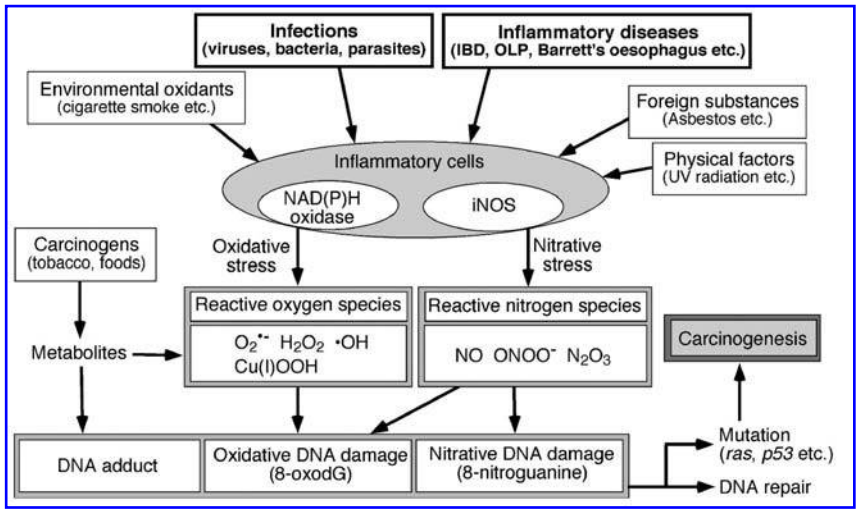

FIG. 1. Proposed mechanisms of oxidative and nitrative DNA damage leading to carcinogenesis induced by various environmental factors.

Excess NO production from inflammatory cells plays a critical role in an enormous variety of pathological processes, including cancer (34, 77). NO reacts with superoxide ($O_2^{\bullet-}$) to form peroxynitrite ($ONOO^-$), a highly reactive species causing nitrative and oxidative DNA damage. $ONOO^-$ can mediate the formation of 8-oxodG (41) and 8-nitroguanine, a marker of nitrative DNA damage (122). Akaike *et al.* have demonstrated the 8-nitroguanine is formed via NO production associated with inflammation in mouse with viral pneumonia (2). 8-Nitroguanine is considered to be not only a marker of inflammation but also a potential mutagenic DNA lesion, leading to carcinogenesis. 8-Nitroguanine formed in DNA is chemically unstable, and thus can be spontaneously released, resulting in the formation of an apurinic site (122). The apurinic site can form a pair with adenine during DNA synthesis, leading to G → T transversions (59) (Fig. 2). Recently, it has been reported that adenine is preferentially incorporated opposite 8-nitroguanine during DNA synthesis, suggesting that G → T transversions can also occur via this mechanism (112). In the $ONOO^-$-treated supF shuttle vector plasmid, which was then replicated in *Escherichia coli*, the majority of mutations occurred at G:C base pairs, predominantly involving G → T transversions (43, 53). Therefore, 8-oxodG and 8-nitroguanine are potentially mutagenic lesions leading to carcinogenesis. Especially, we have proposed the possibility that 8-nitroguanine is a potential biomarker to evaluate the risk of inflammation-related carcinogenesis. Here we discuss the role of oxidative and nitrative DNA dam-

