Effects of Hypochlorous Acid on Oxidative DNA Damage in Human Alveolar Epithelial Cells

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ABSTRACT

Activated neutrophils generate hypochlorous acid (HOCl) via the release of the enzyme myeloperoxidase and hydrogen peroxide (H_2O_2). There is accumulating evidence indicating an association between inflammation and carcinogenesis. However, the underlying mechanisms are largely unknown. In the present study, we found that HOCl induced a slight increase in 8-OHdG DNA lesions, but did not lead to significant DNA strand breakage in human alveolar epithelial cells. In addition, exposure of HOCl to A549 cells did not induce a significant increase in the level of p21 and GADD45 genes. In contrast, H_2O_2, the well-known DNA damaging agent and precursor of HOCl, significantly induced oxidative DNA damage and increased the level of p21 and GADD45 gene expression. Together these results suggest that mutagenic effects of HOCl in human alveolar epithelial cells are unlikely explained by the ability of HOCl to induce the formation of pre-mutagenic 8-OHdG DNA lesions and DNA strand breaks.

Keywords: hypochlorous acid, DNA strand breakage, 8-OHdG, p21, Gadd45, lung cancer

Inflammation induced by biological, chemical, and physiological factors has long been associated with increased risk of human cancer in various organs.[1] Epidemiological studies indicate a strong relationship between inflammation and carcinogenesis. For example, individuals with long-standing extensive ulcerative colitis and Crohn's disease have a significant risk of colorectal cancer, chronic hepatitis B and C infections in the liver predispose to hepatocellular carcinoma, and *Helicobacter pylori* infection has been established to have a causal relationship to gastric cancer.[2] Within the lung, chronic inflammatory diseases, such as idiopathic pulmonary fibrosis, systemic sclerosis, certain pneumoconiosis and chronic obstructive pulmonary disease (COPD), have been implicated to lung carcinogenesis.[3]

Inflammatory processes in the lung are characterized by the influx of neutrophils into the airways. At this moment it is suggested that increased neutrophil accumulation, although necessary as part of the lung's secondary defense systems, is crucially involved in the development of lung cancer.[4] Weitzman and Stossel first demonstrated that activated polymorphonuclear neutrophils (PMNs) are able to cause both mutations and malignant transformations in vitro. Further studies have supported the important role of PMNs in carcinogenesis by the ability of PMNs to induce DNA single strand breaks and DNA base modification. Although the mechanisms whereby inflammation may initiate or promote carcinogenesis has not been fully elucidated, production of DNA damaging reactive oxygen species (ROS) by activated inflammatory cells has been proposed to contribute significantly to inflammation-associated carcinogenesis. Reactive oxygen species are nowadays considered to participate in cancer initiation, promotion and progression. Reactive oxygen species are highly reactive molecules or molecular fragments that are continuously produced in all aerobic organisms, mostly as a consequence of mitochondrial respiratory chain reaction. Besides oxidative phosphorylation, ROS are continuously formed in peroxisomes, the cytochrome P450 system and by inflammatory cells, including neutrophils, eosinophils and macrophages.[5]

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Compared to other organs, the lung represents a unique tissue for oxidant stress because it is directly exposed to higher oxygen concentration. In addition, because of their direct exposure to ambient air, lung cells experience enhanced oxidant stress by environmental irritants and pollutants including oxidants such as cigarette smoke, ozone, and free radical-generating environmental carcinogens. Inhaled particles such as crystalline and those present in cigarette smoke and diesel exhaust can stimulate oxidant generation by inflammatory cells, and it is suggested that pulmonary carcinogenicity upon chronic particle exposure involves an influx and subsequent activation of inflammatory phagocytes.[6]

