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Melatonin ameliorates ER stress-mediated hepatic steatosis through miR-23a in the liver

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ABSTRACT

The endoplasmic reticulum (ER) stress induces hepatic steatosis and inflammation in the liver. Although melatonin ameliorates ER stress-target genes, it remains unknown whether melatonin protects against hepatic steatosis as well as inflammation through regulation of miRNA. MicroRNAs have been identified as pivotal regulators in the field of gene regulation and their dysfunctions are a common feature in a variety of metabolic diseases. Especially, among miRNAs, miR-23a has been shown to regulate ER stress. Herein, we investigated the crucial roles of melatonin in hepatic steatosis and inflammation *in vivo*. Tunicamycin challenge caused increase of hepatic triglyceride and intracellular calcium levels through activation of ER stress, whereas these phenomena were partially disrupted by melatonin. We also demonstrated that expression of miR-23a stimulated with tunicamycin was rescued by melatonin treatment, resulting in reduced ER stress in primary hepatocytes. Overall, these results suggest a new function of melatonin that is involved in ameliorating ER stress-induced hepatic steatosis and inflammation by attenuating miR-23a. Melatonin may be useful as a pharmacological agent to protect against hepatic metabolic diseases due to its ability to regulate expression of miR-23a.

1. Introduction

Endoplasmic reticulum (ER) stress is involved in protein folding, calcium storage, and biosynthesis of macromolecules. Activation of the unfolded protein response (UPR) is triggered by ER stress alleviates ER stress by a feedback regulation and it promotes cell survival [1,2]. In particular, increasing evidence suggests that hepatic ER stress increases in several animal models of non-alcoholic fatty liver disease and in patients with diabetes [3–5]. ER stress has been suggested to induce hepatic triglyceride (TG) accumulation through X-box-binding protein (XBP)-1. The ER also plays an important role in regulating calcium signaling, vesicle trafficking, drug metabolism, and lipid biogenesis [6,7]. Upon ER stress, increased cytosolic calcium (Ca^{2+}) is observed owing to release of Ca^{2+} from the ER lumen in cells [8]. Also, ER-induced UPR has important roles related to increasing inflammation via production of reactive oxygen species (ROS) *in vitro*.

MicroRNAs (miRNAs) are small ~22 nt noncoding RNAs that control gene expression on the post-transcriptional level by down-regulating protein translation and/or degradation [9,10]. miRNAs are known to play a crucial role in regulating cellular functions and metabolic dysfunctions, such as cell death, apoptosis, differentiation, cancer, cardiac hypertrophy, and alcoholic liver disease [10,11]. Recently, miR-23a, a member of the miR-23a~27a~24-2 cluster, was found to act as a key regulator of ER homeostasis and pivotal player in ER-dependent signaling [10,12]. Moreover, it was further

Abbreviations: ACC1, Acetyl-coenzyme A carboxylase; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; Ca^{2+}, Calcium; CHOP, C/EBP homolog protein; ER stress, Endoplasmic reticulum stress; FAS, Fatty acid synthase; Gadd34, Growth arrested and DNA damage-inducible protein 34; Grp78, Glucose regulated protein 78; HDL, High-density lipoprotein; H&E staining, Hematoxylin and eosin (H&E) staining; IRE1α, Inositol-requiring kinase 1α; LDL, Low-density lipoprotein; Mela, Melatonin; p-eIF2α, Phosphor-eukaryotic translational initiation factor α; qPCR, Quantitative polymerase chain reaction; RED, Hydroxymethylglutaryl-CoA reductase; RT, Reverse transcription; SREBP-1, Sterol regulatory element-binding protein-1; TC, Total cholesterol; TG, Triglyceride; TNF-α, Tumor necrosis factor-α; Tuni, Tunicamycin; XBP-1, X-box-binding protein-1.

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established by the increase in cytosolic Ca^{2+} level after overexpression of this cluster [13]. However, a link between miR-23a in response to ER stress and UPR-mediated execution of hepatic steatosis and inflammation in an animal model has not been explored yet.

Melatonin is a molecular found in animals, plants, and microbes [14,15]. Biological actions of melatonin are both receptor-mediated and receptor-independent actions. Also, melatonin easily penetrates cellular membranes [16]. The melatonin receptor influences cell signaling through several second messengers such as cAMP, inositol triphosphate, diacylglycerol, and intracellular Ca^{2+} concentrations. To date, melatonin has been shown to reverse tunicamycin-stimulated ER stress in hepatocellular carcinoma cells to improve cytotoxicity of the drug, resulting in delayed ER stress-mediated cell death [17]. Also, melatonin acts as a potent direct antioxidant [18], and elevates intracellular Ca^{2+} through an inositol 1,4,5-triphosphate independent pathway in human platelets [19]. Although the molecular mechanisms of melatonin are unclear, they may involve at least two parallel transduction pathways; one inhibiting adenylyl cyclase and the other regulating phospholipid metabolism and Ca^{2+} pathway. Despite these findings, it is not clearly understood how melatonin contributes to ER stress-mediated hepatic steatosis and inflammation.