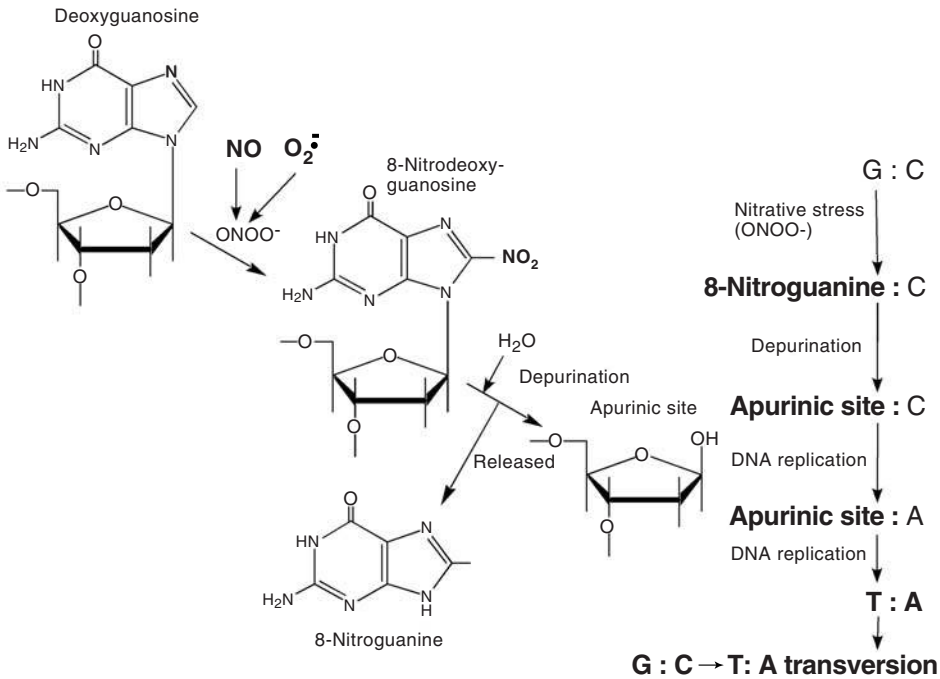

FIG. 2. Proposed mechanism of mutation mediated by 8-nitroguanine formation.

age in carcinogenesis caused by various environmental factors and inflammation.

OXIDATIVE DNA DAMAGE IN RELATION TO CHEMICAL CARCINOGENESIS AND AGING

In 1986, we discovered that the carcinogenic metal, chromium (VI), induced oxidative DNA damage in the presence of hydrogen peroxide (H_2O_2) (49). On the basis of this finding, we have proposed the hypothesis that metal carcinogenesis involves endogenous ROS generation. Other metal compounds, such as cobalt (120), nickel (50), and ferric nitrilotriacetate [Fe(III)-NTA] (40), also directly caused oxidative DNA damage in the presence of H_2O_2. Although the carcinogenicity of copper and iron has not been clear, a recent epidemiological study has demonstrated that the high intakes of copper and iron are associated with the increased risk of colorectal carcinogenesis (103). These findings regarding these metals suggest that free radicals play an important role in carcinogenesis. We demonstrated that copper (II) plus H_2O_2 induced DNA damage at thymine and guanine by generating ROS (121). Copper is an essential component of chromatin (16, 97). Copper accumulates in the liver of Long-Evans Cinnamon rats that spontaneously develop hepatocellular carcinomas (58). A case-cohort study showed a U-shaped relationship between plasma copper levels and risk of breast cancer (82). Iron is the most abundant transition metal ion in the human body. High body stores of iron may increase the risk of cancer in humans (109). Furthermore, renal cell carcinoma was observed in Fe(III)-NTA-treated rats (18). Fe(III)-NTA induced the formation of oxidative DNA lesions including 8-oxodG formation *in vivo* (1, 25). Genetic alterations in the *p15* and *p16* tumor suppressor genes were found in rats treated with Fe(III)-NTA (114).

Carcinogenic chemicals can induce various types of DNA damage including DNA adduct formation and oxidative DNA damage. We found that endogenous metals, particularly copper and iron, catalyzed ROS generation from various organic carcinogens and their metabolites, resulting in oxidative DNA damage (45). Table 1 shows the mutagenicity of various carcinogens and their potentials to induce DNA adduct formation and oxidative DNA damage. Although in 1976, the Ames test was believed to detect approximately 90% of carcinogens, a number of nonmutagenic carcinogens have been detected, and the concordance between this test and the carcinogenicity decreased to approximately 60%. There is a tendency that Ames test-negative carcinogens mainly cause oxidative DNA damage, whereas Ames test-positive carcinogens induce the formation of DNA adducts. Mutagenic carcinogens, such as polynuclear aromatic hydrocarbons [benzo[*a*]pyrene (76) and benz[*a*]anthracene (101, 102)] form DNA adducts predominantly, although these carcinogens are capable of causing oxidative DNA damage. Certain metabolites of benzo[*a*]pyrene (76) and benz[*a*]anthracene (102) induced double-base DNA damage consisting of piperidine-labile and formamidopyrimidine-DNA glycosylase (Fpg protein)-sensitive lesions at the 5′-ACG-3′ sequence (damaged bases are underlined) complementary to a hotspot of the *p53* tumor suppressor gene (Fig. 3). Aromatic nitro and amino compounds [4-aminobiphenyl (69) and heterocyclic amines (66–68)] also caused oxidative DNA damage to some extent. On the other hand, mononuclear compounds, such as benzene metabolites (26, 27, 47, 78), pentachlorophenol (70), *p*-