Upon activation, neutrophils generate a vast amount of oxidants. PMN-generated ROS is suggested to be a major factor involved in the mechanism by which neutrophils induce mutations and promote cancer development. Neutrophils contain the enzyme myeloperoxidase (MPO), which is capable of catalyzing the reaction of chloride with hydrogen peroxide to produce large amounts of hypochlorous acid (HOCl) as an end product of the respiratory burst upon neutrophil activation. Currently, it is generally accepted that the mutagenic capacity of ROS-derived neutrophils is mediated by H_2O_2 through the formation of highly reactive hydroxyl radical ($^{\bullet}OH$). In contrast, until recently HOCl was believed to have no contribution in DNA damage and mutagenesis mediated by activated neutrophils. However, recently we found that hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene mutation frequency was increased in HOCl-exposed A549 human alveolar epithelial cells (Güngör *et al*., Unpublished). In line with this observation, Güngör *et al*. showed that neutrophils were potent inhibitors of nucleotide excision repair (NER) in human pulmonary epithelial cells and MPO-catalysed formation of HOCl was thought to be the most likely ROS responsible for these inhibitory effects. In fact, HOCl is the major oxidant produced by neutrophils, since MPO consumes up to 70% of neutrophil-derived H_2O_2 to generate HOCl. Hypochlorous acid plays an important role in bacterial cell killing, but excessive or misplaced generation of HOCl is known to cause damage to tissues. HOCl is capable to react with a number of biological molecules including DNA, proteins, lipids and cholesterol.[7] In addition, in the presence of superoxide ($O_2^{\bullet-}$) or reduced metal ions, HOCl may generate hydroxyl radicals. Shen *et al*. reported that incubation of DNA with either isolated myeloperoxidase (MPO) or eosinophil peroxidase (EPO), together with plasma levels of halides (Cl- and Br-), and a cell-free $O_2^{\bullet-}$ -generating system resulted in oxidative DNA damage. However, despite the multitude of cellular and extracellular targets with which HOCl can react, its ability to cause DNA damage in intact cells is still poorly investigated. Therefore, our recent findings regarding HOCl-induced mutagenicity, warrant further studies to investigate the oxidative DNA damage effects of HOCl in order to find a possible explanation for the mutagenic effects of HOCl.

Various mechanisms exist to maintain genetic stability of cells facing DNA damage. The cellular response to DNA damage is complex and most of which link to the cell cycle. The inactivation of cell cycle checkpoint control has emerged as a central cause of genetic instability, ultimately leading to increased susceptibility of cells to consequences of DNA damages. Consequently, these unstable cells may contribute significantly to the origin of cancer.[8]

The p53 tumor suppressor is a universal sensor of genotoxic stress that regulates the transcription of genes required for cell-cycle arrest and apoptosis. In response to DNA damage, p53 protein is phosphorylated at its amino-terminus and becomes stabilized upon disruption of an interaction with its negative regulator, MDM2. Upon activation, p53 promotes different interactions with other proteins and with target gene regulatory elements to facilitate cell-cycle arrest, apoptosis, or adaptation in response to DNA damage. Among the transcriptional targets of p53, the cyclin-dependent kinase (CDK) inhibitor $p21^{Cip1}$ and growth arrest and DNA damage (Gadd) genes play a key role in mediating G1 arrest. p21 inhibits growth of proliferating cells by inhibiting G1 cyclin-dependent kinases and proliferating cell nuclear antigen (PCNA)-dependent DNA polymerase activity at DNA replication forks. Like p53, the Gadd genes are induced in cells exposed to genotoxic stress. *Gadds* were originally identified in a genetic screen for early response genes induced by ultraviolet radiation. Each gene encodes a distinct gene product that participates in the cellular response to stress. In the lung, Gadd45 is selectively

expressed in alveolar cells during hyperoxia. Gadd45 binds DNA replication and repair proteins cdc2, p21, and PCNA, suggesting a potential role in cell cycle control and DNA repair. Altogether, it suggested that p21 and Gadd45 may serve to coordinate DNA repair and replication in damaged cells, and are considered as a sensitive indicator of genotoxic stress.[9]

In the present study, we examined oxidative DNA damage induced by HOCl in pulmonary cells. For this purpose, the effects of HOCl on cellular DNA were investigated in A549 human alveolar epithelial cells by measuring both DNA single strand breaks and 8-OHdG DNA lesions. DNA strand breakage was measured by the comet assay which is regarded as a rapid and sensitive method for measuring DNA damage. 8-OHdG was measured as biomarker of oxidative DNA damage using high-performance liquid chromatography with electrochemical detection (HPLC-ECD).[10] As a further potential consequence of the cellular DNA damage, we also investigated whether HOCl alters the expression p21 and Gadd45 using quantitative real time PCR.

METHODS

Cell Culture

A549 cells (human alveolar epithelial cells), purchased from the American Tissue Culture Collection (ATCC), were cultured in DMEM (Sigma, Zwijndrecht, the Netherlands) supplemented with 10% heat inactivated fetal calf serum (FCS, Gibco Invitrogen, Breda, the Netherlands) and 1% penicillin/streptomycin (Sigma, Zwijndrecht, the Netherlands). Cells were maintained at 37°C in a 5% CO_2 atmosphere. Cells were routinely grown in 75 cm^2 cell-culture flasks and were passaged twice a week.