In this study, we revealed that melatonin ameliorates tunicamycin-induced hepatic steatosis and inflammation by attenuating miR-23a in mouse primary hepatocytes and the mouse liver cell line.

2. Materials and methods

2.1. Chemicals

Tunicamycin and melatonin (Sigma–Aldrich, St. Louis, MO, USA) were purchased and dissolved in the recommended solvents.

2.2. Animals

Male, 8-week-old C57BL/6 mice (Jung-Ang Experimental Animals, Seoul, Republic of Korea) were used in the experiment. Melatonin was preadministered interperitoneally to fed mice at 50 mg/kg body weight twice per day for 2 days, and administered interperitoneally with tunicamycin (1 mg/kg) for 24 h. Then, the mice were injected with melatonin for 2 additional days after the tunicamycin treatment. After the treatments, the mice were sacrified, and their livers were collected. All animal experiments were performed in accordance with the rules and regulations of the Institutional Animal Use and Care Committee of Keimyung University School of Medicine.

2.3. Metabolic parameters

Serum was collected from mice and biochemical analyses were performed (Model AU-480; Beckman Coulter, Fullerton, CA, USA) respectively.

2.4. Measurements of hepatic TGs and cholesterol

Hepatic lipids were measured by a modification of a method described previously [20]. Briefly, 100 mg of liver sample was homogenized in 1 ml PBS with a polytron homogenizer at 4 °C and processed immediately to measure TGs and cholesterol. Total lipids were extracted overnight at 4 °C in chloroform-methanol (2:1), dried on a shaker, and stored at −20 °C until lipid measurement. The dried lipid extracts were resuspended in 200 μl of ethanol, and cholesterol and TGs were measured using commercially available kits (Thermo Fisher Scientific, Alexandria, VA, USA). These hepatic lipid data were normalized by liver weight.

2.5. Histological analysis

Liver tissues were fixed in 4% paraformaldehyde and subsequently embedded in paraffin (4–5 μm sections) for hematoxylin and eosin (H & E) staining following standard procedures [3].

2.6. Isolation and culture of primary mouse hepatocytes

Mouse primary hepatocytes were isolated from the livers of 8-week-old male mice. The hepatocytes were used for quantitative polymerase chain reaction (qPCR) and immunoblot analyses. The hepatocyte isolation method was described previously [21].

2.7. Cell culture

AML-12 immortalized mouse hepatocytes were cultured in DMEM/F-12 medium (Gibco-Brl) supplemented with 10% FBS, insulin-transferrin-selenium (Gibco-Brl), dexamethasone (40 ng/ml; Sigma–Aldrich), and antibiotics in a humidified atmosphere containing 5% CO_2 at 37 °C.

2.8. RNA isolation and analysis

Total RNA was isolated from primary hepatocytes and livers of mice using the Trizol method (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized using a SuperScript III First-strand cDNA synthesis kit (Invitrogen) and used for qPCR with a iQ™ SYBR® Green supermix (Bio-Rad, Hercules, CA, USA). mRNA levels were normalized for ribosomal protein L32 gene expression. All qPCR reactions were repeated in triplicate. The qPCR reactions showed simple melting curves indicative of a single amplified product. The following primer sets were used: mouse inositol-requiring kinase 1a (IRE1α): forward, 5′-AACTCCTCTGTCTGCATCC-3′; reverse, 5′-GCCAACTATGTTGATAACTTCC-3′; mouse C/EBP homolog protein (CHOP): forward, 5′-CAGTCATGGCAGCTGAGTCC-3′; reverse, 5′-TAGGTGCCCCCAATTTCATC-3′; mouse glucose regulated protein (Grp) 78: forward, 5′-GAAAGGATGGTTAATGATGCTGAG-3′; reverse, 5′-GTCTTCAATGTCCGCATCCTG-3′; mouse Gadd34: forward, 5′-GACCCCTCCAACTCTCCTTC-3′; reverse, 5′-CTTCCTCAGCCTCAGCATTC-3′; mouse sterol regulatory element-binding protein (SREBP)-1a: forward, 5′-CACAGCGGTTTCGAACG-3′; mouse SREBP-1c: forward, 5′-TGGATTGCACATTTGAAGACAT-3′; mouse SREBP-1 reverse, 5′-GCCAGAGAAGCAGAAGAG-3′; mouse SREBP-2: forward, 5′-ATGGAGAC CCTCACGGA-3′; reverse, 5′-TGCTGTTGTTGCCACTG-3′; mouse fatty acid synthase (FAS): forward, 5′-GCTGCGGAAACTTCAGGAAAT-3′; reverse, 5′-AGAGACGTGTCACTCCTGGACTT-3′; mouse acetyl-coenzyme A carboxylase (ACC) 1: forward, 5′-TGACAGACTGATCGCAGAGAAAG-3′; reverse, 5′-TGGAGAGCCCCACACACA-3′; mouse HMG-CoA Reductase (RED): forward, 5′-CTTGTGGAATGCCTTGTGATTG-3’; reverse, 5′-AGCCGAAGCAGCACATGAT-3’; mouse F4/80: forward, 5′-TGAGATTGTGGAAGCATCCGAG-3′; reverse, 5′-ACAGCAGGAAGGTGGCTATG-3′. mouse tumor necrosis factor (TNF)-α: forward, 5′-CCCTCACACTCAGATCATCTTCT-3′; reverse, 5′-GCTACGACGTGGGCTACAG-3; TaqMan® mmu-miR-23a-3p: 5′-AUCACAUUGCCAGGGAUUUCC-3’; TaqMan® U6: GTGCTCGCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCAAATTCGTGAAGCGTTCCATATTTT; and mouse L32: forward, 5′-ACATTTGCCCTGAATGTGGT-3′; reverse, 5′-ATCCTCTTGCCCTGATCCTT-3′.

