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Lipid Hydroperoxide Induced Oxidative Stress Damage and Antioxidant Enzyme Response in Caco-2 Human Colon Cells

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It has been demonstrated that reactive oxygen species, free radicals, and oxidative products, such as lipid hydroperoxides, participate in tissue injuries and in the onset and progression of degenerative diseases in humans. Studies were conducted using Caco-2 colon carcinoma cells to evaluate cellular damage caused by exposing cells for 30 min to oleic acid hydroperoxides (OAHPx) at concentrations varying from 0 to 25 μM. Cell membrane damage and DNA damage were significantly high even at the lowest concentration of 2.5 μM OAHPx compared to the control. Cell lipid peroxidation, indicated by conjugated diene concentration, increased exponentially with increasing OAHPx concentration. Antioxidant mechanisms in Caco-2 cells were evaluated by measuring catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities. Cellular catalase and GPx activities were not significantly different from each other at 0 to 25 μM OAHPx concentrations. SOD activity decreased with increasing OAHPx concentration. These results show that existing enzymatic antioxidant mechanisms are not sufficient for complete detoxification of 5−25 μM lipid hydroperoxides.

KEYWORDS: Caco-2; intestine; oxidative stress; lipid hydroperoxides; antioxidant enzymes; catalase; superoxide dismutase; glutathione peroxidase; DNA damage; cell membrane damage

INTRODUCTION

Lipid hydroperoxides have been associated with many pathological conditions in humans, such as cancer (*1*) and atherosclerosis (*2*). Lipid hydroperoxides in the body are derived from peroxidized unsaturated fatty acids that are present in cell membranes (*3*) and in dietary fats (*4*). Excessive lipid hydroperoxide concentrations in the gut can contribute to the impairment of mucosal detoxification pathways and enterocyte dysfunction (*4*), leading to the development of digestive tract disease conditions such as inflammation (*5*) and colon cancer (*6*, *7*). Lipid hydroperoxides activate lipoxygenase and cyclooxygenase, which are involved in cellular lipid peroxidation and prostaglandin biosynthesis, respectively, and can act as signal molecules for apoptotic cell death (*5*).

Dietary sources of lipid hydroperoxides include cooked fats and oils, meat, fish, milk and milk products, and eggs (*8*). Lipid peroxide levels (as thiobarbituric acid reactive substances) present in common foods are ≈25−150 nmol/g (*9*), and an intake of 84 g of dietary fat per day could contribute to as much as 1.4 mmol of lipid hydroperoxides (*10*). In reality these levels are expected to be higher because the estimated average daily intake of fat in the American diet is ≈106.7 g (*11*). Direct duodenal infusion of peroxidized lipids in rats resulted in 0.4 μM lipid hydroperoxides in their intestinal mucosa, liver, and kidneys (*12*), whereas plasma levels of lipid hydroperoxides can be as low as 0.1−0.6 pmol/L (*13*). However, in the intestinal mucosa, these levels are much higher and could reach micromolar levels because lipid hydroperoxides may also be generated during food digestion in the acidic gastric fluid that contains absorbed oxygen (*14*). Furthermore, oxidation of dietary fats in the stomach can be enhanced by the presence of metal catalysts that are abundant in foods (*14*, *15*). At low-pH conditions, availability of iron ions is increased due to increased solubility, which would in turn enhance lipid oxidation (*16*). In a simulated gastric fluid system (pH 3), within 2 h of incubation, 8- and 6-fold increases in lipid hydroperoxides were observed in a heated muscle tissue and in heated soybean oil in the presence of myoglobin, respectively (*14*).

Although toxic concentrations of lipid hydroperoxides relevant to lumen conditions have not been reported in humans, the fact that intake of oxidized polyunsaturated fatty acids can disturb the integrity of the intestine by inducing oxidative stress and disrupting redox balance is recognized (*17*). A concentration of 100−200 μM lipid hydroperoxides has been identified to be cytotoxic (*18*), whereas 10−25 μM lipid hydroperoxides induce apoptosis (*19*). Physiologically relevant levels of lipid hydroperoxides that are as low as 1−5 μM have also been known to induce mild oxidative stress in the intestine (*20*).

