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Research Article

Lipid hydroperoxides from processed dietary oils enhance growth of hepatocarcinoma cells

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Linoleic acid, one of the major fatty acid in dietary oils, is an important source for hydroperoxides that may be formed in the presence of oxygen during food processing. Oxidized oils are absorbed in the intestine, transported as chylomicrones to the liver, and may affect unaltered hepatic cells as well as the process of hepatocarcinogenesis. We have studied the effects of linoleic acid hydroperoxides (LOOH) on growth and gene expression of cultured human hepatocellular carcinoma cells (HCC-1.2). The addition of LOOH to the medium of HCC-1.2 carcinoma cells caused dose-dependent cell loss and enhanced lactate dehydrogenase (LDH)-release. Under subtoxic conditions, LOOH induced intracellular hydrogen peroxide production, a decrease of glutathione content, elevated expression of the AP-1 components c-fos and c-jun as well as of the anti-apoptotic enzyme heme oxygenase 1 (HO-1). Furthermore, the cells were pushed by LOOH into the cell cycle as indicated by increased proportion of cells in the S- or G2/M-phase. The unoxidized linoleic acid was not active. Application of SnPPIX, a HO-1 inhibitor, decreased the viability of HCC-1.2 cells, indicating the protective role of HO-1 induction. This is the first evidence that lipid hydroperoxides of dietary origin may be an important driving force for carcinogenesis in the liver.

Keywords: Dietary fat / Fatty acid hydroperoxides / Linoleic acid hydroperoxides / Liver carcinogenesis / Oxidative stress

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1 Introduction

A typical western style diet contains substantial amounts of PUFA that have been treated by heating; *e.g.* frying oils as used in gastronomy are often kept above 100°C for extended periods of time, resulting in the generation of oxidized lipids [1, 2]. Unsaturated fatty acids in fats and oils may even be oxidized at room temperature when exposed to atmospheric oxygen. The reaction is greatly enhanced at elevated temperatures, as observed in our previous study [3]. Accordingly, the majority of fast food products have been found to contain considerable amounts of oxidized lipids [4]. When consumed, oxidized dietary oils are absorbed in the intestine, transported to the liver via chylomicrons, and are incorporated into lipoproteins, as shown by increased oxidized lipid concentrations in the VLDL and LDL fraction [5 – 7]. By these mechanisms, ingested peroxidized dietary oils circulate in the bloodstream, are distributed in the whole body and may reach all organs [6]. Evidence of effects of dietary hydroperoxides on the liver was gained in experiments, which show increased thiobarbituric acid reaction substances (TBARS) and decreased glutathione (GSH) and vitamin E content in this organ after ingestion of dietary oils containing high amounts of lipid peroxides [8]. Taken together, these data support the concept that the liver is strongly exposed to peroxides deriving from processed PUFA.

Lipid peroxidation products are cytotoxic and genotoxic agents [9 – 11]. They are involved in development of arteriosclerosis [12] and are considered to contribute to carcinogenesis in various organs, including the colon [13 – 15]. They may be a major cause, that western style diet is an important risk factor for colorectal carcinogenesis [16 – 19]. We have previously shown that dietary lipid hydroperoxides stimulate expression of COX-2 and, consequently, enhance

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Abbreviations: **HCC**, human hepatocellular carcinoma cells; **HO-1**, heme oxygenase 1; **LH**, unoxidized linoleic acid; **LOOH**, lipid hydroperoxides; **ROS**, reactive oxygen species

the synthesis and release of vascular endothelial growth factor (VEGF) in adenoma and carcinoma cells from the human colon, which in turn may accelerate angiogenesis, one of the growth limiting steps in the formation of colorectal tumors [20]. Hence, linoleic acid hydroperoxides (LOOH) may hypothetically favor growth of colon cancer.

Until now, little attention has been paid to the potential role of PUFA peroxides to hepatocarcinogenesis. However, some hints to such a role do exist, *e.g.* overnutrition, which presumably results in enhanced ingestion of LOOH often leads to the development of the non-alcoholic steatohepatitis (NASH), which in turn is an important risk factor for liver cancer [21, 22].