TABLE 1. DNA ADDUCT FORMATION AND OXIDATIVE DNA DAMAGE INDUCED BY CARCINOGENIC CHEMICALS

		DNA damage		*Ames*	
		Adduct	*Oxidation*	*test*	*References*
Aromatic hydrocarbons					
Mononuclear	Benzene	+	+++	−	26, 27, 78
″	Pentachlorophenol	+	+++	−	70
″	*p*-Dichlorobenzene	−	+++	−	79
″	Caffeic acid	−	+++	−	39
Polynuclear	Benzo[*a*]pyrene	+++	+	+	76
″	Benz[*a*]anthracene	+++	+	+	101, 102
Aromatic nitro and amino compounds					
Mononuclear	*o*-Toluidine	−	+++	−	73
″	*o*-Anisidine	−	+++	−	75
″	Nitrobenzene	−	+++	−	74
Polynuclear	4-Aminobiphenyl	+++	+	+	69
″	MeIQx and IQ	+++	+	+	67, 68
″	PhIP	+++	+	+	66
Others					
	2-Nitropropane	+	+++	±	96
	Benzoyl peroxide	−	+++	−	52
	Homogentisic acid	−	+++	−	29
	Aminoacetone	−	+++	−	28
	Estrogens	−	+++	−	30

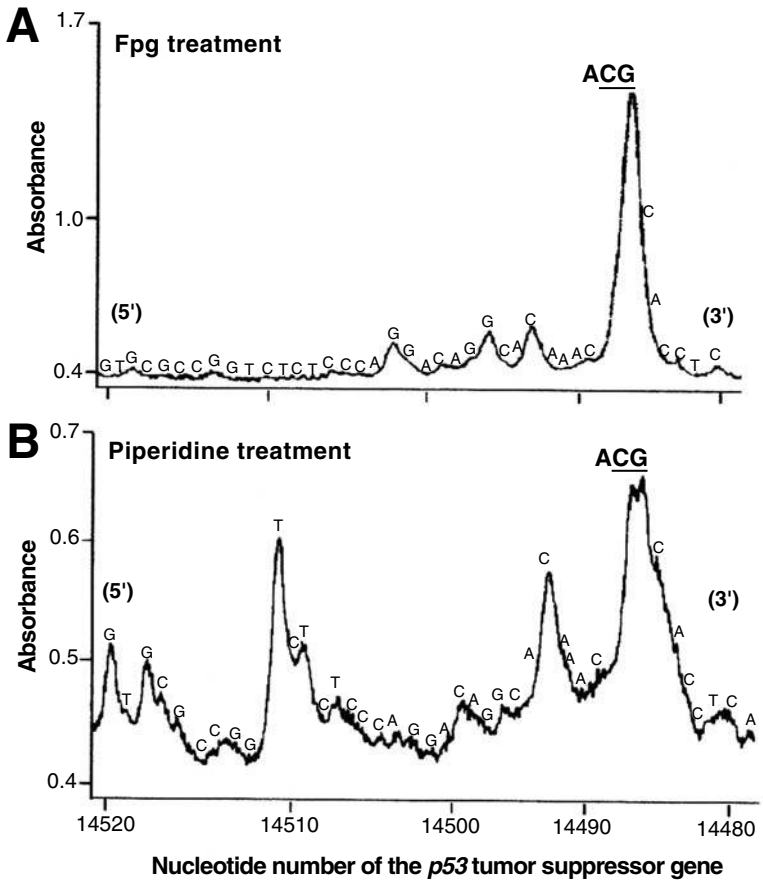

FIG. 3. Site specificity of DNA damage induced by a metabolite of benz[*a*]anthracene, benz[*a*]anthracene–3,4-dihydrodiol. The reaction mixture contained ^{32}P-5′-end-labeled DNA fragment of the *p53* tumor suppressor gene, 20 μ*M*/base calf thymus DNA, 20 μ*M* benz[*a*]anthracene–3,4-dihydrodiol, 100 μ*M* NADH, and 20 μ*M* $CuCl_2$ in 10 m*M* sodium phosphate buffer (pH 7.8) containing 5 μ*M* DTPA. The reaction mixtures were incubated at 37°C for 1 h, followed by the treatment with 6 units of Fpg protein at 37°C for 2 h (**A**) or 10%(v/v) piperidine at 90°C for 20 min (**B**). The treated DNA was electrophoresed on an 8% polyacrylamide/8 *M* urea gel. The autoradiogram was obtained by exposing X-ray film to the gel and then analyzed with a laser densitometer. (Adapted from Reference 102).

dichlorobenzene (79), *o*-toluidine (73), *o*-anisidine (75), nitrobenzene (74), caffeic acid (39), homogentisic acid (a tyrosine metabolite) (29), and aminoacetone (a threonine metabolite) (28) induced oxidative DNA damage predominantly, and thus, these compounds appear to express their carcinogenicity through oxidative DNA damage. In addition, certain metabolites of 2-nitropropane, an aliphatic amino compound, induced oxidative DNA damage in the presence of endogenous metals (96). Benzoyl peroxide plus Cu(I) caused DNA damage specifically at 5′-G of GG and GGG sequences in double-stranded DNA (52). This site specificity is explained by the *ab initio* molecular orbital calculation revealing that a large part of the highest occupied molecular orbital (HOMO) is distributed on the 5′-site guanine of GG sequences in double-helical DNA, and thus, this guanine is easily oxidized (110). Estrogen metabolites (catechol estrogens) induced oxidative DNA damage in the presence of Cu(II) at extremely low concentrations (30). Recently, we demonstrated that the addition of a histone peptide containing a metal-binding site enhanced oxidative DNA damage induced by H_2O_2 plus Cu(II) (63). These findings suggest that metal-mediated oxidative DNA damage contributes to carcinogenesis induced by various environmental chemicals.

