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Metabolism and cytotoxic effects of phosphatidylcholine hydroperoxide in human hepatoma HepG2 cells

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ABSTRACT

In this study, we investigated cellular uptake and metabolism of phosphatidylcholine hydroperoxide (PCOOH) in human hepatoma HepG2 cells by high performance liquid chromatography–tandem mass spectrometry, and then evaluated whether PCOOH or its metabolites cause pathophysiological effects such as cytotoxicity and apoptosis. Although we found that most PCOOH was reduced to PC hydroxide in HepG2 cells, the remaining PCOOH caused cytotoxic effects that may be mediated through an unusual apoptosis pathway. These results will enhance our fundamental understanding of how PCOOH, which is present in oxidized low density lipoproteins, is involved in the development of atherosclerosis.

1. Introduction

Oxidized low density lipoprotein (Ox-LDL) is now recognized as a causative factor in atherosclerosis [1]. Many studies have shown cytotoxic and apoptotic effects of Ox-LDL on monocytes/macrophages, smooth muscle cells and endothelial cells [2], implying that Ox-LDL plays a role in atherosclerosis progression and plaque instability.

Ox-LDL contains predominantly oxidized phosphatidylcholine (PC), which is hypothesized to play a role in the promotion of atherosclerosis [3]. During the course of LDL oxidation, PC is primarily oxidized to PC hydroperoxide (PCOOH), while further modification of PCOOH yields various secondary products such as PC hydroxide (PCOH) and PC with a truncated *sn*-2 acyl group (truncated PC) [3,4]. Among these oxidatively modified PCs, PCOOH was thought to be the most causative agent in terms of atherosclerosis [3,4], but which oxidized PCs show cytotoxic, apoptotic and atherogenic effects remain unclear.

The mechanisms of cellular uptake and metabolism of PCOOH are also unknown. To the best of our knowledge, only one study, by Bao and Williamson, has examined PCOOH metabolism [5]. They incubated human hepatoma cell line (HepG2) with PCOOH, and found that PCOOH was mainly converted to PCOH in the cells. However, there are no subsequent reports concerning PCOOH metabolism in the literature.

Based on these earlier findings, in this study we quantitatively evaluated cellular uptake and metabolism of PCOOH in liver cells and other cultured cells by high performance liquid chromatography–tandem mass spectrometry (LC–MS/MS), and then performed cell culture studies in order to discriminate whether PCOOH or its metabolites (PCOH, truncated PC, etc.) are principally related to pathophysiological processes such as cytotoxicity and apoptosis induction.

2. Materials and methods

2.1. Reagents

1-Palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (16:0–18:2 PC) was purchased from Avanti Polar Lipids (Alabaster, AL). PCOOH was enzymatically synthesized from PC using soybean lipoxygenase-1

Abbreviations: Toc, α-tocopherol; JNK, c-Jun-NH_2-terminal kinase; DAPI, 4′, 6-diamino-2-phenylindole; ESI, electro-spray ionization; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GSH, glutathione; GPx, glutathione peroxidase; GST, glutathione S-transferase; HUVEC, human umbilical vein endothelial cells; BSO, L-buthionine-sulfoximine; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MRM, multiple reaction monitoring; Ox-LDL, oxidized low density lipoprotein; PAzPC, 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine; PONPC, 1-palmitoyl-2-(9′-oxo-nonanoyl)-*sn*-glycero-3-phosohocholine; PC, phosphatidylcholine; PCOOH, phosphatidylcholine hydroperoxide; PCOH, phosphatidylcholine hydroxide; PHGPx, phospholipid hydroperoxide glutathione peroxidase; SM, sphingomyelin.

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and chromatographically purified [6]. The predominant *sn*-2 residue of the purified PCOOH was 13-hydroperoxyoctadecadienoic acid. PCOH was prepared by reducing PCOOH with $NaBH_4$. Sphingomyelin (SM) was obtained from Nagara Science (Gifu, Japan). L-Buthioninesulfoximine (BSO) was from Sigma (Tokyo, Japan). All other reagents were of analytical grade.

2.2. Cells

The human hepatocellular carcinoma cell line HepG2 was obtained from the RIKEN cell bank (Tsukuba, Japan). The monocytic cell line THP-1 was purchased from Dainippon Sumitomo Pharma (Osaka, Japan). The cells were cultured in RPMI-1640 medium (Sigma) containing 0.3 g/l L-glutamine and 2.0 g/l sodium bicarbonate supplemented with 10% fetal bovine serum (FBS) (Dainippon Sumitomo Pharmaceutical, Osaka, Japan), 100 kU/l penicillin (Gibco BRL, Rockville, MD) and 100 mg/l streptomycin (Gibco). Cells were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

2.3. Preparation of test medium

Test samples (PCOOH, PCOH, or PC) were dissolved in methanol at a concentration of 13 mM. The stock solution was diluted with test medium (RPMI 1640 medium containing 0.3% FBS) to achieve the desired final concentration (e.g., 0–50 μM PCOOH). The final concentration of methanol in the test medium was less than 0.4% (v/v), which did not affect cell viability. Other test samples (BSO and α-tocopherol (Toc)) were dissolved in water or ethanol followed by preparation of test medium. Medium with solvent (methanol) alone was similarly prepared and used as the control medium.

