CHOP deficiency attenuates cholestasis-induced liver fibrosis by reduction of hepatocyte injury

Nobuyuki Tamaki,1 Etsuro Hatano,1 Kojiro Taura,1,2 Masaharu Tada,1 Yuzo Kodama,2 Takashi Nitta,1 Keiko Iwaisako,1 Satoru Seo,1 Akio Nakajima,1 Iwao Ikai,1 and Shinji Uemoto1

1Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan; and 2Department of Medicine, University of California San Diego, La Jolla, California

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Recent studies have shown involvement of endoplasmic reticulum (ER) stress in various diseases (34), including neurodegenerative disease (10, 12, 36), diabetes mellitus (6), atherosclerosis (7, 9, 14), heart disease (22), and liver disease (15). CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) is a key component in endoplasmic reticulum (ER) stress-mediated apoptosis. The goal of the study was to investigate the role of CHOP in cholestatic liver injury. Acute liver injury and liver fibrosis were assessed in wild-type (WT) and CHOP-deficient mice following bile duct ligation (BDL). In WT livers, BDL induced overexpression of CHOP and Bax, a downstream target in the CHOP-mediated ER stress pathway. Liver fibrosis was attenuated in CHOP-knockout mice. Expression levels of α-smooth muscle actin and transforming growth factor-β1 were reduced, and apoptotic and necrotic hepatocyte death were both attenuated in CHOP-deficient mice. Hepatocytes were isolated from WT and CHOP-deficient mice and treated with 400 μM glycochenodeoxycholic acid (GCDCA) for 8 h to examine bile acid-induced apoptosis and necrosis. GCDCA induced overexpression of CHOP and Bax in isolated WT hepatocytes, whereas CHOP-deficient hepatocytes had reduced cleaved caspase-3 expression and a lower propropidium iodide index after GCDCA treatment. In conclusion, cholestasis induces CHOP-mediated ER stress and triggers hepatocyte cell death, and CHOP deficiency attenuates this cell death and subsequent liver fibrosis. The results demonstrate an essential role of CHOP in development of liver fibrosis due to cholestatic liver damage.

Apoptosis; necrosis; endoplasmic reticulum stress; transforming growth factor-β1; bile duct ligation

Materials and Methods

Animals and treatment. Mice lacking the CHOP gene (C57BL/6 background) were kindly provided by Profs. Shizuo Akira and Masataka Mori (25). Food and water were allowed ad libitum. Animal protocols were approved by the Animal Research Committee of Kyoto University and all experiments were conducted in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Bile duct ligation. Bile duct ligation (BDL) was performed by surgical ligation of the common hepatic bile duct and the cystic duct in mice aged 6–8 wk old under pentobarbital anesthesia (50 mg/kg). Sham-operated mice underwent a laparotomy without ligation of the common bile duct or the cystic duct. The animals were euthanized on postoperative day 2 or 14. Blood was collected from the vena cava, and serum was separated for measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (T-bil). Some portions of the liver tissue were frozen immediately in liquid nitrogen, and other portions were fixed in 10% neutral-buffered Formalin for subsequent sectioning and mounting on microscope slides.

Liver histology. Formalin-fixed, paraffin-embedded sections were cut at 4-μm thickness and mounted on silanized glass slides. The slides were stained with hematoxylin and eosin, subjected to Masson trichrome staining, or stained with Sirius red (20). Sirius red staining was quantified by image analysis with National Institutes of Health image (Image J), with detection thresholds set for the red color. Images of five nonoverlapping fields were selected at random and captured per section at ×100 magnification. Fields including major arteries or veins were excluded. The degree of labeling in each section was determined from the area within the color range divided by the number of captured image fields.
total area. TdT-mediated dUTP nick-end labeling (TUNEL) analysis was performed on 4-μm sections with an Apoptosis in situ Detection Kit, used according to the manufacturer’s specifications (Wako, Osaka, Japan). The number of TUNEL-positive cells was counted in each section.

