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Research Article

Formaldehyde Increases Free Cholesterol Contents in Hepg2 Cells without Alteration of HMGCR Expression

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1. Abstract

Formaldehyde (FA) is a common environmental pollutant that can cause serious liver damage. The aim of this study is to investigate the effect of FA on cholesterol metabolism in human hepato cellular carcinoma cells (HepG2). After exposure to different concentrations of FA for 24 and 48 h, free cholesterol (FC) contents were measured using the GPO-Trinder method, expression levels of several genes related to cholesterol metabolism were analyzed using RT-q PCR, and protein expression of key factors in cholesterol synthesis were measured using western blotting. Our results showed that intracellular FCs in HepG2 cells significantly increased at 24 and 48 h after exposure to FA at 0.004-0.1 mmol/L, and extracellular FCs greatly decreased at 48 h after exposure to FA at 0.004-0.02 mmol/L. However, mRNA and protein levels did not change in HepG2 cells, and the precise mechanism for FA-induced increase in FCs remains unclear. Our results highlight the necessity of further research to illustrate relevant mechanisms for aldehyde-induced hepato toxicity.

2. Keywords: Formaldehyde; Toxicity; Cholesterol metabolism; HepG2 cells; Cholesterol efflux

3. Abbreviations: FA: Formaldehyde; OA: Oleic Acid; BFA: Brefeldin A; FC: Free Cholesterol; Insig-1: Insulin Induced Gene-1; SREBP-2: Sterol Regulatory Element Binding Protein-2; SCAP: SREBP Cleavage Activating Protein; HMGCR: 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase; FDFT1: Farnesyl-Diphosphate Farnesyl Transferase 1; SM: Squalene Monooxygenase; gp78: Human Glycoprotein 78; LDLR: Low Density Lipoprotein Receptor; ACAT: Acyl-Coenzyme A Cholesterol Acyl Transferase; CES1: Carboxyl Esterase 1; ABCA1: ATP-Binding Cassette Transporter A1; ABCG1: ATP-Binding Cassette Transporter G1

4. Introduction

Formaldehyde (FA), the simplest aldehyde, is easily soluble in polar solvents, such as water and alcohol. With the development of industrialization, it has been widely used in various industrial and consumer products, such as rubber, cosmetics, garments, and gasoline [1-3]. People were exposed to FA by both inhalation and ingestion [3, 4]. Since there are abundant enzymes associated with FA metabolism in the liver, FA can be rapidly metabolized and even produce excess free radicals, leading to oxidative stress [5]. It is reported that the excess mortality among workers in the garment industry could be attributed to occupational formaldehyde exposure [6]. Animal studies and in vitro experiments also show that FA can cause pathological changes in the liver, which are accompanied by significantly increased alanine amino transferase (ALT) and aspartate amino transferase (AST) levels [7-10]. The mechanism of aldehyde-induced liver injury is partial-

ly attributed to oxidative stress and lipid per oxidation [11], but other mechanisms may be involved.

Cholesterol is one of the major membrane lipids in eukaryotic cells, and it also serves as a precursor of steroid hormones, such as gestagens and mineral corticoids [12]. However, excess free cholesterols (FCs) are toxic to cells due to increased lysosomal membrane permeability [13]. Hypercholesterolemia is highly associated with cardiovascular disease and arteriosclerosis [14]. Additionally, an increasing number of studies reveal that abnormal cholesterol levels in hepato cytes induce hepatic steatosis and inflammation, which are critical in the development and progression of non-alcoholic fatty liver disease (NAFLD) [15, 16]. The cellular cholesterol homeostasis can be maintained through different mechanisms, such as uptake of low-density lipoprotein receptor (LDLR), *de novo* biosynthesis from acetyl-Co A, esterification of fatty acids, cholesterol efflux, and apolipoprotein B (Apo B) secretion [17]. The *de novo* biosynthetic pathway of cholesterols in the liver is mainly controlled by sterol regulatory element-binding protein-2 (SREBP-2) that activates the transcription of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), the rate-limiting enzyme in cholesterol biosynthesis [18]. The SREBP-2 activity is regulated through the Insig-1-SCAP-SREBP2 pathway. In sterol-depleted cells, SCAP escorts SREBP-2 from the endoplasmic reticulum (ER) to the Golgi for proteolytic processing and releasing of the NH_2-terminal domains of SREBPs, which can enter the nucleus and modulate target genes involved in cholesterol synthesis (Brown and Goldstein, 1997). In the presence of excess sterols, SCAP can be retained by Insig-1 in the ER, causing a halt to the activation of SREBP-2 and a subsequent decrease in cholesterol synthesis [19].

