Oxidative damage and direct adducts in calf thymus DNA induced by the pentachlorophenol metabolites, tetrachlorohydroquinone and tetrachloro-1,4-benzoquinone

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DNA damage induced by quinoid metabolites of pentachlorophenol (PCP), i.e. tetrachloro-1,4-benzoquinone (Cl4BQ) and tetrachlorohydroquinone (Cl4HQ), was investigated in calf thymus DNA. The 32P-post-labeling assay revealed four major and several minor adducts (3.5 adducts per 105 total nucleotides) that were produced in calf thymus DNA treated with Cl4BQ (5 mM). These DNA adducts were chemically stable even after conditions that induce thermal depurination and are unlikely to undergo depurination/depyrimidination to form apurinic/apyrimidinic (AP) sites. In addition, increases in 8-HO-dG induced by Cl4HQ plus Cu(II) and NADPH were correlated with the formation of AP sites. Concentration-dependent data suggest that oxidative damage is causally involved in the formation of AP sites. Catalase inhibited the formation of DNA damage. These results suggest that oxidative damage is causally involved in the formation of AP sites. Concentration-dependent increases in 8-HO-dG induced by Cl4HQ plus Cu(II) and Cl4BQ plus Cu(II) and NADPH were correlated with the formation of AP sites (0.5 AP sites per 105 nucleotides) and DNA single-strand breaks. The types of DNA damage induced by Cl4HQ plus Cu(II) were similar to those by Cl4BQ plus Cu(II) and NADPH, whereas catalase inhibited the formation of DNA damage. These data suggest that oxidative damage is causally involved in the formation of AP sites. Concentration-dependent increases in 8-HO-dG induced by Cl4HQ plus Cu(II) and Cl4BQ plus Cu(II) and NADPH were correlated with the formation of AP sites (0.5 AP sites per 105 nucleotides) and DNA single-strand breaks. Since hydrogen peroxide alone causes similar DNA damage, these results suggest the involvement of Cu(II) and hydrogen peroxide in the induction of oxidative DNA damage by Cl4HQ/Cl4BQ. The data demonstrate that PCP quinone and hydroquinone induce direct and oxidative base modifications as well as the formation of 5′-cleaved AP sites in genomic DNA. These lesions may have important implications for PCP clastogenicity and carcinogenicity.

Introduction

Pentachlorophenol (PCP), a widely used biocide, has been shown to be carcinogenic in laboratory rats and mice in 2 year chronic bioassays (1,2). To date, the mechanism by which PCP induces carcinogenesis in rodents is not known, but P450 metabolism to quinoid derivatives and oxidative stress are associated with PCP carcinogenesis (3–9).

Pentachlorophenol does not induce point mutations in bacterial and mammalian cells (for review see ref. 10) but does produce chromosomal damage in mammalian cells (4) and possibly in the lymphocytes of workers who are occupationally exposed to it (11). The chromosomal damage induced by PCP may derive primarily from its quinoid metabolites, tetrachlorohydroquinone (Cl4HQ) and tetrachloro-1,4-benzoquinone (Cl4BQ) (5,6,12). Autooxidation and/or enzyme-mediated oxidation of PCP catechol and hydroquinone to the corresponding semiquinones and quinones followed by subsequent reduction of quinones initiate redox cycling cascades and generate reactive oxygen species (ROS), which are believed to be responsible for PCP clastogenicity (3,6,13).

DNA damage resulting from attack by ROS includes base oxidation, deoxyribose damage, strand breaks, apurinic/apyrimidinic (AP) sites and DNA–protein cross-links. It has been shown that ROS can induce AP sites by direct hydrogen abstraction from the sugar moiety of DNA, leading to the formation of 5′-nicked oxidized AP sites (14–16). Cl4HQ and its counterpart, Cl4BQ, are capable of inducing DNA single-strand breaks (3,17,18) and micronuclei (5). It is therefore reasonable to suggest that PCP quinones will lead to the formation of AP sites as well as DNA single-strand breaks by generating ROS via redox cycling. Of the various types of oxidative DNA damage, an increase in 8-hydroxy-2′-deoxyguanosine (8-HO-dG) has been detected in the livers of rats and mice that ingested PCP and in the livers of mice administered Cl4HQ (7,8,19). Oxidized bases and AP sites are repaired by base-excision repair (BER) enzymes. It is not known whether PCP quinones impede the BER process by producing similar DNA lesions.