2.4. Cellular uptake and metabolism of PCOOH

HepG2 cells (1.0×10^6) were pre-incubated with 10% FBS/RPMI-1640 in 10 cm dishes. Twenty-four hours later, the medium was replaced with test medium containing PCOOH. Then, the cells were incubated for 4 h. Similarly, THP-1 cells were treated with test medium. After treatment, the number of cells was evaluated in an OneCell counter (Bio Medical Science, Tokyo, Japan) with an electronic microscope. Cells and medium were then subjected to LC–MS/MS analysis.

2.5. LC–MS/MS analysis

Cells (1.0×10^7) were suspended in 650 μl water, whereas medium (300 μl) was diluted with 300 μl water. The sample (600 μl cell suspension or diluted medium) was then mixed with 2.4 ml chloroform–methanol (2:1, v/v; containing 1.0 mg SM). The mixture was partitioned by centrifugation at 1000 × *g* for 20 min at −20 °C into two phases: chloroform layer (lower organic phase) and methanol–water layer (upper aqueous phase). The lower chloroform layer (lipid fraction) was collected. The remaining aqueous layer was re-extracted with Folch theoretical lower phase [7], and subjected to centrifugation 1000× *g* for 20 min at −20 °C. Lipid fractions were combined and evaporated under nitrogen gas. The dried extract was re-dissolved in 240 μl chloroform–isopropanol (2:1, v/v), and a portion (40 μl) was loaded onto an aminopropyl Sep-Pak cartridge (100 mg) (Waters, Tokyo, Japan) equilibrated with chloroform–isopropanol (2:1, v/v). The cartridge was rinsed with 750 μl chloroform–isopropanol (2:1, v/v), and the oxidized PCs were then eluted with 1.5 ml methanol. The eluent was evaporated, and the residue was dissolved in 200 μl methanol. A final aliquot of 10 μl was injected for LC–MS/MS analysis.

For LC–MS/MS, an ODS column (Atlantis T3 column, 3.5 μm, 2.1 × 100 mm; Waters) was used at 40 °C. The mobile phase consisted of two components: A, water containing 0.1 mM sodium acetate and B, methanol containing 0.1 mM sodium acetate. The gradient profile was as follows: 0–4 min, 70–90% B linear; 4–10 min, 90% B; 10–17 min, 90–100% B linear; 17–30 min, 100% B; 30–30.1 min, 100–70% B linear; 30.1–35.0 min 70% B. The flow rate was 0.2 ml/min. Oxidized PCs were analyzed using a 4000 QTRAP LC–MS/MS System (AB SCIEX, Tokyo, Japan). MS/MS parameters were optimized with synthesized PCOOH and PCOH standards under electro-spray ionization (ESI) (positive). Oxidized PCs in cells and medium were determined using the multiple reaction monitoring (MRM) mode as follows: PCOOH, *m*/*z* 812.4 > 541.4; PCOH, *m*/*z* 796.5 > 613.4 [8]. Extraction efficiencies of PCOOH and PCOH standards spiked with cell suspension (or medium) were over 90%. Truncated PCs were analyzed using MRM transitions from the literature [9]: 1-palmitoyl-2-(9′-oxo-nonanoyl)-*sn*-glycero-3-phosohocholine (PONPC; *m*/*z* 740.6 > 634.5) and 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine (PAzPC; *m*/*z* 664.6 > 201.0).

2.6. Cell cytotoxic assay

HepG2 cells (1.0×10^4) were pre-incubated with 10% FBS/RPMI-1640 in 96-well plates. Twenty-four hours later, the medium was replaced with test medium containing 50 μM PCOOH, PCOH or native non-oxidized PC for 24 h. Then, the cells were observed under a microscope, and the number of viable cells was evaluated using WST-1 reagent (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. In brief, 10 μl WST-1 reagent was added to the medium, and incubated at 37 °C for 2 h. Absorbance (450/655 nm) of the medium was measured with a microplate reader. In addition to these experiments, test medium containing either 50 μM PCOOH + 100 μM BSO (glutathione (GSH) synthesis inhibitor) or 50 μM PCOOH + 100 μM BSO + 50 μM Toc was prepared, and cell cytotoxicity assays were performed. Cellular GSH levels were evaluated by using GSH-Glo™ Glutathione Assay (Promega, Madison, WI).