So far, the mechanisms underlying the hepato toxicity of formaldehyde, especially its disturbance of cholesterol metabolism, remain unclear. In this work, we hypothesized that excess free cholesterols (FCs) may contribute to FA-induced liver injury. HepG2 cell, derived from a human hepato blastoma, retains many genotypic and phenotypic characteristics of normal hepato cytes (such as synthesis of lipoproteins and expression of lipoprotein receptors), and it has been recognized as a valuable and informative model system for studying hepatic function [20, 21]. To test our hypothesis, HepG2 cells were treated with FA, and several key genes and proteins involved in cholesterol metabolism and transport were investigated using various methods.

5. Materials and Methods

5.1. Cell Culture

HepG2 cells were purchased from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). Cells were routinely cultured in Dulbecco's modified eagle's medium-high glucose (DMEM-HG) (Hyclone, Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (Sijiqing, Hangzhou, China) in a humid atmosphere of 5% CO_2 at 37℃ The media were changed every two days.

5.2. Formaldehyde Treatment.

FA was purchased from Sigma-Aldrich (St. Louis, MO, USA). When cells reached 80% confluency, they were detached with 0.25% trypsin. Cells were seeded at a density of 3×10^6 cells/ml in multi well plates and cultivated for 24 h. Then, cells were treated with different concentrations of FA (0, 0.004, 0.02, and 0.1 mmol/l) in fresh DMEM media based on the previous study [11]. Oleic acid (OA, 1 mmol/l) and Brefeldin A (BFA, 2.5μg/ml) were used as positive controls. Each treatment was repeated in triplicate.

5.3. Measurement of FC Contents

FC contents were determined with GPO-Trinder method using a commercial kit according to the manufacturer's instructions (Apply gen Technologies, Beijing, China). FC was extracted by organic solvents with some modification [11, 22]. Cells were seeded in 6-well plates and cultured overnight. Then, the cells were treated with different concentrations of FA for 24 and 48 h, respectively. At specific time points, the media were collected for the measurement of extracellular FCs. After treatments, cells were washed with phosphate-buffered saline (PBS) and detached with 0.25% trypsin for the measurement of intracellular FCs. The absorbance of each well was measured at 550 nm on a micro plate reader (Bio-Rad, CA, USA). Protein contents were measured using a BCA assay kit (Boster Biological Technology, Ltd., Wuhan, China). The results were expressed as mg FCs/mg cell protein. The experiments were repeated six times.

5.4. Expression of Genes Related to Cholesterol Metabolism

The mRNA expression of several key genes related to cholesterol metabolism, including Insig-1, SCAP, SREBP-2, HMGCR, and LDLR, were determined by reverse transcription-quantitative polymerase chain reaction (RT-q PCR) using primers purchased from Takara, as shown in (Table 1). After treatments, cells were harvested and total RNA was extracted using TRIzol reagent (Invitrogen, American) according to the manufacturer's instructions. The RNA concentration was determined based on the absorbance at 260 nm. C DNA was reversely transcribed with the Prime Script RT Reagent Kit (Takara, Japan) using 500 ng of RNA as a template, and mRNA expression of genes mentioned above were determined using SYBR Green PCR Master Mix (Takara, Japan). All q PCR experiments were

performed in triplicate on a Bioer Line Gene 9600 quantitative PCR detection system (Hangzhou, China). The relative mRNA levels were normalized to β-actin as an internal reference using the $2^{-\Delta\Delta X_T}$ method [23].