In the present study, we have investigated whether fatty acid hydroperoxides also affect hepatocarcinoma cells. Heme oxygenase-1 (HO-1), an inducible enzyme, catalyzes oxidative degradation of heme to form biliverdin, carbon monoxide and free iron [23]. Biliverdin is reduced to bilirubin, which possesses well-documented radical trapping properties [24]. Due to its anti-apoptotic effects, HO-1 may enhance tumor growth, as described for hepatoma cells [25, 26]. Here, we show the induction of this enzyme in cultured human hepatocarcinoma cells by LOOH as well as the influence of these fat components on cell cycle, intracellular formation of reactive-oxygen species (ROS) and gene expression patterns. Thus, we propose that the ingestion of dietary lipid peroxides is a neglected but possibly important factor contributing to the progression of liver cancer.

2 Materials and methods

2.1 Cell lines

HCC-1.2 hepatocarcinoma cell line was established in our laboratory (Eisenbauer, M. *et al*., submitted). The cell line was kept under standard tissue culture conditions using RPMI medium containing 10% FCS and has a doubling time of ~24 h. For treatment, the cells were seeded at 2.5×10^5 cells/well into a 24-well plate and grown for 48 h until they reached ~80% confluence.

2.2 Measurements of lactate dehydrogenase

Lactate dehydrogenase (LDH) release into the medium was measured using an enzyme detection kit obtained from Roche Diagnostics according to the manufacturer's instructions. Samples were analyzed in duplicates. Triton X100-treated cultures were used as a positive control.

2.3 Synthesis and application of linoleic acid hydroperoxides

LOOH was synthesized according to [27] and characterized as described earlier [28]. Briefly, linoleic acid was oxidized for 72 h at room temperature in the dark. The oxidation mixture was dissolved in petroleum ether (boiling range 40–60°C) and extracted four times with water/methanol (1:3 v/v). The obtained aqueous methanol was then extracted four times with light petroleum. The methanolic phase was then evaporated under reduced pressure. The two most likely positions of the oxygen on the peroxidized linoleic acid are positions 9 and 13, leading to two different structural hydroperoxide isomers in about equal proportions. The concentration of hydroperoxides was calculated using an extinction coefficient of 233 nm = 25 250 M^{-1} cm^{-1} in ethanol. LOOH stock solutions were prepared in ethanol and stored in liquid N_2 to protect from further oxidation. Immediately before use, unoxidized linoleic acid (LH)- and LOOH-stocks were diluted into serum free medium containing 1mg/mL BSA and dispersed by sonication for three times 5 s. The final concentration of ethanol in the culture medium was less than 0.05%.

2.4 Measurements of intracellular hydrogen peroxide

For quantification of H_2O_2 induction, subconfluent HCC-1.2 cells were harvested, washed and suspended in Hanks balanced salt solution (HBSS) containing 1% FCS at a concentration of 1×10^5 cells/mL. The cells were incubated with LOOH in the presence of 20 μM dihydrofluorescein diacetate (Fluka) for 2 h at 37°C. Treatment with 1 mM H_2O_2 was used as positive control. The fluorescein formation in the cells was monitored by a Coulter Epics XL flow cytometer using EXPO32 software. Each probe was handled in duplicates.

2.5 Quantification of intracellular GSH

Cells were incubated with indicated concentrations of LOOH or LH for 24 h, washed twice with ice-cold PBS and harvested using a rubber policeman. Cell pellets were collected by centrifugation at 4°C and sonicated on ice in 100 μL 50 mM PBS (pH 7.2) containing 1 mM diethylene triamine pentaacetic acid (DTPA, Sigma). After 15 min centrifugation at $10\,000 \times g$ and 4°C, supernatant was collected and frozen at −20°C until further processing. GSH content in deproteinated samples was determined using Glutathione Assay Kit (Cayman Chemical, USA) according to the manufacturer's instruction. The kit applies enzymatic recycling method, using glutathione reductase, for the quantification of GSH. GSH reacts with DTNB (Ellman's reagent, 5,5′-dithiobis-2-nitobenzoic acid) and the yellow colored product 5-thio-2-nitrobenzoic acid (TNB) is built. The mixed disulfide GSTNB is reduced by glutathione reductase to recycle the GSH and to produce more TNB. The TNB production rate is directly proportional to this reaction, which is in turn directly proportional to the concentration of GSH in the sample. The kinetics of absorbance changes in the wells at 405 nm was measured each