Immunohistochemistry. The specimens were fixed in 10% formalin and embedded in paraffin. Subsequently, they were deparaffinized, and endogenous peroxidase was quenched with 0.3% hydrogen peroxide in methanol at room temperature for 15 min. The antigen was retrieved by incubation in citric acid buffer at 90°C for 20 min. After being blocked, the sections were incubated with primary antibody-recognizing α-smooth muscle actin (α-SMA) (no. ab5694; Abcam, Cambridge, UK) at 1:200 dilution overnight at 4°C and then with labeled polymer in an Envision + System HRP (Dako Japan, Tokyo, Japan) at room temperature for 1 h. The sections were examined after incubation with a Liquid DAB Substrate Chromogen System (Dako Japan) for 5 min.

Isolation and culturing of hepatocytes. Under pentobarbital anesthesia, mice liver was perfused for 5 min with SC-1 solution consisting of (all in mg/l) 8,000 NaCl, 400 KCl, 88.7 NaH2PO4, 2,380 HEPES, 120.45 Na2HPO4, 70 MgCl2, 190 EGTA, and 900 glucose (pH 7.25), followed by digestion at 37°C for 15 min with 0.03% collagenase dissolved in SC-2 solution consisting of (all in mg/l) 8,000 NaCl, 400 KCl, 88.7 NaH2PO4, 120.45 Na2HPO4, 2,380 HEPES, 350 NaHCO3, and 560 CaCl2·2H2O (pH 7.25). After collagenase perfusion, the liver capsule was cut, and the cells were dispersed in Gey’s balance salt solution (GBSS)-B consisting of (all in mg/l) 8,000 NaCl, 370 KCl, 210 MgCl2·6H2O, 70 MgSO4·7H2O, 120 Na2HPO4, 30 K2HPO4, 991 glucose, 227 NaHCO3, and 225 CaCl2·2H2O (pH 7.25). The cell suspension was filtered through a steel mesh and centrifuged at 50× g for 15 min. Then, the supernatant was discarded, the cell pellet was resuspended in GBSS-B solution, and the washing procedure was repeated three times.

The isolated hepatocytes were cultured on six-well plates coated with type I collagen at a cell density of 5 × 105 cells/well with Dulbecco’s modified Eagle’s medium with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO2-95% air. The medium was replaced with serum-free Dulbecco’s modified Eagle’s medium 6 h after plating. Twelve hours later, 400 μM glycodeoxycholic acid (GCDCA) (Nacalai Tesque, Kyoto, Japan) was added, and the cells were incubated for 8 h. After being stained with bixinemizide H33342 fluorescein dihydrochloride DMDO solution (Hoechst 33342) (Nacalai Tesque) and propidium iodide (PI) (Calbiochem, San Diego, CA), the cells were examined with a fluorescence microscope. Images of five nonoverlapping fields at ×200 magnification were selected at random, and the cells were counted. PI-positive cells were considered as dead cells, and the PI-positive ratio was calculated as the percentage of PI-positive cells among Hoechst 33342-positive cells.

Quantitative real-time RT-PCR. Total RNA was extracted from the liver tissues or cultured hepatocytes with TRIZol (Invitrogen Japan, Tokyo, Japan). Total RNA (2 μg) was reverse transcribed to cDNA with an Omniscript RT Kit (Qiagen in Japan, Tokyo, Japan) with oligo(dT)12-18 primers (Invitrogen Japan). Real-time RT-PCR was performed with a QuantiTect SYBR Green PCR Kit (Qiagen) and an ABI PRISM 7700 Sequence Detection System (Applied Biosystems Japan, Tokyo, Japan). RT-PCR was performed with an initial step at 95°C for 15 min, followed by 40 cycles at 95°C for 30 s, 56°C for 30 s, and 72°C for 1 min with a final step at 72°C for 10 min. Specific primers used are listed in Table 1. The expression levels were calculated after conversion to numerical values by ABI PRISM 7700 SDS software and are shown as ratios relative to the expression level of GAPDH.