5.5. Detection of Protein Expression by Western Blotting

After treatments, cells were collected and lysed in loading buffer containing 50 mM Tris-HCl, pH 6.8, 5% glycerol, 5% β-mercaptoethanol, 5% sodium dodecyl sulfate (SDS), and 0.25% bromophenol blue [11]. Cell pellets were collected by centrifugation (9,500 rpm for 10 min at 4°C), and protein concentrations were determined using the TCA method. The proteins (10-20 μg per well) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% polyacrylamide gels and transferred electronically to a nitrocellulose membrane (Thermo Scientific, USA). The membranes were blocked with 5% skim milk in phosphate-buffered saline containing 0.05% Tween-20 (PBST) for 1 h at room temperature and then incubated with appropriate primary antibodies overnight at 4°C. The primary antibodies included rabbit anti-human HMGCR (1:1,000, ab174830, Abcam), rabbit anti-human Insig-1(1:1,000, 22115-1-AP, Protein tech), rabbit anti-human SREBP2 (1:1,000, ba30682, abcam), and rabbit anti-human gp78 (1:1,000, A3717, AB colonal). After washing three times with PBST, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (goat anti-rabbit IgG, 1:5,000, BA1050, Boster) in TBST for 1 h at room temperature. The proteins were detected using enhanced chemiluminescence (ECL, Thermo Scientific). β- actin was used as a loading control. After the films were developed, the optical density of all protein bands was analyzed using Image-Pro plus 6.0 software (Media Cybernetics, Bethesda, MD, USA).

5.6. Statistical Analysis

All data are presented as mean ± standard deviation. Statistical analysis was conducted using SPSS v18.0 (IBM Corp., NY, USA). One-way analysis of variance (ANOVA) was used with a Dunnett's test to compare the means of various groups. A *P* value of less than 0.05 was considered statistically significant.

6. Results

6.1. Effects of FA on Intracellular and Extracellular FC

Compared with the control, FA (0.004 - 0.1 mmol/L) and OA (1 mmol/L) significantly increased intracellular FC levels in HepG2 cells at 24 h and 48 h (Table 2). OA and BFA significantly increased the extracellular FC levels at 24 h, but did not change them at 48 h (Table 3). On the other hand, FA at all concentrations had no impact on the extracellular FC levels at 24 h, but FA at 0.004 and 0.02 mmol/L significantly increased the extracellular FC levels at 48 h (Table 3).

Cells were treated with various concentrations of formaldehyde for 24 and 48 h. Following treatment, the cells were harvested and lysed with tryptase, and intracellular FC levels were measured using the

Table 1: Primers used for gene expression by RT-qPCR

Genes	Primer sequences
β-actin-F	TGGCACCCAGCACAATGAA
β-actin-R	CTAAGTCATAGTCCGCCTAGAAGCA
SREBP-2-F	CTATGGTGGGCACTAGGAATGAG
SREBP-2-R	GAGGGGCAGGAGAGAAAGAAA
SCAP-F	AGCTCGCTGCTCATGTCTGT
SCAP-R	CTAACCCAATAACCACCACAAGG
Insig1-F	GTGTGTGTATCACCAGTGGGTCAA
Insig1-R	TATCGCAGTGTGGGAACCAAGA
HMGCR-F	GCCTGGCTCGAAACATCTGAA
HMGCR-R	CTGACCTGGACTGGAAACGGATA
FDFT-1-F	AAGATGACATGACCATCAGTGT
FDFT-1-R	CACTGTTTGGTATTTCTCAGCC
SM-F	CTGACCTTTATGATGATGCAGC
SM-R	CAGGCTTTTCTTAGTTGATGCA
LDLR-F	CTGGTCAGATGAACCCATCAAAGA
LDLR-R	TCATTGCAGACGTGGGAACAG
ACAT-F	TAACAGCTGCCAATGCCAGTACA
ACAT-R	GGTTCTACAGCAGCGTCAGCAA
CES1-F	TGAAACCCAAGACGGTGATAGGA
CES1-R	CGAGCAAAGTTGGCCCAGA
ABCA1-F	TTTTTGCTCAGATTGTCTTGCC
ABCA1-R	TGTACTGTTCGTTGTACATCCA
ABCG1-F	CTCCTATGTCAGGTATGGGTTC
ABCG1-R	AAAATCCCGAGTACGATGAAGT

Table 2: Effect of formaldehyde treatment on intra-hepatocelluar FC levels in HepG2 cells (means ± standard deviations, n=6)