In addition to carcinogenesis, oxidative DNA damage contributes to aging. We have reported that in cultured cells exposed to UVA radiation, oxidative stress induced the acceleration of telomere shortening, which has been considered to play the important role in aging (80). UVA irradiation with an endogenous photosensitizer riboflavin efficiently induced 8-oxodG formation at consecutive guanines in the ^{32}P-5′-end-labeled DNA fragments containing telomeric sequence (5′-TTAGGG-3′). Therefore, site-specific oxidative DNA damage appears to contribute to aging (51).

DNA DAMAGE IN RELATION TO INFLAMMATION-RELATED CARCINOGENESIS

Today, experimental and epidemiological evidence indicates that a variety of infectious agents constitute one of the main causes of cancer (14, 35). The International Agency for Research on Cancer (IARC) has estimated that approximately 18% of cancer cases worldwide is attributable to infectious diseases (35). The burden of cancer caused by infectious agents is shown in Table 2. Viruses are principal infectious agents, and bacterial and parasitic infections contribute to carcinogenesis to a lesser extent. Inflammation can be induced by chronic infection and many other physical, chemical, and immunological factors (14, 77). It has been hypothesized that many malignancies arise from areas of infection and inflammation (4, 14).

ROS and RNS play a key role in inflammation-mediated carcinogenesis. We demonstrated that carcinogenic nickel compounds induced oxidative DNA damage in rat lungs

Table 2. The Burden of Cancer Caused by Infectious Agents Worldwide

Infectious agent	*IARC classification**	*Cancer site*	*Number of cancer cases*	*% of cancer cases worldwide*
Bacterial infection				
H. pylori	1	Stomach	490,000	5.4
Viral infection				
HPV	1, 2A	Cervix and other sites	550,000	6.1
HBV, HCV	1	Liver	390,000	4.3
EBV	1	Lymphoma		
		Nasopharyngeal carcinoma	99,000	1.1
HHV-8	2A	Kaposi sarcoma	54,000	0.6
HTLV-1	1	Leukemia	9,000	0.1
Parasitic infection				
Schistosoma haematobium	1	Bladder	2,700	0.1
Liver flukes				
Opisthorchis viverrini	1	Cholangiocarcinoma	800	
Clonorchis sinensis	2A			
		Total infection-related cancers	1,600,000	17.7
		Total cancers in 1995	9,000,000	100

Adapted from 2003 IARC "World Cancer Report" (35).
*Group 1, carcinogenic to humans; Group 2A, probably carcinogenic to humans.

through inflammation (48). Akaike *et al.* have demonstrated that 8-nitroguanine is formed in association with inflammation in mice with viral infection (2). In relation to inflammation-related carcinogenesis, we examined the formation of 8-nitroguanine in addition to 8-oxodG using biopsy samples obtained from patients with inflammatory diseases and animals under inflammatory conditions. To examine the distribution of 8-nitroguanine, we produced highly sensitive and specific anti-8-nitroguanine antibody without cross reaction, as shown in Fig. 4 (86). Here we discuss the role of nitrative and oxidative DNA damage in carcinogenesis arising from various inflammatory conditions.

Oxidative and nitrative DNA damage induced by infectious agents

Liver fluke infection and cholangiocarcinoma. Infection with the liver fluke *Opisthorchis viverrini* (OV) is a major risk factor of cholangiocarcinoma, especially in the northeastern region of Thailand (23, 38) and has been evaluated to be carcinogenic to humans (Group 1) (38). Infection with this parasite can be repeatedly caused by eating raw or undercooked fish containing the infective stage of the fluke. A majority (approximately 70%) of OV-induced cholangiocarcinoma occurs in the intrahepatic bile ducts, and the remainder occurs in the extrahepatic duct (118). However, the mechanism by which OV induces cholangiocarcinoma remains to be understood.