The intestinal epithelium has a very rapid cell turnover rate (*21*). Because the tissue homeostasis is balanced by cell proliferation and cell death, inducing shifts in the redox balance by oxidants, such as lipid hydroperoxides, can enhance mutagenic and apoptotic responses (*9*). Administration of hydro-

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peroxy and hydroxyl fatty acids in rats stimulated DNA synthesis and induced ornithine carboxylase activity consistent with enhanced cellular proliferation (*22*). Moreover, rats developed mucosal hypertrophy of the colon when given peroxidized ethyl linoleate (*23*).

To ensure cellular integrity and tissue homeostasis, the intestine exhibits several defense mechanisms, such as maintaining high concentrations of cellular antioxidant enzyme levels and inducing cell death by apoptosis to discard injured enterocytes (*9*). According to Aw et al. (*12*), mucosal glutathione (GSH) plays a major role in removing hydroperoxides from the intestine. In a healthy system where GSH levels are sufficient, intracellular metabolism of absorbed hydroperoxides is increased, thereby reducing luminal retention of hydroperoxides (*24*). Bile is a rich source for GSH, which helps maintain mucosal GSH to promote peroxide removal in the intestine (*25*).

The susceptibility of the gastrointestinal tract to oxidative damage is determined by its antioxidant status and the imbalance in oxidant/antioxidant content in gastric mucosa. Although the intestine is the primary site of action for dietary oxidative agents, very little information is available on how lipid hydroperoxides affect the cellular antioxidant mechanisms in the intestine. Previously we reported the damaging effects of hydrogen peroxide to the human intestine (*26*) using the human colon carcinoma cell line, Caco-2. This study is focused on evaluating the extent of damage caused by lipid hydroperoxides (5−25 μM) and the changes in cellular antioxidant enzymes in response to the oxidative stress induced in the intestine.

MATERIALS AND METHODS

A human colon carcinoma cell line (Caco-2) was obtained from the American Type Culture Collection (Rockville, MD). Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen Corp. (Carlsbad, CA). L-Glutamine, penicillin with streptomycin, trypsin with ethylenediaminetetraacetic acid (EDTA), and phosphate-buffered saline (PBS) were purchased from Fisher Scientific (Fair Lawn, NJ). Trypan blue, nonessential amino acid solution, xanthine, hypoxanthine, nitro blue tetrazolium, diethylenetriaminepentaacetic acid, lactate dehydrogenase (LDH) based TOX-7 kit, and glutathione peroxidase cellular activity assay kit CGP-1 were obtained from Sigma Chemical Co. (St. Louis, MO). Hydrogen peroxide, chloroform, cyclohexane, and ethanol were purchased from VWR International (Bridgeport, NJ). The Micro BCA protein assay kit was purchased from Pierce Biotechnology (Rockford, IL).

Culture and Oxidation of Caco-2 Cells. Caco-2 cells were grown in DMEM supplemented with 20% FBS, 1% L-glutamine, 1% nonessential amino acids, and 50 units/mL penicillin with 50 μg/mL streptomycin. The cell cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were seeded onto collagen-coated 25 or 75 cm^2 area culture flasks. Oxidation was induced by exposing Caco-2 cells to 2.5−25 μM oleic acid hydroperoxides (OAHPx) in FBS-free DMEM or PBS, supplemented with 1% L-glutamine and 1% nonessential amino acids, for 30 min on the fourth day of culturing after confluency had been reached. Cells were harvested by a brief (6 min) trypsinization and then centrifuged (Beckman GS-15R centrifuge, Beckman, Palo Alto, CA) at 200*g* for 5 min.

Preparation of OAHPx Solutions. Lipid hydroperoxides were generated by oxidation of oleic acid under fluorescent light for 21 days. The total hydroperoxide content was quantified using the peroxide value of oxidized oleic acid (*27*). Preliminary studies showed that oxidation products other than lipid hydroperoxides were extremely low and were not in significant amounts in the oxidized oleic acid samples. Stock solution of peroxidized lipid emulsions was prepared by sonicating 80 μmol of oxidized oleic acid in PBS at pH 7.4 containing 19 mmol L^{-1} sodium taurocholate (*12*). The stock emulsion was diluted with FBS-free DMEM before exposure to cells to attain needed OAHPx concentrations. The final sodium taurocholate content of the medium in all treatments was adjusted to be the same.