Western blot analysis. Frozen liver tissues or cultured hepatocytes were homogenized in lysis buffer containing 50 mM Tris·HCl, 2% sodium dodecylsulfate, and 10% glycerol and then boiled for 2 min. After the concentration of the sample was determined, 0.1% bromophenol blue and 6% 2-mercaptoethanol were added. Then 20 μg of each sample was electrophoresed and transferred onto Immobilon membranes (Millipore, Billerica, MA). The membranes were blocked and incubated at 4°C overnight with the following primary antibodies and dilutions: anti-GADD153 (CHOP) antibody (no. sc-793; Santa Cruz Biotechnology, Santa Cruz, CA) at 1:200; anti-phosphorylated c-Jun (p-c-Jun) antibody (no. sc-63132-R; Santa Cruz Biotechnology) at 1:500; anti-transforming growth factor-β1 (TGF-β1) antibody (no. 5559–100; BioVision, Mountain View, CA) at 1:200; anti-cleaved caspase-3 antibody (no. 9661; Cell Signaling Technology, Danvers, MA) at 1:200; anti-GAPDH antibody (no. sc-20357; Santa Cruz Biotechnology) at 1:500; and anti-α-tubulin antibody (no. CP06; Calbiochem) at 1:1,000. After being washed, the membranes were reacted with secondary antibodies conjugated with horseradish peroxidase. Chemiluminescence was detected with an ECL Plus Western Blotting Detection System (Amersham, Buckinghamshire, UK), used according to the manufacturer’s instructions. The intensity of the bands was quantified with imaging analysis software Quantity One (Bio-Rad Laboratories, Hercules, CA) and normalized with GAPDH or α-tubulin as an internal control.

Statistical analysis. All data are expressed as means ± SE, and the statistical significance of a difference between groups was assessed by Mann-Whitney U-test. P values <0.05 were regarded as statistically significant.

RESULTS

BDL causes transient induction of CHOP in mouse liver. BDL was performed in wild-type (WT) mice to determine if cholestasis is accompanied by induction of CHOP, which is known to be upregulated by ER stress. The mice were euthanized on postoperative day 2 or 14. Quantitative real-time RT-PCR demonstrated a 3.7-fold upregulation in CHOP mRNA on day 2 in WT mice (Fig. 1A). In Western blot analysis, CHOP expression was barely detectable in sham-operated mice but was elevated on day 2 in BDL mice (Fig. 1B). CHOP expression returned to the baseline level by day 14. These results indicate that cholestatic liver injury is accompanied by transient induction of CHOP. As predicted, the induction of CHOP expression was completely abolished in CHOP-deficient mice (data not shown). To ascertain whether CHOP-mediated ER stress pathway was specifically blocked in CHOP KO mice, the expressions of other ER stress markers were...

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<th>Genes</th>
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<tr>
<td>CHOP</td>
<td>5′-GGGAGTGGACCACTCATGTTT</td>
<td>5′-GGCCAGTGACCCAGAACG</td>
</tr>
<tr>
<td>GADPH</td>
<td>5′-ATGGCTGATCCCACTGTGAC</td>
<td>5′-GGCCAGTGACCCAGAACG</td>
</tr>
<tr>
<td>GRP78</td>
<td>5′-CTCCACAGCACCTGAGTTA</td>
<td>5′-ATGGCTGATCCCACTGTGAC</td>
</tr>
<tr>
<td>Bax</td>
<td>5′-GGGAGTGGACCACTCATGTTT</td>
<td>5′-GGCCAGTGACCCAGAACG</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>5′-GGCCAGTGACCCAGAACG</td>
<td>5′-GGCCAGTGACCCAGAACG</td>
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CHOP, CCAAT/enhancer-binding protein (C/EBP) homologous protein; GRP, glucose-regulated protein.
CHOP deficiency attenuates cholestasis-induced liver fibrosis

KO was slightly weaker than in WT (Fig. 1, to almost the same level, though GRP78 expression in CHOP induced liver fibrosis. Sirius red staining showed that fibrosis was reduced in CHOP KO mice on day 14 after BDL [4.08 ± 0.52% (WT) vs. 2.36 ± 0.22% (KO) of a microscope field, \( P < 0.05 \)] (Fig. 2, A–C).