Table 1. PCR-primers and products

Gene		Primer	Annealing	Cycles
b-Actin	Sense	5′-GCACTCTTCCAGCCTTCCTT-3′	55°C	22
	Antisense	5′-CGCTCAGGAGGACCAATGAT-3′		
HO-1	Sense	5′- AAGATTGCCCAGAAAGCCCTGGAC-3′	55°C	32
	Antisense	5′-AACTGTCGCCACCAGAAAGCTGAG-3′		
c-fos	Sense	5′-CCTGTCAAGAGCATCAGCAGCATGG-3′	55°C	29
	Antisense	5′-GAGTACAGGTGACCACCGGAGTGC-3′		

5 min for 30 min. Then, a calibration curve was created with slopes of GSH standards. From the sample slope the concentration of GSH was calculated.

2.6 Cell number

Cell number was determined via measuring the uptake of neutral red into the lysosomes of viable cells. Cultures were incubated with neutral red at a concentration of 50 μg/mL in serum-free medium for 2 h at 37°C. The medium was removed; the cells were washed twice with PBS and finally incubated with 1% acetic acid in 70% ethanol. The concentration of dye was measured photometrically at 562 nm. The number of viable cells was determined in triplicates.

2.7 Measurements of cell survival

For measurements of survival, cells were treated with 50 μM LH or LOOH in serum-free medium containing 1 mg/mL BSA. After 3-h treatment, medium was removed and new serum-free medium was added for another 21 h in the absence or presence of 1 μM SnPPIX, an inhibitor of HO-1. Cells were harvested by trypsinization and supernatants with floating cells were added. After centrifugation and two washing steps with PBS, the cells were resuspended in medium containing 300 ng/mL propidium iodide and analyzed by flow cytometry. The percentage of propidium iodide-stained cells was determined.

2.8 Analysis of the cell cycle

Cells were seeded into the 24-well plates at 4×10^5 cells/well, grown for 48 h and treated with different concentrations of LOOH for 24 h. Then, the cells were harvested using trypsin, washed twice with PBS and fixed with 70% ethanol. The pellets were washed with PBS, treated with RNase A for 0.5 h at 37°C and stained with propidium iodide at a concentration of 20 μg/mL. DNA content was analyzed by FACS using Coulter Epics XL flow cytometer using EXPO32 software.

2.9 Isolation of RNA and RT-PCR

RNA isolation was performed using a standard Trizol-extraction protocol (Life Technologies, Gibco BRL). Purity and quantity of the RNA was determined using agarose gel electrophoresis and photometry. cDNA synthesis was performed on 2 – 5 μg of total RNA with oligo-dT primers for 1 h at 42°C using MMLV reverse transcriptase (Sigma, St. Louis, MO).

Genes of interest were amplified from cDNA samples by standard PCR cycles consisting of 1-min denaturation (94°C), 30-s annealing and 1-min synthesis (72°C). Details for individual genes are given in Table 1. Products were separated on 6% acrylamide gels using 0.5 μg/mL ethidium bromide for staining. GelDoc 2000 system and the program Quantity One 4.2.1. (Bio-Rad Laboratories, USA) were used for quantitation.

2.10 Real-time PCR

A focused PCR array type APHS-033 (Superarray Bioscience, Frederick, USA) was used for analysis of gene expression in HCC-1.2 hepatocarcinoma cells. Prior to experiments, RNA was cleaned up by the RNeasy Kit (Qiagen). A DNase treatment step was included as recommended by the manufacturer. Negative control wells confirmed that the system was clean from both genomic DNA and external DNA contaminations. Real time PCR was performed according to the manufacturers' instructions. ΔCt method was used for calculations.

2.11 Statistics

All experiments were performed at least three times. If not indicated otherwise, data are expressed as mean ± SEM, and statistical differences were determined using ANOVA with significance considered as $p < 0.05$.