Cell Membrane Damage. Cells were grown to confluence in 25 cm^2 culture flasks and washed with PBS prior to use. Different concentrations of OAHPx (0−25 μM) in PBS supplemented with 1% L-glutamine and 1% nonessential amino acids were used to induce oxidation. After 30 min of exposure, PBS from each flask was collected. Damage to cell membrane by OAHPx was studied by measuring the release of lactic acid dehydrogenase (LDH) from injured cells. LDH leakage into PBS and total LDH activity (LDH leakage to PBS plus LDH in remaining cells) were measured with an in vitro cytotoxicity assay kit, lactate dehydrogenase based TOX-7 (Sigma ChemicalCo.), and corrected by the activity already present in the medium of untreated cells. The assay is based on the reduction of NAD to NADH by LDH. NADH is utilized to convert a tetrazolium dye in the assay kit to a colored compound with an absorption maximum at 490 nm. The intensity of the color is indicative of LDH activity in the assay medium, and the LDH activity was measured spectrophotometrically (Beckman Coulter DU800 spectrophotometer, Beckman Coulter Inc., Fullerton, CA) at 490 nm. LDH activity in the PBS supernatant was determined as a percentage of the total LDH activity.

Preparation of Cell Lysates. Culture medium was decanted, and cells were washed with 5−10 mL of PBS. The cells were harvested by a brief trypsinization. Cell suspensions were centrifuged at 200*g* for 5 min and washed twice with 5 mL of PBS. Supernatants were discarded and cell pellets resuspended in 5 mL of PBS at 0 °C and then placed on ice. Cells were lysed using a minibead beater (Biospec Products, Bartlesvillle, OK) for 10 s at 4200 rpm. The lysates were centrifuged (Beckman GS-15R centrifuge) at 14000*g* for 10 min at 4 °C and supernatants immediately used for lipid peroxidation and antioxidant enzyme assays.

Lipid Peroxidation Assay. Lipid peroxidation was assayed by measuring conjugated dienes in cell lysates. Conjugated dienes were quantified according to the method described by Buege and Aust (*28*). One milliliter of cell lysate in PBS was mixed thoroughly with 5 mL of chloroform/methanol (2:1) solution, followed by centrifugation (Beckman GS-15R centrifuge) at 1000*g* for 5 min until phase separation was achieved. Most of the upper layer was removed by suction, and 3 mL of the lower chloroform layer was transferred to a test tube. The chloroform layer was removed under nitrogen infusion, and the lipid residue was dissolved in 1.5 mL of cyclohexane. The absorbance of the solution at 233 nm was measured (Beckman Coulter DU800 spectrophotometer) against a cyclohexane blank at 233 nm. Conjugated dienes were reported as absorbance at 233 nm.

Catalase Assay. Catalase was assayed spectrophotometrically at 25 °C by following the extinction of H_2O_2 at 240 nm (*29*). The catalase activity per milliliter of the cell lysate was calculated as the reduction of H_2O_2 (mmol min^{-1} mL^{-1}). Nonenzymatic H_2O_2 decomposition (baseline) was subtracted from each determination.

Superoxide Dismutase (SOD) Assay. A modified version of the Nishikimi et al. (*30*) method was used to detect SOD activity in cell lysates. In this method, superoxide radicals were generated using a xanthine oxidase/hypoxanthine system, and the potential of the cell lysates to scavenge superoxide radicals was measured spectrophotometrically. The reaction mixture contained 1 mL of 3 mM hypoxanthine, 1 mL of 100 mIU xanthine oxidase, 1 mL of 12 mM diethylenetriaminepentaacetic acid, 1 mL of 178 μM nitro blue tetrazolium, and 1 mL of the cell lysate. All solutions were prepared in PBS. The absorbance of the mixtures at 560 nm was recorded initially at 0 min and thereafter at 5 min intervals up to 30 min. Superoxide radical-scavenging capacities (percent) of the cell lysates at the end of 30 min were calculated with the equation

$$Y = [1 - (A/B)] \times 100$$

where Y = percentage of superoxide radicals scavenged, A = absorbance of the medium containing cell lysate at 30 min, and B = absorbance of the medium without cell lysate at 30 min (blank).