2.7. Mitochondrial membrane potential

HepG2 cells (1.0×10^4) were seeded onto Poly-D-lysine-coated coverslips set in a 24-well plate and cultured for 24 h. Cells were treated with test medium for 4–24 h. After treatment, the medium was replaced with fresh medium containing 200 nM MitoTracker Red (Cambrex Bio Science Walkersville, Inc., Walkersville, MD) and incubated for 30 min at 37 °C. After washing with cold phosphate-buffered saline, the cells were fixed with 2% paraformaldehyde for 15 min. The cells were then stained with 4′,6-diamino-2-phenylindole (DAPI) solution (5 μg/ml) for 15 min. Intracellular localization of the probes was determined by a fluorescent microscope.

2.8. Western blot analysis

After a 24 h pre-incubation of HepG2 cells (1.0×10^6) with 10% FBS/RPMI-1640 in a 10 cm dish, the cells were treated with the test medium for 4–24 h. Cells were sonicated in ice-cold CelLytic™ M (Sigma) including 1.0% protease inhibitor cocktail. After the homogenates were centrifuged at 15,000× *g* for 15 min at 4 °C, the supernatant was collected, and the protein concentration was measured. Cellular proteins (10 μg/well) were separated by SDS-PAGE (8–14% gel). After blocking with skim milk, the membranes were incubated with primary antibodies for p53, phospho-p38 (*Thr180*/*Tyr182*), phospho-c-Jun-NH_2-terminal kinase (JNK)

(*Thr183*/*Tyr185*), JNK, cleaved caspase-3 (*Asp175*), Rock-1 (*H85*) (Santa Cruz Biotechnology, Santa Cruz, CA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Cell Signaling Technology, Beverly, MA), followed by secondary antibodies (anti-mouse or anti-rabbit IgG) conjugated with horseradish peroxidase (Cell Signaling Technologies). Target proteins were visualized using an Immobilon Western Chemiluminescent HRP Substrate (Millipore, Darmstadt, Germany), and images were captured using the ChemiDoc MP system (Bio-Rad, Hercules, CA).

2.9. Cell cycle analysis by flow cytometry

HepG2 cells (4.0×10^5) were pre-incubated with 10% FBS/RPMI-1640 in 6-well plates for 24 h, and then incubated with test medium for 4–24 h. The cells were washed, harvested, and subjected to centrifugation at 1000× *g* for 5 min at 4 °C. The cells were fixed with ice-cold 70% ethanol (700 μl) for the subsequent DNA staining with 10 μM propidium iodide. The cells were analyzed by flow cytometry (COULTER EPICS® XL-MCL™, Beckman Coulter, Brea, CA). Cell populations in each cell cycle phase were analyzed using ModFit LT 3.2 software (Verity Software House, Inc., Topsham, ME).

2.10. Statistical analysis

The data are expressed as means ± SD. We performed statistical analysis using a one-way ANOVA, followed by the Tukey test for multiple comparisons. Differences were considered significant at $P < 0.05$.

3. Results

3.1. LC–MS/MS evaluation of cellular uptake and metabolism of PCOOH

Analysis of standard PCOOH by MS/MS with flow injection showed an intense molecular ion at *m*/*z* 812.4 $[M + Na]^+$. Product ion scanning was then conducted for this ion, and PCOOH-specific fragment ions (e.g., *m*/*z* 541.4) were identified (Fig. 1A). Similarly, MS/MS analysis was performed for PCOH (Fig. 1B). Thus, the identified ions (*m*/*z* 541.4 for PCOOH and *m*/*z* 613.4 for PCOH) allowed for selective detection of the analytes by LC–MS/MS with MRM. In the MRM chromatogram (Fig. 1C), standard PCOOH was clearly detected at 17.2 min, followed by PCOH at 17.3 min.

As shown in Fig. 2A and B, LC–MS/MS revealed that when HepG2 cells were incubated with 50 μM PCOOH for 4 h, a considerable amount of PCOH (25.6 pmol/1.0×10^5 cells) was detected in the MRM chromatogram. Meanwhile, non-metabolized PCOOH was found in the cells only at low levels (0.7 pmol/1.0×10^5 cells). The conversion of PCOOH into PCOH occurred in dose-dependent fashion (Supplementary Fig. S1A). There were no detectable levels of other oxidized PCs, including truncated PC (PONPC and PAzPC). Based on these results and also medium data (Fig. 2C and D), in HepG2 cells PCOOH is actually taken up and metabolized to PCOH, rather than other oxidized PCs. As we expected, similar metabolism patterns were observed for PCOOH-treated THP-1 cells (Fig. 2E and F), as well as for other cell lines, including DLD-1 (human colon adenocarcinoma), MCF-7 (human breast cancer), human coronary artery endothelial cells, and HUVEC (human umbilical vein endothelial cells) (data not shown). On the other hand, when PCOOH