Because activation of hepatic stellate cells (HSCs) is related to hepatic fibrosis, we stained the liver sections immunohistochemically for \( \alpha \)-SMA, a marker of activated HSCs (Fig. 2, D and E). There was a significant reduction in the number of \( \alpha \)-SMA-positive cells in CHOP-deficient mice. The expression of mRNA for TGF-\( \beta \)1, a potent profibrogenic cytokine, was determined by quantitative real-time RT-PCR; a 2.5-fold up-regulation in TGF-\( \beta \)1 mRNA was found in WT mice, whereas the induction in expression was completely abolished in CHOP KO mice (Fig. 2F). In Western blot analysis, TGF-\( \beta \)1 was barely detectable in sham-operated mice and was elevated after BDL in WT mice (Fig. 2G). In CHOP KO mice, induction of TGF-\( \beta \)1 expression was attenuated compared with WT mice, resulting in a statistically significant difference in TGF-\( \beta \)1 levels between WT and CHOP-deficient mice on day 14 (Fig. 2H).

CHOP deficiency rescues hepatocytes from apoptotic and necrotic death after BDL. Focal necrotic spots were seen in livers 14 days after BDL (Fig. 3, A and B). These spots were identified as clearly demarcated and less eosinophilic areas with loss of nuclear staining. There were fewer focal necrotic spots in CHOP KO mice than in WT mice [269.7 ± 38.6 (WT) vs. 77.1 ± 20.0 (KO) per 1-cm\(^2\) section, \( P < 0.05 \)]. The percentage areas of focal necrosis were 14.6 ± 3.0 and 29.0 ± 2.9% of whole sections in CHOP KO and WT mice, respectively (\( P < 0.05 \)) (Fig. 3C). Necrosis spots were not evaluated on day 2 because they were not clearly demarcated at this time point. Liver sections 2 days after BDL were evaluated for the presence of apoptotic cells by TUNEL staining (Fig. 3, D and E). There were fewer TUNEL-positive cells in CHOP KO mice than in WT mice on day 2 [3.63 ± 0.85 (WT) vs. 0.99 ± 0.28 (KO) per 1,000 hepatocytes, \( P < 0.05 \)] (Fig. 3F). There were less than 0.5 TUNEL-positive cells per 1,000 hepatocytes on day 14 in both WT and CHOP KO mice. We assessed serum transaminase levels after BDL (Table 2). The elevation of transaminases preceded histological evidence of necrosis; we observed drastic elevation of transaminases on day 2, while necrosis areas were not yet histologically definite. The transaminase levels decreased by day 7 and day 14 when we observed well-demarcated necrotic areas on histology. CHOP KO mice showed significantly lower transaminase levels at day 7 [AST, 547.8 ± 73.6 (WT) vs. 256.7 ± 3.8 (KO), \( P \leq 0.05 \); ALT, 470.2 ± 49.7 (WT) vs. 255.3 ± 8.7 (KO), \( P \leq 0.05 \)]. They showed consistently lower transaminase levels on day 2 and day 14, as well, although the differences were not statistically significant.

Bax, a downstream target of CHOP that plays an important role in the CHOP-mediated apoptosis pathway (11, 24), was elevated threefold on day 2 after BDL and remained at a higher level than in sham-operated mice on day 14 (Fig. 3G). In CHOP KO mice, elevation of Bax expression was not observed. Bcl-2, one of the factors that inhibit ER stress-mediated apoptosis, was also elevated in WT mice. The induction of Bcl-2 was suppressed in CHOP KO mice (Fig. 3H). These results demonstrate that CHOP deficiency results in less cholestatic liver injury-induced hepatocyte death by either apoptosis or necrosis.