2.9. Immunoblotting

Liver extracts and primary hepatocytes lysates were analyzed for immunoblotting as described [21]. Twenty mg of protein extracts were separated on a 6–10% SDS–PAGE column at a constant voltage of 100 V using Bio-Rad blotting system. Proteins were transferred onto nitrocellulose membranes subjected to 340 mA of constant electric current for 1.5 h. Membranes were blocked with 5% non-fat milk and incubated at 4 °C with anti-Grp78, anti-IREα, anti-p-eIF2α, anti-CHOP, anti-XBP-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-β-actin (Sigma–Aldrich, USA), and anti-FAS; (Cell signaling, USA), and then The blots were developed using the Super Signal Kit from Pierce (Amersham Bioscience, Piscataway, NJ, USA).

2.10. Measurement of intracellular calcium

Intracellular Ca^{2+} concentrations were evaluated as previously described [22]. Briefly, mouse primary hepatocytes were seeded on coverslips that formed the base of a 6-well plate and were treated

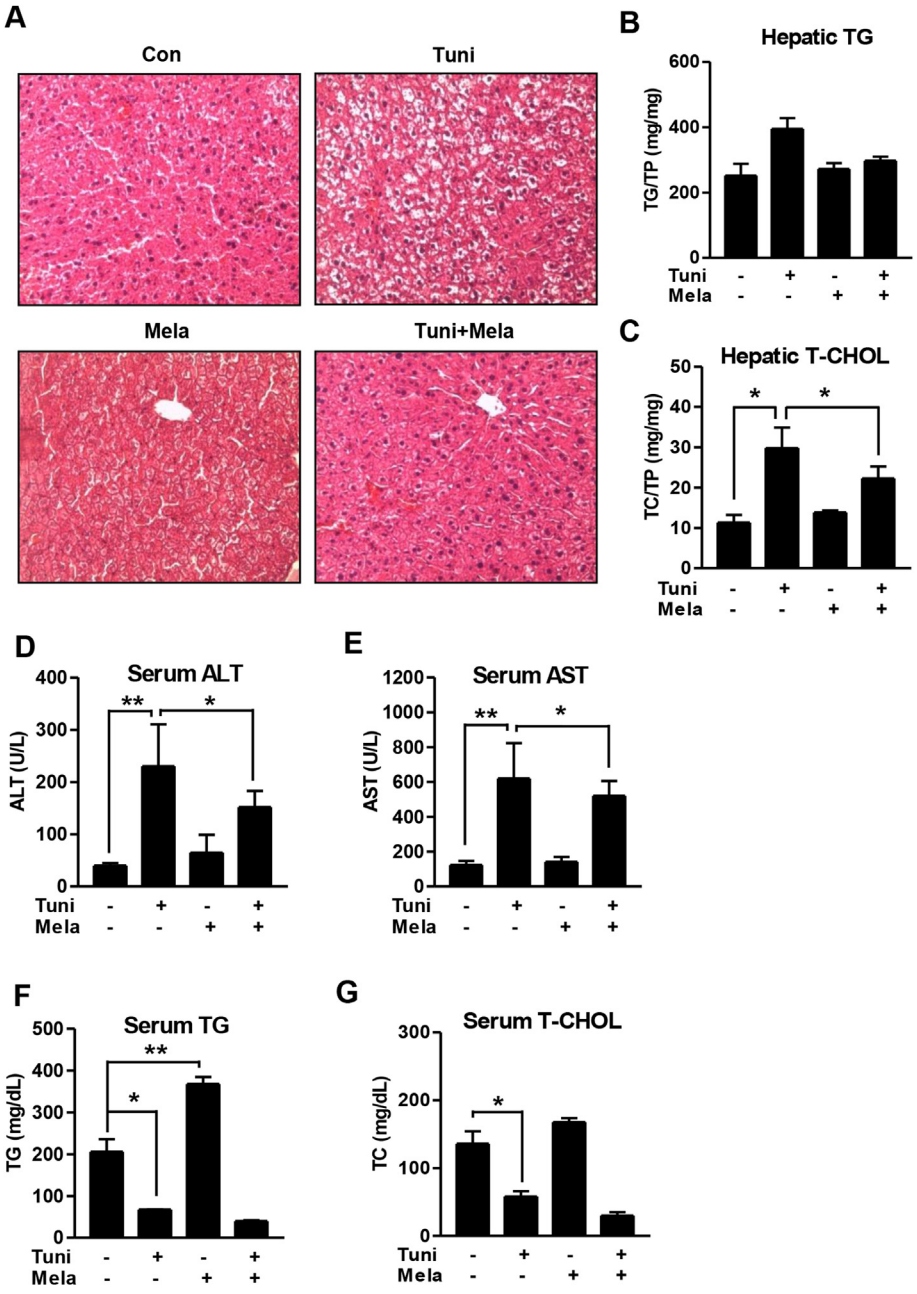

Fig. 1. Melatonin ameliorates tunicamycin-induced lipid homeostasis in the liver and effect of melatonin on tunicamycin-mediated serum lipid parameters. (A) H&E staining of liver. Melatonin (Mela; 50 mg/kg body weight) was pretreated to feed C57BL/6 mice for 2 days, and administered with tunicamycin (Tuni; 1 mg/kg) for 24 h. Then, the mice were continuously injected with melatonin for 2 days after the tunicamycin treatment. Measurement of hepatic triglyceride (TG) (B) and total cholesterol (T-CHOL) (C) concentrations were analyzed from tunicamycin and melatonin stimulated mice. All mice were separated into experimental groups (n = 5 mice per group) $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. untreated control and Tuni-treated mice. (D–G) Analysis of serum lipid parameters in tunicamycin-fed mice with or without melatonin treatment as mentioned in above. Levels of serum ALT, AST, triglycerides (TG), and total cholesterol (T-CHOL) were measured using an automated blood analyzer. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. untreated control or individual-treated mice.

