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Single injection of naked plasmid encoding α-melanocyte-stimulating hormone protects against thioacetamide-induced acute liver failure in mice

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Abstract

Oxidative stress has been implicated in the propagation of acute liver injury. The aim of our study was to investigate whether gene transfer of α-melanocyte-stimulating hormone (α-MSH), a potent anti-inflammatory peptide, could prevent fulminant hepatic failure in mice. Acute liver damage was induced by intraperitoneal administration of thioacetamide. Hydrodynamics-based gene transfection with α-MSH expression plasmid via rapid tail vein injection was initiated 1 day prior to intoxication. The mortality in the α-MSH-treated mice was significantly lower compared to the vehicle group 3 days after injury. Liver histology significantly improved and TUNEL-positive hepatocytes decreased in the treated mice. The degradation of IκBα, endogenous inhibitor of nuclear factor κB, and upregulation of inducible nitric oxide synthase and tumor necrosis factor-α mRNA levels were prevented in the α-MSH-treated group, indicating decreased oxidative stress and inflammation. These results suggest α-MSH gene therapy might protect against acute hepatic necroinflammatory damage with further potential applications.

Keywords: Fulminant hepatitis; α-MSH; Gene delivery; Thioacetamide

Fulminant hepatitis is associated with a very high mortality and oxidative stress contributes to its pathogenesis [1,2]. Free radicals are toxic to various cells, including hepatocytes and initiate a reactive oxygen species (ROS)-mediated cascade causing hepatocyte cell death, leading to acute hepatitis. An animal model of fulminant hepatic failure (FHF) induced by the hepatotoxin thioacetamide (TAA) has been described [2–4]. TAA is a thiono-sulfur-containing compound endowed with liver-damaging and carcinogenic activities. Shortly after administration, it undergoes extensive metabolism to thioacetamide *S*-oxide, and is further metabolized by cytochrome P-450 monooxygenase. Its products include sulfene, and thioacetamide *S*-oxide, a very reactive compound. The binding of this metabolite to tissue macromolecules may be responsible for hepatocyte cell death [3,5].

α-Melanocyte-stimulating hormone (α-MSH) is a 13-amino acid neuroimmunomodulatory peptide that arises through proteolytic processes of the proopiomelanocortin precursor molecule (POMC). α-MSH is produced in

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several tissues, including the pituitary gland, gut, brain, circulatory system, and skin [6,7]. It plays a role in the control of melanogenesis and endocrine metabolism, and has strong anti-inflammatory effects [6,8]. The peptide's anti-inflammatory actions are mediated via a family of melanocortin receptors. Binding of α-MSH to the receptor activates adenylate cyclase, which results in an increase of cAMP in the cytoplasm [9,10]. In turn, this event modulates a signal transduction that prevents dissociation of the IκB–NF-κB (nuclear factor κB) complex, thus averting translocation of NF-κB into the nucleus and the subsequent transcription of proinflammatory cytokines such as TNF-α, IL-1, and IL-6 [10–12]. A previous study demonstrated that α-MSH reduced endotoxin-induced intestinal injury and liver inflammation [13,14]. Therefore, the α-MSH peptide is a powerful drug candidate with potential therapeutic applications for the treatment of inflammatory diseases. It may be possible to utilize the broad therapeutic properties of α-MSH to create gene therapy-based disease treatments such as encephalomyelitis and inflammatory bowel disease [15,16].

In this study, we describe a hydrodynamics-based in vivo transfection procedure utilizing a single, systemic administration of naked α-MSH expression plasmid that results in significant high levels of exogenous α-MSH expression in mice. To delineate the nature and mechanism(s) of the hepatoprotection via α-MSH gene therapy, we evaluated the effects of α-MSH on murine fulminant hepatic failure in terms of prevention of TAA-induced apoptosis, abrogation of inflammatory cytokine upregulation, and prevention of IκBα degradation.