An alternative hypothesis for the formation of AP sites by PCP quinoids involves depurination/depyrimidination of PCP quinone–DNA adducts. It has been shown that PCP induces DNA adducts in the livers of rats and mice (19,20). These adducts could be removed enzymatically, or non-enzymatically due to their chemical instability, as observed in estrogen and PAH quinone DNA adducts (21,22) to form AP sites.

The formation of AP sites in genomic DNA as a result of PCP quinoids has not been investigated thus far. To examine the hypothesis that ROS generated by PCP quinoids are the main source for the parallel formation of 8-HO-dG, DNA single-strand breaks and AP sites, we analyzed the formation of oxidative and direct DNA damage induced by PCP quinoids in calf thymus DNA.

Abbreviations: 8-HO-dG, 8-hydroxy-deoxyguanosine; AP, apurinic/apyrimidinic; ASB, aldehyde reactive probe–slot-blot; BER, base-excision repair; Cl4BQ, tetrachloro-1,4-benzoquinone; Cl4HQ, tetrachlorohydroquinone; dG, 2′-deoxyguanosine; ESA, electrochemical array detector; NC, nitrocellulose; PCP, pentachlorophenol; RAL, relative adduct levels; ROS, reactive oxygen species.

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Materials and methods

Chemicals

Cl$_2$HQ (99%) and Cl$_4$BQ (98%) were from Aldrich (Milwaukie, WI). Micrococcal nuclease, nuclease P1, potato apyrase (Type VII) and spleen phosphodiesterase (Type I) were purchased from Sigma (St Louis, MO). Whatman chromatography paper was from Fisher Scientific (Pittsburgh, PA). T4 polynucleotide kinase was purchased from Altech (Deerfield, IL). [$\gamma$-32P]ATP (sp. act. >7000 Ci/mmol) was from ICN Pharmaceutical (Irvine, CA). Kodak XAR-5 film was obtained from Eastman Kodak ( Rochester, NY) for autoradiography. Reagents used for AP sites assay by aldehyde reactive probe-slot-blot (ASB) assay were as described by Nakamura and Swenberg (23). All other chemicals were purchased from Sigma, Aldrich or Fisher unless stated otherwise, and used without further purification.

Reaction of calf thymus DNA with Cl$_2$HQ and Cl$_4$BQ

To determine the induction of various types of DNA damage by PCP-derived quinoid metabolites, calf thymus DNA was incubated with PCP quinoids, i.e. Cl$_2$HQ and Cl$_4$BQ, under physiological conditions. The incubation medium (final volume 0.5 ml) consisted of 50 mM phosphate-buffered saline (PBS; pH 7.4) and 500 µg calf thymus DNA. The calf thymus DNA used in this study was pretreated with 100 mM methoxylamine (Sigma) to reduce the background number of AP sites (24). Nakamura et al. (24). In brief, DNA in PBS was incubated with 1 mM aldehyde reactive probe at 37°C for 10 min. After ethanol precipitation, DNA was resuspended in TE buffer (10 mM Tris–HCl pH 7.4, 1 mM EDTA). The DNA concentration was measured by a UV spectrophotometer, and the DNA solution was then dissolved in TE buffer. Heat-denatured DNA was immobilized on a nitrocellulose (NC) membrane (Hybond-C Super, Amersham Pharmacia Biotech). The NC membrane was soaked with 5× SSC (0.75 M NaCl and 0.075 M sodium citrate) at 37°C for 15 min and then baked in a vacuum oven. The membrane was pre-incubated with 10 ml Tris- NaCl buffer containing bovine serum albumin and then incubated in the same solution containing streptavidin-conjugated horseradish peroxidase at room temperature. After rinsing the NC membrane, the enzymatic activity on the membrane was visualized by enhanced chemiluminescence reagents. The nitrocellulose filter was then exposed to X-ray film, and the developed film was analyzed using a scanning densitometer.

Detection of residual AP sites was performed as described by Nakamura et al. (16). DNA and Escherichia coli exonuclease III (Exo III) (New England Biolabs, MA) in 10 mM Tris–HCl/ KOH were incubated at 37°C for 1 min, immediately followed by addition of 0.1 vol 100 mM EDTA. The sample was incubated with 100 mM putrescine in the reaction buffer at 37°C for 30 min, immediately followed by the ASB assay. The three-cleavage assays were performed as described by Nakamura et al. (16). For the three-cleavage assay, DNA, 10 mM EDTA and 100 mM putrescine were incubated in 10 mM Tris–HCl/KOH at 37°C for 30 min and immediately analyzed by the ASB assay.