Glutathione Peroxidase (GPx) Assay. GPx activity was measured using the GPx cellular activity assay kit CGP-1 (Sigma Chemical Co.). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPx, which is

then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPx activity. The activity of GPx per milliliter of the cell lysate was calculated as the decrease in NADPH (μmol min^{-1} mL^{-1}).

DNA Damage by Comet Assay. The comet assay was performed using Trevigen's comet assay reagent kit for single-cell electrophoresis assay (Trevigen Inc., Gaithersberg, MD). Cells (1×10^5) were suspended in 1 mL of ice-cold PBS. Fifty microliters of the cell suspension was combined with 500 μL of prewarmed low melting point (LMP) agarose, and 75 μL of this mixture was immediately pipetted onto a CometSlide. Slides were placed flat at 4 °C in the dark for 30 min for gelling. After completion of gelling, slides were transferred into a prechilled lysis solution (2.5 M sodium chloride, 100 mM EDTA, pH 10, 10 mM Tris base, 1% sodium lauryl sarcosinate, and 1% Triton X-100) and placed at 4 °C for 50 min. Slides were then incubated in a fresh electrophoresis buffer (0.3 M NaOH, 1 mM EDTA, pH 13) for 40 min at room temperature to allow unwinding of DNA. Electrophoresis was carried out at room temperature in fresh electrophoresis buffer for 40 min at 1 V/cm and 300 mA. After electrophoresis, slides were gently rinsed by dipping several times in distilled water and then immersed in 70% ethanol for 5 min and air-dried. Slides were stored with desiccant at room temperature prior to analysis. Slides were stained with SYBR green and viewed by an Olympus AX70TRF microscope digital camera system (Olympus Optical Co. Ltd.). Digital images of DNA were analyzed, and DNA damage was quantified by measuring the tail moment using NIH Image software available at http://rsb.info.nih.gov/ij/. About 100−150 cells were scored per sample. Damage is represented by an increase of DNA fragments that have migrated out of the cell nucleus in the form of a characteristic streak similar to the tail of a comet (*31*). DNA damage can be assessed using different parameters, such as tail length, relative tail fluorescence intensity, and tail moment (*31*, *32*).

Statistical Design. A general linear fixed effects model blocked by cell passage number, which corresponds to the age of cells, was used in all test systems. All experiments were repeated four times. Statistical analysis was conducted using Statistical Analysis System (SAS) software version 8.02 (SAS Institute Inc., Cary, NC) with analysis of variance (ANOVA) followed by Tukey's HSD test for significant differences. A p value of ≤ 0.05 was considered to be statistically significant unless stated otherwise. Nonlinear modeling was used to explain cellular damage and antioxidant enzyme activity. Parameter estimation was performed using the Marquardt's Compromise method (*33*). The best model that fits the data was chosen by comparing Akaike's Information Criterion (AIC), −2 log likelihood, Bayesian Information Criterion (BIC), and mean squared error (MSE) obtained for each model. The significance of parameters was confirmed by calculating their 95% confidence intervals and the correlation matrix.

RESULTS

Cell Damage. Lactic acid dehydrogenase enzyme activity in culture medium, which is an indicator of a leaky cell membrane due to damage, was significantly higher than that of the control at 2.5 μM OAHPx (**Table 1**). Cell treatments up to 3.5 μM OAHPx showed <10% of LDH activity. Concentrations >5 μM OAHPx resulted in an LDH activity of >15%. A maximum of 50% LDH activity was attained at 15 μM OAHPx. The increase in LDH activity with increasing concentrations of OAHPx followed a four-parameter logistic model [$Y = \delta + (\alpha - \delta)/(1 + \exp(-\kappa(X - \tau)))$] (**Figure 1**). The model shows a maximum rate of LDH leakage (τ) at 6.4 μM OAHPx. In other words, the increments by which the rate of LDH activity increases with increased OAHPx concentration become smaller after 6.4 μM OAHPx. The corresponding LDH activity at this point is 24%. Conjugated dienes, which are indicators of cell lipid peroxidation, continuously increased with increasing OAHPx concentrations. A significant increase compared to that of the control was detected at the 5 μM OAHPx level.

