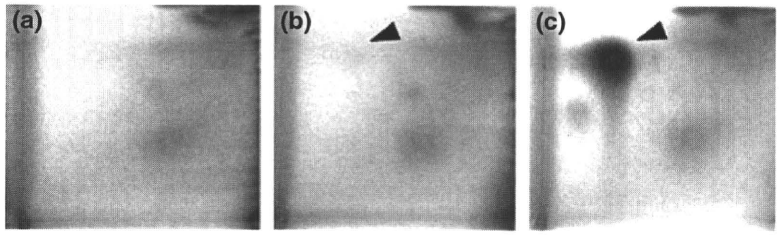

Fig. 1. Autoradiograms of 2-amino-3-methylimidazo-[4,5-*f*]quinoline (IQ)-DNA adducts the livers of 0 (a), 0.001 (b) and 100 (c) p.p.m. IQ-treated groups at week 4. Arrowheads indicate IQ-DNA adduct. The imaging plates were exposed for 3 h (a) and 24 h (b and c).

Table 3. IQ-DNA adduct and 8-OHdG formation in liver DNA

Group	IQ (p.p.m.)	No. rats	Adduct level ($\times 10^{-7}$ ntd)	8-OHdG ($\times 10^{-5}$ dG)
1	0	5	UDL	0.23 ± 0.07
2	0.001	5	UDL	0.25 ± 0.05
3	0.01	5	0.045 ± 0.02	0.24 ± 0.07
4	0.1	5	0.1 ± 0.004	0.32 ± 0.10
5	1	5	1.7 ± 0.07	0.24 ± 0.08
6	10	5	12.7 ± 0.07	0.22 ± 0.07
7	100	5	107.0 ± 0.07	0.23 ± 0.08

IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; ntd, nucleotide; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; UDL, under the detectable limit.

that suppression of cell cycle progression by $p21^{Cip/WAF1}$ followed by DNA repair is at least one of the mechanisms responsible for the observed no-effect of low doses of IQ in rats in the present model.

It is known that the vast majority of DNA damage is repaired by base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR).[34] APE-1 plays an essential role in the BER repair process by cleaving the phosphodiester backbone.[35] The activities of two different heterodimeric complexes, MSH2-MSH3 and MSH2-MSH6, belonging to the MMR system are mainly responsible for the post-replicative repair of mismatches.[36] We found that IQ significantly increased the expression levels of APE-1 but not MSH2 and MSH3 at doses of 10 and 100 p.p.m. in the liver. It has also been reported that IQ has no effect on expression of ERCC1, which is a key molecule in the NER process.[37] These findings suggest that BER rather than MMR or NER responds to IQ-induced DNA damage.

GADD45 is involved in a variety of growth regulatory mechanisms, including DNA repair, growth arrest and apoptosis.[38] It is induced by genotoxic and certain other cell stresses by p53-dependent and independent pathways.[39,40] GADD45 expression was significantly induced in the 100 p.p.m. group. The fact that significant induction of APE-1 and GADD45 was observed only at the highest doses of 10 and/or 100 p.p.m. indicate the IQ-induced DNA damage response is dose-dependent. Moreover, the fact that in the groups with low doses expression of APE-1 and GADD45 were not affected and that there was a significant but moderate induction of $p21^{Cip/WAF1}$ imply that normal physiological levels of these genes are sufficient to repair the DNA damage caused by low doses of IQ. However, the expression levels of these genes are all increased by higher carcinogenic doses of IQ. A reasonable explanation of the no-effect of low doses of IQ and the carcinogenicity of high doses of IQ is that carcinogenicity is the consequence of a disruption in the balance between DNA damage and repair and between abnormal cell proliferation and apoptosis or cell cycle regulation.

Our results show that p53 gene expression is not induced by administration of IQ. Furthermore, p53-deficient mice do not show higher susceptibility to IQ-induced liver carcinogenesis than wild type mice.[41] These results suggest that p53 does not have a significant impact on the carcinogenicity of IQ.

DNA adduct formation by metabolic activation of IQ is believed to play an important role in the carcinogenicity of IQ.[42] Formation of IQ-DNA adducts in the liver showed a linear dose-dependency and proved to be one of the most sensitive end-points for the detection of exposure to IQ. Adduct formation was detectable in groups administered far lower doses of IQ compared with detection of other end-points such as cell proliferation and preneoplastic lesion induction. That IQ-DNA adduct formation was not detected in the 0.001 p.p.m. group was most likely due to the detection limit of the assay. It should be noted that DNA adduct is a premutagenic lesion and not necessarily correlated to the frequencies of mutation and cancer induced by genotoxic compounds. For example, it is known that IQ forms DNA adducts in the kidneys and stomach of both rats and monkeys, but does not induce tumors in these organs.[43,44] Our present findings of a linear dose-response of IQ-DNA adduct formation and a nonlinear carcinogenic dose-response to IQ administration support the idea that IQ-DNA adducts do not necessarily lead to mutation and formation of cancerous lesions. Our results are also in line with previous results on HCA including MeIQx[1,18,45] and PhIP.[24] These results can be explained, at least in part, by the actions of gene products such as $p21^{Cip/WAF1}$, GADD45 and APE-1 and the other repair genes for DNA damage. Moreover, in the case of MeIQx, it has been suggested that formation of DNA adducts alone might not be sufficient to produce cancers and that the MeIQx-induced genetic alterations in the liver are enhanced by liver regeneration induced by high doses of MeIQx itself.[1] Therefore, while IQ-DNA adduct formation is important in IQ carcinogenicity, high levels of adduct formation are likely required and other factors such as cell proliferation can affect the balance between DNA damage and repair and lead to fixation of DNA mutations into the cell's genome.