To study the amount of heat-labile DNA bases, calf thymus DNA pre-exposed to Cl$_4$BQ was incubated at 70°C for 2 h in PBS. The number of AP sites induced by heat incubation was measured by the ASB assay.

Analysis of 8-HO-dG by HPLC-ECD

Quantitative analysis of 8-HO-dG was based on a HPLC/ electrochemical detection method (27). DNA (100 µg) was hydrolyzed enzymatically to deoxyribonucleosides using deoxyribonuclease I, spleen phosphodiesterase, snake venom phosphodiesterase and alkaline phosphatase. The digest was separated by reversed phase HPLC, and 8-HO-dG quantitated using an electrochemical array detector (ESA, Chelmsford, MA). In a serial array of electrodes at increasing potential (200, 300, 375, 450, 525, 600 mV), 8-HO-dG was present in DNA digests, with maximum oxidation occurring at 300 mV. Quantitative analysis of 8-HO-dG and 2′-deoxyguanosine (dG) was based upon peak area relative to the calibration curves of the corresponding authentic standards (0.992 < $r^2 < 0.999$).

DNA single-strand break assay

DNA single-strand breaks were assayed using agarose gel electrophoresis as previously described by Freeman et al. (28) and Chen et al. (29), with modifications. Briefly, 20 µg calf thymus DNA (dissolved in deionized water) was denatured at 100°C for 3 min in the presence of 5 µl 10 mM NaOH, 95% formamide, 0.05% bromophenol blue and 0.05% xylene cyanol. The denatured DNA was electrophoresed on a 0.7% agarose gel with 0.5 µg/ml ethidium bromide in 40 mM Tris–borate buffer containing 1 mM EDTA. The DNA was visualized under UV irradiation and was photographed with a Polaroid camera.
DNA damage induced by PCP metabolites

Statistical analysis
All data are expressed as mean ± SD. The significance of differences in the results was evaluated with ANOVA, followed by Dunnett’s multiple comparison test.

Results
DNA damage in calf thymus DNA induced by tetrachloro-1,4-benzoquinone with and without addition of Cu(II) and NADPH

In view of the recent studies indicating that depurination of quinone–DNA adducts of polyaromatic hydrocarbons and estrogen give rise to the formation of AP sites (21,22), we tested the hypothesis that the reactive quinone metabolites of PCP, i.e. Cl4BQ and Cl4HQ, induce the formation of AP sites in calf thymus DNA via alkylation of genomic DNA and its subsequent depurination/depyrimidination. We incubated 500 µg calf thymus DNA with 1–5 mM Cl4BQ for 2 h under physiological conditions. When Cl4BQ-modified DNA was assayed for the presence of direct DNA adducts by 32P-post-labeling method, four major adducts and several minor adducts adjacent to these were detected (Figure 1). The relative adduct levels (RAL) were estimated to be 3.5 ± 0.93 per 105 total nucleotides following incubation of calf thymus DNA with 5 mM Cl4BQ. The relative percentages of the adducts (major and adjacent minor adducts) were estimated as follows: 1, 4%; 2, 9%; 3, 67%; 4, 20%. No DNA adducts were detected in control DNA under the same conditions (Figure 1A).

To induce depurination of DNA adducts, the Cl4BQ-modified DNA was subjected to neutral thermal hydrolysis (in PBS, pH 7.4; heated at 100°C for 20 min). Results indicated that all four major adducts remained intact after the treatment (Figure 1C and D). This result suggested that these DNA adducts are chemically stable even after conditions that induce thermal depurination and that they are unlikely to undergo depurination/depyrimidination to form AP sites.

To determine if Cl4BQ directly induces AP sites in DNA, we measured Cl4BQ-modified DNA for the presence of AP sites using the ASB assay. Results indicated that Cl4BQ induced a 2- to 3-fold increase in the number of AP sites in calf thymus DNA over control (Figure 2B) and that the increase was concentration dependent. To determine if AP sites induced by Cl4BQ are excisable by a combination of class II AP endonuclease and β-elimination, we incubated the modified DNA with E.coli Exo III and putrescine, which cleave the DNA 5’ and 3’ to the AP site, respectively (16). After the incubation, the number of AP sites was reduced approximately 67 and 72% in control and Cl4BQ-treated DNA, respectively (P > 0.05) (Figure 3). This result indicated that more than two-thirds of the total number of AP sites induced by Cl4BQ were cleared by class II AP endonuclease and β-elimination.