We investigated DNA damage in the liver of hamsters with single and repeated OV infection as a model of inflammation-related carcinogenesis in humans. We were first in reporting that 8-nitroguanine is formed in relation to inflammation-related carcinogenesis using this animal model (90). Double immunofluorescence staining revealed that 8-oxodG and 8-nitroguanine were formed in inflammatory cells and epithelium of bile ducts (86, 87). The immunoreactivities of 8-oxodG and 8-nitroguanine in inflammatory cells were most prominently observed on days 21 and 30, respectively (86). It is noteworthy that these DNA lesions still remained in the epithelium of bile ducts on day 180. The formation of 8-nitroguanine and 8-oxodG increased in the epithelium of bile ducts in the order of three-time infection > two-time infection > single infection. This may be explained by the fact that repeated infection increased iNOS expression in the epithelium of bile ducts in the same order (87). Proliferating cell nuclear antigen (PCNA), which functions as a cofactor for DNA polymerase δ, is associated with DNA replication and long-patch base excision repair (20, 99). In our study, PCNA accumulated in the epithelium of bile ducts after repeated OV infection, supporting the hypothesis that cell proliferation was promoted by inflammation-mediated DNA damage (87). Recently, we reported that OV antigen induces inflammatory response, including iNOS expression, through Toll-like receptor (TLR)-2-mediated pathway (89). We found that the formation of 8-oxodG and 8-nitroguanine occurred to a much greater extent in cancerous tissue than in noncancerous tissue in intrahepatic cholangiocarcinoma patients, and that these DNA lesions contribute to tumor progression (88). In conclusion, more frequent OV infection can induce the iNOS expression in the epithelium of bile ducts and subsequently cause nitrative and oxidative damage to nucleic acids, which may participate in every step of cholangiocarcinoma development, including initiation, promotion and progression.

Helicobacter pylori *infection and gastric cancer.* The presence of the Gram-negative bacterium, *Helicobacter*

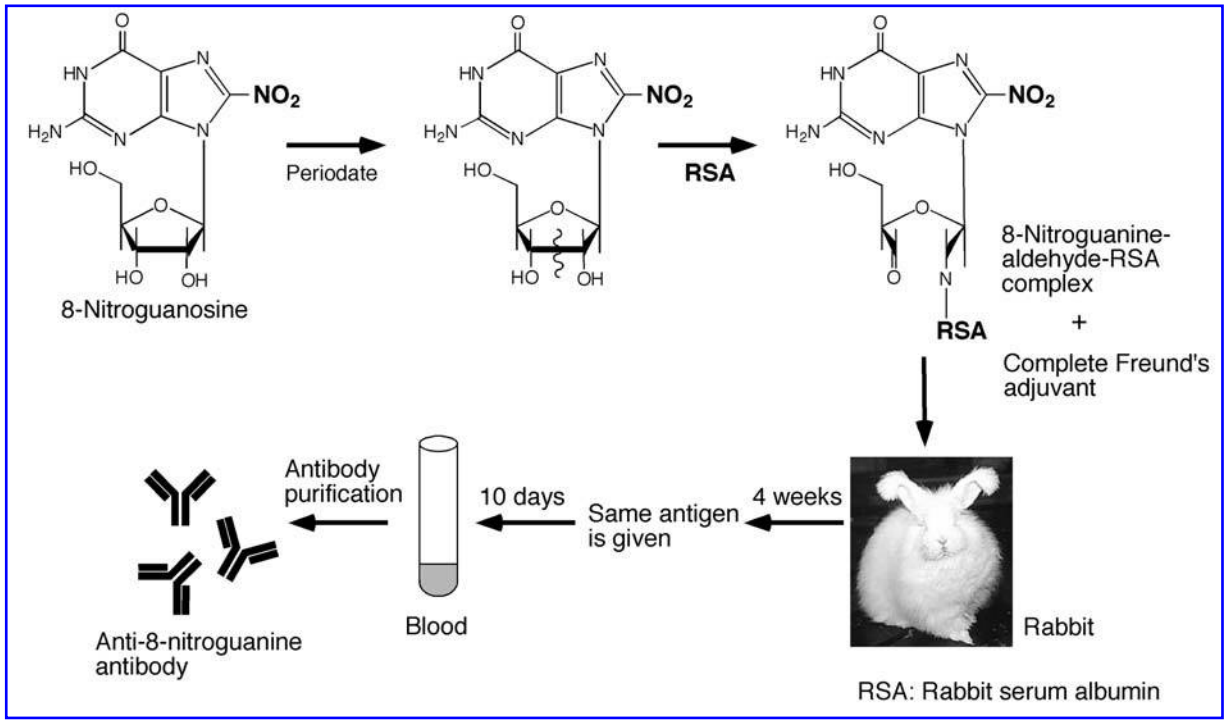

FIG. 4. Production of polyclonal anti-8-nitroguanine antibody. 8-Nitroguanosine was incubated with sodium metaperiodate and then conjugated with rabbit serum albumin (RSA). The 8-nitroguanine-aldehyde–RSA conjugate mixed with Freund's complete adjuvant was injected in rabbit intracutaneously. After 4 weeks, the same antigen was given and the blood was taken 10 days later. We purified the anti-8-nitroguanine antibody by affinity chromatography. Specificity of the purified antibody was examined by a dot immunobinding assay and absorption test (86).

