INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is now recognized as an important health concern (Angulo, 2002). NAFLD is characterized by hepatic lipid accumulation that starts with steatosis and progresses to non-alcoholic steatohepatitis (NASH) with progressive fibrosis. The histopathological features of NASH include evidence of steatosis, liver cell injury, a mixed inflammatory lobular infiltrate, and variable degrees of fibrosis (Ludwig et al., 1980). The pathogenic mechanism of steatosis is related to fatty acid metabolism in the liver (Donnelly et al., 2005). In the pathogenesis of NASH, a “two hit” theory has been proposed. Fat accumulation in the liver is the “first hit”. Fat makes the liver vulnerable to endotoxins and ischemic reperfusion damage, impairs liver regeneration (Uesugi et al., 2001) and causes hepatic insulin resistance (Kim et al., 2001). Acyl-CoA: diacylglycerol acyltransferase 2 (DGAT2) is the final step and rate limiting reaction in triglyceride synthesis (Choi et al., 2007; Yu et al., 2005; Wang et al., 2010). Reactive oxygen species (ROS) together with tumor necrosis factor (TNF) α and monocyte chemoattractant protein (MCP) -1 represent the “second hit” (Jou et al., 2008). Fatty acids can deliver both hits. In general, chronic liver injury can be improved by diet and exercise, but fundamental medications for such liver injury have not been established.

Estrogen binds estrogen receptor and contributes to
downstream biological reactions. Estrogen receptor exists as two subtypes, estrogen receptor α and β. In the liver, estrogen receptor α is the principally expressed form, and estrogen receptor β is expressed at only low levels (Kuiper et al., 1997). Estrogen-treated mice are reportedly protected from the injurious effects of hepatic ischemia and reperfusion, and estrogen protected mice from diethylnitrosamine-induced hepatocarcinogenesis (Harada et al., 2004; Naugler et al., 2007). These results suggest that estrogen plays a protective role in the pathology of hepatotoxicity. However, there has been little information concerning the potential functional role of estrogen after the onset of hepatotoxicity in steatosis and NASH.

Tamoxifen (TAM) is a selective estrogen receptor modulator (SERM) that can act as either an estrogen agonist or an estrogen antagonist, depending on the tissue (Dhingra, 1999). TAM acts as an antagonist in the mammary gland, but acts as an agonist in the uterus, bone tissue, and liver (Osborne and Fuqua, 1994; Mitlak and Cohen, 1997; Cosman and Lindsay, 1999). We previously reported that TAM plays a protective role against drug-induced and chemical-induced acute liver injuries (Yoshikawa et al., 2012). However, the effects of TAM on chronic liver injury, including steatosis and NASH, remain to be addressed.

In this study, we investigated whether TAM plays a protective role in mouse models of steatosis and NASH. Furthermore, we elucidated the factors that attenuate liver injury by administration of TAM.

MATERIALS AND METHODS

Materials

TAM was obtained from Wako Pure Chemical Industries (Osaka, Japan). Primers were commercially synthesized at Hokkaido System Sciences (Hokkaido, Japan). Monoclonal antibodies against anti-Thr202/Tyr204-phosphorylated extracellular signal-regulated kinase (ERK) 1/2, anti-Thr180/Tyr182-phosphorylated p38 mitogen-activated protein kinase (MAPK), and anti-Thr183/Tyr185 phosphorylated c-Jun N-terminal kinase (JNK) 1/2 were purchased from Cell Signaling Technology (Beverly, MA, USA). Monoclonal antibodies against ERK1/2 and JNK1/2 and the polyclonal antibodies against p38 MAPK, eukaryotic initiation factor 2 (eIF2α) and anti-Ser51-phosphorylated eIF2α were also obtained from Cell Signaling Technology. IRDye680-labeled goat anti-rabbit or anti-mouse secondary antibody and Odyssey Blocking Buffer were from Li-COR Biosciences (Lincoln, NE, USA). All other reagents were of the highest grade commercially available.