Groups	FC (mmol/g)	
	24 h	48 h
Negative control	0.2894±0.0665	0.3753±0.1365
0.004mmol/L FA	0.3905±0.1187*	0.4260±0.0330*
0.02mmol/L FA	0.3850±0.0612*	0.5679±0.0813*
0.1mmol/L FA	0.3794±0.0703*	0.4895±0.0618*
1mmol/L OA	0.5481±0.0425*	0.5666±0.0337*
2.5μg/ml BFA	0.2908±0.0866	1.2551±0.5750*

Table 3: Effect of formaldehyde treatment on extra-hepatocelluar FC levels of HepG2 cells (means ± standard deviations, n=6)

Groups	FC（mmol/L）	
	24h	48h
Negative control	0.1773±0.0274	0.3256±0.0707
0.004 mmol/L FA	0.2502±0.0696	0.2182±0.0880*
0.02 mmol/L	0.2220±0.0669	0.2290±0.0846*
0.1 mmol/L	0.1645±0.0385	0.3101±0.0940
1mmol/L OA	0.3245±0.0909*	0.4140±0.1011
2.5 μg/ml BFA	0.2968±0.0520*	0.3158±0.0942

GPO-Trinder method. * Compared to the control, $P<0.05$.

HepG2 cells were treated with various concentrations of formaldehyde for 24 and 48 h. Following treatment, the supernatants were collected and extracellular FC levels were measured using the GPO-Trinder method.* Compared to the control, $P<0.05$.

6.2. Effects of FA on mRNA levels of Insig1, SCAP, and SREBP2

SREBP-2 is synthesized as a membrane-bound precursor that requires a two-step proteolytic cleavage to be activated, which controls the transcriptional level of HMGCR. Compared with the control, BFA significantly increased the levels of Insig-1, SCAP and SREBP-2 at 24 h and 48 h after treatment, and OA did not affect the levels of Insig-1, SCAP and SREBP-2 at 24 h but significantly decreased the levels of SCAP and SREBP-2 at 48 h (Figure 1). In contrast, FA at 0.004-0.1 mmol/L did not affect mRNA levels of Insig-1, SCAP and SREBP-2 (Figure 1).

6.3. Effects of FA on Mrna Levels of Cholesterol Synthesis-Related Genes

Cholesterol synthesis is regulated by SREBP2 at the transcriptional level, which involves many enzymes. HMGCR is the rate-controlling enzyme of the mevalonate pathway that produces cholesterol and other isoprenoids. Farnesyl-diphosphate farnesyl transferase 1 (FDFT1) catalyzes the two-step conversion of farnesyl pyrophosphate to squalene, which is subsequently converted to the squalene-2,-3-epoxide by squalene monooxygenase (SM). Compared with the control, BFA significantly increased the mRNA levels of HMGCR, SM, and FDFT-1 at 24 h and 48 h after treatment (Fig. 2). OA and FA did not affect the mRNA levels of these genes.

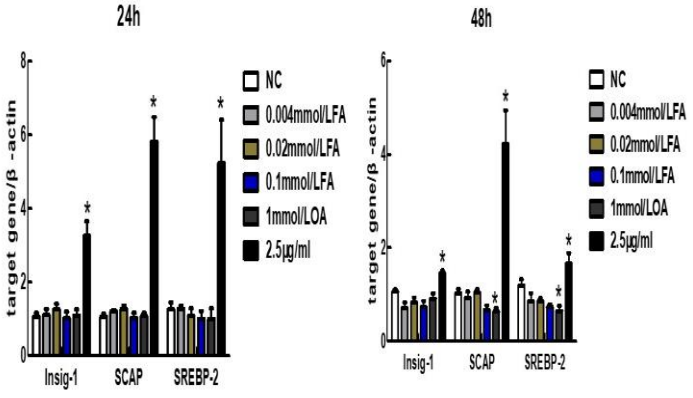

Figure 1: Expression levels of Insig-1(A), SCAP(B) and SREBP-2(C) genes in HepG2 cells (n=3). HepG2 cells were treated with FA, OA and BFA for 24 and 48 h. * Compared to the control, P<0.05.