3 Results

3.1 Cytotoxicity of fatty acid hydroperoxides

HCC-1.2 hepatocarcinoma cell lines were exposed to various concentrations of LOOH and LH for 24 h. LOOH lowered the number of viable cells and increased the release of intracellular LDH to the medium in a dose-dependent way (Figs. 1a and b). LH exerted no significant effect, indicating that the cytotoxic effect of LOOH was mediated by its hydroperoxide moiety and not by its fatty acid backbone. In

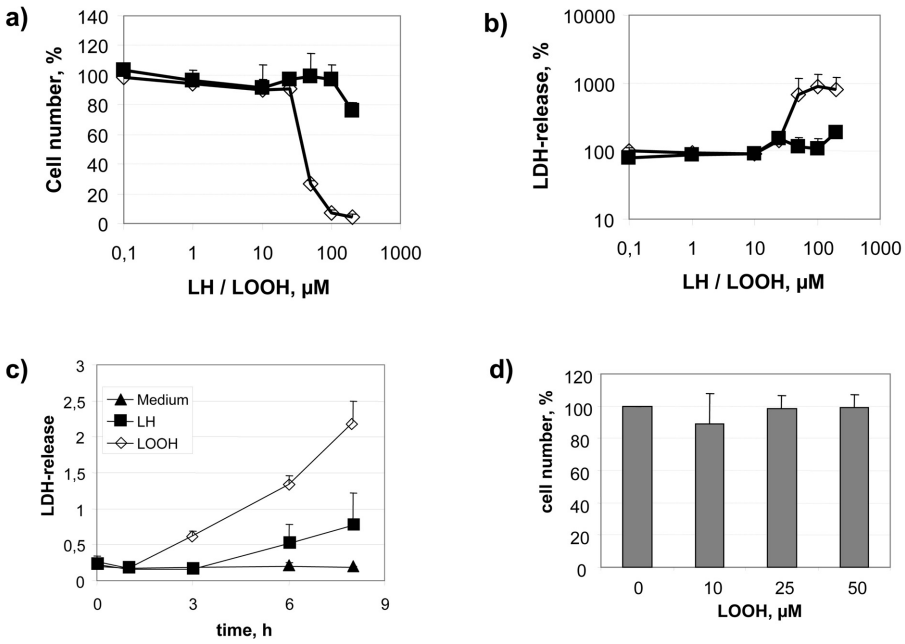

Figure 1. Cytotoxicity of linoleic acid hydroperoxides. HCC-1.2 cells were treated with the indicated concentration of linoleic acid (LH, ■) or linoleic acid hydroperoxides (LOOH, ⋄) for 24 h (a and b). Alternatively, 50 µM LH or 50 µM LOOH was applied to the cells for the indicated periods of time (c and d). The data represent results from three independent experiments.

a time-course study application of 50 µM LOOH induced no cell loss (Fig. 1c) and a marginal LDH release at 3 h (Fig. 1d). These conditions were chosen for further analyses.

3.2 Intracellular ROS production and depletion of cellular glutathione by LOOH

Formation of intracellular H_2O_2 in HCC-1.2 hepatocarcinoma lines was determined by incubating the cells with LOOH in the presence of dihydrofluorescein diacetate and subsequent detection of an intracellular fluorescence by FACS analysis. The generation of H_2O_2 was significantly increased at all concentrations of LOOH applied (Fig. 2a).

Oxygen radicals react with cellular antioxidants and may cause their depletion. Glutathione is a water-soluble antioxidant, which either can react directly with radicals or may serve as a cofactor for antioxidative enzymes, the glutathione peroxidases. The latter are known to reduce hydrogen peroxide or lipid peroxides. Thus, we also investigated the influence of LOOH on intracellular glutathione level. As shown in Fig. 2b, the glutathione content was reduced after treatment with LOOH. LH, however, did not exhibit this prooxidative effect.