Materials and methods

Animal grouping and plasmid injection. Male C57BL6 mice weighing 18–22 g (6–8 weeks old) were provided by the National Science Council, Taiwan. All animal procedures were performed according to approved protocols and in accordance with recommendations for the proper use and care of laboratory animals. The recombinant α-MSH expression plasmid was a kind gift from Dr. Hedley (Zycos, Lexington, MA, USA) [17]. In brief, the fusion construct that encoded the 13 amino acids of α-MSH in-frame with the first domain of mouse serum albumin driven by a human cytomegalovirus promoter (pCMV-MoLFα) was cloned. The construct that encoded only the first domain of mouse serum albumin (pCMV-SMo195) was used as a vehicle control. These plasmids were purified using an EndoFree Plasmid Giga Kit (Qiagen, Valencia, CA, USA). Mice were divided into MoLFα and vehicle groups, and plasmid of pCMV-MoLFα and pCMV-SMo195 was, respectively, injected into these mice (each group $n = 15$). Plasmid was administered to mice via rapid injection of a large amount of solution through the tail vein by a modified hydrodynamics-based gene transfer technique. Briefly, under isoflurane anesthesia, mice were intravenously (tail vein) injected with 150 μg plasmid in 500 μl saline within 5 s, immediately followed by manual massage of the liver as adapted from Liu and Huang [18]. These mice were sacrificed by overdose of anesthesia before and 1, 2, 3, and 4 days after gene transfection. Blood samples were collected for analysis of α-MSH gene expression (each time point $n = 3$). The amount of plasma α-MSH was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Phoenix Pharmaceuticals, Belmont, CA, USA) according to the user's manual.

Thioacetamide-induced acute liver injury. Acute liver injury was induced by intraperitoneal injection of thioacetamide (TAA, 200 mg/kg) (Sigma–Aldrich, St. Louis, MO, USA). This dose was determined due to our previous study [19]. Sham-treated mice were treated with the same volume of phosphate-buffered saline as a control ($n = 6$). One day before animals were TAA-treated, 36 mice were divided into MoLFα and vehicle groups, and plasmid DNA of pCMV-MoLFα and pCMV-SMo195 was, respectively, injected into these mice. After 3 days, surviving mice were killed by overdose of anesthesia, and blood samples were collected. Serum alanine aminotransferase (ALT) levels were obtained to evaluate the severity of hepatic injury. Liver samples were rapidly removed for histopathological and biochemical examination. Another 20 mice were grouped the same as described above. One day after TAA administration, surviving mice were sacrificed for blood and liver sample collection.

Histopathological and immunohistochemical examination. Livers were removed and fixed overnight in 10% buffered formalin. Ten-micrometer sections were stained with hematoxylin–eosin for histological evaluation. Immunohistochemical staining was carried out using antibodies to inducible nitric oxide synthase (iNOS) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and α-smooth muscle actin (α-SMA) (DakoCytomaton, Glostrup, Denmark). Sections were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 1 h. Positive expression was detected with diaminobenzidine (DAB) (Sigma–Aldrich). Three random sections of each liver were examined. Furthermore, hepatocyte apoptosis was detected by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) using an apoptosis detection kit (Oncogene Research Products, Cambridge, MA, USA). TUNEL staining was performed according to the manufacturer's instructions.

Immunoblotting of IκBα. Liver samples were homogenized in lysis buffer with complete protease inhibitor cocktail tablets (Roche, Mannheim, Germany). For analysis of IκBα protein expression after intoxication, 20 μg of protein extracts was electrophoresed on a 10% acrylamide SDS–PAGE gel and immunoblotted onto PVDF membranes. Membranes were blocked for 1 h at room temperature and incubated overnight with a 1:1000 dilution of IκBα and α-tubulin antibodies (Santa Cruz Biotechnology). α-Tubulin antibody was used as the internal control. Antibody binding was detected using an HRP-linked IgG. Bands were visualized using an ECL detection system (Amersham–Pharmacia Biotech, Little Chalfont, England). Band intensities were quantified by using an image analyzer (Densitograph AE-6900M, Atto, Tokyo, Japan).