Animal treatments

Female ICR (7 weeks old, 27-29 g) were obtained from SLC Japan (Shizuoka, Japan). Animals were housed in a controlled environment (temperature 25 ± 1°C, humidity 50 ± 10%, and 12-hr light/12-hr dark cycle) in the institutional animal facility with access to food and water ad libitum. Mice were fed a normal diet, high-fat-diet (HFD) or methionine and choline deficient diet (MCDD) for 10 weeks. After 9 weeks of diet, the mice were divided into 2 groups. TAM (1 mg/kg) dissolved in saline was intraperitoneally administered for 5 consecutive days. We previously reported that administration of TAM for 5 days demonstrated hepatoprotective effects against chemical-induced acute liver injuries (Yoshikawa et al., 2012). Twelve hours after the final administration of TAM, the mice were sacrificed. The liver was fixed in buffered neutral 10% formalin and used for immunohistochemical staining. The degree of liver injury was assessed by hematoxylin-eosin (H&E) staining and the plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined using Fuji DRI-CHEM 4000V (Fuji Film Med. Co., Tokyo, Japan). Animal maintenance and treatment were conducted in accordance with the National Institutes of Health Guide for Animal Welfare of Japan, as approved by the Institutional Animal Care and Use Committee of Kanazawa University, Japan.

GSH level

Livers (50 mg) were homogenized with ice-cold 5% sulfosalicylic acid and centrifuged at 8,000 × g at 4°C for 10 min. The GSH concentration in the supernatant was measured as described previously (Tietze, 1969).

Lipid peroxidation measurement

Lipid peroxidation was measured using Aldelect Lipid Peroxidation Assay kit (Enzo Life Sciences, NY, USA). In brief, for each reaction, 10 μl of probucol and 640 μl of diluted R1 reagent (1:3 of methanol: N-methyl-2-phenylindole) were added to 10 mg of liver homogenate and mixed with 150 μl of 12 M HCl. Each reaction was incubated at 45°C for 60 min and centrifuged at 10,000 × g for 10 min. The supernatant was used to measure malondialdehyde (MDA) formation at 586 nm.

Real-time reverse transcription (RT)-PCR

RNA from mouse liver was isolated using RNAiso (Takara Bio, Shiga, Japan) according to the manufacturer’s instructions. Carnitine palmitoyl transferase-1 (CPT-1), acyl-CoA: diacylglycerol acyltransferase 2 (DGAT2), fatty acid synthase (FASN), monocYTE chemoattractant protein (MCP) -1, sterol regulatory element-binding protein-1
(SREBP-1), tumor necrosis factor (TNF) α, and glyceraldehyde-3-phosphate dehydrogenase (Gapdh) were quantified by real-time RT-PCR. The primer sequences used in this study are shown in Table 1. The reverse transcription process, total RNA (4 μg) and 150 ng random hexamer were mixed and incubated at 70°C for 10 min. The RNA solution was added to a reaction mixture containing 100 units of ReverTra Ace, reaction buffer and 0.5 mM dNTPs in a final volume of 40 μl. The reaction mixture was incubated at 30°C for 10 min, 42°C for 1 hr and heated at 98°C for 10 min to inactivate the enzyme. The real-time RT-PCR was performed using the Mx3000P real-time PCR system (Stratagene, La Jolla, CA, USA). The PCR mixture contained 1 μl of template cDNA, SYBR Premix Ex Taq solution and 8 pmol forward and reverse primers. Amplified products were monitored directly by measuring the increase of the dye intensity of the SYBR Green I (Molecular Probes, Eugene, OR, USA) that binds to the double-strand DNA amplified by PCR.

Immunoblot analysis
SDS-polyacrylamide gel electrophoresis and immunoblot analysis were performed. Mouse liver homogenates (50 μg) were separated on 10% polyacrylamide gels and electrotransferred onto polyvinylidene difluoride membranes, Immobilon-P (Millipore Corporation, Billerica, MA, USA). The membranes were probed with the monoclonal antibodies against anti-Thr202/Tyr204-phosphorylated ERK1/2, anti-Thr180/Tyr182-phosphorylated p38 MAPK, and anti-Thr183/Tyr185-phosphorylated JNK1/2, rabbit anti-human GAPDH antibodies, and mouse anti-KDEL antibodies and incubated with IRDye680-labeled goat anti-rabbit or anti-mouse IgG secondary antibody diluted with PBST. The Odyssey Infrared Imaging system (Li-COR Biosciences, Lincoln, NE, USA) was used for the detection. The relative expression levels were quantified using ImageQuant TL Image Analysis software (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

Statistical analysis
Data are presented as the mean ± S.D. Comparisons of 2 groups were made with an unpaired, two-tailed Student’s t-test. Comparisons of multiple groups were made with ANOVA followed by Dunnett or Turkey test. A value of P < 0.05 was considered statistically significant.