with either tunicamycin or melatonin for 6 h. The cells were washed twice with the Kreb–Ringer bicarbonate buffer to wash out unbound dye in the medium containing the cells. Images were obtained by confocal microscopy (LSM 5 EXCITER, Carl Zeiss, Jena, Germany), and fluorescent signals were measured at 488 and 525 nm excitation and emission wavelengths, respectively.

2.11. Statistical analysis

Results are expressed as mean ± standard deviation. Differences between groups were detected by one-way analysis of variance or a paired Student's *t*-test. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Tunicamycin-induced hepatic lipid profiles are mediated by melatonin

To confirm whether tunicamycin-induced lipid accumulation was altered by melatonin, we evaluated the physiological relevance of melatonin on ER stress-induced metabolic diseases in an animal experiment. In agreement with the histological analysis, tunicamycin-treated mice accumulated lipid and showed the destroyed liver architecture. However, the increased hepatic lipid accumulation was decreased markedly following melatonin treatment (Fig. 1A). Next, the effect of melatonin on tunicamycin-induced hepatic lipid metabolism was evaluated by determining the levels of hepatic TGs and total cholesterol (TC). As shown in Fig. 1B and C, tunicamycin significantly elevated hepatic TG (56%, $p < 0.05$) and TC levels (26%, $p < 0.05$), and this phenomenon was markedly reduced (25% into both TG and TC level, $p < 0.05$) by melatonin. These results demonstrate a novel crucial role of melatonin for regulating tunicamycin-mediated hepatic lipid metabolism *in vivo*.

Next, we determined whether melatonin plays a role in tunicamycin-mediated serum parameters in an *in vivo* experiment. The tunicamycin-treated group showed elevated levels of alanine aminotransferase (ALT, 57%, $p < 0.01$) and aspartate aminotransferase (AST, 5-fold, $p < 0.01$), and the increases of ALT (33%, $p < 0.05$) and AST (16%, $p < 0.05$) were decreased in following melatonin treatment (Fig. 1D, E). Otherwise, levels of TG (67%, $p < 0.05$) and total cholesterol (T-CHOL, 57%, $p < 0.05$), (Fig. 1F, G), were dramatically reduced by Tunicamycin treatment. Overall, our