Cell Exposure

Confluent A549 cells were washed with HBSS, followed by exposure to HOCl (final concentrations ranging from 0 to 100 μM) in HBSS (37 °C). The negative control group was treated with a vehicle control (HBSS) and the positive control group was treated with H_2O_2. After incubating at 37°C for 15 minutes, the cells were harvested by trypsination for analyses of toxicity and DNA damage. Each individual exposure was repeated twice to ensure reproducibility. To examine the effects of HOCl exposure on DNA damage response, cells were exposed to HOCl in serum-free medium for 15 minutes, followed by washing and further incubation for up to 4 hours.

Cytotoxicity Assay

Cytotoxicity of HOCl or H_2O_2 in A549 cells was determined using trypan blue (0,04%, Sigma) and cells were counted in an Burker counting chamber.[11] Viable cells will maintain membrane integrity and will not take up trypan blue. Cells with compromised cell membranes take up trypan blue, and were counted as dead. At least 100 cells were counted and the number of viable cells was calculated as percentage of the total cell population.

Comet Assay

DNA strand breakage in A549 cells was determined by single cell gel electrophoresis/ alkaline comet assay, as described by Knaapen *et al*. [12]. Microscope slides were coated with a layer of 1.5% agarose. A549 cells were harvested and suspended in HBSS. Subsequently, 25 μl of the cell suspension (2-10^6 cells/ml) was mixed with 75 μl 0.65% low melting point agarose. This mixture was added to the precoated slides and covered with a cover glass. Following solidification (45 minutes, 4°C), cover glasses were removed and slides were immersed in lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris-base, 250 mM NaOH, pH 10; 10% DMSO and 1% Triton X-100) and stored at 4°C. After 18 h, slides were placed in an electrophoresis tank filled with buffer (300 mM NaOH, 1 mM EDTA, pH 13, 4°C) for 20 minutes. Electrophoresis was conducted at 300 mA and 25 V for 20 minutes. Subsequently, slides were neutralized by repeated washing (3-10 minutes) with neutralization buffer (90 mM Tris, 90 mM Borate, 2 mM EDTA, pH 7.5). Finally, slides were immersed in ethanol and allowed to dry under air. All steps described were performed in the dark/dimmed light to prevent additional DNA damage. Dried slides were stained with ethidium bromide (10 mg/ml) and

comets were visualized using a Zeiss Axioskop fluorescence microscope. Samples were tested in two independent incubations within each single experiment. On every single slide, 50 cells were analyzed randomly and the comet tail moment (a product of the DNA fraction in the tail and the tail length) was measured. The mean value of the tail moment in a particular sample was taken as an index of DNA damage in this sample.

Isolation of DNA and measurement of 8-oxodG by HPLC-ECD

After incubation, cells were harvested by trypsination and lysed for 2 hours at 37°C in SET-SDS-Prot K solution (75 mM NaCl, 25 mM EDTA, 0,5 mg/ml proteinase K, 1% SDS), followed by incubation with 0,1 mg/ml RNAse-A and 100 U/ml RNAse T for 1 hour at 37°C. DNA was extracted using a standard phenol-chloroform method. The DNA was then dissolved in 2 mM Tris-HCl (pH 7.4) at a final concentration of 0.5 mg/ml. Oxidative DNA damage was measured using HPLC-ECD, as described previously, and was expressed as the ratio of 8-Oxo-2-deoxyguanosine (dG).

Quantitative Real Time PCR

Quantitative real time PCR was applied to assess the effects of HOCl on the expression of DNA damage response genes. A549 cells were seeded in 28 cm^2 dishes and treated with HOCl. After 15 minutes, HOCl was removed and cells were further incubated in DMEM for 0 to 4 hours. After incubation, A549 cells were lysed in 1 ml Trizol® (Gibco Invitrogen, Breda, the Netherlands). Total RNA was isolated using phenol-chloroform method. The RNA yield was quantitated by UV spectrometry and then first strand cDNA was generated from 0,5 µg of total RNA using iScript™ cDNA synthesis kit (BIO-RAD, Veenendaal, the Netherlands). cDNA was used as the template for real time PCR to analysis mRNA expression of following genes: p21 and Gadd45. Real time PCR was performed with a MyiQ Single Colour real time PCR detection system (BIO-RAD, Veenendaal, the Netherlands) using SYBR© Green Supermix (BIO-RAD), 5 µl diluted cDNA and 0,3 µM primers (Table 1) in a total volume of 25 µl. PCR was conducted in the following condition: denaturation at 95°C for 3 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60°C for 45 seconds. After PCR, a melt curve temperature (60-95°C) was produced for product identification and purity. PCR efficiency of all primer sets, as assessed by the use of cDNA dilution curves, was approximately 100%. Data were analyzed using the MyiQ Software System (BIO-RAD) and were expressed as relative gene expression (fold increase), using the $2^{-\Delta\Delta Ct}$ method and employing â-actin as house keeping genes.