Table 1. Lactate Dehydrogenase (LDH) Activity in the Culture Medium of Caco-2 Cells Treated with Oleic Acid Hydroperoxide (OAHPx)

OAHPx concn (μM)	LDH activity (%) in culture medium[a]
0	0 a
1	0 a
2	2.2 ± 0.2 a
2.5	5.7 ± 0.6 b
3	8.2 ± 0.6 bc
4	11.7 ± 0.6 cd
5	15.5 ± 2d
7.5	30.3 ± 3.2e
10	41.8 ± 4.1 f
12.5	48.3 ± 4.2 g
15	50.7 ± 4.5 g
20	50.8 ± 3.8 g
25	51.1 ± 4.5 g

[a] Values are the mean ± SD of four determinations. Means having different letters are significantly ($p < 0.05$) different from one another.

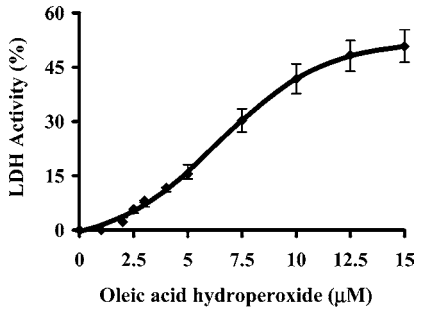

Figure 1. LDH activity in the culture medium (as a percentage of total cell LDH activity) of Caco-2 cell cultures incubated with different concentrations of OAHPx as depicted by a four-parameter logistic model. Each point represents the mean ± SD of four determinations.

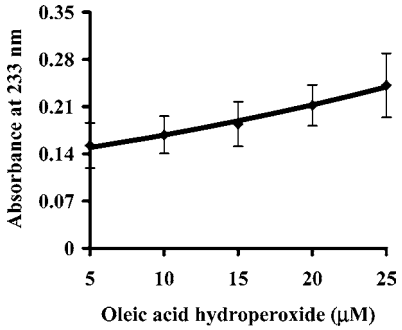

Figure 2. Conjugated dienes (absorbance at 233 nm) in Caco-2 cell cultures incubated with different concentrations of OAHPx as depicted by an exponential model. Each point represents the mean ± SD of four determinations.

Production of conjugated dienes at 5−25 μM OAHPx increased exponentially [$Y = \alpha(\exp(\kappa X))$] as shown in **Figure 2**. The model predicts a maximum 0.0236 increase in absorbance at 233 nm per 5 μM increment of OAHPx. **Table 2** shows the model equations developed to predict cell damage.

DNA Damage. Strand breaks of DNA, as measured by the tail moments using the comet assay, increased with increasing OAHPx concentrations (**Figure 3**). Significant DNA damage compared to the control was detected in cells treated with OAHPx at levels as low as 2.5 μM OAHPx, which also corresponded with the observed LDH damage. Damage to DNA in cultures treated with 15−25 μM OAHPx was significantly higher than that at 2.5−10 μM OAHPx concentrations. The

Table 2. Model Equations Developed To Depict Cell Damage and SOD Activity Induced by Oleic Acid Hydroperoxide[a]

	predicted model	pseudo R^2
LDH activity[b]	$Y = -4.236 + (52.516 + 4.236)/[1 + \exp((-0.408) \times (X - 6.4223))]$	0.9994
conjugated dienes[c]	$Y = 0.1326 \times \exp(0.0236X)$	0.9999
SOD activity[d]	$Y = 18.314 + (26.7102 - 18.314)/[1 + \exp((5.501) \times (Z - 3.013))]$	1.0

[a] X = oleic acid hydroperoxide concentration (μM). [b] Y = LDH activity in the culture medium as a % of total cell LDH activity. [c] Y = conjugated dienes as the absorbance at 233 nm. [d] Y = SOD activity as the % of superoxide radicals scavenged and Z = log value of oleic acid hydroperoxide concentration (μM).

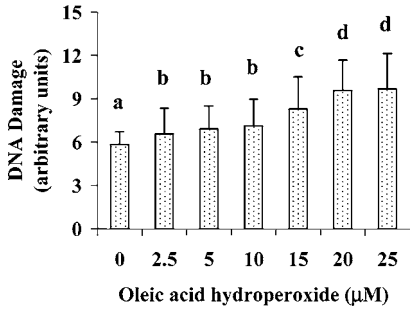

Figure 3. DNA damage (tail moments) measured by comet assay in Caco-2 cell cultures incubated with different concentrations of OAHPx. Each point represents the mean ± SD of four determinations. Different letters indicate significantly different observations ($p < 0.05$).