Fig. 1. Bile duct ligation (BDL) causes transient induction of CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) and other endoplasmic reticulum (ER) stress markers in mouse liver. Quantitative real-time RT-PCR (A) and Western blot analysis (B) of CHOP, quantitative real-time RT-PCR of glucose-regulated protein (GRP)78 (C), and Western blot analysis of phosphorylated c-Jun (p-c-Jun) (D). Data are shown as means ± SE (\( n = 4 \)). KO, knockout; WT, wild-type; Sham, sham-operated.

determined. GRP78 and p-c-Jun, a downstream target of JNK (13), were induced on day 2 in both WT and CHOP KO mice to almost the same level, though GRP78 expression in CHOP KO was slightly weaker than in WT (Fig. 1, C and D). Additionally, we determined the expression of cleaved caspase-12. It was induced slightly on day 14 but not on day 2 in both WT and CHOP KO mice (data not shown). These data confirm that ER stress was induced by BDL in both WT and CHOP KO mice and that CHOP deficiency specifically blocked CHOP-mediated ER stress pathway.

CHOP deficiency attenuates hepatic fibrosis caused by BDL. We investigated the effect of CHOP deficiency on cholestasis-induced liver fibrosis. Sirius red staining showed that fibrosis was reduced in CHOP KO mice on day 14 after BDL [4.08 ± 0.52% (WT) vs. 2.36 ± 0.22% (KO) of a microscope field, \( P < 0.05 \)] (Fig. 2, A–C).
GCDCA induces CHOP in hepatocytes in vitro. GCDCA, a toxic bile acid that accumulates in cholestasis, was used to induce bile acid-induced cell death in a primary culture of hepatocytes. Hepatocytes were isolated from WT mice and treated with 400 μM GCDCA. Quantitative real-time RT-PCR indicated a 5.3-fold upregulation in CHOP mRNA from baseline in WT hepatocytes 8 h after addition of GCDCA (Fig. 4A). In Western blot analysis, CHOP expression was undetectable in untreated hepatocytes and elevated in GCDCA-treated hepatocytes (Fig. 4B). We determined the expression of glucose-regulated protein (GRP)78, another ER stress marker. It was induced by GCDCA in both WT and KO hepatocytes to the same level (Fig. 4C). This result confirms that ER stress was induced by GCDCA in both WT and CHOP KO mice and that GCDCA specifically blocked CHOP-mediated ER stress pathway in CHOP KO mice. The expression of Bax and Bel-2 was induced in GCDCA-treated hepatocytes of WT mice, whereas the induction was weaker in CHOP KO mice (Fig. 4D, E and F). CHOP deficiency attenuates cleavage of caspase-3 and necrotic cell death induced by GCDCA in cultured hepatocytes. To ascertain a causative role for CHOP deficiency in the reduced hepatocyte death observed in the BDL model, bile acid-induced cell death was compared in primary hepatocytes from WT and CHOP-deficient mice. Western blot analysis showed that induction of cleaved caspase-3 by GCDCA was significantly suppressed in CHOP-deficient mice (Fig. 5A and B) and that PI staining showed less hepatocyte death [27.5 ± 1.5% (WT) vs. 11.4 ± 1.3% (KO), P < 0.05] (Fig. 5C and D). These results indicate that bile acid-induced necrotic and apoptotic cell death are attenuated in CHOP KO mice.