RNA purification and reverse-transcription polymerase chain reaction. The expressions of iNOS and tumor necrosis factor-α (TNF-α) mRNA were analyzed by the RNA purification and reverse-transcription polymerase chain reaction (RT-PCR) technique. Total RNA was extracted from harvested liver tissues with chloroform and the TRIzol reagent (Molecular Research Center, Cincinnati, OH, USA). RNA was then reverse-transcribed into cDNA. A 20-μl reverse-transcription reaction mixture containing 1 μg of total RNA, 1× PCR buffer (10 M Tris–HCl, pH 8.3, 0.05 M KCl), 300 μM deoxynucleoside triphosphate, 1 U of RNase inhibitor, 2.5 μM oligo$(dT)_{16}$, and 10 U M-MLV reverse transcriptase was incubated at 42 °C for 1 h, heated to 95 °C for 5 min, and then quickly chilled to 5 °C for 5 min. PCR was performed at a final concentration of 1× PCR buffer, 1.0 μM each of the 3′ and 5′ primers, and 10 U of Advan-Taq Plus DNA polymerase (Clontech, Palo Alto, CA, USA) in a total volume of 50 μl. The mixture was amplified for 32 cycles in a thermal cycler (Stratagene, La Jolla, CA, USA). The iNOS cDNA amplification profile involved denaturation at 94 °C for 45 s, annealing at 58 °C for 1 min, and extension at 72 °C for 45 s for 35 cycles. TNF-α amplification profile involved denaturation at 94 °C for 45 s, annealing at 61 °C for 45 s, and extension at 72 °C for 45 s for 33 cycles. 18S ribosomal RNA (18S) was

amplified to verify equal loading. The 5′ and 3′ primers for iNOS, TNF-α, and 18S were the same as described in a previous report [20]. The expected product lengths of iNOS, TNF-α, and 18S were 426, 307, and 200 bp, respectively. Amplification products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. The gel was scanned at a NucleoVision imaging workstation (NucleoTech, San Mateo, CA, USA) and quantified using GelExpert release 3.5.

Statistical analysis. All data are presented as means ± standard error of mean (SEM). Statistical analyses were performed using Kaplan–Meier survival analysis and one-way ANOVA followed by *t* test. *p* values less than 0.05 were considered significant.

Results

Serum α-MSH levels after gene transfection

The presence of α-MSH in serum was analyzed with an ELISA kit (minimum detectable concentration = 0.15 ng/ml). As shown in Fig. 1, a single, systemic administration of the pCMV-MoLFα plasmid resulted in marked expression of α-MSH in vivo. Levels of the α-MSH peptide in the circulation reached as high as 1.58 ± 0.47 and 1.18 ± 0.28 ng/ml 1 and 2 days following plasmid injection, respectively. The circulating level of α-MSH declined rapidly; however, a significant amount of 0.52 ± 0.24 and 0.36 ± 0.18 ng/ml was still found 3 and 4 days after plasmid injection (each time point, $n = 3$) (Fig. 1). Baseline and levels in the vehicle group mice were all below the detection limit.

Survival rate and serum aminotransferase change following acute TAA administration

The lethality study was conducted to examine if α-MSH increased the survival of mice challenged with TAA (200 mg/kg). After 3 days, only five mice of the vehicle group mice survived (27.7%), while 15 in the MoLFα group (83.3%). There were statistically significant differences between the MoLFα and vehicle groups ($p = 0.001$ by Log-Rank test, Kaplan–Meier survival analysis) (Table 1). Indeed, α-MSH prevented increases in the serum ALT level after TAA administration. There were statistically significant differences in the MoLFα group as compared to the vehicle group (day 1: 319 ± 60.5 vs. 1782 ± 118.2, ***$p < 0.001$; day 3: 179 ± 29.4 vs. 535 ± 86.6, *$p < 0.05$; each time point, $n = 3$) (Fig. 2).