Gene	Sequence 5'→3'
β -actin	Forward primer: CCTGGCACCCAGCACAAT
	Reverse primer: GCCGATCCACACGGAGTACT
p21	Forward primer: GCAGACCAGCATGACAGATTTC
	Reverse primer: GGATTAGGGCTTCCTCTT
Gadd45	Forward primer: CGACCTGCAGTTTGCAATATGA
	Reverse primer: CCCCCACCTTATCCATCCTT

Table 1. Primer sequences for quantitative real time PCR

Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM) from 2 independent experiment, unless stated otherwise. Statistical analysis was performed using SPSS version 11.5 for Windows. Differences between experimental groups were analyzed using one-way ANOVA followed by multiple comparison evaluations using Dunnett's method to test differences between treatments and control. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Cell Viability

Since HOCl is known to induce cytotoxic effects in mammalian cells (33), we evaluated the viability of cells upon exposure to HOCl and H_2O_2. This was done to exclude the possibility of DNA damage induction as a secondary mechanism following possible HOCl-induced cell toxicity. H_2O_2 was used as a positive control to examine the feasibility of HOCl to induce oxidative DNA damage. Figure 1 shows the cell viability of A549 cells immediately following exposure to concentrations of HOCl and H_2O_2 from 0 to 200 µM for 15 minutes. Exposure of A549 cells to concentration of HOCl from 0 to 200 µM had no significant effect on cell viability as more than 90% of cells were viable upon exposure. Similarly, exposure of cells to H_2O_2 did not result in any significant loss of cell viability.

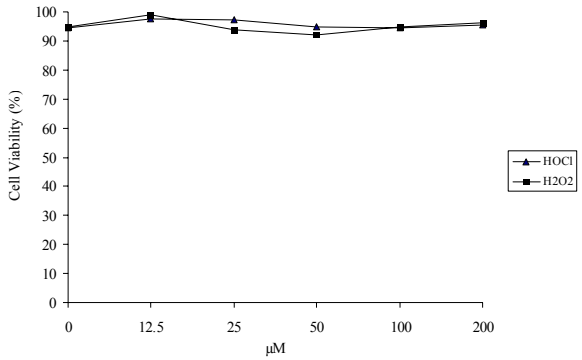


Figure 1. Cell viability of A549 cells incubated with HOCl and H_2O_2 for 15 minutes. Viability was tested by trypan blue dye exclusion assay. Data are expressed as percentage of viable cells (%) from one experiment.

Oxidative DNA Damage

To investigate whether HOCl could lead to oxidative DNA damage, cells were exposed to 0 to 100 µM of HOCl for 15 minutes. A significant increased of 8-oxodG formation in DNA isolated from A549 cells was only detected upon treatment with 50 µM of HOCl, compared to level observed in DNA from unexposed cells (Fig. 2). A higher concentration of HOCl (100 µM) did not result in significant increased levels of 8-OHdG. As was expected, H_2O_2 exposure, used as a positive control, resulted in a significant increased formation of 8-OHdG.

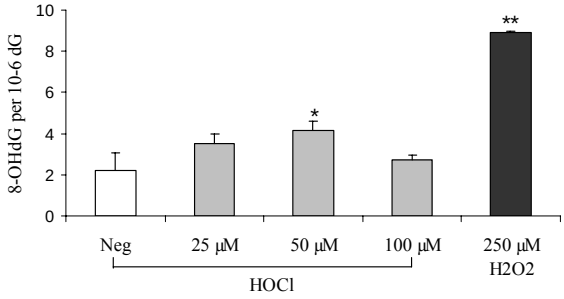


Figure 2. Formation of 8-OHdG in A549 cells after HOCl and H_2O_2 exposure for 15 minutes and 30 minutes, respectively. Levels were determined by HPLC-ECD as described in 'Materials and Methods and presented as the number of 8-OHdG residue per 10^6 deoxyguanosine (dG). Data points are mean ± SEM (n=2). *$p<0.05$ and **$p<0.01$ as compared to unexposed cells.