It has been demonstrated *in vitro* that IQ is more efficiently metabolized and activated by CYP1A2 than by CYP1A1 or CYP1B1.[46] However, limited *in vivo* data are available. In a study by McPherson *et al.*[47], no significant induction in mRNA expression level or activity of either CYP1A1 or CYP1A2 were reported in the livers of rats receiving 300 p.p.m. IQ in the diet for 52 weeks, but these enzymes were significantly increased after daily administration of 20 mg/kg b.w. IQ by oral gavage for 3 days; in the average adult rat, a dose of 300 p.p.m. IQ in the diet is approximately equivalent to administration of 20 mg/kg b.w. IQ by oral gavage. The results of the present study revealed that IQ significantly induced CYP1A2 expression at doses from 0.01 to 10 p.p.m., but CYP1A2 was not induced in the 100 p.p.m. group. The lack of effect of 100 p.p.m. IQ on CYP1A2 expression is consistent with the results in rats receiving 300 p.p.m. IQ in the diet for 52 weeks.[47] Significant increases in CYP1A1 expression in the 100 p.p.m. group provide an alternative mechanism that can compensate for decreased CYP1A2 activity. However, as noted above, in apparent contrast to our results, in the study by McPherson *et al.*,[47] administration of 300 p.p.m. IQ over the course of 52 weeks did