To test whether heat-labile bases are introduced by Cl4BQ, the number of AP sites in DNA exposed to Cl4BQ was quantitated after neutral thermal hydrolysis at 70°C for 2 h. The number of AP sites in DNA treated with Cl4BQ after heat incubation was equivalent to that in control (data not shown). This finding, along with data on DNA adducts detected by...
32P-post-labeling, suggests that Cl4BQ did not introduce a significant amount of heat-labile base lesions. To investigate the origin of Cl4BQ-induced AP sites, we assayed the Cl4BQ-modified DNA for increases in 8-OH-dG and DNA single-strand breaks. The amount of 8-OH-dG present in the control calf thymus DNA was high, which is probably due to an artifact of commercial isolation. Even compared with the high amount of 8-OH-dG in the control DNA, increased 8-OH-dG was detected in DNA treated with 1–5 mM Cl4BQ (P < 0.05) (Figure 2A) whereas no significant increases were observed in DNA single-strand breaks (Figure 4, lanes 7–8). This finding indicates parallel formation of AP sites and 8-OH-dG in Cl4BQ-modified DNA and suggests that AP sites induced by Cl4BQ may be due to deoxyribose damage directly caused by oxidative stress.

It has been shown that quinones undergo redox cycling in the presence of reducing equivalents (i.e. NADPH) and metal ions [e.g. Cu(II)] to generate ROS (6,30). To determine whether Cl4BQ mediates the formation of ROS and the production of AP sites in genomic DNA, we incubated calf thymus DNA with 1–10 μM Cl4BQ in the presence of 100 μM NADPH and 100 μM Cu(II). Results demonstrated that Cl4BQ plus NADPH and Cu(II) greatly increased the number of AP sites over control even at 1 μM (Figure 5B). This finding suggested that without the addition of Cu(II) for the presence DNA adducts and metal ions enhanced redox cycling of Cl4BQ to form AP sites.

In the presence of NADPH and Cu(II), parallel increases of 8-OH-dG and DNA single-strand breaks were detected in calf thymus DNA treated with Cl4BQ. The concentration of 8-OH-dG in DNA treated with Cl4BQ at 1 μM with the inclusion of Cu(II) and NADPH was 4.5-fold greater than that of the corresponding control (56 ± 8.1 versus 13 ± 2.4 per 10^5 dG) (Figure 5A). In addition, a dose-dependent induction of DNA fragmentation by Cl4BQ plus NADPH and Cu(II) was observed (Figure 4, lanes 4–6). These findings were in good agreement with results reported by Naito et al. (6) derived from similar reaction conditions. This finding confirmed that inclusion of NADPH and Cu(II) enhanced Cl4BQ to undergo redox cycling to generate ROS which is capable of inducing base modification, sugar damage, AP sites and DNA single-strand breaks.
DNA damage induced by PCP metabolites

Fig. 5. Formation of (A) 8-HO-dG and (B) AP sites in calf thymus DNA treated with 1–10 µM Cl4HQ, 100 µM Cu(II) and 100 µM NADPH under physiological condition at 37°C for 2 h. Data represent the mean ± SD of three determinations. *Statistically significant difference (P < 0.05) from control [DMSO plus Cu(II) and NADPH].

Fig. 6. Formation of (A) 8-HO-dG and (B) AP sites in calf thymus DNA treated with 300 µM Cl4HQ plus 100 µM Cu(II) under physiological condition at 37°C for 2 h. Data represent the mean ± SD of three determinations. *Statistically significant difference from control [DMSO plus Cu(II)] (P < 0.05).

Fig. 7. Concentration-dependent formation of 8-HO-dG and AP sites in calf thymus DNA treated with 0.5–300 µM Cl4HQ and 100 µM Cu(II). The data for 8-HO-dG and AP sites are normalized to the number of total nucleotides and each point represents the mean ± SD of three determinations.

the involvement of hydrogen peroxide. Increases in the number of AP sites by Cl4HQ plus Cu(II) were dose-dependent at concentrations ranging from 0.5 to 300 µM Cl4HQ (Figure 7).