6.4 Effects of FA on Mrna Levels of Regulatory Genes Involved in Cholesterol Homeostasis

Many factors are involved in cholesterol regulation, including LDLR, acyl-coenzyme A: cholesterol acyl transferase (ACAT), carboxylesterase 1(CES1), and so on. LDLR plays a vital role in exogenous cholesterol uptake, which specifically binds cholesterol-rich LDL molecules and transfers exogenous cholesterols into hepatocytes. ACAT is an intracellular enzyme responsible for the esterification of free cholesterol (FC) with long-chain fatty acyl-Co A derivatives, thereby preventing the accumulation of excess FCs [44, 54]. CES1 is the most abundant cholesteryl ester hydrolase in the liver [24, 25]. We analyzed the effects of FA on the mRNA expression of LDLR, ACAT, and CES1. As shown in (Figure 3), BFA significantly increased LDLR mRNA expression levels at 24 h but decreased it at 48 h following treatment. In addition, BFA also significantly increased ACAT mRNA expression levels at both 24 h and 48 h following treatment, which may lead to a decrease in intracellular FC levels. OA signifi-

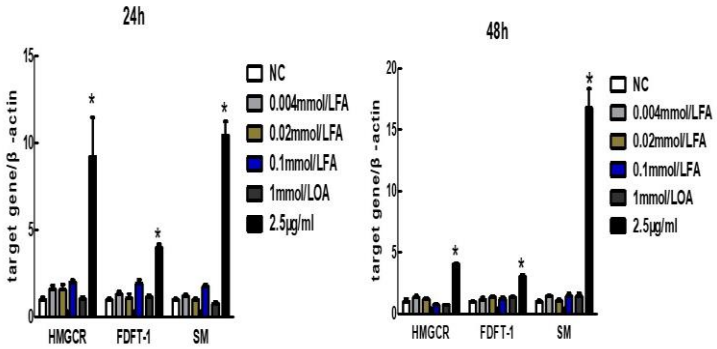

Figure 2: Expression levels of HMGCR, FDFT-1 and SM genes in HepG2 cells (n=3). HepG2 cells were treated with FA, OA and BFA for 24 and 48 h. * $P<0.05$, as compared to the control.

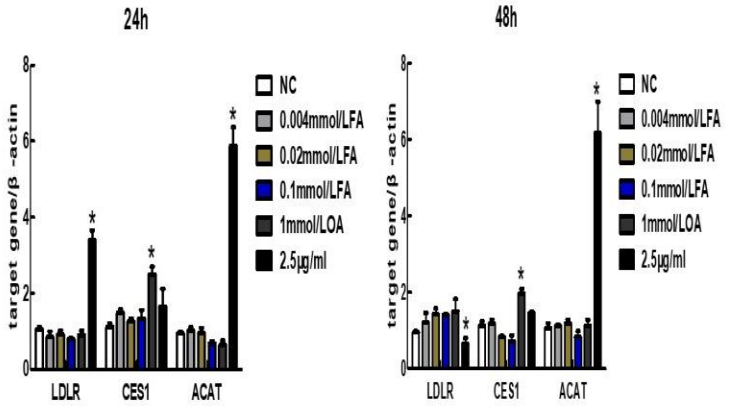

Figure 3: Expression levels of LDLR, CES1 and ACAT genes in HepG2 cells (n=3). HepG2 cells were treated with FA, OA and BFA for 24 and 48 h. *P<0.05, compared to the control.

cantly increased CES1 mRNA expression levels at 24 h and 48 h following treatment, which may lead to an increase in intracellular FC levels. Different from these two positive controls, FA did not change mRNA expression levels of ACAT, and CES1, suggesting that FA does not affect uptake and esterification of cholesterol, as well as hydrolysis of cholesteryl ester.

6.5. Effects on FA on Protein Levels of Insig-1, SREBP2, HMGCR, and Gp78

Furthermore, the protein levels of Insig-1, SREBP2, HMGCR, and glycoprotein 78 (gp78) were detected using western blotting. Both BFA and OA significantly increased the protein levels of SREBP2 and HMGCR at 24 and 48 h following treatment (Figure 4), but they had no influence on gp78 protein levels. Compared with the positive controls, FA (0.004–0.1 mmol/L) did not change the protein levels of Insig-1, SREBP2 and HMGCR.