DISCUSSION

Cholestasis is a major cause of liver fibrosis, and hepatocyte death is a key initiator that triggers a hepatic fibrotic response (30). However, the mechanism through which cholestasis causes hepatocyte death is largely unknown. A recent study by Mencin et al. (21) showed that increased susceptibility to cholestasis-induced hepatocyte injury in α-1 antitrypsin Z protein mice is associated with a higher level of ER stress, and bile acid-induced hepatocyte death has also been shown to be accompanied by ER stress (31). These studies suggest the possible involvement of ER stress in cholestasis-induced...
hepatocyte injury, but the causative role of ER stress has yet to be shown clearly.

In the present study, we used mice deficient for CHOP, an essential factor in the ER stress pathway, to investigate the role of CHOP and ER stress in cholestasis-mediated hepatocyte injury and subsequent liver fibrosis. In response to ER stress, three independent factors (CHOP, JNK, and caspase-12) are known to be activated (15). We confirmed that BDL induces these factors in WT mice and that CHOP-mediated pathway is specifically blocked in CHOP KO mice. Our results show that development of liver fibrosis after BDL was attenuated in CHOP-deficient mice. Decreased induction of TGF-β1, a particularly potent profibrogenic cytokine, may be a key factor in alleviation of the fibrogenic response. Immunoreactivity for α-SMA, an indicator for HSC activation, was also inhibited in CHOP-deficient mice, suggesting that inhibition of TGF-β1 induction results in reduced activation of HSCs, which leads to reduced collagen deposition.

Table 2. Serum transaminase and total bilirubin levels after BDL.

<table>
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<tr>
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<th>AST (U/l)</th>
<th>ALT (U/l)</th>
<th>T-bil (mg/dl)</th>
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<tr>
<td>Sham</td>
<td></td>
<td></td>
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<tr>
<td>Day 2</td>
<td>56.3±13.5</td>
<td>33.8±11.2</td>
<td>0.1±0.0</td>
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<tr>
<td>Day 14</td>
<td>43.0±3.4</td>
<td>13.5±1.7</td>
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<tr>
<td>WT</td>
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<tr>
<td>Day 2</td>
<td>7.756±2.757</td>
<td>3.460±1.407</td>
<td>10.6±1.0</td>
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<tr>
<td>Day 7</td>
<td>547.8±73.6</td>
<td>470.2±49.7</td>
<td>10.7±1.2</td>
</tr>
<tr>
<td>Day 14</td>
<td>1,150±445</td>
<td>674±201</td>
<td>13.6±2.3</td>
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<td>CHOP KO</td>
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<tr>
<td>Day 2</td>
<td>4,479±3,083</td>
<td>2,719±1,734</td>
<td>10.5±1.9</td>
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<tr>
<td>Day 7</td>
<td>256.7±3.8</td>
<td>255.3±8.7</td>
<td>16.2±1.7</td>
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<tr>
<td>Day 14</td>
<td>349±74.8</td>
<td>159±64.1</td>
<td>19.4±1.9</td>
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Data are shown as means ± SE (n = 4). BDL, bile duct ligation; Sham, sham-operated; WT, wild-type; KO, knockout; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-bil, total bilirubin.
TGF-β1 is secreted by Kupffer cells, HSCs, and hepatocytes in the liver, and its secretion is stimulated upon engulfment of dead hepatocytes (2, 5, 35). Hepatocyte death also causes direct activation of HSCs through binding of apoptotic cell-derived DNA to toll-like receptor 9 (33). These findings indicate that hepatocyte death plays a key role in activation of HSCs and development of liver fibrosis. In fact, hepatocyte apoptosis has a direct and causative role in hepatic fibrogenesis since it has been shown that genetically induced hepatocyte apoptosis is sufficient to generate a fibrotic response (3, 4, 30). Therefore, we consider that the reduced TGF-β1 induction in

**Fig. 4.** Glycochenodeoxycholic acid (GCDCA) induces CHOP and GRP78, another ER stress marker, in hepatocytes in vitro. Hepatocytes isolated from mice were cultured in the presence of 400 μM GCDCA for 8 h, and quantitative real-time RT-PCR (A) and Western blot analysis (B) of CHOP were performed. Quantitative real-time RT-PCR analysis of GRP78, Bax, and Bcl-2 expression was also performed (C–E). Data are shown as means ± SE (n = 4).