DNA Strand Breakage

DNA single strand breaks in human alveolar epithelial cells were analyzed by comet assay. Among various assays for measuring DNA damage, the single-cell gel electrophoresis (Comet) assay is a sensitive and powerful method for determining DNA strand breakage. The alkaline version of the comet assay is capable for measuring single-stranded breaks, secondary DNA breaks at alkali-labile sites as well as transient breaks induced by DNA repair enzymes[14]. Figure 3 shows that there is no significant induction of DNA strand breakage in cells exposed to HOCl for 15 minutes. By contrast, a considerable amount of DNA strand breakage was observed in cells exposed to equimolar doses of the positive control H_2O_2.

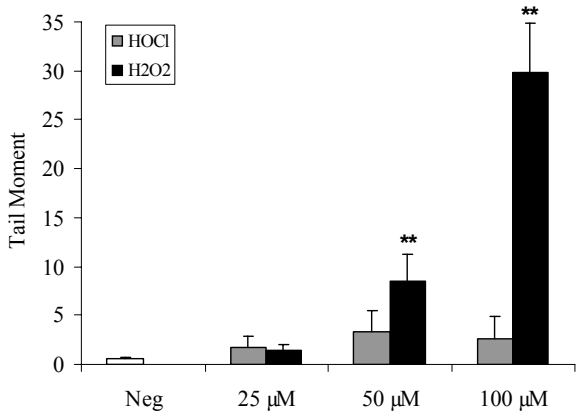
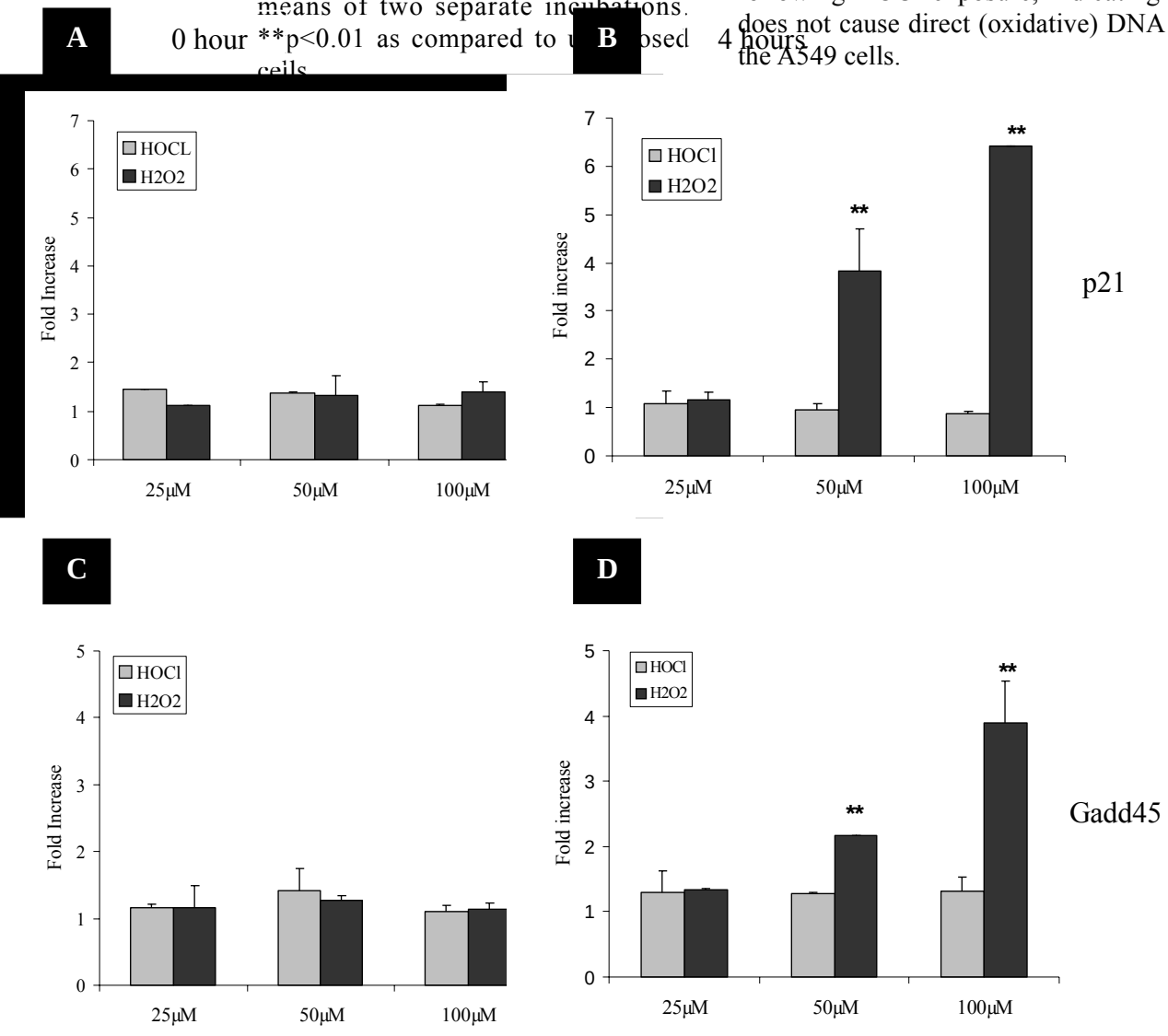