3.3 LOOH pushes hepatocarcinoma cells into the S- and G2/M-phase of the cell cycle

Intracellular ROS formation is known to stimulate cell proliferation [29]. Since we observed intracellular ROS production as well as GSH depletion in LOOH-treated hepatocarcinoma cells, we studied the impact of LOOH on the cell

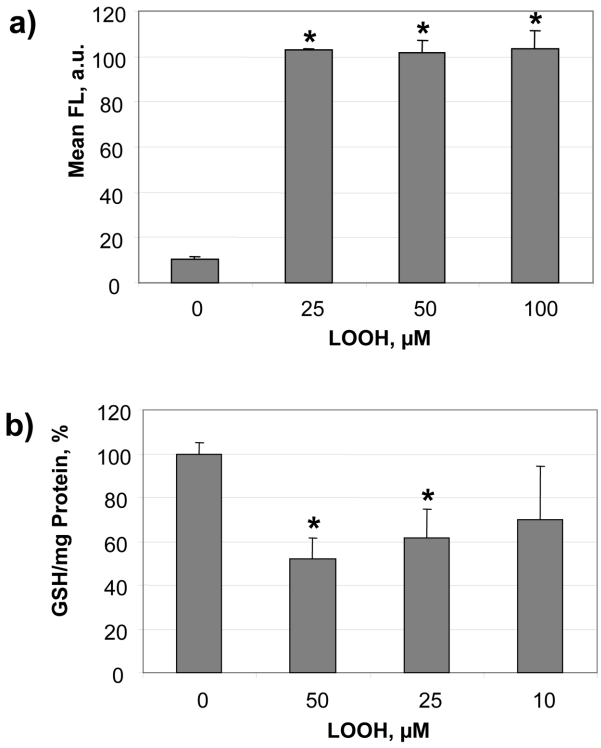

Figure 2. Oxidative stress in HCC-1.2 hepatocarcinoma cells under the treatment with linoleic acid hydroperoxides. Intracellular hydrogen peroxide in HCC-1.2 cells after incubation with indicated LOOH concentrations (a). The data were obtained in three independent experiments. GSH content in lysates obtained from the cells treated with the indicated concentration of LOOH or with 50 µM LH for 24 h (b). The data represent results from three independent experiments (* $p < 0.05$).

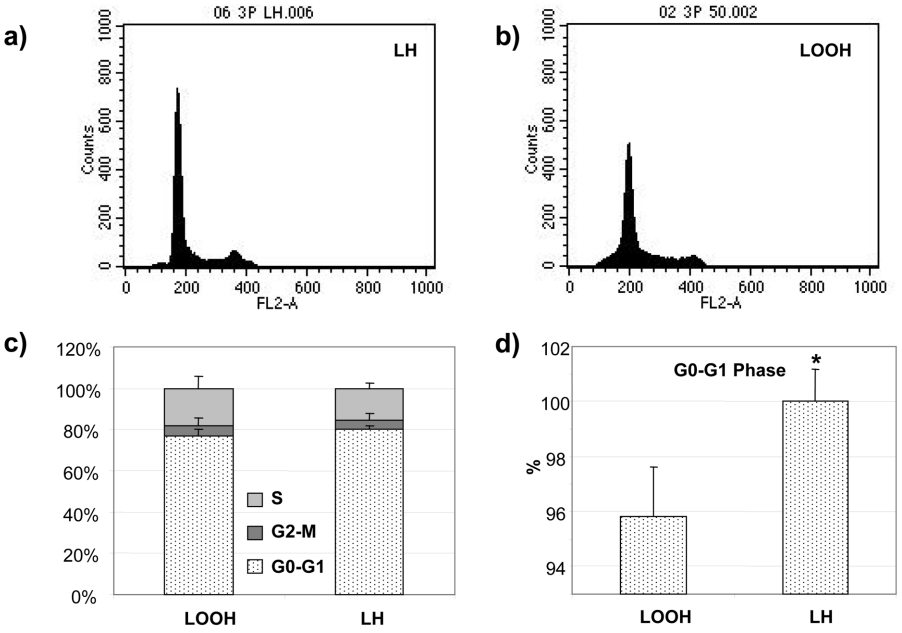

Figure 3. Influence of LOOH treatment on the cell cycle. HCC-1.2 cells were treated with 50 μM linoleic acid (LH) or 50 μM linoleic acid hydroperoxides (LOOH) for 24 h. The amount of cells in G0/G1, G2/M and S phases of the cell cycle was determined as described in Section 2. Typical histograms are given after the treatment with LH (a) or with LOOH (b). Distribution of cells between G0/G1, G2/M and S phases of the cell cycle and relative amount of the cells in G0/G1 phase are shown in (c) and (d). The data represent results from five independent experiments (* $p < 0.05$).

cycle. The percentage of LOOH-exposed hepatocarcinoma cells in G0/G1 phase decreased and the percentage of cells in G2/M- and S-phase increased when compared with exposure to LH (Fig. 3).