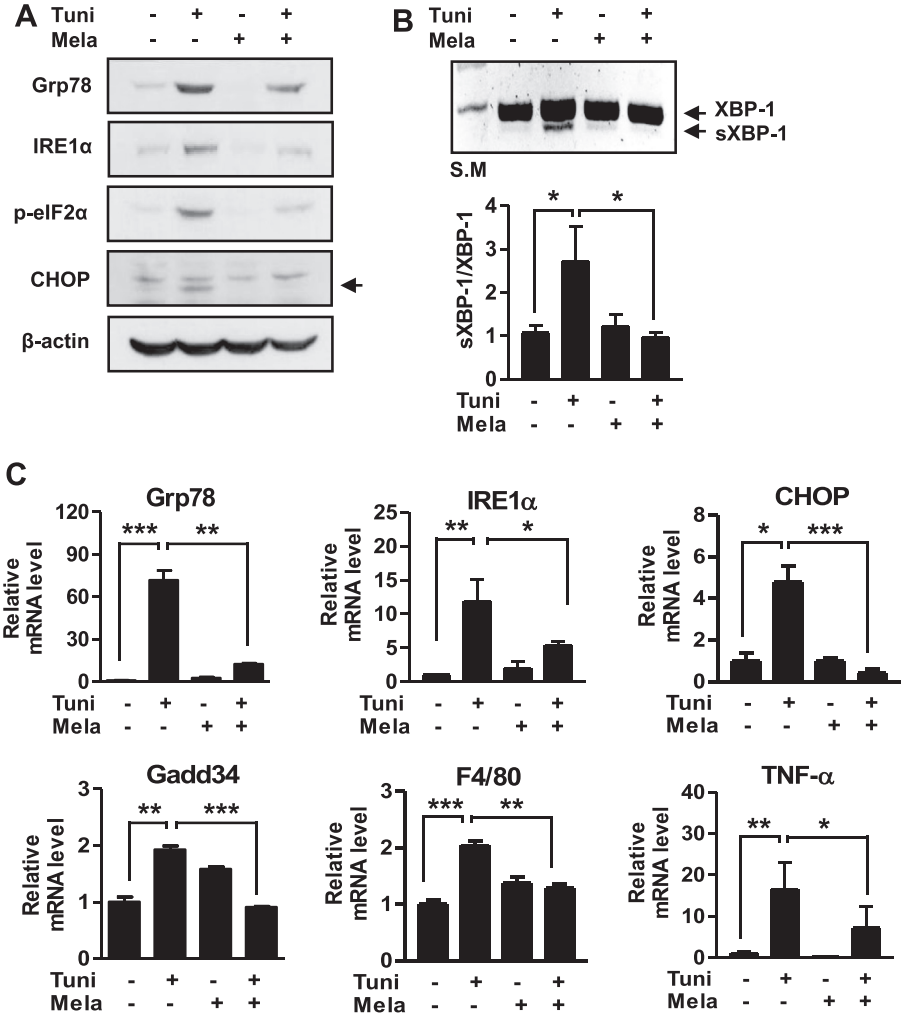

Fig. 2. Melatonin improves tunicamycin-induced ER stress and inflammation in the liver. (A) Effect of Melatonin on ER stress. C57BL/6 mice were injected intraperitoneally with either tunicamycin or melatonin, as described in Fig. 1. Arrow indicates a CHOP protein. (B) Melatonin induces cleavage of XBP-1. Spliced XBP-1 was detected by real-time PCR (upper panel) and PCR band intensity of sXBP-1 normalized to total XBP-1 (lower panel). (C) qPCR analysis for ER stress marker genes and inflammatory genes. All mice were separated into experimental groups (n = 5 mice per group). S.M., size marker. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. untreated control or Tuni-treated mice.

results suggest that melatonin partially plays an important role in improving tunicamycin-induced hepatic diseases.

3.2. ER stress responses and pro-inflammatory markers are altered by melatonin

To elucidate a link between melatonin and tunicamycin-induced hepatic disease, we assessed the role of melatonin in tunicamycin-induced metabolic dysfunction in the liver. The increased mRNA levels of ER stress markers, Grp78, IRE-1α, p-eIF2α, CHOP, and spliced XBP-1 (64%, $p < 0.05$) by tunicamycin were dramatically repressed by melatonin treatment (Fig. 2A, B). To further determine whether melatonin improved tunicamycin-mediated liver injury, we confirmed the mRNA levels of ER markers and inflammation by qPCR analysis. mRNA expressions of both the ER markers including Grp78 (71.8-fold, $p < 0.001$), IRE1α (11.5-fold, $p < 0.01$), CHOP (4.8-fold, $p < 0.05$), Gadd34 (1.9-fold, $p < 0.01$) and inflammatory genes such as F4/80 (2-fold, $p < 0.001$) and TNF-α (16.6-fold, $p < 0.01$) in the tunicamycin-induced metabolic dysfunction group were higher than those expression levels in the control groups, whereas this stimulatory effect of tunicamycin was markedly repressed by melatonin treatment (Fig. 2C). Taken together, these findings show that melatonin improves tunicamycin-stimulated ER stress and liver inflammation.