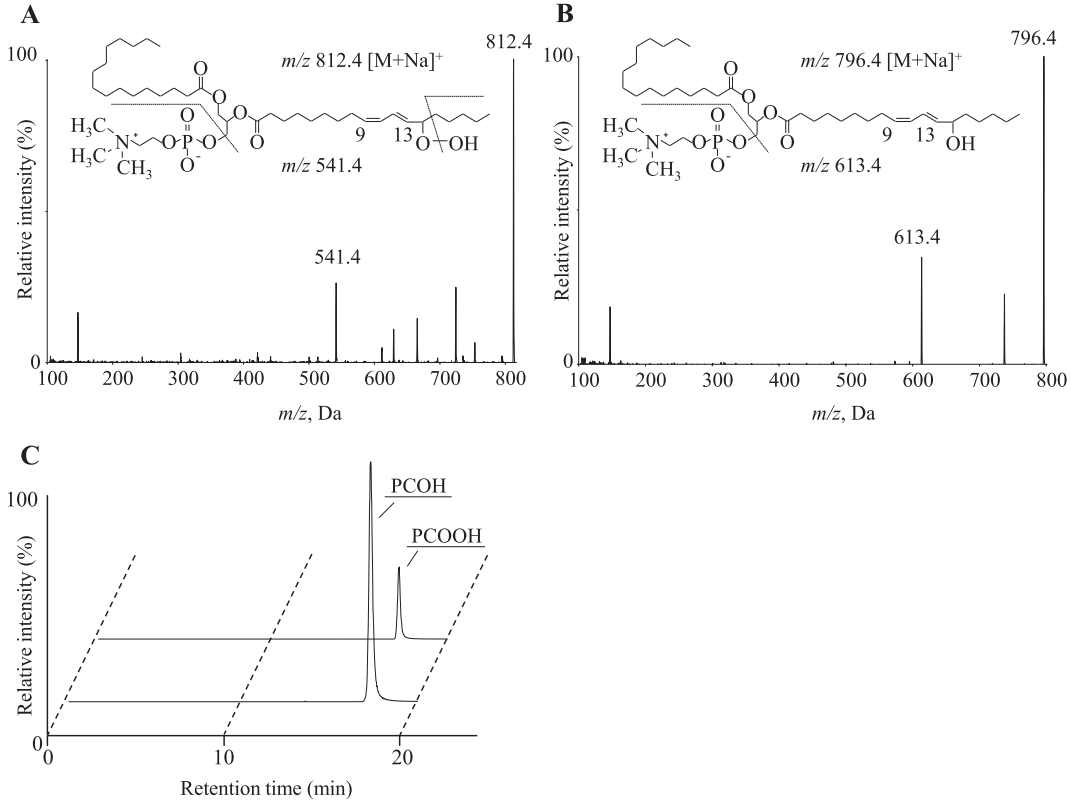

Fig. 1. Q1 mass spectra of PCOOH and PCOH in positive ESI mode. The product ion mass spectra of sodiated PCOOH (*m*/*z* 812.4 $[M + Na]^+$) (A) and PCOH (*m*/*z* 796.4 $[M + Na]^+$) (B) are shown. Standard PCOOH or PCOH (1 μM in methanol) was infused directly into the MS/MS apparatus at a flow rate of 10 μl/min. Standard PCOOH and PCOH (each 1 pmol) were analyzed by LC–MS/MS with MRM using *m*/*z* 812/541 and *m*/*z* 796/613, respectively (C).