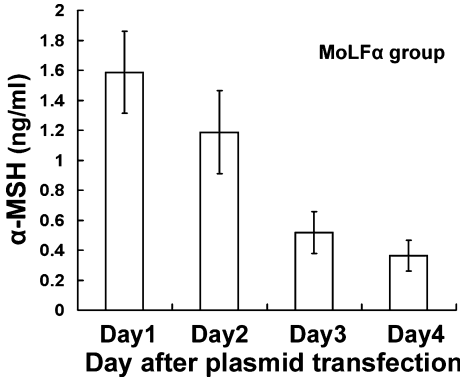

Fig. 1. Circulating α-MSH levels in mice following a single injection of the naked plasmid. Mice were rapidly injected via a tail vein with 500 μl saline containing 150 μg plasmid of pCMV-MoLFα (MoLFα group) and pCMV-SMo195 (vehicle group), respectively. Plasma was collected before and 1, 2, 3, and 4 days after plasmid transfection as indicated, and α-MSH levels were determined by a specific ELISA kit. Baseline and levels in the vehicle group mice were all below the detection limit (0.15 ng/ml). Data are presented as means ± SEM ($n = 3$ for each time point).

Table 1
The mortality cohort after TAA (200 mg/kg) administration in mice

	Day 0	Day 1	Day 2	Day 3	Survival[b] (%)
MoLF group	18[a]	16	15	15	83.3
Vehicle group	18	10	8	5	27.7

[a] Numbers of surviving animals in each group at each time point are shown.

[b] A statistically significant difference in survival was found between the MoLFα and vehicle groups ($p = 0.001$ by Log-Rank test, Kaplan–Meier survival analysis).

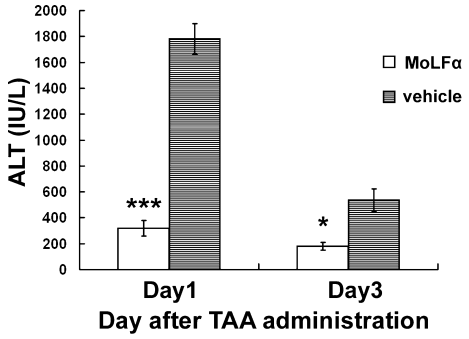

Fig. 2. Attenuation of acute hepatitis in mice. Levels of serum transaminase (ALT) 1 and 3 days after TAA (200 mg/kg) administration were shown. Significant differences were found between the MoLFα and vehicle groups (day 1: 319 ± 60.5 vs. 1782 ± 118.2, ***$p < 0.001$; day 3: 179 ± 29.4 vs. 535 ± 86.6, *$p < 0.05$). Data are expressed as means ± SEM. Statistical analysis was by one-way ANOVA and $n = 3$ for each time point.

Necroinflammatory responses in the liver following acute TAA administration

We next addressed the question of whether pCMV-MoLFα gene delivery prevents necroinflammatory responses following acute TAA administration. As expected, massive necrosis in the pericentral area with infiltration of inflammatory cells was observed in the liver 1 and 3 days following acute TAA administration. α-MSH largely prevented this TAA-induced necroinflammatory change in the liver (Fig. 3). Moreover, TAA induced apoptosis in the liver as demonstrated by TUNEL staining. TUNEL-positive cells were