3.3. Melatonin-mediated inhibitory effect of hepatic lipid accumulation is independent of regulation of de novo lipogenesis

De novo lipogenesis is a major pathway of several mechanisms to induce development of hepatic steatosis. To verify if role of melatonin is involved in regulation of ER stress-mediated lipid accumulation, the expression of lipogenic genes was measured by qPCR analysis. As shown in Fig. 3A, mRNA levels of SREBP-1a (66%, $p < 0.01$) and SREBP-1c (72%, $p < 0.01$) which are key transcription factor for expressions of lipogenic genes, and FAS (42%, $p < 0.05$), were decreased markedly in tunicamycin-treated mice, but not those of SREBP-2 (56%, $p < 0.01$) which is major regulator of cholesterol metabolism, and its target gene, RED (37%, $p < 0.05$). Moreover, levels of SREBP-1a (68%, $p < 0.01$), and SREBP-1c (61%, $p < 0.01$), and ACC1 (41%, $p < 0.05$) mRNA were decreased by melatonin-treatment only. Protein level of FAS was also decreased in tunicamycin-treated mice (Fig. 3B). These results suggest that melatonin might be involved in improving tunicamycin-mediated hepatic steatosis without affecting *de novo* lipogenesis.

3.4. Melatonin ameliorates ROS-stimulated miR-23a and Ca^{2+} concentration

Since miRNAs play a pivotal role in regulating ER stress-dependent signaling and Ca^{2+} homeostasis machinery [12,23], we

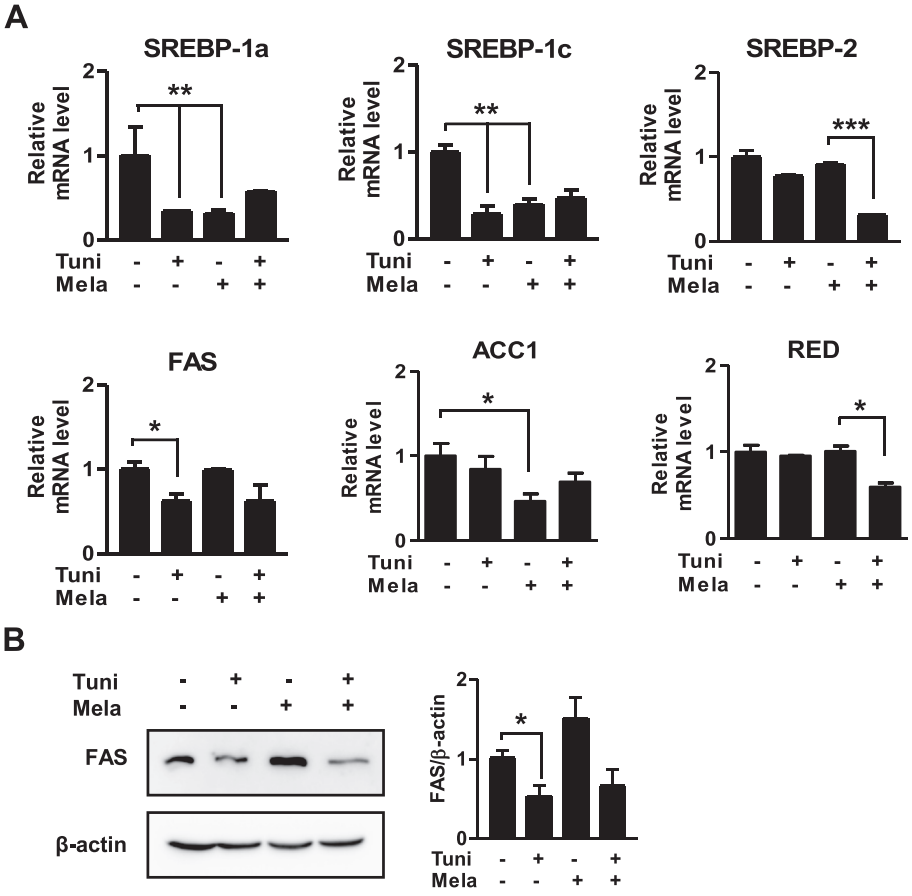

Fig. 3. Melatonin regulates tunicamycin-mediated hepatic lipid metabolism in the liver. Tissue extracts were isolated from livers of the indicated groups and assessed by qPCR analysis (A) and Western blot analysis (B) for lipogenic genes with various primers and antibodies. mRNA and protein levels were normalized to L32 and/or β-actin levels. All mice were separated into experimental groups (n = 5 mice per group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. untreated control or individual ligand-treated mice.

have attempted to identify a functional link between melatonin and ER stress using miRNAs. Among miRNAs related to ER stress signaling, miR-23a expression was activated by tunicamycin and disrupted by melatonin treatment (Fig. 4A). However, miR-24-2 and miR-27a expressions which are another members of mir-23a~27a~24-2 cluster induced in ER stress and apoptosis were not changed by both tunicamycin and melatonin (data not shown).