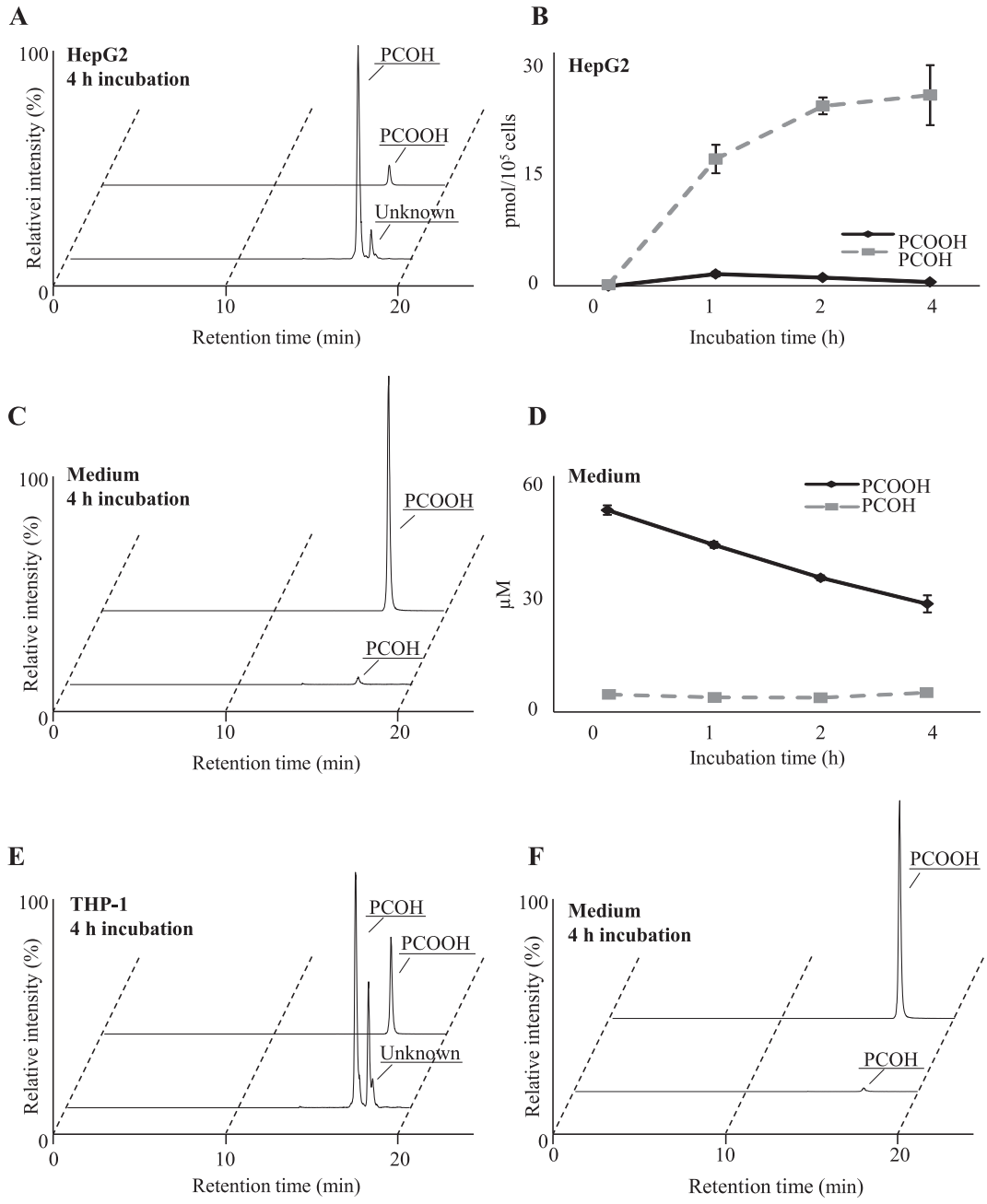

Fig. 2. Cellular uptake and metabolism of PCOOH in HepG2 cells (A–D) or THP-1 cells (E, F). After these cells were incubated with 50 μM PCOOH for 0–4 h, PCOOH and PCOH were extracted from the cells and medium. Extracts were analyzed by LC–MS/MS with MRM to determine the amount of PCOOH and PCOH in cells (A, B, E) and medium (C, D, F). Values are means ± SD ($n = 3$).

was incubated in medium alone (without cells) for 4 h, PCOOH had somewhat decreased (data not shown), perhaps due to decomposition [5]. However, this has probably no connection with our finding (cellular conversion of PCOOH into PCOH), because there were no detectable levels of PCOH after incubation of PCOOH in medium alone.

3.2. Evaluation of physiological effects of PCOOH and PCOH

Since cellular metabolism of PCOOH was defined, cell culture studies were performed in order to discriminate whether PCOOH or PCOH have physiological effects. HepG2 cells were treated with 50 μM PCOOH, PCOH or native non-oxidized PC for 24 h, and the number of viable cells was evaluated using a WST-1 assay. We found that PCOOH reduced cell viability by 50% (Fig. 3A). The reduction appeared from 50 μM PCOOH treatment (Supplementary Fig. S1B). In contrast, no decrease in cell viability was observed for PCOH- and PC-treated HepG2 cells. To evaluate the mechanism of PCOOH-induced cell death, HepG2 cells were treated with 50 μM PCOOH + 100 μM BSO, an inhibitor of GSH production (Supplementary Fig. S2A) that is a substrate for the PCOOH-decomposing enzyme, phospholipid hydroperoxide glutathione peroxidase (PHGPx), or 50 μM PCOOH + 100 μM BSO + 50 μM Toc, a well-known antioxidant molecule. Cell viability was markedly