**Fig. 5.** CHOP deficiency attenuates cleavage of caspase-3 and necrotic cell death caused by GCDCA in cultured hepatocytes. Western blot analysis of cleaved caspase-3 at 8 h after addition of 400 μM GCDCA was performed (A), and it was analyzed densitometrically (B). The cells were stained with Hoechst 33342 and propidium iodide (PI) and then examined by fluorescent microscopy (C) to determine the percentage of PI-positive cells (D). Data are shown as means ± SE (n = 5).
CHOP-deficient mice is, at least in part, attributable to decreased hepatocyte death.

To clarify the causative role of CHOP deficiency in reduced hepatocyte death, we isolated hepatocytes and induced cell death with GCDCDA, a toxic bile acid that accumulates in cholestasis and directly yields hepatocyte death in vitro (28). Both necrosis and apoptosis caused by GCDCDA were inhibited in CHOP-deficient hepatocytes. This result suggests that, in our BDL model, decreased hepatocyte death in CHOP KO mice was the primary reason for attenuation of liver fibrosis, rather than a secondary consequence of other changes in these mice. Our results also indicate that the CHOP-mediated ER stress pathway plays an essential role in bile acid-induced hepatocyte injury. Further studies are necessary to elucidate the mechanism by which CHOP induces hepatocyte death, but Bax is one of the potential targets for CHOP. Bax is located downstream of CHOP and induces apoptosis through its translocation into mitochondria (11, 24). This is consistent with our results showing that CHOP deficiency attenuated the expression of Bax. Bcl-2 was also induced in WT mice in both in vivo and in vitro models. A study has shown that Bcl-2 expression was observed in hepatocytes of BDL rats and that Bcl-2-positive hepatocytes isolated from BDL rats were resistant to induction of apoptosis by GCDCDA (17). The overexpression of Bcl-2 is probably an adaptive phenomenon to resist apoptosis by toxic bile acids.

Interestingly, loss of CHOP inhibited not only apoptosis but also necrosis in both BDL mice and GCDCDA-treated hepatocytes. One might be skeptical of this observation since CHOP is known to be a mediator of ER stress-induced apoptosis but not of necrosis. The recently proposed concept of “necrapoptosis” might account for this paradoxical finding (18, 19). Features of both apoptosis and necrosis frequently coexist in the same tissue and even in a single cell; necrosis develops when rapid onset of the mitochondria permeability transition causes ATP depletion, whereas apoptosis develops when this transition occurs without exhaustion of ATP. A feature commonly observed in cells undergoing apoptosis is so-called secondary necrosis, in which necrotic cell death with breakdown of the plasma membrane permeability barrier occurs as apoptosis progresses. Such secondary necrosis may develop as a consequence of ATP depletion because of mitochondrial failure during apoptosis (19); that is, apoptotic stimuli such as toxic bile acids may cause necrosis depending on the circumstances. Although GCDCDA is known to induce apoptosis in isolated hepatocytes (28, 32), it also causes necrosis at a higher concentration (1). In the BDL model, we observed apoptosis mainly in the acute phase and necrosis in the chronic phase. Bile acids accumulate gradually, and, consequently, their toxicity to hepatocytes becomes more severe over time in this model. Therefore, an increased concentration of toxic bile acids in the later phase may have resulted in necrosis, whereas, in the early phase, the bile acids induced apoptosis. The observation that CHOP deficiency attenuated necrosis and apoptosis implies that both can be triggered by the same stimulus.

In conclusion, our results show that CHOP plays an essential role in hepatocyte injury and subsequent liver fibrosis due to cholestasis. Further understanding of the mechanism of CHOP-mediated hepatocyte death may allow for effective intervention in this pathway for prevention of liver fibrosis induced by cholestasis.

ACKNOWLEDGMENTS

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