Table 3. Antioxidant Enzyme Activity in Caco-2 Cells Treated with Oleic Acid Hydroperoxides

OAHPx (μM)	catalase activity[b]	SOD activity[c]	GPx activity[d]
0	1.08 ± 0.05 a	36.2 ± 2.8 a	0.514 ± 0.02 a
5	1.08 ± 0.07 a	26.7 ± 1.4 b	0.513 ± 0.037 a
10	1.16 ± 0.01 a	26.5 ± 1.4 b	0.506 ± 0.018 a
15	1.06 ± 0.04 a	25.4 ± 1.3 b	0.505 ± 0.007 a
20	0.94 ± 0.04 a	22.7 ± 1.2 bc	0.466 ± 0.025 a
25	0.93 ± 0.02 a	20. 4 ± 1.2 c	0.437 ± 0.009 a

[a] Results are mean values of four determinations ± standard deviation. Means within a column having different letters are significantly ($p < 0.05$) different from one another. [b] Decrease in H_2O_2 (mmol L^{-1} min^{-1} mL^{-1}). [c] Superoxide radical scavenged (%). [d] Decrease in NADPH (μmol L^{-1} min^{-1} mL^{-1}).

observed increase of DNA damage and conjugated diene production followed similar patterns with a 0.97 correlation coefficient (r).

Antioxidant Enzyme Activity. Catalase, SOD, and GPx enzyme responses were different from each other depending on OAHPx concentrations as shown in **Table 3**. Catalase and GPx enzymes did not show changes in activities in cultures treated with 5−25 μM OAHPx compared to those of the control cultures. SOD activity was significantly reduced at 5 μM OAHPx compared to that of the control, and it continued to decrease with increasing concentrations of OAHPx. Although there were no distinguishable differences in SOD activity in cells treated with 5−20 μM OAHPx, a significant decrease was observed at 25 μM OAHPx compared to that of 15 μM OAHPx. The decrease in SOD activity could be explained using a four-parameter logistic model [$Y = \delta + (\alpha - \delta)/(1 + \exp(-\kappa(X - \tau)))$] (**Table 2**) developed with nonlinear regression. The model predicted a 7% decrease ($\alpha - \delta$) in SOD activity when the OAHPx concentration was increased from 5 to 25 μM and a maximum rate of decrease in SOD activity at 20.34 μM OAHPx.

DISCUSSION

Lipid peroxides, which are primary products of lipid oxidation, have been associated with the toxicity of oxidized polyunsaturated fatty acids (*34*). Although direct consumption of cytotoxic hydroperoxide concentrations (100−200 μM) is an unlikely event in humans, high intake of polyunsaturated fatty acids together with foods containing metal catalysts could give rise to the accumulation of hydroperoxide levels capable of generating oxidative stress in the lumen (*14*). Oxidative stress is an important physiological stimulus that affects the redox status in a cell, which then can lead to cell transitions from a quiescent state to a proliferative or an apoptotic state (*35*). Earlier studies (*20*) show that exposing Caco-2 cells to subtoxic low levels (1−5 μM) of lipid hydroperoxides for 6 h induced cell transition from a quiescent state to a proliferative state that was mediated by peroxide-induced disruption of cellular redox balance. However, increasing the exposure time to 24 h resulted in growth arrest (*20*). Similarly, exposure of Caco-2 cells to 5−25 μM lipid hydroperoxides for 1 h disrupted intestinal homeostasis, contributing to cell apoptosis, which could not be reversed even after recovery of redox balance to normal levels (*19*). These studies collectively showed that exposure of cells to shorter durations of low levels of hydroperoxides stimulates cell proliferation, whereas prolonged mild stress induced by low levels of hydroperoxides, or short duration severe oxidative stress induced by high concentrations of hydroperoxide, caused the progression of cell apoptosis. In the current study, severe membrane damage occurred at lower OAHPx concentrations, such as 10 μM. These results suggest that levels >5 μM can give rise to cell death by necrosis rather than apoptosis.