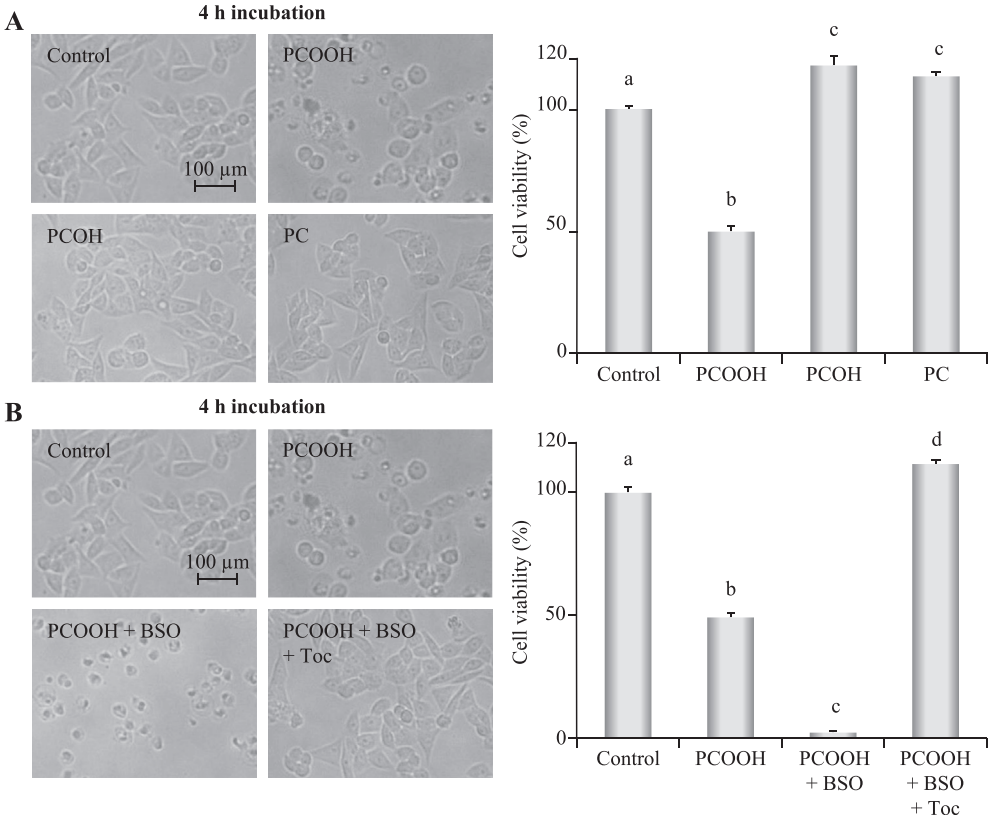

Fig. 3. Effects of PCOOH and PCOH on HepG2 cell viability. After a 24 h incubation of HepG2 cells with test medium containing 50 μM PCOOH, PCOH or native non-oxidized PC, cells were observed under a microscope and the number of viable cells was evaluated using the WST-1 method (A). In addition to these experiments, test medium containing either 50 μM PCOOH + 100 μM BSO (GSH synthesis inhibitor) or 50 μM PCOOH + 100 μM BSO + 50 μM Toc was prepared, and a cell cytotoxicity assay was performed (B). Values are means ± SD ($n = 6$). Means without a common letter differ, $P < 0.05$.

decreased in PCOOH + BSO-treated HepG2 cells, and the cytotoxicity was attenuated by Toc treatment (Fig. 3B). Neither BSO nor Toc affected cell viability (data not shown). Therefore, although most PCOOH was converted to PCOH in HepG2 cells, the remaining PCOOH would be a particularly biologically active molecule, and PCOOH-induced cell death could be closely related to changes in cellular oxidative stress levels.

3.3. Evaluation of what type of cell death is induced by PCOOH

Since PCOOH, but not PCOH, caused cytotoxicity in HepG2 cells, we investigated what type of cell death (e.g., apoptosis) was induced by PCOOH. As expected, hallmarks of apoptosis (e.g., lower mitochondrial membrane potential and higher levels of phosphorylated JNK and p38) were observed, especially in PCOOH + BSO-treated HepG2 cells (Supplementary Fig. S2B, Fig. 4A). These changes could be clearly attenuated by Toc treatment. Unexpectedly, however, p53 expression was decreased after a 24 h incubation of HepG2 cells with PCOOH + BSO. PCOOH did not affect levels of cleaved caspase-3 and Rock-1. In flow cytometry analysis, accumulation of cells in the G2/M phase was observed, especially in PCOOH + BSO-treated HepG2 cells (Fig. 4B). These results suggest that PCOOH induces cell death via an unusual apoptosis pathway, possibly through mitotic catastrophe and/or the recently discovered ferroptosis pathway [10,11].

4. Discussion

In the present study we evaluated cellular uptake and metabolism of PCOOH in HepG2, THP-1 and other cell lines using LC–MS/MS, and elucidated PCOOH metabolism in terms of the reductive conversion of PCOOH to PCOH in the cells (Fig. 2). We performed cell culture studies to determine whether PCOOH or its metabolites are principally related to pathophysiological processes, which showed that while most PCOOH was converted to PCOH, the remaining PCOOH caused cytotoxic effects, possibly through an unusual apoptosis pathway (Figs. 3 and 4). On the other hand, the concentrations of PCOOH have been shown to range from 50 to 230 nM in plasma of healthy subjects [12]. Thus, the concentrations (0–50 μM) used in this study are relatively high. Meanwhile, PCOOH reached about 10 μM in blood of patients [12]. Topical PCOOH accumulation, such as in atherosclerotic lesions, is also conceivable. We therefore think that PCOOH concentrations used in this study represents a physiologically attainable concentration.