pylori (H. pylori) is associated with not only chronic atrophic gastritis and peptic ulcer but also gastric adenocarcinoma and non-Hodgkin's lymphoma [mucosa-associated lymphoid tissue (MALT) lymphoma] (83). *H. pylori* infection has been evaluated to be carcinogenic to humans (Group 1) (36). The mechanisms by which *H. pylori* infection causes gastric cancer have been investigated. Lipopolysaccharide (LPS), which is a component of Gram-negative bacteria including *H. pylori,* is a ligand of TLR4. TLR4 is involved in activation of the transcription factor nuclear factor-κB (NF-κB) (61). Another study has revealed that TLR2 and TLR5, but not TLR4, are required for *H. pylori*-induced NF-κB activation by epithelial cells (107). A recent report has shown that NF-κB functions as a tumor promoter in inflammation-associated cancer (85). NF-κB is involved in regulation of iNOS expression (111). Thus, it is necessary to examine whether *H. pylori*-mediated iNOS expression leads to 8-nitroguanine formation in gastric epithelial cells and this DNA lesion contributes to carcinogenesis.

We performed a double immunofluorescence labeling study and demonstrated that the intense immunoreactivities of 8-nitroguanine and 8-oxodG were observed both in gastric gland epithelial cells and inflammatory cells in patients with *H. pylori* infection (60). On the other hand, in gastritis patients without *H. pylori* infection, these immunoreactivities were observed in inflammatory cells but not in gastric gland epithelial cells. This finding is supported by the previous study demonstrating that the formation of 8-oxodG is increased in the gastric epithelium of *H. pylori*-infected patients (3, 84). It has been reported that the expression of iNOS mRNA and protein was significantly increased in *H. pylori*-positive gastritis compared to *H. pylori*-negative gastritis (21). Specific cytokines are considered to participate in *H. pylori*–induced iNOS expression in gastric mucosa. Cag-positive *H. pylori* strain induces an intense inflammatory responses including interleukin (IL)-8 production from epithelial cells and subsequent production of tumor necrosis factor (TNF-α) from inflammatory cells (83). These cytokines have been reported to participate in iNOS expression in gastric mucosa (57, 115). Collectively, the host immune response to *H. pylori* mediated by cytokines, resulting in iNOS expression, may lead to an increase in the accumulation of 8-nitroguanine and 8-oxodG in gastric epithelium.

Several studies have demonstrated that PCNA is an independent prognostic factor for gastric cancer in patients with *H. pylori* infection (99). In our study, the accumulation of PCNA was significantly higher in gastric gland epithelial cells in patients with *H. pylori* infection compared to those without infection (60). These results suggest that nitrative and oxidative DNA damage in gastric epithelial cells and their proliferation by *H. pylori* infection may lead to gastric carcinoma.

Hepatitis C virus infection and liver cancer. Hepatitis C virus (HCV) is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma throughout the world (37, 93). HCV infection has been evaluated as a

Group 1 carcinogen by IARC (37). It is generally accepted that hepatocellular carcinoma arises through a multistep process of genetic alterations in hepatocytes during chronic hepatitis C (CHC) (7, 11, 33, 72). It has been demonstrated that 8-oxodG is accumulated in hepatocytes from patients with chronic viral hepatitis (55, 105). Several studies on patients with chronic HCV have shown that hepatic iron overload is attributable to liver injury and that iron depletion improved serum aminotransferase levels (42, 44) and normalized elevated hepatic 8-oxodG level (44). Therefore, iron overload may be involved in generation of reactive species to form mutagenic DNA lesions. Alternatively, HCV core protein is capable of inducing oxidative stress in cultured cells and animals (81), and this protein has been demonstrated to participate in hepatic carcinogenesis in transgenic mice (65). Moreover, a HCV-encoded nonstructural protein (NS3) has been reported to participate in generation of oxygen radicals from phagocytes via activation of NADPH oxidase (9, 116). However, the mechanism of HCV infection-induced hepatitis followed by hepatocarcinogenesis via DNA damage is still unclear.