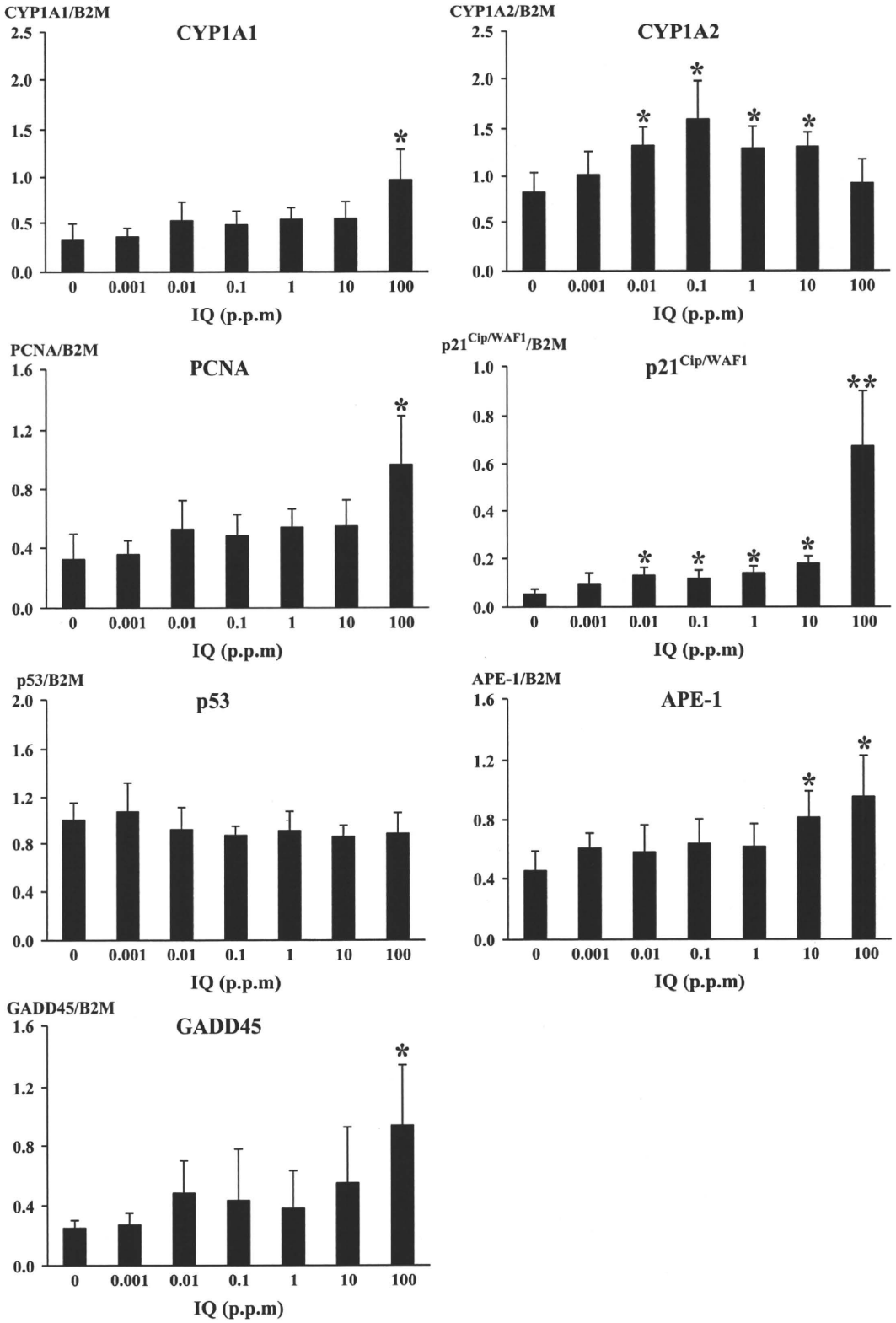

Fig. 2. Relative mRNA expression in the livers of rats at week 16. *Significantly different from 0 p.p.m. **Significantly different from all other groups. APE-1, AP endonuclease-1; B2M, beta-2-microglobulin; GADD45, growth arrest and DNA damage-inducible protein 45; PCNA, proliferating cell nuclear antigen.

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Table 4. Development of ACF in the colons of rats administered IQ for 16 weeks

Group	IQ (p.p.m.)	No. rats	Size of ACF				
			1	2	3	≥4	Total
1	0	240	0.08 ± 0.28	0.12 ± 0.32	0.06 ± 0.25	0.08 ± 0.29	0.33 ± 0.64
2	0.001	240	0.12 ± 0.36	0.08 ± 0.29	0.10 ± 0.32	0.09 ± 0.30	0.39 ± 0.69
3	0.01	240	0.15 ± 0.41	0.15 ± 0.42	0.06 ± 0.24	0.06 ± 0.24	0.43 ± 0.77
4	0.1	240	0.11 ± 0.33	0.11 ± 0.35	0.06 ± 0.25	0.08 ± 0.27	0.36 ± 0.63
5	1	240	0.15 ± 0.45	0.10 ± 0.30	0.10 ± 0.33	0.05 ± 0.23	0.41 ± 0.80
6	10	240	0.19 ± 0.48*	0.16 ± 0.41	0.07 ± 0.25	0.09 ± 0.40	0.50 ± 0.86
7	100	120	1.48 ± 1.46*	1.29 ± 1.51*	0.70 ± 0.93*	0.72 ± 1.01*	4.19 ± 3.34*

*Significantly different from group 1. ACF, aberrant crypt foci; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline.

not induce CYP1A1. Therefore, it is reasonable to postulate that the dose-relationship between IQ and induction of CYP1A1 is not a simple dose-response. CYP1B1 does not appear to be involved in the metabolism of IQ at doses up to 100 p.p.m. in rats. The findings described above demonstrate the importance of taking into account dosage, duration and route of exposure in interpretation of the data on metabolic activation of IQ. Further studies on the dose-response relationships between chronic IQ exposure and the protein expression levels and activities of detoxifying enzymes, especially at doses relevant to human exposure, would provide further insight into the role of metabolic activation in IQ carcinogenicity.

Oxidative DNA damage does not appear to play a role in IQ-induced carcinogenesis. In the present study, no significant changes in 8-OHdG levels or Ogg1 expression levels in the livers of IQ-treated rats were observed. Our results are consistent with the recent findings in IQ-treated Big Blue rats that oxidative stress was not responsible for the initiation of IQ-induced carcinogenesis in the liver and colon.[37] In this respect, IQ is different from MeIQx, in which oxidative DNA damage plays an important role in liver carcinogenesis.[48]

In summary, the present study provides the first experimental data on the carcinogenicity of low doses of IQ in both the liver and colon of the test animal and compares the effect of IQ at the cellular level with its carcinogenic effect. Our findings support the idea that there is a practical threshold that should be considered when evaluating the risk of genotoxic carcinogens. To this end, further accumulation of data, especially mechanistic data, should be promoted to facilitate not only an understanding of the carcinogenic effects of low doses of genotoxic carcinogens but also to establish an accurate means of quantitative risk assessment.

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Disclosure Statement

The authors have no conflict of interest.