ER stress tunicamycin-induced ROS generation was reduced after treating with the antioxidant NAC suggesting that tunicamycin-stimulated ER stress is associated with ROS production. Similar to NAC treatment, increased mRNA levels of Grp78 and CHOP by tunicamycin were decreased with melatonin treatment 29% and 42% ($p < 0.05$), respectively and also in Grp78 protein level (Fig. 4B, C).

As melatonin is involved in Ca^{2+} homeostasis through regulation of ER stress, we tried to verify the potential molecular mechanism between melatonin and Ca^{2+} release in primary hepatocytes. As expected, intracellular Ca^{2+} level was elevated (61%, $p < 0.01$) by tunicamycin in primary hepatocytes (Fig. 4D). This stimulatory effect of tunicamycin was reduced (48%, $p < 0.01$) by melatonin. These results show that melatonin improves ER stress and scavenges ROS by inhibiting miR-23a expression, resulting in decrease of intracellular Ca^{2+} level owing to attenuation of ER stress signaling.

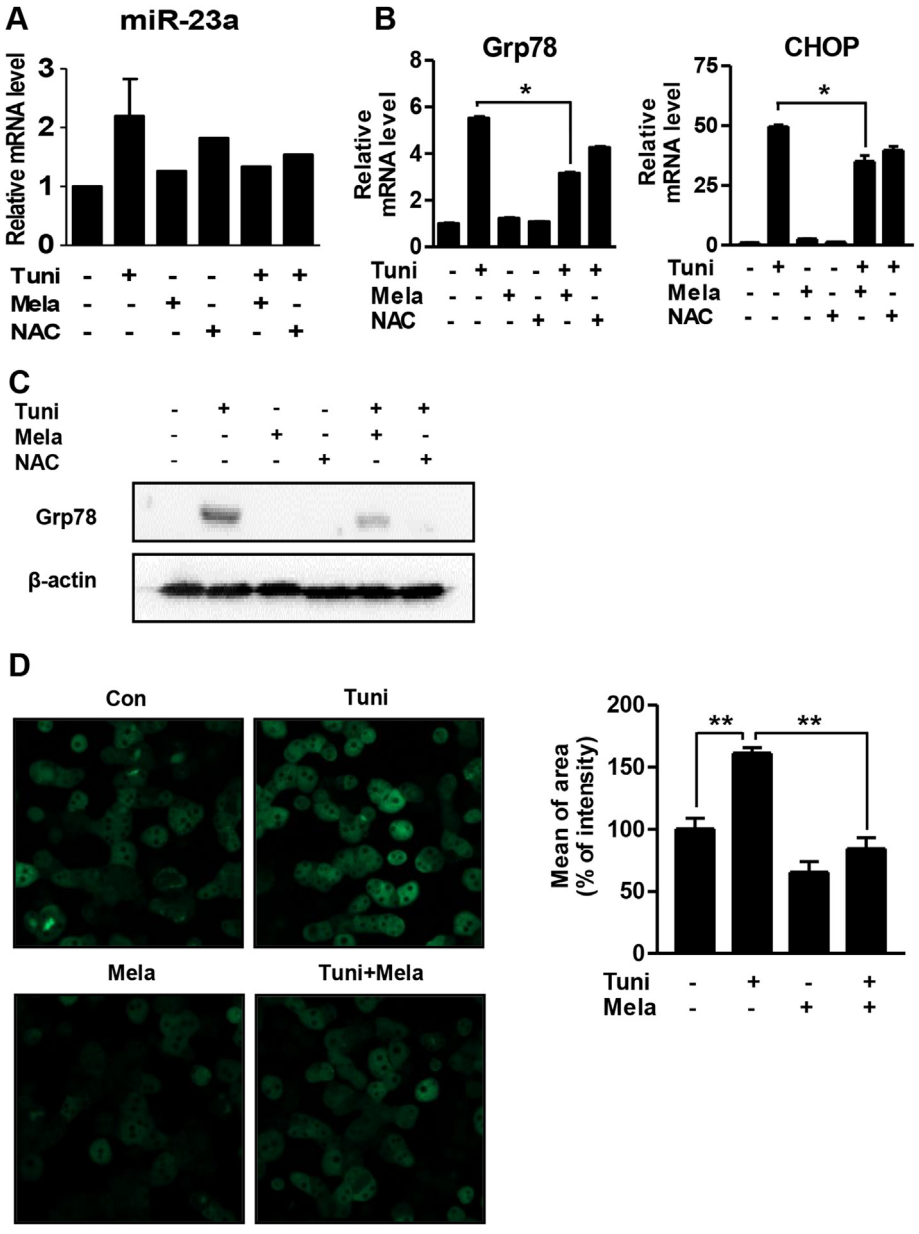

Fig. 4. Effect of melatonin on ER stress-related miR-23a expression and intracellular Ca^{2+} concentration in the liver. (A) Regulation of miR-23a expression by tunicamycin and melatonin. Mouse primary hepatocytes were exposed with either melatonin (50 μg/ml) or tunicamycin (1 μg/ml) for 6 h (B) qPCR analysis. After the tunicamycin, melatonin and NAC treatments for 6 h in AML12 cell line for qPCR analysis. (C) Effect of melatonin on Induction of Grp78 protein. Protein samples were prepared with each ligand for 12 h treatment, and then assessed by immunoblotting with anti-Grp78. (D) Measurement of Ca^{2+} level. Mouse primary hepatocytes were treated with either tunicamycin or melatonin for 6 h under various conditions. Ca^{2+} release and fluorescence intensity was analyzed by confocal laser microscopy and LSM 3 EXCITER software. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. untreated control or Tuni-treated cells.

4. Discussion

Recent studies have indicated that miRNAs might be involved in the control of ER stress signaling [12,24]. The connection between UPR signaling in ER and the miRNAs expression has discovered in the regulation of protein homeostasis. A number of miRNAs have been reported to positively or negatively regulate ER stress response through specific targets with unknown mechanism.

In the present study, we showed that tunicamycin induced hepatic steatosis. Moreover, mRNA expressions of lipogenic genes were repressed in tunicamycin challenged mice. It has been known for a decade that ER stress can lead to altered lipid metabolism and hepatic TG accumulation. Werstuck et al. demonstrated that homocysteine-induced ER stress leads to hepatic steatosis and altered cholesterol and TG biosynthesis in cultured human hepatoma cells and livers of hyper-homocysteinemic mice [25]. Although the physiological role of ER stress in the regulation of hepatic lipogenesis remains controversial [3,26], it is established that several components of the UPR signaling pathway are involved in regulating lipid metabolism [27,28]. Our results demonstrate that SREBP-1c and FAS mRNA expression were decreased dramatically by tunicamycin *in vivo*. Based on these findings, we speculate that *de novo* lipogenesis or hepatic lipogenesis is not involved in tunicamycin-stimulated TG accumulation in the liver and melatonin ameliorates steatosis through regulation of other factors except SREBPs.