RESULTS
Effects of TAM on hepatic injury in steatosis or NASH model mice
Mice were fed HFD or MCDD for 10 weeks. The plasma ALT and AST levels in steatosis and NASH mice models were significantly increased compared with CTL mice. Treatment with TAM (1 mg/kg, i.p., 5 days) resulted in significantly decreased ALT and AST levels in both mouse models compared with TAM-unadministered CTL mice (Fig. 1A). Hepatocytes in the HFD-fed mice exhibited different sizes of lipid droplets and inflammation.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>CPT-1</td>
<td>FP</td>
<td>5'-GCA TAC CAA AGT GGA CCC CT-3'</td>
</tr>
<tr>
<td></td>
<td>RP</td>
<td>5'-TGC TCT GCA AAC ATC CAG CC-3'</td>
</tr>
<tr>
<td>DGAT2</td>
<td>FP</td>
<td>5'-CAT GAA GAC CCT CAT CGC CG-3'</td>
</tr>
<tr>
<td></td>
<td>RP</td>
<td>5'-GTG ACA GAG AAG ATG TCT TGG-3'</td>
</tr>
<tr>
<td>FASN</td>
<td>FP</td>
<td>5'-GCT CCT CGC TTG TCG TCT G-3'</td>
</tr>
<tr>
<td></td>
<td>RP</td>
<td>5'-GAT CCT TCA GCT TTC CAG AC-3'</td>
</tr>
<tr>
<td>MCP-1</td>
<td>FP</td>
<td>5'-TGT CAT GCT TCT GGG CCT G-3'</td>
</tr>
<tr>
<td></td>
<td>RP</td>
<td>5'-CCT CTC TCT TGA GCT TGG TG-3'</td>
</tr>
<tr>
<td>SREBP-1</td>
<td>FP</td>
<td>5'-GAA CAG ACA CTG GCC GAG ATG-3'</td>
</tr>
<tr>
<td></td>
<td>RP</td>
<td>5'-AGG AGG CCA GAG AAG CAGAAG-3'</td>
</tr>
<tr>
<td>TNF-α</td>
<td>FP</td>
<td>5'-TGT CTC AGC CTC TCT TCA TCC C-3'</td>
</tr>
<tr>
<td></td>
<td>RP</td>
<td>5'-TGA GGG TCT GGG CCA TAG AAC-3'</td>
</tr>
<tr>
<td>Gapdh</td>
<td>FP</td>
<td>5'-AAA TGG GGT GAG GCC GGT-3'</td>
</tr>
<tr>
<td></td>
<td>RP</td>
<td>5'-ATT GCT GAC AAT CTT GAG TGA-3'</td>
</tr>
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</table>

Table 1. Sequences of primers used for real-time RT-PCR analysis in this study

FP, forward primer; RP, reverse primer.
livers of mice fed MCDD exhibited macrovesicular steatosis, ballooning degeneration of hepatocytes, and lobular inflammation (Fig. 1B). No effect of TAM on CTL mice was observed (data not shown). Thus, feeding with HFD and MCDD for 10 weeks induced steatosis and NASH in mice. TAM-administration decreased the accumulated fat and inflammation in the livers of both mouse models. The attenuation of hepatic steatosis resulted in decreased
hepatic inflammation in TAM-administered mice.

**Effects of TAM on oxidative stress in the livers of steatosis or NASH model mice**

Glutathione (GSH) detoxifies ROS produced in the mitochondrial electron transport chain (Okabe et al., 1994). The ratios of GSH/glutathione disulfide (GSSG) and MDA are often used as markers of cellular toxicity and oxidative stress and appear to increase during NAFLD progression (Caballero et al., 2010). To investigate whether the oxidative stress was modulated by TAM, GSH/GSSG ratio and MDA levels in the liver were measured (Fig. 2). The ratios of GSH/GSSG in the steatosis and NASH model mice were significantly decreased compared with CTL mice, but TAM treatment produced no effect on the ratio of GSH/GSSG in the livers of the mice. MDA levels were significantly increased in steatosis and NASH model mice compared with CTL mice, but TAM had no effect on MDA (Fig. 2). These results suggest that administration of TAM is not likely to affect oxidative stress.