Figure 3. DNA strand breaks induced by HOCl and H_2O_2. A549 cells were exposed to different concentrations of HOCl and H_2O_2 in HBSS at 37°C for 15 and 30 minutes, respectively. DNA strand breaks were examined using comet assay and are presented as tail moments (a product of the DNA fraction in the tail and the tail length). Values are expressed as means of two separate incubations. **p<0.01 as compared to unexposed cells.

Cellular Response to DNA Damage

To investigate additional evidence of DNA damage, the expression of p21 and Gadd45 was evaluated using real time PCR in cells exposed to HOCl. To asses the effects of HOCl exposure on the cellular DNA damage response, A549 cells were initially exposed to HOCl and H_2O_2 for 15 minutes followed by washing and further incubation of the cells in serum-free medium for 0 and 4 hours. Analysis of gene expression immediately upon exposure of A549 cells to HOCl and H_2O_2 (i.e. directly following an incubation of 15 minutes) did not result in significant changes in p21 and Gadd45 gene expression (Fig 4A and C). The mRNA levels of p21 and GADD45 were still not significantly changed after a 4 hours recovery period in cells exposed with HOCl (Fig. 4B and D). In contrast, exposure of human alveolar epithelial cells to equimolar amounts of H_2O_2 caused a dose-dependent induction of p21 and GADD45 gene expression. These data are in line with the absence of a clear induction of DNA single strand breaks or 8-OHdG following HOCl exposure, indicating that HOCl does not cause direct (oxidative) DNA damage in the A549 cells.