ER stress including the UPR pathway has demonstrated links between inflammation and stress signaling through the nuclear factor (NF)-κB and c-Jun N-terminal kinases (JNK) pathways [29,30]. Previous studies have shown that melatonin attenuated ER stress responses by modulating UPR and prevented inflammation through regulating NF-κB, and hypoxia-inducible factor [31,32]. Based on these findings, we investigated the inflammatory response with tunicamycin and melatonin in mouse liver and primary hepatocytes. Tunicamycin-induced TNF-α expression decreased following melatonin treatment in both mouse liver and primary hepatocytes (Fig. 2). Moreover, F4/80, a macrophage marker, also increased following tunicamycin treatment and decreased by melatonin treatment with tunicamycin. But melatonin alone did not affect transcription of genes. These findings suggest that melatonin attenuates the inflammatory response by inhibiting ER stress and that it may also play a role in regulating macrophage infiltration in the liver.

We showed that ER stress markers decreased with melatonin treatment both *in vivo* and *in vitro*. Moreover, because melatonin did not completely eliminate the induction of ER stress markers, we hypothesize that melatonin does not regulate any one component among the ER stress pathways but that it controls upstream of ER stress signaling. Previous reports have demonstrated that miR-23a which is the first member of the mir-23a~27a~24-2 cluster stimulates ER stress and tunicamycin increases Ca^{2+} concentration and that intracellular Ca^{2+} deposits induce ER stress [12,13,33]. In our study, the roles of disturbances in miR-23a gene expression and Ca^{2+} release with melatonin were elucidated in primary hepatocyte. These findings indicate that the increase of miR-23a expression and intracellular Ca^{2+} concentration are involved in hepatic diseases. Even though we showed prevented effect of melatonin on ER stress-triggered hepatic disturbances through controlling miR-23a machinery and Ca^{2+} concentration, it could not rule out the possibility that melatonin may also depend on other unknown mechanisms of protein stability, degradation, and other miRNAs family to regulate the protection pattern of tunicamycin-mediated liver dysfunction, and it is still remains unknown whether miR-23a may be involved in releasing Ca^{2+} from ER or mitochondria to cytosol under both the molecular mechanism for miR-23a machinery and Ca^{2+} homeostasis into the cell.

In conclusion, our results demonstrate that melatonin plays a pivotal role in ER stress-activated hepatic steatosis and inflammation through controls of miR-23a machinery and Ca^{2+} homeostasis. Thus, melatonin may be used as a pharmacological agent to protect from hepatic metabolic dysfunction induced by ER stress.

Conflict of interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgments

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