A defective cell membrane cannot prevent toxic components from entering the cell. Increases in cell lipid peroxidation and extracellular LDH activity as observed in this study confirm that cell exposure to OAHPx results in a leaky cell membrane. DNA damage, as quantified by the comet assay that measures the single- and double-strand breaks of DNA (*31*), positively correlated with the increase in lipid peroxidation as shown in our results. This increased DNA damage with increasing concentrations of OAHPx suggests the capacity of hydroperoxides to initiate genetic mutations. Due to the rapid turnover rate of intestinal cells (*20*), the possibility of carrying a defective DNA to the next cell generation is more probable compared to cells with low turnover rates. To prevent defective DNA from moving into the next generation, cells exhibit preventative mechanisms, such as arresting cell growth to allow time for DNA repair or inducing apoptosis (*36*). This prevents propagation of damaged DNA in the cell cycle.

In a human gastric system that contains effective antioxidant enzyme systems (*37*), GPx is the most important enzyme in removing lipid hydroperoxides (*4*). During the reduction of hydroperoxides, reduced GSH is first oxidized to glutathione disulfide (GSSG) and then regenerated via glutathione reductase (GR) at the expense of NADPH (*38*). Therefore, the activity of GPx depends on the availability of cellular GSH and NADPH and the activity of GR. Antioxidant enzymes have been shown to vary among individuals depending on their age and sex (*39*), dietary intakes (*40*), lifestyle habits, such as smoking and alcohol consumption (*41*, *42*), level of physical activity (*43*), and health condition (*44*). Enzyme activities have also been shown to differ among tissues within an individual (*37*). GPx, compared to SOD and catalase, is the enzyme most affected by dietary supplements and smoking (*40*). This implies that intake of dietary antioxidant supplements or foods containing high antioxidant levels may enhance the activity of GPx, making hydroperoxide detoxifi-

cation activities more effective. The effect of dietary supplements may also be due to the presence of selenium, which is needed for GPx activity (*45*).

It was observed in this study that in Caco-2 cells treated with 5−25 μM OAHPx, the activity of GPx did not show significant differences when compared to that of the control that had no lipid hydroperoxides in the medium. However, when subjected to hydrogen peroxide, GPx activity gradually increased with increasing concentrations of H_2O_2 (*26*). Furthermore, despite the unchanged antioxidant enzyme activities, Caco-2 cells showed significant cell lipid peroxidation, membrane damage, and DNA damage by 5−25 μM hydroperoxides. Concentrations as low as 10 μM produced extensive membrane damage as indicated by LDH leakage. These results show that existing antioxidant enzyme mechanisms are not capable of completely detoxifying 5−25 μM lipid hydroperoxide concentrations. However, it should be noted that although Caco-2 cells mimic the intestinal environment, they do not represent exact conditions found in the intestinal epithelium because of the absence of nervous and endocrine functions.

Catalase, which is responsible for removing hydrogen peroxide, did not show any significant differences in activity when treated with OAHPx compared to that of the control, whereas catalase activity increased in Caco-2 cells when exposed to H_2O_2 (*26*). SOD, the enzyme responsible for removing superoxide radicals in living systems, showed a significant decrease when treated with OAHPx and continued to decrease with increasing OAHPx concentrations. This could be due to the effects of breakdown products of lipid hydroperoxides. A similar effect on SOD activity was also observed when cells were exposed to H_2O_2, but the decreasing effect was explained by a three-parameter polynomial model (*26*), whereas with OAHPx oxidation a logistic model was a better fit to explain the cellular SOD activity.

In biological systems, lipid hydroperoxide can undergo decomposition, forming peroxyl, hydroxyl, and alkoxyl radicals in the presence of superoxide and other free radicals and metal ions (*46*). The combined effects of these free radicals and lipid hydroperoxides together would determine the activities of the antioxidant enzymes in living cells. Further research is necessary to determine the mechanisms underlying the observed changes in enzyme activity induced by OAHPx.

In summary, this study showed extensive cellular damage as indicated by increased LDH leakage, cell lipid peroxidation, and DNA damage by 5−25 μM hydroperoxides. The antioxidant enzyme catalase and glutathione peroxidase activities did not significantly change, whereas superoxide dismutase decreased with increasing hydroperoxide concentrations, indicating that self-defense enzymatic antioxidant mechanisms are insufficient to protect the intestine from lipid hydroperoxide-mediated oxidative injury.

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