We investigated DNA damage in liver biopsy specimens of patients with CHC and the effect of interferon treatment. The immunoreactivities of 8-nitroguanine and 8-oxodG were strongly detected in the liver from patients with CHC, but not in control livers (nonalcoholic fatty liver) (32). 8-Nitroguanine accumulation was found in not only infiltrating inflammatory cells but also hepatocytes particularly in the periportal area. The accumulation of 8-nitroguanine and 8-oxodG increased with inflammatory grade. iNOS expression was observed in the cytoplasm of hepatocytes and Kupffer cells in CHC patients. In the sustained virological responder group after interferon therapy, the accumulation of 8-nitroguanine and 8-oxodG in the liver was markedly decreased (32). Our results are consistent with the previous reports showing that the expression of iNOS in hepatocytes has been observed in patients with chronic hepatitis (62) and hepatocellular carcinoma (94). Taken together, these findings indicate that 8-nitroguanine is a useful biomarker to evaluate the severity of HCV-induced chronic inflammation leading to hepatocellular carcinoma. 8-Nitroguanine could also be used for the evaluation of the efficacy of CHC treatment.

Oxidative and nitrative DNA damage induced by noninfectious agents

Inflammatory bowel disease and colon cancer. Ulcerative colitis and Crohn's disease, which are referred to as inflammatory bowel diseases (IBD), are well known as chronic inflammatory diseases in lower bowel. These diseases lead to long-term impairment of intestinal structure and function (6, 91). Epidemiological studies have shown that the incidence of colorectal cancer in IBD is greater than the expected incidence in the general population (13, 19, 56). Although the precise mechanisms of the pathogenesis of IBD have not been clarified, a large number of immunological abnormalities has been noted in patients with IBD (6, 91). Inflammation occurs as a result of either excessive functions of effector T cells, such as T helper type 1 (Th1) and 2 (Th2) cells or deficient function of regulatory T cells.

On the basis of the hypothesis that the imbalance of helper and regulatory T cell functions plays the key role in IBD pathogenesis, we prepared a mouse model of IBD. For induction of IBD, C.B-17 SCID mice were injected intraperitoneally with purified $CD45RB^{high}CD4^{+}$ T cells (mainly consisting of Th1 and Th2 cells lacking regulatory T cells) as described previously (92). This IBD mouse model showed that the body weight increased with aging to a lesser extent than nontreated controls and that the intestine was shortened. Pathological findings of this mouse model, which showed severe inflammation in colon tissues, were similar to IBD patients. Double immunofluorescence technique revealed that both 8-nitroguanine and 8-oxodG were formed mainly in epithelial cells of the IBD mouse model (17). When the tissue sections were pretreated with RNase, 8-nitroguanine immunoreactivity was more clearly observed in the nuclei of epithelial cells, suggesting that 8-nitroguanine is formed mainly in genomic DNA. The formation of 8-nitroguanine in the nuclei was confirmed by electron microscopic immunohistochemistry. iNOS, PCNA, and p53 proteins were also expressed in the colon epithelium. We also demonstrated that 8-nitroguanine was formed in colon epithelium of patients with ulcerative colitis (unpublished data). Relevantly, several studies have shown that iNOS is expressed in the epithelial cells in colitis patients (31, 106, 119). In noncancerous colon tissues from patients with ulcerative colitis, iNOS protein levels were positively correlated with p53 serine 15 phosphorylation levels (31). These results suggest that nitrative DNA damage, as well as oxidative DNA damage, participates in colon carcinogenesis in patients with IBD.

Oral lichen planus and oral cancer. Oral lichen planus (OLP) is a chronic inflammatory mucosal disease (100). Several pathological features indicate that OLP is immunologically-mediated inflammatory response, including an intense, band-like infiltrate of predominantly T-lymphocytes subjacent to epithelium. The basal epithelial cells are the target for immune destruction by cytotoxic T lymphocytes (15, 117). The most important complication of OLP is development of oral squamous cell carcinoma (OSCC) (64, 95). However, DNA damage associated with OLP and OSCC has not been investigated.

We demonstrated that the accumulation of 8-nitroguanine and 8-oxodG was apparently observed in oral epithelium of biopsy specimens of patients with OLP and OSCC, whereas no immunoreactivity was observed in normal oral mucosa (Table 3) (12). Colocalization of 8-nitroguanine and iNOS was found in oral epithelium of OLP and OSCC patients. Immunoreactivity of 3-nitrotyrosine, which is formed by protein tyrosine nitration and considered to be a biochemical marker for inflammation, was also observed in oral epithelial cells. Accumulation of p53 was more strongly observed in oral epithelium in OSCC than OLP, whereas there was no p53 accumulation in normal oral mucosa (Table 3). Our findings demonstrate that iNOS-dependent DNA damage in OLP may lead to p53 accumulation in not only OLP but also OSCC. It is concluded that the formation of 8-nitroguanine and 8-oxodG may contribute to development of oral cancer from OLP.