7. Discussion

Our previous study found that FA could inhibit the viability of HepG2 and increase extracellular TG levels, indicating that FA may cause damage to HepG2 cells through interference with lipid metabolism [11]. In this work, we further observed if FA could interfere with cholesterol metabolism in order to illustrate the mechanism by which FA causes liver damage. It is reported that excess FC in hepatocytes cause liver injury or inflammatory and pro fibrotic effects by increasing the production of reactive oxygen species [26]. Here, we found that FA significantly increased intracellular FC levels at 24 and 48 h after treatment (Table 2), suggesting that FA disturbed the FC metabolism. The disturbed FC metabolism may cause damage to the liver through mitochondrial dysfunction and lysosome impairment [15, 27-28].

Among various mechanisms involved in cholesterol metabolism, the *de novo* synthesis of cholesterol and its uptake from the serum are considered to be most important. It is reported that SREBP-2 could specifically activate cholesterol synthesis [29]. However, our results showed that FA did not affect the mRNA and protein expression of Insig-1, SCAP, and SREBP-2 in HepG2 cells (Figure1), indicating that FA exposure increased FC levels through additional mechanisms other than the Insig-1/SCAP/SREBP-2 pathway.

Cholesterol synthesis is tightly regulated by some factors, such as HMGCR, FAFT-1, and SM. HMGCR exist in all tissues, but it is most predominantly expressed in the liver [30]. As a membrane-bound enzyme in the ER, HMGCR is the rate-limiting enzyme in cholesterol synthesis [31]. Increased HMGCR contents are associated with FC levels and the severity of liver disease [32]. The intracellular cholesterol level regulates the feedback of HMGCR and inhibits HMGCR expression at the transcriptional level through the Insig-1/SCAP/SREBP2 pathway [33]. In this study, FA did not increase HMGCR mRNA and protein levels (Fig. 2 and Fig. 4), but increased intracellular FC levels. In addition, FA did not affect Insig-1 and nSREBP-2 protein levels. These results suggest that FA has no influence on HMGCR. It is known that HMGCR is a highly regulated protein, either transcriptionally or post-translationally. Post-translational modulations are responsible for the stability of HMGCR, which involve many factors, including RNF145, gp78, Hrd1, TRC8, [34-36]. Gp78, an ER-membrane anchored ubiquitin ligase, can promote the degradation of several mis folded proteins in the ER by recruiting ubiquitin-conjugating enzyme (E2), and it can also degrade HMGCR, ApoB-100, and Insig-1/2, thereby affecting cholesterol homeostasis [19, 37]. Many chemicals can also affect the stability of HMGCR, such as statin. Statin can competitively bind to the catalytic domain of HMGCR to block cholesterol synthesis, and it can also inhibit the ubiquitination of HMGCR via gp78 to increase the stability of HMGCR [37]. In this work, we found that FA could not affect the levels of gp78 protein (Figure 4), confirming that FA had no influence on HMGCR.

FDFT1 plays a key regulatory role in cholesterol synthesis by directing farnesyl pyrophosphate to either sterol or non-sterol branches of the isoprenoid pathway [39]. FDFT1 catalyzes the conversion of two molecules of farnesyl pyrophosphate to squalene in two steps. SM catalyzes the epoxidation of squalene to 2, 3-oxidosqualene (squalene epoxide) in the first oxygenation step in sterol biosynthesis, and it is also considered as one rate-limiting enzyme of this pathway

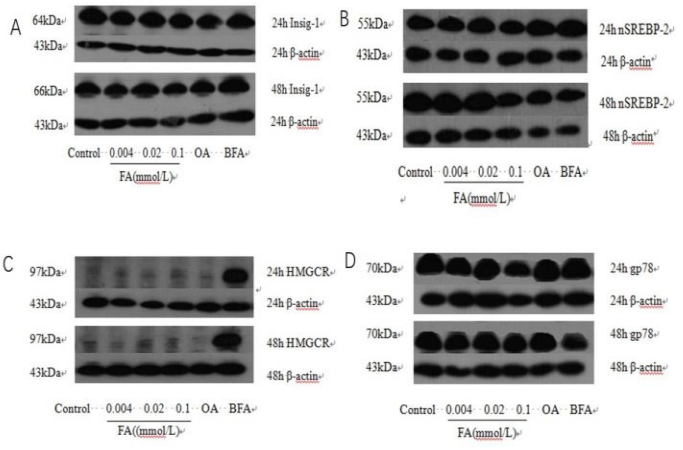

Figure 4: Effects of formaldehyde on HMGCR protein expression in HepG2 cells (mean ± SD, n=3). After treatment with various concentrations of formaldehyde, OA and BFA for 24 and 48 h, cells were harvested and lysed with sample buffer. Protein levels were determined using western blot. β-actin were used as a loading control. * P<0.05, compared to the control.