**Effects of TAM on hepatic mRNA expression of SREBP-1, FASN, CPT-1 and DGAT2 in steatosis or NASH model mice**

The expression of genes involved in fatty acid metabolism in the liver was investigated. The expression of SREBP-1, a transcription factor that activates genes involved in lipogenesis, was unchanged in the steatosis and NASH model mice. The administration of TAM did not affect the expression of SREBP-1 in either mouse models. The expressions of FASN, an enzyme involved in fatty acid synthesis, was significantly decreased in the steatosis and NASH model mice compared with the CTL mice. In CTL mice, the expression of FASN was significantly increased by TAM, but TAM did not alter FASN expression in the steatosis and NASH model mice compared with the TAM-unadministered mice. In the steatosis model mice, the expression of CPT-1, a rate-limiting regulator of mitochondrial β-oxidation and mitochondrial fatty acid import, was significantly increased in the steatosis model mice compared with the CTL mice. The administration of TAM significantly decreased CPT-1 compared with the TAM-unadministered steatosis model mice. In the NASH model mice, the expression of CPT-1 was significantly decreased compared with the CTL mice. However, the administration of TAM did not affect the expression of CPT-1 compared with TAM-unadministered NASH model mice (Fig. 3). The expression of DGAT2, a rate limiting enzyme in triglyceride synthesis, was significantly decreased in CTL and both mouse models by TAM. These results suggest that TAM may be involved in decreasing the hepatic fat accumulation in both mouse models, and may inhibit β-oxidation of fatty acid in the steatosis model mice.

![Fig. 2](image-url)  
**Fig. 2.** Effects of tamoxifen (TAM) on the ratios of glutathione (GSH)/glutathione disulfide (GSSG) and on oxidative stress in steatosis or non-alcoholic steatohepatitis (NASH) model mice. The ratios of GSH/GSSG and the malondialdehyde (MDA) levels were measured 12 hr after the last administration of TAM. Data are shown as the mean ± S.D. of results from 4-5 mice. Differences compared to the control (CTL) mice were considered significant at *p < 0.05, **p < 0.01 and ***p < 0.001.
Effects of TAM on hepatic mRNA expression of TNFα and MCP-1 in steatosis or NASH model mice

The proinflammatory cytokine TNFα and the chemokine MCP-1 are known to play an important role in the development and progression of hepatic inflammation (Anstee and Goldin, 2006). To investigate whether TAM affected the production of inflammatory cytokines and chemokines, hepatic mRNA of TNFα and MCP-1 was measured. In our previous study (Higuchi et al.,...
we confirmed that the expression levels of mRNA and proteins were similar for cytokines and chemokines. Thus, changes in mRNA levels were tracked in the present study. As shown in Fig. 4, the expression of TNFα and MCP-1 were significantly increased in the steatosis and NASH model mice compared with the CTL mice. Treatment with TAM significantly decreased TNFα and MCP-1 compared with TAM-unadministered mice (Fig. 5). These results suggested that the attenuation of liver injury by the administration of TAM is related to the decreased expression of inflammatory factors.

**Effects of TAM on MAPK signaling pathways in steatosis or NASH model mice**

The phosphorylation of MAPK is a major component of many intracellular signaling pathways. To clarify the MAP kinase activation status, the phosphorylation of ERK1/2 (44/42 kDa), p38 MAP kinase (43 kDa), and JNK1/2 (46/54 kDa) in liver homogenates was assessed by immunoblot analysis. No significant alteration was observed in the phosphorylation of ERK in either mouse models compared to the CTL mice. Treatment with TAM resulted in a significant increase in the ERK phosphorylation in the CTL mice and both mouse models compared with the TAM-unadministered mice. However, no change was observed in the phosphorylation of p38 in either mouse models compared with the CTL mice. In addition, treatment with TAM did not affect the phosphorylation of p38. The steatosis and NASH model mice exhibited significant increases in JNK phosphorylation compared to CTL mice. Treatment with TAM produced a significant decrease in the JNK phosphorylation in the NASH model mice compared with the TAM-unadministered mice (Fig. 5). These results suggest that the attenuation of liver injury in both mouse models by treatment with TAM is related to MAPK and ERK phosphorylation in particular.