Table 3. Correlation of Immunoreactivities of 8-Nitroguanine, 8-OxodG, iNOS, and p53 in the Basal Layer of Oral Epithelium in OLP Patients and Normal Mucosa

Immuno-reactivity grading	*8-nitroguanine*			*8-oxodG*			*iNOS*			*P53*		
	NM	*OLP*	*OSCC*	*NM*	*OLP*	*OSCC*	*NM*	*OLP*	*OSCC*	*NM*	*OLP*	*OSCC*
−	15	0	0	15	0	0	15	3	0	15	3	0
+	0	1	0	0	1	1	0	4	1	0	7	1
++	0	6	2	0	6	2	0	3	0	0	1	0
+++	0	4	1	0	4	0	0	1	2	0	0	2
P	0.00001			0.00001			0.00127			0.00038		

NM = normal mucosa ($n = 15$); OLP = oral lichen planus patients ($n = 11$); OSCC = oral squamous cell carcinoma patients ($n = 3$). The following scores were assigned to each specimen according to the degree of staining: −, negative; +, <25%; ++, 25%–50%; and +++, >50% of the cells in tissue sections. The significant difference between NM and OLP was analyzed by Chi-square test.

CONCLUSION

We have investigated the mechanisms of oxidative and nitrative DNA damage induced by various carcinogenic chemicals and inflammatory conditions. We have demonstrated that monocyclic chemicals and many other chemicals induce oxidative DNA damage rather than DNA adduct formation. On the other hand, polycyclic chemicals can induce oxidative DNA damage to some extent in addition to DNA adduct formation. Therefore, the contribution of these carcinogens to each type of DNA lesion seems to be dependent on their chemical structures and properties.

In relation to inflammation-related carcinogenesis, we examined the formation of 8-nitroguanine and 8-oxodG in human samples and animals. It is noteworthy that DNA damage was specifically induced at sites of carcinogenesis under various inflammatory conditions. In human samples, 8-nitroguanine formation was observed in gastric gland epithelial cells of patients with *H. pylori* infection (60) and in hepatocytes of patients with chronic hepatitis C (32). 8-Nitroguanine was also formed in oral epithelium of OLP and OSCC patients (12). Moreover, in hamsters infected with the liver fluke OV causing cholangiocarcinoma, 8-nitroguanine formation was induced in bile duct epithelium (87). 8-Nitroguanine formation was also found in colonic gland epithelial cells of mouse model of IBD (17). Therefore, 8-nitroguanine could be used as a potential biomarker to evaluate the risk of inflammation-related carcinogenesis. Recently, 8-nitroguanosine has been reported to be a highly redox-active molecule that strongly stimulates $O_2^{\bullet -}$ generation from NADPH-dependent reductases (98). 8-Nitroguanine may be a cofactor for redox reaction and cell signaling implicated in diverse physiological and pathological events (123). More importantly, experimental evidence has suggested that 8-nitroguanine is a mutagenic DNA lesion, which preferentially leads to G → T transversions (112, 122), in addition to 8-oxodG (8, 104). Indeed, G → T transversions have been observed *in vivo* in the *ras* gene (5) and the *p53* tumor suppressor gene in lung and liver cancer (33, 113). These findings imply that DNA damage mediated by ROS and RNS may participate in carcinogenesis via activation of protooncogenes and inactivation of tumor suppressor genes. In conclusion, oxidative and nitrative DNA damage could be promising biomarkers to evaluate the risk of carcinogenesis induced by a wide variety of chemicals and inflammatory conditions.

ABBREVIATIONS

CHC, chronic hepatitis C; Fe(III)-NTA, ferric nitrilotriacetate; HCV, hepatitis C virus; H_2O_2, hydrogen peroxide; HOMO, highest occupied molecular orbital; IARC, International Agency for Research on Cancer; IBD, inflammatory bowel diseases; IL, interleukin; iNOS, inducible nitric oxide synthase; Iz, 2,5-diamino-4H-imidazol-4-one; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; NO, nitric oxide; $O_2^{\bullet -}$, superoxide; OLP, oral lichen planus; $ONOO^-$, peroxynitrite; OSCC, oral squamous cell carcinoma; OV, *Opisthorchis viverrini*; Oz, 2,2,4-triamino-5-(2H)-oxozolone; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; PCNA, proliferating cell nuclear antigen; RNS, reactive nitrogen species; ROS, reactive oxygen species; RSA, rabbit serum albumin; Th, T helper; TLR, Toll-like receptor; TNF, tumor necrosis factor.

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