3.4 LOOH induces heme oxygenase 1

Different oxidants were found to induce HO-1. This in turn enhances tumor growth via improved protection against intracellular prooxidative stress and a subsequently lowered apoptotic activity and accelerated cell cycle [25, 26, 30]. We therefore tested whether LOOH induces HO-1 in hepatocarcinoma cells. After 3 h of exposure to LOOH, the expression of HO-1 mRNA was considerably increased but remained unaffected after LH (Fig. 4a).

The HO-1 gene contains a binding site for the transcription factor AP-1 in the promoter region, implicating transcriptional regulation by AP-1. We investigated the mRNA expression of c-fos and c-jun, the components of AP-1. At the time of the maximal response of HCC-1.2 cells to LOOH, c-fos was up-regulated as determined by RT-PCR (Fig. 4b). When applying quantitative RT-PCR c-fos and c-jun were found to be induced by factors of 4.45 and 20.8 respectively ($n = 2$, LOOH *vs*. LH: $p < 0.05$). This suggests that LOOH up-regulates HO-1 via induction of AP-1.

3.5 LOOH may protect hepatocarcinoma cells from apoptosis via induction of bcl-2 and HO-1

We observed toxic effects at high LOOH concentrations on hepatocarcinoma cells (Figs. 1a and b). However, subtoxic LOOH concentrations up-regulated the anti-apoptotic bcl-2 in hepatocarcinoma cells 9.24-fold ($n = 2$, LOOH *vs*. LH) as measured by quantitative RT-PCR. Thus, low concentrations seem to prepare cells for protection against apoptosis.

HO-1 has been suggested to protect from apoptosis and the observed induction of HO-1 in the hepatocarcinoma cells by LOOH may therefore be an essential part in the anti-apoptotic defense of LOOH-exposed cells. We therefore tested whether inhibition of this enzyme lowers survival of the hepatocarcinoma cells. In fact, inhibition of HO-1 activity increased considerably the number of non-viable HCC-1.2 cells (Fig. 4c).

4 Discussion

Fatty acid peroxides are formed in dietary fats and oils after exposure to oxygen and heating greatly accelerates this process. Accordingly, lipid peroxides are usual components of western style diet [1, 2, 4, 31]. The role of dietary fat for the development and further growth of HCC has not yet been clarified.

Some decomposition of lipid hydroperoxides takes place in stomach, but is incomplete and at higher concentrations hydroperoxides pass into the intestines [32]. The animal study of Staprans *et al*. [6] used radioactively labeled linoleic acid in native and oxidized form. They showed that oxidized dietary lipids are delivered to the liver in the same way as non-oxidized lipids and are utilized for VLDL synthesis, in which form they were found in blood plasma. Accordingly, a part of the intragastically applied oxidized linoleic acid was found in chylomicrones of mesenteric lymph in rat [6]. The chylomicron fraction of human volunteers fed with oxidized oils also contains increased amounts of oxidized lipids for up to 8 h after ingestion [7]. These