**Effects of TAM on ER stress in the liver of steatosis and NASH model mice**

ER stress is caused by the accumulation of unfolded and misfolded proteins in the ER lumen and is associated with hepatic steatosis (Harding et al., 2000). To investigate the mechanism of the protective effects of TAM in the mouse models, we analyzed ER stress and signaling pathways. The levels of Bip, an ER chaperone and a stress sensor protein, were increased in the liver of the steatosis and NASH model mice compared with the CTL mice. Treatment with TAM significantly decreased the
expression of Bip in the steatosis model mice but not in the NASH model mice compared with the CTL mice. In addition, the levels of phosphorylated eIF2α were increased in the liver of the steatosis and NASH model mice compared with the CTL mice, whereas treatment with TAM resulted in significantly decreased eIF2α phosphorylation compared with the TAM-unadministered mice (Fig. 6). These results suggest that the attenuation of liver injury by treatment with TAM is associated with ER stress signaling.

DISCUSSION

Steatosis and NASH are now recognized as important health concerns, and their incidences have been rising. NASH develops in patients with metabolic syndrome, and can be simulated by chronic chemical intoxication as well as by the MCDD model in experimental animals. Furthermore, it is postulated that both lipid peroxidation and ROS induce the release of TNFα and MCP-1, which may contribute to NASH (Pessayre et al., 2004). In general, the HFD feeding induces hepatic steatosis without progression to steatohepatitis in rodents (Vetelainen et al., 2007). Estrogen has been shown to protect against HFD-induced steatosis and is suggested to play a protective role in liver injury (Riant et al., 2009; Chow et al., 2011). TAM is a SERM that can act as an estrogen agonist in the liver (Oborne and Fuqua, 1994). We previously reported that TAM has a hepatoprotective effects against various drug-induced and chemical-induced acute liver injuries (Yoshikawa et al., 2012). However, there is little information about the effects of TAM on steatosis and NASH. In this study, we created steatosis and NASH model mice and investigated the effects of TAM after the onset of these liver diseases. TAM decreased the plasma ALT and AST levels in both mouse models. In addition, histopathological analysis demonstrated that TAM decreased the accumulation of fat and inflammation in the livers in both mouse models. These results indicate that TAM attenuated the liver injury in the steatosis and NASH model mice.

ROS promote oxidative damage and contribute to tissue destruction in a wide variety of diseases (Te et al.,...
The ratios of GSH/GSSG and MDA levels are often used as a measurement of cellular toxicity and oxidative stress and appear to be increased in the progression of NAFLD (Caballero et al., 2010; Chowdhry et al., 2010). In the present study, the ratios of GSH/GSSG in the steatosis and NASH model mice were significantly decreased compared with CTL mice. In addition, MDA was significantly increased in both mouse models, but TAM had no effects on the ratio of GSH/GSSG or on MDA levels. These results indicated that TAM may not be involved in the oxidative stress, suggesting that another pathway may play a role in the attenuation of the liver injury in the steatosis and NASH model mice.

Fat accumulation in the liver can be recognized as the “first hit” in the pathogenesis of NASH. In addition, the fatty acid increase by the administration of TAM is considered one of the causes of steatosis (Cole et al., 2010). In this study, the administration of TAM in the steatosis and NASH model mice did not change FASN and SREBP-1 levels, which are involved in fatty acid synthesis. However, administration of TAM decreased DGAT2 expression in CTL mice and both mouse models. DGAT2 is the rate-limiting enzyme catalyzing the final step in triglyceride synthesis and exerts an important role in the development of fatty liver diseases (Choi et al., 2007; Yu et al., 2005; Wang et al., 2010). Thus, these results suggested that TAM did not increase but instead decreased the fat accumulation after the onset of steatosis and NASH.

Fatty acid β-oxidation occurs in both mitochondria and peroxisomes, where oxygen is available to enable the formation of ROS. In NASH, the fatty acid overload plays an important role in ROS generation as a result of electron leakage during mitochondrial β-oxidation in energy production (Haque et al., 2010). Levels of CPT-1, a rate-limiting regulator of mitochondrial β-oxidation through its role in mitochondrial fatty acid import, were increased in the steatosis model mice, whereas administration of TAM significantly decreased CPT-1 levels compared to the TAM-unadministered mice. These results suggested that TAM may be related to the suppressed β-oxidation of fatty acid in the steatosis model mice.