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Review

In Vitro and *In Vivo* Genotoxicity Induced by Fullerene (C_{60}) and Kaolin

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Nanomaterials are being utilized for many kinds of industrial products, and the assessment of genotoxicity and safety of nanomaterials is therefore of concern. In the present study, we examined the genotoxic effects of fullerene (C_{60}) and kaolin using *in vitro* and *in vivo* genotoxicity systems. Both nanomaterials significantly induced micronuclei and enhanced frequency of sister chromatid exchange (SCE) in cultured mammalian cells. When ICR mice were intratracheally instilled with these nanomaterials, DNA damage of the lungs increased significantly that of the vehicle control. Formation of DNA adducts in the lungs of mice exposed to nanomaterials were also analyzed by stable isotope dilution LC-MS/MS. 8-Oxodeoxyguanosine and other lipid peroxide related adducts were increased by 2- to 5-fold in the nanomaterial-exposed mice. Moreover, multiple (four consecutive doses of 0.2 mg per animal per week) instillations of C_{60} or kaolin, increased *gpt* mutant frequencies in the lungs of *gpt* delta transgenic mice. As the result of mutation spectrum analysis, G:C to C:G transversions were commonly increased in the lungs of mice exposed to both nanomaterials. In addition, G:C to A:T was increased in kaolin-exposed mice. In immunohistochemical analysis, many regions of the lungs that stained positively for nitrotyrosine (NT) were observed in mice exposed to nanomaterials. From these observations, it is suggested that oxidative stress and inflammatory responses are probably involved in the genotoxicity induced by C_{60} and kaolin.

Key words: nanomaterials, genotoxicity, fullerene (C_{60}), kaolin, DNA adducts

Introduction

Recently, nanomaterials are being utilized for cosmetics and industrial products, and applications in medicine are under consideration. The assessment of genotoxicity and safety of nanomaterials is therefore of concern. One reason behind this is the asbestos crisis (1). Some nanomaterials are not only nano-sized particles, but also asbestos shape-like fibers, and the carcinogenic potential of such nanomaterials has attracted much attention over the years. Moreover, it is thought that nano-sized particles can be taken up in cells and cause intracellular damage (2,3). With this background, we here investigated induction of *in vitro* and *in vivo* genotoxicity using fullerene (C_{60}) and kaolin as examples. To clarify the mechanisms of mutations due to these nanomaterials, we analyzed the formation of DNA adducts in the lungs of mice after exposure. Here, we briefly summarize our data and also discuss mechanisms of genotoxicity induced by nanomaterials.

Size Distribution in Suspensions of Nanomaterials

The size distribution of nanomaterials used in the present study was analyzed by dynamic light scattering (DLS) as described previously (4). The most abundant sizes were at 234.1 ± 48.9 and 856.5 ± 119.2 nm for C_{60} and 357.6 ± 199.4 nm for kaolin, respectively.

In Vitro Genotoxicity Test

Micronucleus test: The micronucleus genotoxicity/clastogenicity test is widely used for assessment of environmental substances and medicinal chemicals. Here, we investigated the micronucleus inducing activity of C_{60} and kaolin using human lung carcinoma A549

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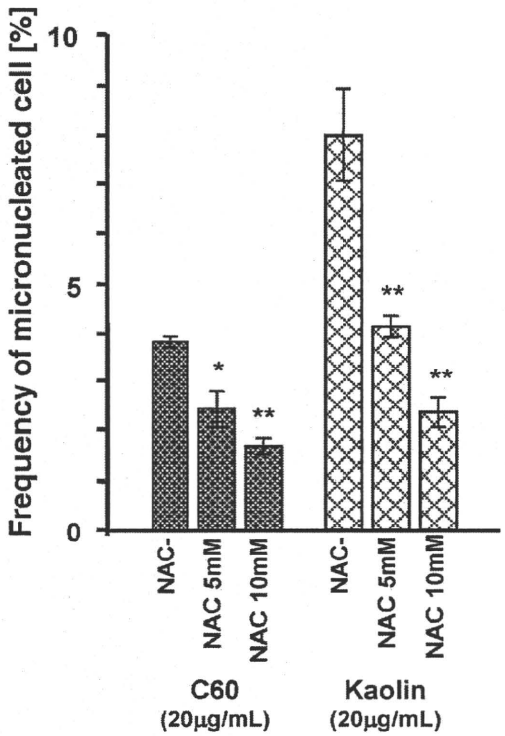

Fig. 1. Effects of anti-oxidative agents on the micronucleus inducing activity of nanoparticles. Values represent the means of three experiments ± SD. Asterisks (*, ** for $p<0.05$ and $p<0.01$, respectively) indicate significant differences from cells without NAC in the Student's t-test. Concentrations of nanoparticles in $\mu g/cm^2$ are given in parentheses.