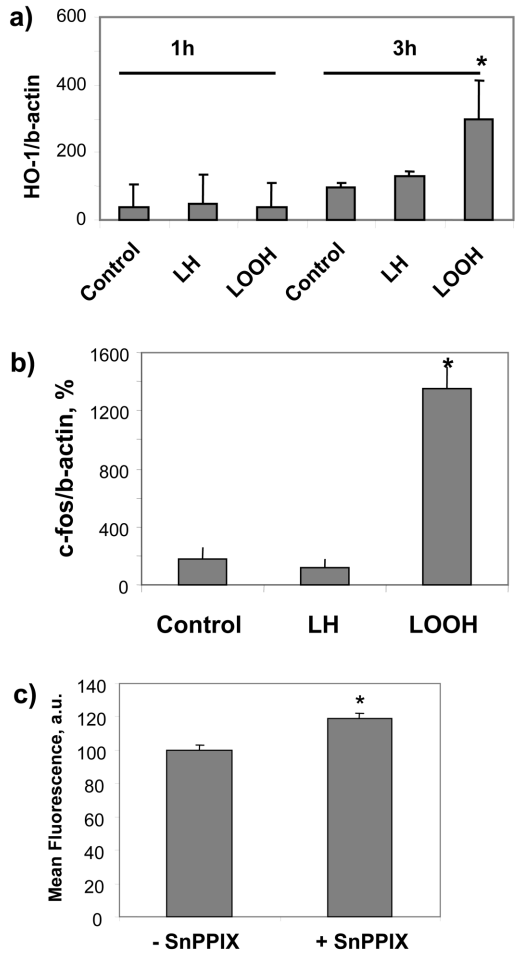

Figure 4. LOOH induces heme oxygenase -1 and the AP-1 component c-fos. HCC-1.2 cells were seeded at 2.5×10^5 cells/well into a 24-well plate, grown for 48 h and treated with 50 μM linoleic acid (LH) or 50 μM linoleic acid hydroperoxides (LOOH). Expression of HO-1 (a) and c-fos (b) was analyzed by RT-PCR as described in Section 2. Non-viable cells were quantified by propidium iodide staining as described in Section 2 (c). The data represent results from three independent experiments (* $p < 0.05$).

observations strongly imply that the liver as a lipid "repackaging" organ is strongly exposed to oxidized dietary oils.

Since lipid hydroperoxides are thermally unstable and decompose at high temperature or in presence of transition metals, their amounts in the diet strongly depend on the oxidation conditions of the oil as well as on the time point of measurements. In our own study [3], we heated corn oil at 100°C and followed the time course of hydroperoxide formation. We found lipid hydroperoxide concentrations ranging from 1 to 250 mM (corresponding to 280 ng/g and 70 mg/g, respectively) and a time course with an initial increase followed by decomposition (unpublished observations). Hageman *et al*. [31] found much less content of LOOH in several heat-treated oils as the maximum concentration we have found in our experiments. This discrepancy could be explained on the basis of heat induced degradation of lipid hydroperoxides so that the data of Hageman *et al*. do not necessarily represent the maximum concentration of hydroperoxides, which can be reached in the diet.

Estimations of the amount of hydroperoxides that are transported from the intestine to the liver with lymphatic fluid can be performed on the basis of literature data. We assumed that the concentration of hydroperoxides in fat reaches 250 mM [3]. Since fat is usually consumed with other dietary components, the concentration in stomach will be ca. ten times less and can be estimated as 25 mM. If 95% of hydroperoxides were decomposed in stomach [32], a concentration of 1.25 mM would still pass to the intestine. Animal experimental data of Penumetcha *et al*. [33] using radioactively labeled LOOH demonstrated that about 50% of LOOH can be taken up from lumen and esterified by intestinal epithelial cells within 2 h of exposure. If only 6–7% of lumen hydroperoxide concentration (1.25 mM) would be incorporated into the chylomicrones, then 50 μM hydroperoxide concentration in the lymphatic fluid would be reached. Thus, in our opinion, the hydroperoxide concentrations used in our study can be reached physiologically.

Another support in favor of these estimations comes from the study of Staprans *et al*. [7], who found 46 nmol/μmol triglyceride of conjugated diene in chylomicrons of human volunteers fed oxidized oil diet. Unfortunately, this study did not prove the presence of hydroperoxy groups in conjugated dienes, but the indirect evidences such as increased susceptibility of chylomicrones to copper mediated oxidation *in vitro* support the idea that it was the case. Assuming the average molecular weight of triglycerides to be 850 g/mol and the concentration of triglycerides in lymph fluid to be 1 mg/mL, the estimated concentration of lymph hydroperoxides calculated from these data should be approximately 50 μM.

However, lipid hydroperoxides may also be formed endogenously during inflammation. Most frequently, hepatocellular carcinoma arises in chronically inflamed livers suffering from viral hepatitis B or C infection. In this process, mesenchymal cells are activated and produce oxygen radicals, which can interact with PUFA of cellular membranes forming lipid peroxides. A correlation was found between end products of lipid peroxidation and pathological features of hepatitis C patients [34]. Steatotic areas in the liver of hepatitis C patients also showed an increase of lipid peroxidation products [35].