To confirm the identities of AP sites after treatment with Cl4HQ plus Cu(II), the modified calf thymus DNA was incubated with *E. coli* exonuclease III and putrescine followed by the ASB assay as described above. After incubation, the number of AP sites was reduced by approximately 85 and 84% in control and Cl4HQ plus Cu(II)-treated DNA, respectively. This finding indicated that the majority of the AP sites induced by Cl4HQ plus Cu(II) in calf thymus DNA were excisable by class II AP endonuclease and β-elimination. To further determine whether the AP sites induced by Cl4HQ plus Cu(II) in calf thymus DNA were present as 5′-incised AP sites, DNA was incubated with putrescine followed by the ASB assay. A significant reduction of AP sites was detected after putrescine treatment (Figure 8). These results indicated that Cl4HQ plus Cu(II) produced predominantly (85%) 5′-cleaved AP sites in calf thymus DNA.

In parallel, we investigated the effects of Cl4HQ exposure on the induction of 8-HO-dG and DNA single-strand breaks in calf thymus DNA. Cl4HQ (up to 1 mM) did not increase the concentration of 8-HO-dG over controls (P > 0.05), whereas the inclusion of 100 µM Cu(II) increased 8-HO-dG 3-fold in Cl4HQ-treated DNA over the corresponding control [DMSO plus Cu(II)] (P < 0.001) (Figure 6A), suggesting that Cu(II) catalyzes the autooxidation of Cl4HQ. Addition of 5 U catalase to the incubates reduced the concentrations of 8-HO-dG to levels comparable with that of control. The increases in
oxidative DNA damage, 8-HO-dG, was detected in the livers of PCP-treated mice, and these results were in good agreement with Naito et al. (6), who reported previously that ROS was generated during the Cu(II)-catalyzed autooxidation of Cl₂BQ.

Discussion

Direct DNA adducts and oxidative DNA damage have been implicated in PCP mouse liver carcinogenesis (3,6,7,20). PCP quinoids are reactive electrophiles capable of alkylating genomic DNA (19,20). It has been shown that labile bulky DNA adducts of estrogen and polycyclic aromatic hydrocarbon quinones are subsequently depurinated to form intact AP sites (21,22). In addition to direct DNA adducts, redox cycling of PCP quinoids to generate ROS is believed to play an important role in PCP carcinogenesis. The most commonly analyzed biomarker of oxidative DNA damage, 8-HO-dG, was detected in the livers of Fischer 344 rats that had been administered PCP for 27 weeks (10). Recent findings in Fischer 344 rats that had been administered PCP for 27 weeks also revealed a statistically significant increase in 8-HO-dG in hepatic DNA over control (19). This finding seemed to contradict the carcinogenicity of PCP in these two species, where PCP induces liver tumors in mice, but not in rats (1,2). In addition to oxidized bases, it is likely that other types of oxidative DNA lesions may be involved.

In an effort to understand both the direct and oxidative damage induced in DNA, we analyzed the relationships between the formation of oxidative and direct DNA adducts in calf thymus DNA under physiological conditions. When calf thymus DNA was treated with Cl₂BQ, several quinone–DNA adducts were detected by the 32P-post-labeling assay. In parallel, increases in 8-HO-dG and AP sites were observed in Cl₂BQ-modified calf thymus DNA. We tested whether the AP sites induced by Cl₂BQ were derived from chemical depurination/depyrimidination of some of the PCP quinone–DNA adducts due to their instability. When we treated Cl₂BQ-modified DNA by neutral thermal hydrolysis, we did not observe any loss of quinone–DNA adducts by the 32P-post-labeling assay (Figure 1C and D), or a significant increase in the number of AP sites. Results from our analyses do not support the hypothesis that depurination/depyrimidination of PCP quinone–DNA adducts was the source of AP sites.

The origin of the treatment-related increases in AP sites and 8-HO-dG in Cl₂BQ-modified DNA is not obvious. This process may not involve ROS since Cl₂BQ is unlikely to mediate the formation of ROS in the absence of reducing equivalents, such as NADPH. It is well known that Cl₂BQ can serve as an oxidizing agent to mediate hydride transfer from carbon to oxygen due to its high redox potential (31). Cl₂BQ appears to directly abstract hydrogen on the C4-’position of deoxyribose and C8-position of guanine to produce AP sites and 8-HO-dG, respectively, where it reduces to Cl₂HQ in this process. However, these increases in the formation of AP sites and 8-HO-dG in genomic DNA induced by a high concentration of Cl₂BQ (5 mM) are unlikely to be of biological significance.