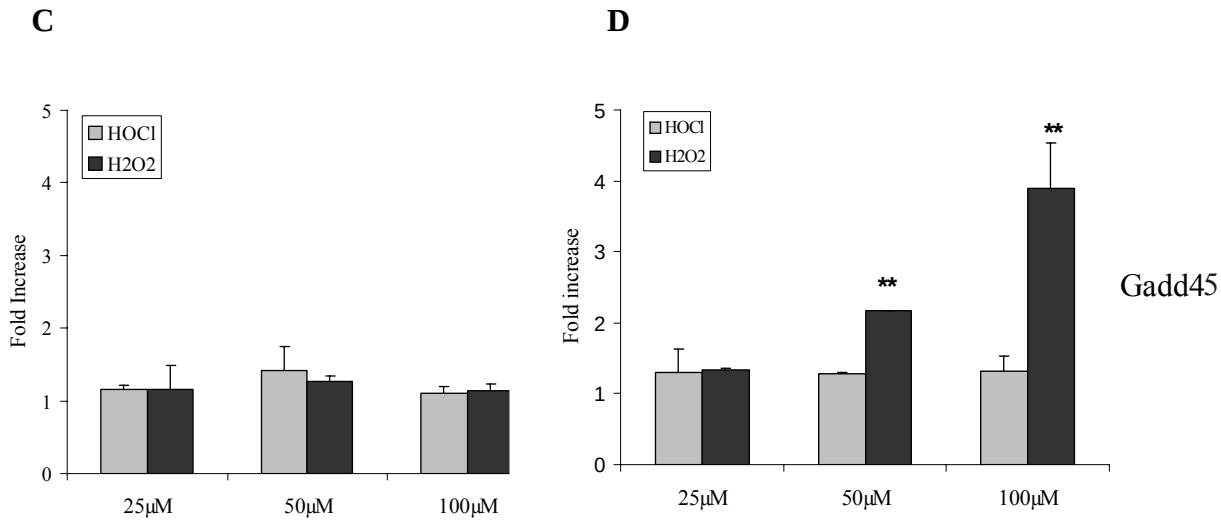


Figure 4. A549 cellular response to DNA damage. Cells were exposed to HOCl and H_2O_2 for 15 minutes in serum-free medium at 37°C. Level of p21 and GADD45 mRNAs were assessed by quantitative real time PCR. The figure shows p21 gene expression at 0 hour (A) and 4 hours following 15 minutes of incubation with HOCl (B); and Gadd45 gene expression at 0 hour (C) and 4 hours (D) following HOCl exposure. Data points are expressed as fold increase of gene expression compared to unexposed cells (1.00). **$p<0.01$ as compared to unexposed cells.

DISCUSSION

Neutrophils are a major source of oxidants in the inflamed lungs, and the constant release of ROS by these cells provides a plausible mechanism by which inflammatory processes and pulmonary carcinogenesis might be related. HOCl is likely the major strong oxidant produced by neutrophils, since MPO consumes up to 70% of neutrophil-derived H_2O_2 to generate HOCl and this may provide a possible explanation for the observed association between inflammation and lung cancer risk. HOCl has been shown to attack nucleotides and individual DNA bases in vitro, and to inactivate various DNA repair enzymes, processes that could lead to mutagenesis, thereby contributing to the increased risk of cancer. Infact, recently Güngör *et al*. found that HOCl exposure increased the mutations in the HPRT gene of A549 cells (Unpublished). In order to find possible explanations for these mutagenic effects of HOCl, we investigated oxidative DNA damage of HOCl in human alveolar epithelial cells. In the present study, we examined the ability of HOCl to promote oxidative DNA base modification through the formation of 8-OHdG. The level of 8-OHdG in DNA has long been used as a biomarker of oxidative DNA damage and is considered to play important role in carcinogenesis.[10] There was a slight but statistically significant increase in the 8-OHdG levels in cells treated with 50 µM of HOCl compared. However, higher concentration of HOCl tend to cause a lower level of 8-oxodG. The explanation for this effect is not clear, but might be caused by the degradation of some DNA base lesions at higher concentration of HOCl. Indeed, this event has already been demonstrated when HOCl reacts with isolated DNA. Additionally, several studies have observed the lack of purine damage products such as 8-OHdG after exposure with HOCl. They found that HOCl leads to a wider pattern of DNA base modification including oxidation of pyrimidine bases and chlorination of cytosine as well as xanthine and hypoxanthine production, but little oxidation of purines. Moreover, 5-chlorouracil (5-CU), a chlorination product of HOCl-mediated damage to nucleobases, seems to be a specific marker of HOCl-induced oxidative DNA damage. Therefore, the ideal way to investigate the effects of HOCl in DNA base modification is to measure not only 8-OHdG but also other types of DNA base modification. Taken together, the unique pattern of DNA base modification indicates that hydroxyl

radicals are not the major contributors to DNA damage induced by HOCl.[15]

In addition to oxidizing DNA bases, ROS may cause DNA strand breakage owing to free radical attack of the DNA sugar-phosphate backbone. DNA strand breaks may cause mutagenic and clastogenic effects which may ultimately lead to cancer development. In accordance with oxidative DNA damage results, we did not detect significant DNA strand breakage in human alveolar epithelial cells after treatment with HOCl. Our findings are consistent with those reported previously for gastric cells and murine macrophage-like tumor cells, whereas others reported a slight increased of DNA strand breakage in Chinese Hamster B14 cells. This discrepancy might be caused by the difference in sensitivity of target cells or HOCl concentration and duration of incubation used in HOCl exposure. As already observed in other studies, equimolar doses of H_2O_2, applied as a positive control, induced a substantial DNA strand breakage in A549 cells. It is believed that H_2O_2-induced DNA strand breakage is mediated by the formation of highly reactive ÿOH. Hydrogen peroxide is relatively stable and is shown to cross cellular membranes, and can penetrate the nucleus where •OH can be generated in the vicinity of the DNA molecule via the Fenton reaction.[16]