In the first half of this study, we examined how PCOOH is absorbed and metabolized in cultured cells. LC–MS/MS provides useful structural information for the analytes, even in the presence of background contaminants found in complex biological systems. Several PCOOH metabolites from human plasma and tissues were previously examined and identified using LC–MS/MS with MRM [13]. Here we applied this analytical method to analyze oxidatively modified PCs such as PCOOH, PCOH, PONPC, PAzPC and others that might be present in the cells or the culture medium. The detection of these oxidized PCs by LC–MS/MS was reproducible and not altered by the storage of cellular and medium extract samples below −30 °C for 1 month (data not shown). For reference, before extraction of PCOOH (or PCOH) from cells and media, we added SM, which is effective in preventing possible degradation of analyte during extraction procedures.

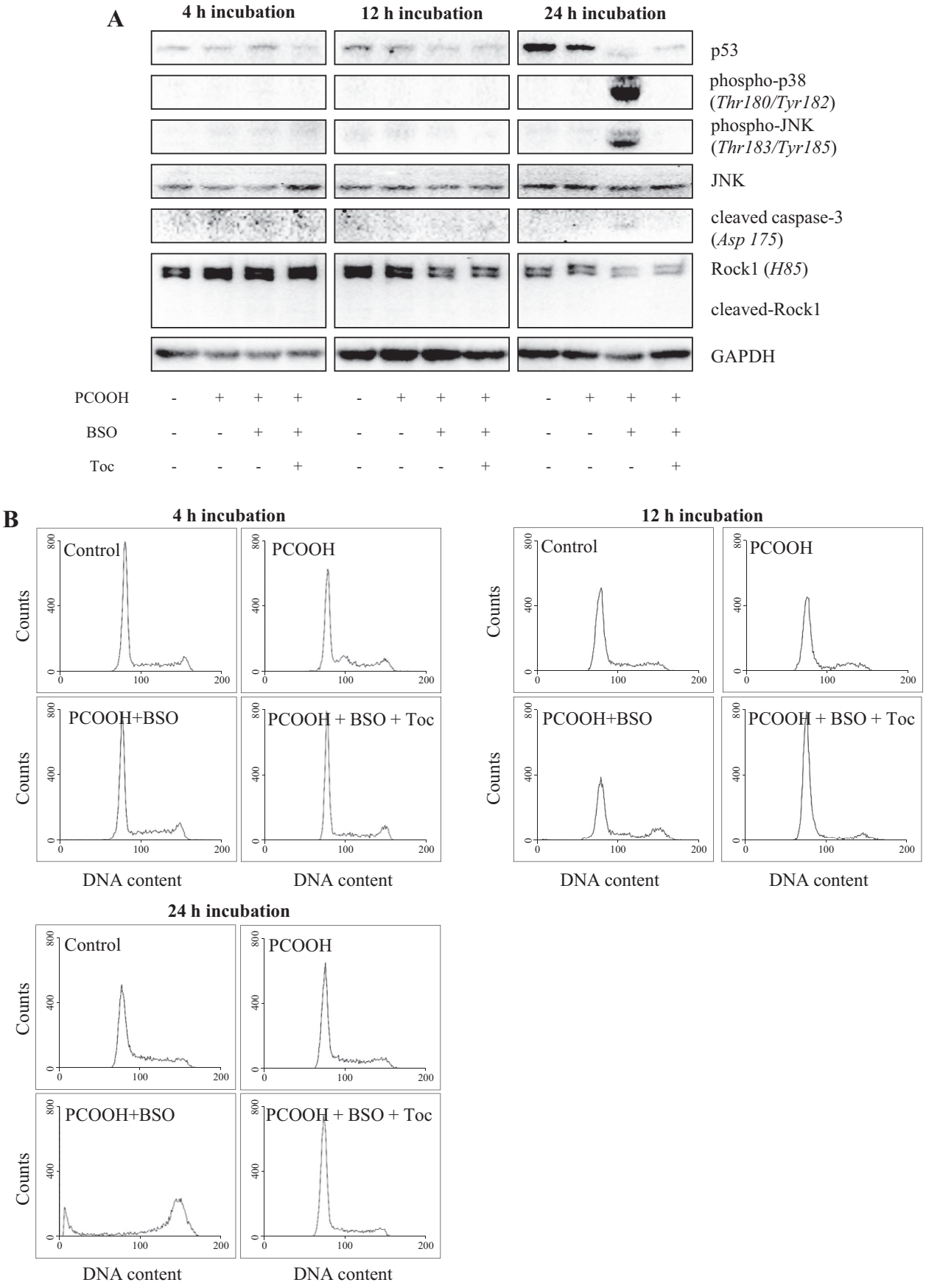

Fig. 4. Effect of PCOOH on apoptotic protein expression and cell cycle in HepG2 cells. After a 4–24 h incubation of HepG2 cells with test medium (containing 50 μM PCOOH, 50 μM PCOOH + 100 μM BSO, or 50 μM PCOOH + 100 μM BSO + 50 μM Toc), expression levels of p53, phospho-p38 (*Thr180*/*Tyr182*), phospho-JNK (*Thr183*/*Tyr185*), JNK, cleaved caspase-3 (*Asp175*), Rock-1 (*H85*) and GAPDH were evaluated by western blot analysis (A). Cell cycle was analyzed by flow cytometry (B).