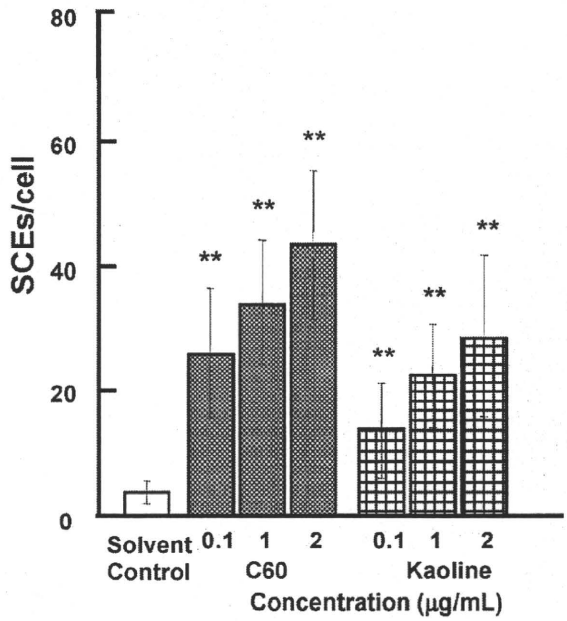

Fig. 2. Sister chromatid exchange (SCE) in CHO AA8 cells following treatment with C60 or kaolin for 1 h. The values represent the means of three experiments ± SD. Asterisks (**) indicate a significant difference ($p<0.01$) from control (treatment with 0.005% (v/v) Tween-80) cells in the Student's t-test.

cells (4). Six-hours treatment with 200 μg/mL kaolin caused growth inhibition of 60% whereas, C_{60} at the same concentration was without effect. C_{60} and kaolin particles both increased the number of micronucleated cells. The background frequency of micronucleated cells was 0.7% to 1.0%, and this rose to 10% and 5% with 200 μg/mL of C_{60} and kaolin, respectively, the increase being statistically significant in both cases. To investigate the effects of an anti-oxidative agent on the micronucleus induction, we conducted tests with or without *N*-acetyl cysteine (NAC) using Chinese hamster ovary CHO-AA8 cells. As shown in Fig. 1, the frequency of micronucleated cells was decreased significantly in the presence of NAC. With 20 μg/mL of C_{60} and kaolin for 6 h without NAC the results were 3.8% and 8%, respectively, but in the presence of 10 mM NAC these decreased to 1.7% and 2.3%. From this observation, oxidative stress might be involved in the genotoxicity induced by nanoparticles. Furthermore, it is known that photoexcited C_{60} produces reactive oxygen species (5) and in the present experiments, the cells and C_{60} were not shielded from visible light completely. Therefore, reactive oxygen species might contribute to micronucleus-induction in C_{60}-treated cells. On the other hand, biologically relevant features of kaolin are unclear and further studies will be required to elucidate genotoxic mechanisms.

Sister chromatid exchange (SCE) test: SCE is also used for mutagenic testing of many products. While the mechanisms responsible for SCE are not completely understood, they involve breakage of both DNA strands, followed by exchange of whole DNA duplexes. This occurs during the S phase and is efficiently induced by mutagens that form DNA adducts or that interfere with DNA replication. To investigate SCE inducing activity of nanoparticles, we examined CHO-AA8 cells following 1 h treatment with C_{60} and kaolin (Fig. 2). The SCE frequencies in cells treated with 2.0 μg/mL of C_{60} and kaolin were approximately 11 and 7 times higher than the control level, respectively ($P<0.01$ at 0.1 μg/mL or higher concentrations). C_{60} demonstrated stronger genotoxic/clastogenic potency than kaolin. Cozzi *et al.* earlier reported that H_2O_2-treatment produced reactive oxygen species and induced SCE in CHO cells, and antioxidants, such as ascorbic acid and β-carotene, reduced the frequency (6). In the present study, the results of the micronucleus test indicated involvement of reactive oxygen species so that they might contribute to SCE induction as well.

In Vivo Genotoxicity Test

Comet assay: The comet assay is known as a standard simple and sensitive technique for evaluation of

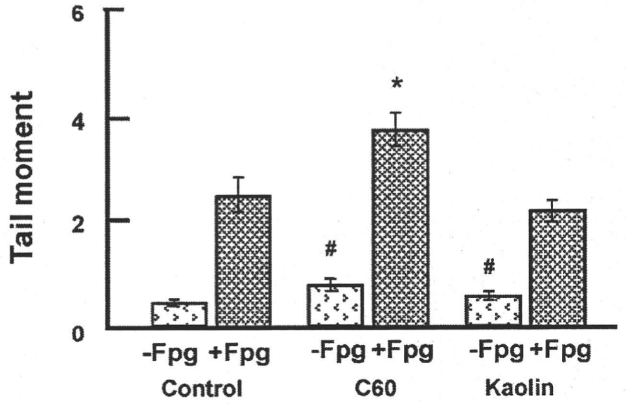

Fig. 3 DNA damage measured by comet assay in lungs of C57BL/6J mice intratracheally instilled with particles, with or without FPG treatment. Male mice were treated at a dose of 0.2 mg of particles per animal, and sacrificed 3 h after particle administration. The values represent the means of data for five animals ± SE. An asterisk (*) denotes $p < 0.01$ from that of control (+FPG) and a sharp (#) denotes $p < 0.01$ from that of control (-FPG) in a Dunnett's test after one-way ANOVA of Tail Moment.