We have used LOOH as model compound. In the presence of transition metals, LOOH forms alkoxy radicals in a Fenton-type reaction. Alkoxy radicals rapidly rearrange giving carbon-centered radicals, which can be detected by spin trapping EPR [28]. At higher concentrations, LOOH-derived radicals induce lipid peroxidation which leads to cell damage, apoptosis and necrosis [3]. This could stimulate growth of preneoplastic lesions by selective prolifera-

tion and inflammation [36]. In our cell model, the long incubation times of 24 h combined with high LOOH concentrations of 100 μM exhibited toxic effects. Similarly, toxic effects were observed in animal models after feeding with oxidized oils [37, 38]. Again, in our cell model shorter incubation times and lower doses of LOOH did not decrease cell number but influenced cell proliferation and gene expression.

LOOH were found to increase intracellular formation of hydrogen peroxide. Hydrogen peroxide can arise from spontaneous or catalyzed dismutation of superoxide radicals, which, in turn, can be formed from the rearrangement of lipid hydroperoxy radicals [39]. Intracellular hydrogen peroxide from superoxide radicals formed by NOX1, the catalytic moiety of the superoxide generating NADPH oxidase of phagocytes, was shown to increase mitotic rate, cell transformation and tumorigenicity of carcinoma cells [29]. Consequently, the effects of LOOH in our model system can be mediated at least partially by intracellular superoxide formation. To clarify this question, additional studies with catalase and/or SOD transfections would be necessary.

We observed increased H_2O_2 production and decreased GSH levels in the HCC-1.2 cells following incubation with LOOH. These findings implicate the development of oxidative stress under LOOH treatment, which agrees with our earlier studies on biological and model lipid membranes. Superoxide and/or hydrogen peroxide possess important signaling functions, *e. g*. they regulate the activity of signaling proteins such as protein tyrosine kinases [40], protein tyrosine phosphatases [41] and peroxiredoxins [42] and affect cell proliferation, differentiation and migration [43]. The observed increase of the number of hepatocarcinoma-cells in G2/M- and S-phase could be due to intracellular production of oxygen radicals.

The interplay between cancer cell GSH content and apoptosis is controversial. GSH is necessary for cell growth and severe GSH depletion is associated with cell death [44]. In contrast, "mild" GSH depletion would lead to oxidation of the SH groups of protein cysteine residues and affect protein function. *E. g*., tumor suppressor and transcription factor p53, contains 12 cysteine residues in its amino acid sequence and the oxidation of some of these inhibits p53 function [45, 46]. Since p53 is known to promote apoptosis, repression of its activity could allow the cell to escape from the p53-mediated apoptotic pathway.

Redox-sensitive transcription factors and pathways can be activated by oxidants, leading to alterations in the gene expression profile [47]. Activator protein AP-1 belongs to this group of transcription factors. Like NFkB and Nrf2, AP-1 transactivates HO-1 [30]. In our study, we could show for the first time that also LOOH is able to induce HO-1 possibly via induction of c-fos and c-jun, the two components of AP-1. In agreement with our findings, several other studies have demonstrated the induction of HO-1 by a variety of oxidative stimuli [30]. The growth of many tumors depends on HO-1 [23], which is considered as a valuable target in anti-tumor therapy. The anti-apoptotic action of HO-1 is partially attributed to the antioxidant properties of bilirubin. Carbon monoxide can also contribute to the cytoprotective properties of HO-1 by inhibiting both p53 and release of cytochrome c from mitochondria [23]. HO-1 inhibitors enhanced the response of tumor cells to photodynamic therapy [48] as well as retarded *in vivo* development of tumors derived from hepatoma cells [23, 30]. Whether hepatic HO-1 is activated by consumption of oxidized oils and whether it facilitates tumor formation and growth *in vivo* remains to be elucidated.

In conclusion, our data suggest that LOOH accelerates the cell cycle and lowers the apoptotic activity via HO-1 induction in hepatocarcinoma cells. Hypothetically, therefore, lipid hydroperoxides of dietary origin may enhance hepatocarcinogenesis.

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