To test the hypothesis that ROS is the main source of AP sites induced by Cl₂BQ, we treated calf thymus DNA with a much lower concentration of Cl₂BQ (1 μM) in the presence of Cu(II) and NADPH. Under this condition, we did not detect direct DNA adducts (data not shown) but did observe parallel increases in the formation of 8-HO-dG, AP sites and DNA single-strand breaks over the corresponding control (Figures 4 and 5). The increase in 8-HO-dG and AP sites induced by 1 μM Cl₂BQ plus NADPH and Cu(II) was estimated to be 2-fold, respectively, greater than those by a high dose of Cl₂BQ (5 mM) alone. As metal ions are always present in cells, it is likely that the presence of metal ions and reducing equivalents promotes the redox cycling of Cl₂BQ to generate ROS and initiate oxidative DNA damage.

When calf thymus DNA was treated with Cl₂HQ and Cu(II), parallel induction of 8-oxo-dG, AP sites and DNA single-strand breaks was observed. In addition, catalase completely inhibited the damage induced by Cl₂HQ plus Cu(II), suggesting that Cu(II) and hydrogen peroxide is involved in the process. These results were in good agreement with Naito et al. (6), who reported previously that Cl₂BQ plus Cu(II) and NADPH and Cl₂HQ plus Cu(II) induce parallel formation of 8-HO-dG and DNA fragmentation. Furthermore, levels of direct DNA adducts induced by a high dose of Cl₂HQ (1 mM) were estimated to be 500-fold less than those of 8-HO-dG and AP sites by a low dose of Cl₂HQ (10 μM) plus Cu(II). Overall, this evidence supports the notion that AP sites in calf thymus DNA induced by Cl₂BQ/C1₂HQ in the presence of Cu(II) and/or NADPH are primarily mediated by ROS. Regression analysis of the parallel formation of AP sites and 8-HO-dG indicated that there was a linear relationship of the levels of 8-HO-dG and the number of AP sites induced by Cl₂BQ plus Cu(II) and NADPH and Cl₂HQ plus Cu(II) (r² = 0.977) (Figure 9). The ratios of 8-HO-dG to AP sites (per nucleotide) were estimated to be 1:1.6. Figure 10 depicts the proposed pathway leading to the formation of AP sites, 8-HO-dG and DNA adducts in calf thymus DNA induced by redox cycling of PCP quinoids and Cu(II) and the subsequent formation of Cu(I) and H₂O₂.

Furthermore, results of the AP site cleavage assay indicate that ~65% of AP sites in calf thymus DNA treated with Cl₂HQ...
Fig. 9. The regression analysis of 8-HO-dG and the number of AP sites in calf thymus DNA treated with Cl₄BQ plus Cu(II) and NADPH (■), and Cl₄HQ plus Cu(II) (●). The data for 8-HO-dG and AP sites are normalized to the number of total nucleotides and each point represents the mean ± SD of three determinations.

Fig. 10. Diagram of the proposed pathway leading to the formation of various types of DNA damage by redox cycling of PCP quinoids.

plus Cu(II) were cleaved by reacting with putrescine (Figure 8). This suggests that the predominant form of AP sites induced by Cl₄HQ plus Cu(II) are cleaved on the 5’ side of the AP sites. This finding is in good agreement with our previous investigation in calf thymus DNA treated with H₂O₂ and FeSO₄, where 5’-cleaved AP sites accounted for two-thirds of all AP sites (16). Taken together, these results provide evidence that in addition to base modifications, the 5’-cleaved AP sites induced by PCP quinone/hydroquinone-generated ROS in genomic DNA represent a major type of oxidative damage to the DNA backbone. Oxidative DNA damage has been reported in the liver of both rats and mice treated with PCP (8,19), but liver tumors were observed in mice only, whereas mesotheliomas occurred in male rats (1,2). The species difference in susceptibility to PCP-induced hepatocarcinogenicity between rats and mice may be attributed to differences in genetic predisposition, such as the high frequency of mutation in Ha-ras and c-myc oncogenes in B6C3F₁ mouse liver (32,33).

Differences in the expression of base excision repair enzymes for oxidative DNA damage may also contribute to this discrepancy, since mesothelial cells are susceptible to asbestos-induced oxidative DNA lesions due to the deficiency in BER enzymes for oxidative DNA damage (34,35). In summary, we suggest that ROS-mediated damage is the predominant type of DNA damage induced by PCP quinoids. The 5’-cleaved AP sites induced by ROS arising from PCP quinoids may explain, in part, PCP-induced clastogenicity and carcinogenesis.

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References


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