DNA damage. The types of damage usually detected are single and double strand breaks. The pH (usually between neutral and alkaline pH) of the lysis condition can be adjusted depending upon the type of damage. Under alkaline conditions, AP sites and others where excision repair takes place are detected as DNA damage. We here evaluated DNA damage induced by particles using the comet assay under alkaline conditions. The values for DNA tail moment in the lungs with single-particle treatment at 0.2 mg/body for 3 h were measured, and DNA damage was significantly increased, around 2-fold, as compared with the vehicle control, and its intensity was C_{60} > kaolin. When we examined the effects of oxidation of purines, DNA damage was analyzed by formamidopyrimidin-glycosilase (FPG)-modified comet assay. DNA damage induced by kaolin was not changed, whereas DNA damage caused by C_{60} was elevated up to 1.7 fold compared with the vehicle control (Fig. 3). In addition, Jacobsen *et al.* also reported that C_{60} significantly increased the level of FPG sensitive sites/oxidized purines determined by the comet assay using the E1-Mutatrade markMouse lung epithelial cell line (7). From these findings, it seems that oxidative damage would be partly involved in the induction of DNA damage by C_{60}, although other changes responsible for DNA damage might be induced by kaolin.

Oxidative and lipid peroxide related DNA adduct formation: DNA adducts, formed by reactions with exogenous or endogenous agents, are known to induce gene mutations. Reactive oxygen species (ROS) are one type of endogenous agent that can produce oxidative DNA adducts such as 8-oxo-2′-deoxyguanosine (8-oxodG), a widely recognized and utilized biomarker of oxidative stress, and a major mutagenic lesion producing predominately G to T transversion mutations (8). In addition, ROS generate lipid hydroperoxides to yield heptanon-etheno (Hε)-adducts, such as HεdG, HεdA and HεdC via 4-oxo-2-nonenal (4-ONE) (9). These adducts can lead to mutations, if not repaired. We examined whether these oxidative and lipid peroxide related DNA adducts were induced in the lungs of mice by intratracheally instilled nanomaterials. 8-OxodG and three kinds of Hε-adducts were analyzed in the lungs of ICR mice 3, 24, 72 and 168 h after intratracheal instillation of 0.2 mg/body of C_{60} or kaolin, and quantified by the stable isotope dilution LC-MS/MS method described by Chou *et al.* (10). Compared with a vehicle control, DNA adduct levels were increased by about 2- to 5-fold in the lungs of mice 24 h after injection of nanoparticles (Fig. 4). The increases were time dependent until 72 h then gradually decreased within 168 h of injection (data not shown). Related to this, oxidative DNA damage was induced by intratracheal instillation of C_{60} or kaolin in the comet assay with FPG treatment, as described above. In addition, Folkmann *et al.* reported that oral gavage of C_{60} increased the levels of 8-oxodG in the liver and the lungs of F344 rats (11). Moreover, Tsurudome *et al.* described increased 8-oxodG levels induced by intratracheally instilled diesel exhaust particles in the lungs of F344 rats, and 8-oxoguanine DNA glycosylase 1 (OGG1) mRNA was also overexpressed (12). The decreased DNA adducts in the present study at 168 h may have been a result of a repair enzyme such as OGG1. This is the first observation that Hε-lipid peroxide related DNA adducts are increased by nanoparticles. Such adducts could clearly contribute to nanomaterial-induced DNA damage and mutation. Our findings suggest involvement of ROS generation, although differences between C_{60} and kaolin still require clarification.

***gpt* Mutations in the lungs of *gpt* transgenic mice:** Transgenic *gpt* delta mice are a useful model system for detecting both point mutations and large deletions (< 10 kb) (13). λEG10 transgenes carrying gpt (detection of point mutations) and *red*, *gam* (detection of deletion) genes have been integrated into mouse chromosome 17, and point mutations and deletions observed in any tissues can be detected as 6-thioguanine (6-TG) resistant colonies and Spi^- plaques, respectively. To examine *in vivo* mutagenicity of nanoparticles, *gpt* delta transgenic mice were exposed to C_{60} and kaolin at four different doses by intratracheal instillation, and *gpt* mutations were analyzed. The background *gpt* mutant frequency (MF) in lungs was $10.3 \pm 0.53 \times 10^{-6}$. MFs were significantly increased by 2 to 3-fold to $30.75 \pm 3.32 \times 10^{-6}$ ($p = 0.019$) for C_{60} and $19.30 \pm 4.82 \times 10^{-6}$ ($p = 0.002$) for kaolin (4).