The limited level of HOCl-induced oxidative DNA damage and DNA strand breakage, as observed in the present study, may also be explained by the preferable interaction of HOCl with H_2O_2. The rate constant for reaction of HOCl with H_2O_2 is about 1000-fold faster than that for the reaction with DNA.[17] Indeed, Knaapen *et al*. suggested the presence or generation of endogenous, intracellular H_2O_2 in type II epithelial cells because they found that DNA strand breakage and induction of 8-OHdG was possible without extracellularly added H_2O_2. More specifically, Ye *et al*. [18] demonstrated that H_2O_2 was present in A549 cells, and that its concentration dramatically increased after exposure to the transition metal chromium. In addition, DNA damage induced by neutrophils is inhibited when neutrophil-derived H_2O_2 is consumed by MPO. Such a mechanism implies that MPO actually protects lung cells from neutrophil-elicited DNA damage by conversion of the stable and genotoxic H_2O_2 into the more cytotoxic HOCl.

To investigate additional evidence of DNA damage, we examined the cellular response after exposure with HOCl. Cells have evolved multiple mechanisms in the maintenance of genetic integrity upon DNA damage, most of which link to the cell cycle. Cell cycle checkpoints represent integral component of DNA repair and replication. By arresting the cell cycle, checkpoints presumably allow cells to repair DNA. In order to signal cell cycle arrest, checkpoint control pathways must sense DNA damage and then transmit the signal to several biochemical pathways that respond to DNA damage and restore the DNA structure. The p53 tumor suppressor protein is a central DNA damage checkpoint and clearly required for a complete DNA damage response. It has been known for years that the half-life of the otherwise short life p53 is increased several fold in cells treated with DNA-damaging agents. An increase in p53 concentration leads to the transcriptional activation of genes which modulate cell cycle control and DNA repair, including p21 and Gadd45 genes.[8] However, in the current study we did not find significant changes in the expression of both genes in cells after HOCl exposure. These results indicate that HOCl may not stimulate the p53-dependent cellular DNA damage response. Another possible explanation might be caused by the bolus doses of HOCl used in this experiment because Vile *et al*. previously showed that HOCl was effective to increase p53 levels only when it was produced as a flux, mimicking its production by neutrophils. This suggests that when generated continuously at low concentration, HOCl can act on specific target molecules involved in the regulation of p53 levels.[19] At the same concentration, H_2O_2 treatment significantly increases the gene expression of p21 and Gadd45 in A549 cells, which is in accordance with its ability to induce oxidative DNA damage.

There are several possible mechanisms by which HOCl can promote mutation. It should be emphasized that HOCl is very reactive and able to react with any cellular target before it reaches the nucleus and DNA need not always be attacked directly to raise levels of mutagenic oxidation products. In fact, the primary cellular target of HOCl appears to be proteins. HOCl-protein interactions result in side-chain modification, backbone fragmentation, and cross-linking, which may

ultimately to disturbed enzyme function. HOCl indeed has been identified as a potent inhibitor of DNA strand break repair, base excision repair (BER) through inhibition of poly (ADP-ribose) polymerase (PARP) activity, as well as nucleotide excision repair (NER) in benzo[a]pyrene-diolepoxide (BPDE)-exposed human alveolar epithelial cells. HOCl could also induce indirect genotoxicity via lipid peroxidation which generates many products including aldehydes such as malondialdehyde (MDA) and trans-4-hydroxy-2-nonenal (4-HNE). Previously, it reported that 4-HNE and MDA treatment of cells greatly inhibited NER for both UV-light and BPDE-induced DNA damage. Additionally, these two types of aldehydes are able to form DNA adducts and these aldehyde-induced DNA adducts have been shown to be mutagenic in human cells. With regard to HOCl, lipid peroxidation has been shown to be caused by thyl- and nitrogen-centered radicals, which are products of HOCl reaction with amino acids.[20] Together these results lead to propose that damage to DNA and DNA repair machinery are the most possible mechanisms caused by HOCl that could contribute to its mutagenicity and carcinogenicity.

CONCLUSION

In conclusion, the studies presented here demonstrated that HOCl induced a slight increase in the formation of 8-OHdG DNA lesions and did not lead to significant DNA strand breakages in human alveolar epithelial cells. The conclusion is obviously limited by our inability to detect additional types of HOCl-induced DNA base modification such as oxidation of pyrimidine base and chlorination of cytosine. We suggest that there are additional mechanisms such as lipid peroxidation or oxidation/modification of proteins involved in DNA repair, which may play a role in mutagenicity of HOCl. Future studies in this area are warranted since these might serve as a powerful tool to elucidate the association of neutrophil infiltration and lung carcinogenesis.

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