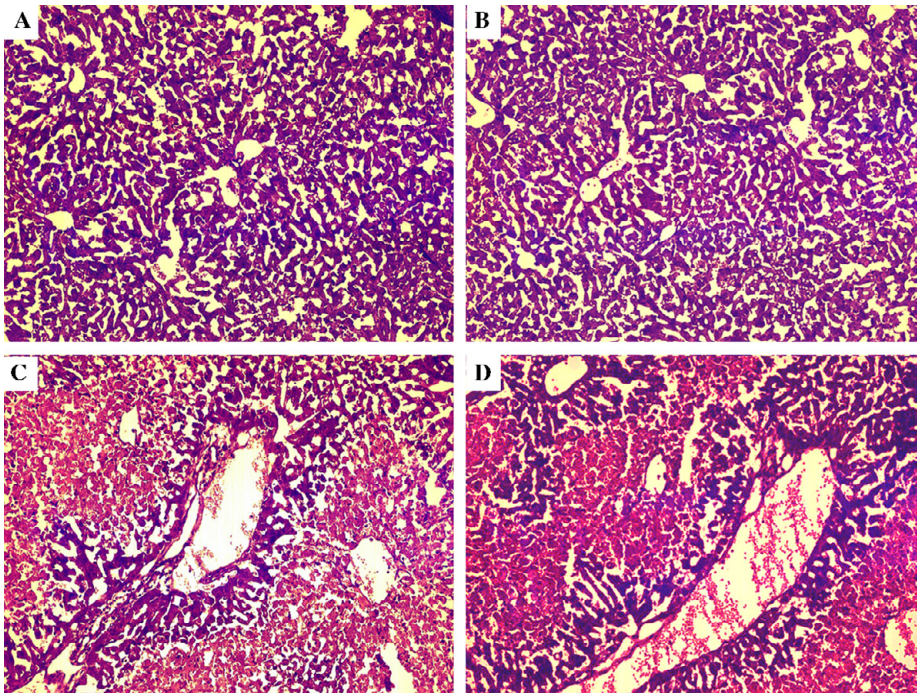

Fig. 3. Liver histology (H&E) of mice with fulminant hepatic failure induced by thioacetamide (200 mg/kg). Mice were killed 1 and 3 days after TAA administration, and their livers were fixed. Severe liver necrosis and inflammation around the central veins could be noted in the vehicle group. Only minimal hepatic necrosis and inflammation were present in the MoLFα group. (A) MoLFα day 1; (B) MoLFα day 3; (C) vehicle day 1; and (D) vehicle day 3. Original magnification: 100×.

observed in the pericentral area coinciding with the destructive hepatic sinusoidal architecture. There were significantly fewer TUNEL-positive cells in the MoLFα group than in the vehicle group. These results indicated that α-MSH inhibits TAA-induced apoptosis in mice (Fig. 4).