A considerable amount of PCOH was detected in MRM chromatograms after incubation of HepG2 cells (or THP-1 and other cells) with PCOOH (Fig. 2), while non-metabolized PCOOH was found in the cells only at low levels. These results are in agreement with those of previous studies that also used HepG2 cells [5]. In addition, we found that there were no detectable levels of other oxidized PCs in PCOOH-treated cells. The following conclusions can be inferred from these results: *1*) PCOOH is rapidly taken up by HepG2 cells and metabolized mainly to PCOH; and *2*) a significant amount of PCOH and a small amount of PCOOH are present in cells even after 4 h cultivation. Based on these results, we are now investigating the mechanism of PCOOH uptake and incorporation into cells (e.g., facilitated diffusion). As a point of reference, after incubation of HepG2 cells with PCOOH, a small amount PCOH appeared in the cell culture medium, suggesting that cells release this PCOOH metabolite.

The reduction of lipid hydroperoxides, including PCOOH, into their two-electron reduction products (i.e., lipid hydroxides) is thought to be mediated by at least three enzymes: glutathione peroxidase (GPx), glutathione S-transferase (GST), and peroxiredoxin. Five isoforms of GPx have been identified, with GPx4 (i.e., PHGPx) considered to be an important enzyme for the reduction of PCOOH to PCOH [14]. Indeed, Bao and Williamson [5] measured PHGPx activity by colorimetric methods in PCOOH-treated HepG2 cells, and speculated that 99.5% of the reduction of PCOOH into PCOH in HepG2 cells depended on this PHGPx activity. To build on this earlier study [5], here we investigated gene and protein expression of PHGPx in PCOOH-treated HepG2 cells, but evaluation of whether PCOOH affects gene and protein expression of PHGPx was difficult due to the low expression levels of PHGPx in HepG2 cells (data not shown). This finding may be related to the fact that PHGPx is highly expressed in testes, but not in other tissues, including liver [15]. Therefore, further study is needed to evaluate whether PHGPx indeed plays an important role in reducing PCOOH to PCOH in HepG2 and other cultured cells.

In the second half of this study, we evaluated the cytotoxicity of PCOOH or PCOH and found that PCOOH, but not PCOH, caused cytotoxic effects (Figs. 3 and 4). Considering the finding that PCOOH + BSO caused significant HepG2 cell death that could be clearly prevented by Toc, PCOOH-induced cell death seems to be closely related to oxidative stress changes. In addition, we found that PCOOH induced lower mitochondrial membrane potential, higher levels of phosphorylated JNK and p38, lower levels of p53, and increased G2/M phase accumulation, suggesting that PCOOH induces cell death via an unusual apoptosis pathway. To date, there are three types of cell death: apoptosis, necrosis and autophagy [16]. In addition to these forms of cell death, ferroptosis and mitotic catastrophe [17] were recently reported as non-apoptotic cell death pathways. Ferroptosis involves the production of iron-dependent reactive oxygen species, and is morphologically, biochemically and genetically distinct from apoptosis, necrosis and autophagy [10]. Yang et al. [11] performed cell culture studies based on the notion that BSO treatment depletes cellular GSH and therefore inhibits GPx, and found that GPx inhibition using BSO enhanced ferroptotic cell death induced by erastin. In this study, similar phenomena were observed, that is, BSO actually caused GSH depletion in HepG2 (Supplementary Fig. S2A), which enhanced cell death induced by PCOOH. Thus, PCOOH may cause oxidative stress and induce cell death in HepG2 cells through a pathway that involves ferroptosis. On the other hand, mitotic catastrophe has been reported to occur in p53-deficient cancer cells, which induces G2 phase arrest [17]. Similar phenomena were observed in PCOOH + BSO-treated HepG2 cells, suggesting that another possible mechanism by which PCOOH induces cell death involves mitotic catastrophe. Additional studies will be needed to evaluate these possibilities.

Besides the cytotoxicity of PCOOH observed here (Figs. 2–4), we previously found that THP-1 cell adhesion to intracellular adhesion molecule-1 was increased in the presence of PCOOH [18]. The result indicates that PCOOH in Ox-LDL may be involved in monocyte adherence to the arterial wall during the initiation of atherosclerosis. In another experiment, we found that PCOOH stimulated angiogenic responses in HUVECs [19]. These previous findings [18,19] together with our present results (Figs. 2–4) will enhance our fundamental understanding of the involvement of PCOOH in Ox-LDL in the development of atherosclerosis, although further studies are needed to investigate atherogenic processes (e.g., cytotoxicity, apoptosis, mitotic catastrophe, ferroptosis, and angiogenesis) of PCOOH in Ox-LDL.

Conflict of interest

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.063.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.063.

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