[40, 41]. Here, we found that FA did not affect the mRNA levels of FDFT1 and SM (Figure 2), showing that FA could not enhance cholesterol synthesis through these factors. But we found that BFA could greatly increase the mRNA expressions of HMGCR, FDFT-1 and SM, especially SM mRNA expression by 10 folds at 24 h and 17 folds at 48 h, respectively. BFA is regarded as an intracellular trafficking inhibitor to inhibit cholesterol efflux by inhibiting basolateral membrane (BLM) delivery and dimerization of transcobalamin II receptor (TC II-R) in Caco-2 cells [42-43, 45]. In contrast, our result showed that BFA significantly increased intracellular levels of FC in HepG2 cells at 48 h following treatment, but did not increase FC levels in the media, i.e., extracellular levels of FC (Figure 2) and we also found that BFA significantly increased the mRNA expressions of HMGCR and FDFT-1 and SM. Therefore, these results suggest that BFA might not inhibit the efflux of cholesterol but increase FC synthesis in HepG2 cells.

In addition to cholesterol synthesis, intracellular cholesterol levels are also regulated in part by cholesterol uptake via the LDLR pathway and cholesterol esterification. LDLR is a cell membrane glycoprotein found primarily in hepatocytes, and it can specifically recognize apoB-100 of LDL particles and deliver LDL into cells [46]. Additionally, the increased transcriptional activity of LDLR may enhance the re-uptake of LDL, leading to an increase in intracellular FC contents [47]. In this study, we found that FA did not change LDLR mRNA expression in HepG2 cells, suggesting that the increase in intracellular FC levels induced by FA exposure is not due to the promotion of LDL uptake. In addition, FA did not affect mRNA expression levels of ACAT or CES1, although OA significantly increased CES1 mRNA expression. CES1 is a neutral hydrolase for cholesterol ester and triglyceride and plays an important role in hepatic lipid mobilization [48]. A few studies have shown that CES1 prevents lipid accumulation in liver and macrophages [50]. Hepatic CES1 can also facilitate the entry of cholesterol into the bile acid synthetic pathways for sterol elimination [51]. Our results suggest that OA could increase intracellular FC levels in HepG2 cells by increasing CES1 expression. We also found that FA could increase intracellular FC levels, but could not increase the expression of cholesterol synthesis-related genes and proteins, as well as uptake of cholesterol from extracellular sources. In order to interpret the reasons for increased cholesterol levels induced by FA, we further observed if FA could affect cholesterol efflux because excess cholesterol can be exported from the cell via cholesterol transporters (ABCG1 and ABCA1) [42]. ABCG1 effluxes excess cholesterol from cells to HDL particles, while ABCA1 effluxes cholesterol to lipid-poor apolipoprotein AI (apoAI) [52]. Interestingly, we found that FA significantly increased mRNA expressions of ABCG1 and ABCA1 (Figure 5), which might counteract the increase in intracellular cholesterol in HepG2 cells. In addition, other factors might be involved in intracellular cholesterol transport, such as caveolin, which regulates the intracellular cholesterol transport in a complex process involving caveolae, ER, and Golgi complex [53]. Intracellular trafficking of caveolins is key to the regulation of cholesterol homeostasis in cells [56]. It is reported that ethanol can increase the expression of CAV-1 in HepG2 cells and affect cholesterol metabolism [56]. Caveolins might interact with excess FC induced by FA exposure.

In conclusion, our results showed that FA exposure could significantly increase intracellular FC in HepG2 cells, which may cause damage to the liver. But FA did not increase FC by increasing cholesterol synthesis and uptake of cholesterol or decrease of cholesterol efflux. The precise mechanisms for the increment of intracellular FC need to be further studied.

8. Funding Source

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