Oxidative stress leads to inflammatory responses, including TNFα and MCP-1 (Hotamisligil, 2010). In addition, these proinflammatory cytokines and chemokines are associated with the development and progression of hepatic inflammation (Anstee and Goldin, 2006). The expressions of TNFα and MCP-1 were increased in the steatosis and NASH model mice. Treatment with TAM significantly decreased TNFα and MCP-1 in both mouse models compared with the TAM-unadministered mice.
It has been reported that estrogen attenuates the expressions of TNFα and MCP-1 via the estrogen receptor in female mice (Huang et al., 2008). Thus, these results indicated that TAM would exert a hepatoprotective effect against inflammation in the steatosis and NASH model mice.

The phosphorylation of MAPK, which is required for enzyme activity, activates signaling cascades, the downstream effects of which have been linked to the regulation of cellular apoptosis, an inflammatory response (Seger and Krebs, 1995). The activation of these regulatory MAPKs such as JNK and p38, through phosphorylation, usually leads to cell death and inflammation (Martindale and Holbrook, 2002; Ura et al., 2001). In contrast, MAPK family members such as ERK exert protective and anti-apoptotic effects upon activation by an upstream kinase and regulate a number of major cellular functions, such as cell proliferation, differentiation and inflammation (Czaja et al., 2003). Furthermore, ERK activation is reported to decrease DGAT2 expression (Wang et al., 2010; Tsai et al., 2007). Increased JNK phosphorylation has been reported in steatosis and NASH model mice (Hirosumi et al., 2002). Furthermore, estrogen reportedly increases ERK phosphorylation (Alvaro et al., 2002). In this study, we demonstrated that TAM increased in the extent of ERK phosphorylation, which may lead to increased regeneration of the liver and reduced fat accumulation and inflammation by decreasing the expression of DGAT2, TNFα and MCP-1 in steatosis and NASH. In addition, treatment with TAM decreased JNK phosphorylation in the NASH model mice. These results suggested that ERK activation by TAM is required for the attenuation of hepatotoxicity in the steatosis and NASH model mice, and inhibition of JNK activation resulted in the attenuation of inflammation and apoptosis in NASH model mice.

It has been suggested that endoplasmic reticulum (ER) stress is associated with steatosis and NASH (Puri et al., 2008; Maniratanachota et al., 2005). Furthermore, apoptosis and inflammation are key features in the progression of NASH and are both linked to ER stress (Malhi and Kaufman, 2011). The accumulation of misfolded proteins in the lumen of the ER and fat accumulation activate several signaling cascades. The initial outcomes of ER signaling are the transcription of ER chaperones, such as Bip, and a reduction in protein translation, which diminishes the stress in the lumen of the ER. eIF2α expression leads to an increase in the activation of the CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP), which activates proapoptotic pathways (Ohoka et al., 2005). In this study, the expression of Bip was increased by ER stress in both mouse models, whereas treatment with TAM decreased the expression of Bip in the steatosis model mice. In addition, TAM decreased eIF2α phosphorylation in both mouse models. The decrease in fat accumulation in the liver by the administration of TAM is thought to diminish ER stress, including decreasing Bip and eIF2α, leading to attenuation of the liver injury (Fig. 7).

In this study, we revealed that TAM has a protective effect on the liver injury of the steatosis and NASH model mice. However, in clinical practice, there have been reports that patients with breast cancer developed NASH upon the administration of TAM (Murata et al., 2000). The causes were associated with obesity, but the detailed mechanism has not been elucidated. In addition, fatty acid increase upon the administration of TAM is considered one of the causes of steatosis (Chowdhry et al., 2010). However, in this study, the administration of TAM after the onset of steatosis and NASH model mice did not increase fatty acid synthesis, but did decrease fat accumulation (Figs. 1, 3). Thus, further investigation is required to determine why the effects of TAM differ under each condition between mice and humans.

NAFLD is more common in male than female in human, which implicates estrogens as potentially protective, or indicates that androgens may aggravate NASH (Loomba et al., 2009). We previously reported that TAM had hepatoprotective effects against chemical-induced acute liver injuries in female mice but not in male mice, suggesting that TAM may not affect steatosis and NASH.
Effect of tamoxifen on steatosis and NASH model mice

model in male mice. Therefore, female mice was investigated in this study.

In conclusion, our findings clearly demonstrated that the hepatoprotective effects of TAM against steatosis and NASH are mediated through the inhibition of inflammation. Furthermore, TAM increased the activation of ERK1/2, leading to a decrease in the accumulated fat, increase in anti-apoptosis and regeneration in liver. The present study provides new information concerning the potential therapeutic effects of TAM on steatosis and NASH via activation of ERK1/2.

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