Moreover, we examined the mutational characteris-

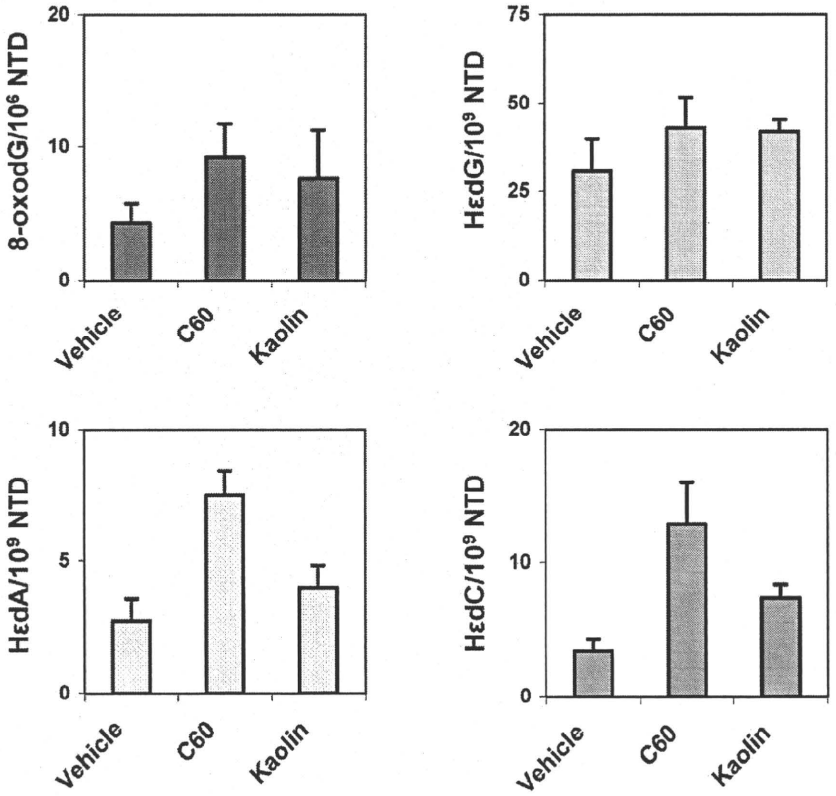

Fig. 4. Oxidative and lipid peroxide related DNA adduct formation in the lungs of ICR mice induced by nanoparticle exposure. DNA was extracted from lungs of mice 24 h after intratracheal instillation of 0.2 mg/body of C60 or kaolin, and digested enzymatically. Control animals were exposed to saline containing 0.05% Tween80. The 8-oxodG and 3 kinds of Hε-adducts were quantified by the stable isotope dilution LC-MS/MS method described by Chou *et al.* (10).

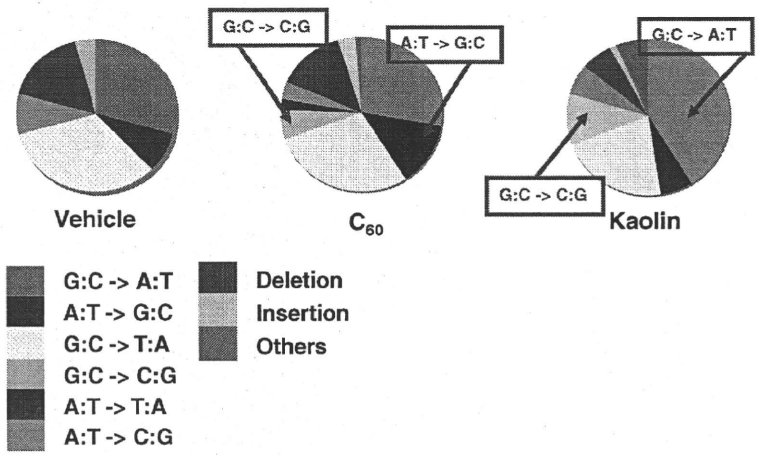

Fig. 5. Classification of *gpt* mutations from the lungs of control and nanoparticle treated mice.

tics induced by particles by PCR and DNA sequencing analysis of 6-TG resistant mutants. Classes of mutations found in the *gpt* gene are shown in Fig. 5. Interestingly, G:C to C:G transversions were increased in common with both particle treatments. Since these mutations were commonly increased regardless of the constituents (i.e., C_{60} is graphite and kaolin is aluminum silicate), the mechanisms might be the same. It has been reported that various oxidative stresses caused by sunlight, UV radiation, hydrogen peroxide and peroxy radicals frequently induce G:C to C:G transversions in various *in vitro* assay systems (14–17). Moreover, a variety of ox-

idative lesion products of guanine other than 8-oxodG, including imidazolone (Iz), oxazolone (Oz), spiroiminodihydantoin (Sp) and guanidinohydantoin (Gh), have been reported (18–24). Three such molecules, Oz, Sp and Gh are now thought to be key causes G to C transversions with translesion synthesis systems (22–25). Therefore, it is suggested that G:C to C:G transversions induced by C_{60} and kaolin could involve Oz, Sp and Gh formation. In addition, G:C to A:T transitions were also significantly increased by instillation of kaolin but not C_{60}. In general, G to A (or C to T) transitions have commonly been observed in spontaneous and chemically-induced mutants, and deamination of guanine or 5-methylcytosine might be involved. Burney *et al.* reported that nitric oxide induces DNA damage. NO· can form N_2O_3, and direct by this agent can lead to DNA deamination via diazonium ion formation (26). Moreover, nitric oxide is produced by activated macrophages in inflamed organs. In fact, test substance-phagocytized macrophages and granulomas were frequently observed in the lungs of mice (4).

Immunohistochemical Analysis of Inflammation Factors

In order to confirm enhancement of nitric oxide production by C_{60} and kaolin, we examined immunohistochemical staining of an inflammation factor, nitrotyrosine (NT), in the lungs of *gpt* delta mice treated with these nanoparticles using the same procedure reported previously (27) with minor modification. As shown in Fig. 6, the pattern of NT staining corresponded to the areas of inflammation within lung parenchyma. In the case of C_{60} exposure, many regions of the lungs stained positively (data not shown), and intense NT staining was localized in test substance-phagocytized macrophages and granulomas. Similarly, staining with NT antibodies was observed in macrophages and alveolar epithelial cells in the lungs of mice exposed to kaolin, although to a lesser extent as compared with C_{60}.

Conclusion

Our results clearly demonstrated that both *in vitro* and *in vivo* genotoxicity are induced by C_{60} and kaolin. However, the mechanisms have yet to be fully clarified, and oxidative stress might be at least partly involved. There are a number of ways in which reactive oxygen species (ROS) could be generated: i) nanoparticles might trigger ROS production by iron-catalysed Fenton reactions; ii) nanoparticles could accumulate in cells due to phagocytosis, then enhance the production of ROS by NADPH oxidase (28,29). Recently, innate immune activation through Nalp3 inflammasomes has been suggested to play an important role in pulmonary fibrotic disorders of silicosis and asbestosis (30,31). It has been reported that proinflammatory cytokines, such as interleukin 1β are key molecules for pneumoconiosis. At

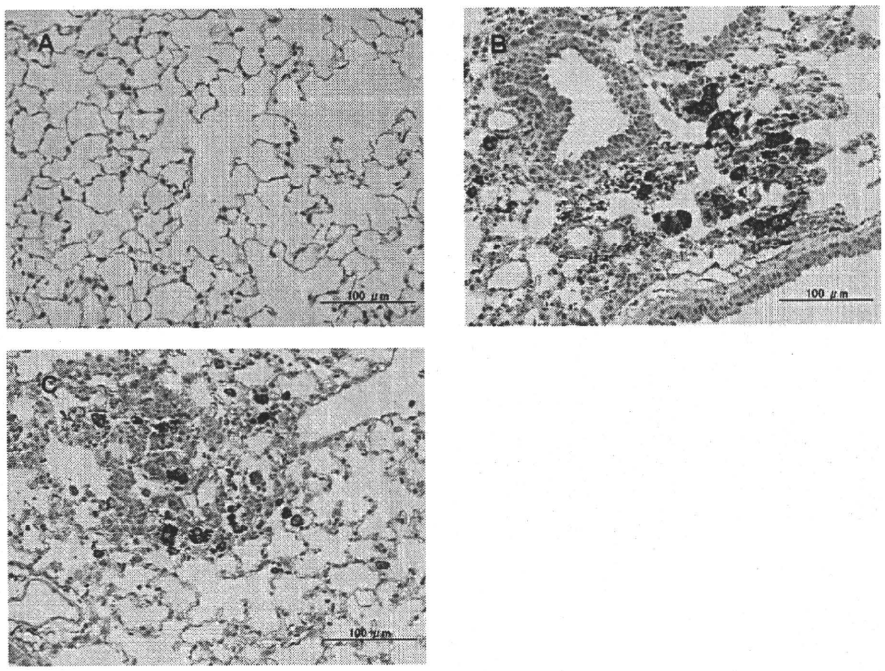

Fig. 6. Immunohistochemical localization of nitrotyrosine (NT). Since C_{60} is brown in color, we used an SG substrate kit (Vector Laboratories, USA) for peroxidase, with positive cells stained dark blue-gray. A: alveolar region in a control mouse, with no significant staining for NT. B: alveolar region in a mouse exposed to C_{60}, with positive macrophages phagocytizing test substance and epithelial cells. The brown colored material is C_{60}. C: alveolar region in a mouse exposed to kaolin. Note intense staining for NT in the granulomatous region.

present, no data are available for activation of the Nalp3 inflammasome pathway by C_{60} and kaolin. However, it is likely that both nanoparticles can activate in the same way as asbestos and silica, because oxidative stress was increased in the lungs of treated mice. Further studies of the mechanisms of genotoxicity are needed.

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