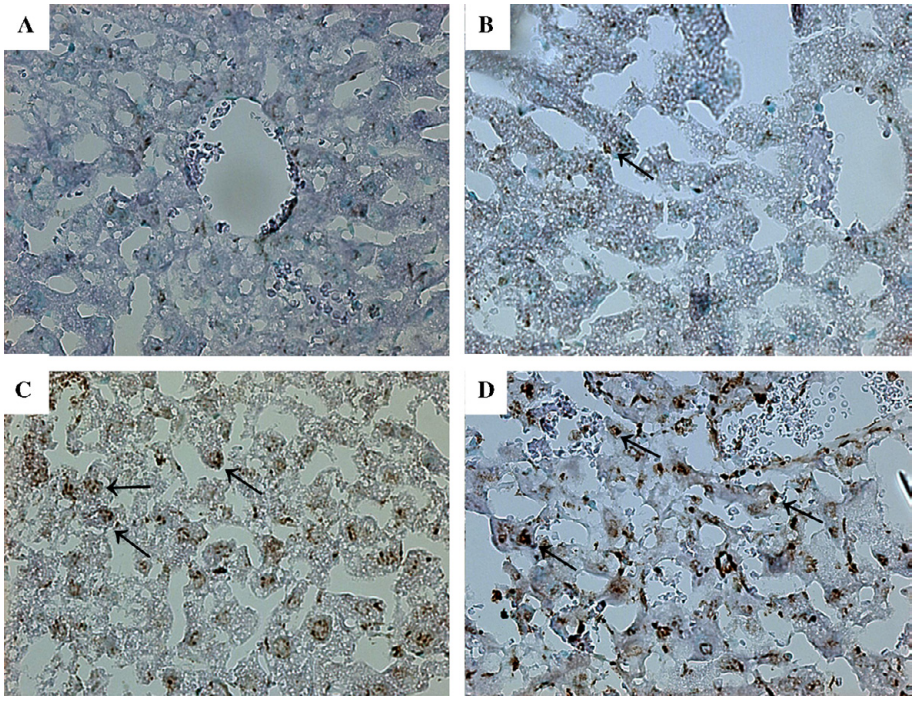

Fig. 4. α-MSH inhibition of TAA (200 mg/kg)-induced hepatocyte apoptosis in mice. TUNEL staining showed that TUNEL-positive cells were observed in pericentral areas of the vehicle group, where these cells were hardly detected in the MoLFα group of mice. Arrows indicate TUNEL-positive cells. (A) Sham-treated; (B) MoLFα day 3; (C) vehicle day 1; and (D) vehicle day 3. Original magnification: 400×.

Western blot analysis of IκBα

Degradation of IκBα is an important step in the activation of NF-κB. To explore the possible hepatoprotective effect of α-MSH, we detected changes in IκBα after intoxication using immunoblot analysis. In the vehicle group, IκBα degradation was evident 1 day after TAA administration compared to the sham group. There was a statistically significant difference in the IκBα expression between the MoLFα and vehicle groups (day 1, $^{**}p < 0.01$) (Fig. 5). This degradation was prevented in the MoLFα group mice, indicating inhibition of NF-κB activation by α-MSH.

iNOS and TNF-α mRNA expressions following acute TAA Administration

α-MSH is known to inhibit iNOS and TNF-α production activated by β-amyloid and interferon γ [21]. The mRNA expressions of iNOS and TNF-α in the liver 1 and 3 days after TAA administration were detected using RT-PCR (Fig. 6A). The level of iNOS mRNA in the vehicle group increased up to 5.4-fold as compared to that of the sham group 3 days after the TAA injection. α-MSH administration attenuated this upregulation. There were statistically significant differences between the MoLFα and vehicle groups (day 1, $^{*}p < 0.05$; day 3, $^{***}p < 0.001$) (Fig. 6B). The level of TNF-α mRNA also increased up to 2.7-fold as compared to the sham group 3 days after TAA injection. There were statistically significant differences between the MoLFα and vehicle groups (day 3, $^{**}p < 0.01$) (Fig. 6B). Localization of iNOS in the liver after TAA administration was also detected by immunohistochemical staining. Intense specific staining for iNOS was observed in the pericentral

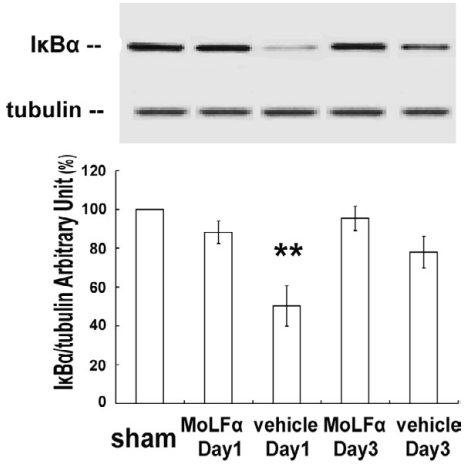

Fig. 5. Western blot analysis of IκBα changes in the liver after TAA (200 mg/kg) administration in mice. Representative photographs are shown. IκBα degradation was evident 1 day after TAA administration in the vehicle group compared to the MoLFα group (day 1, $^{**}p < 0.01$). Immunoblotting with α-tubulin was used as the internal control (three separate experiments).

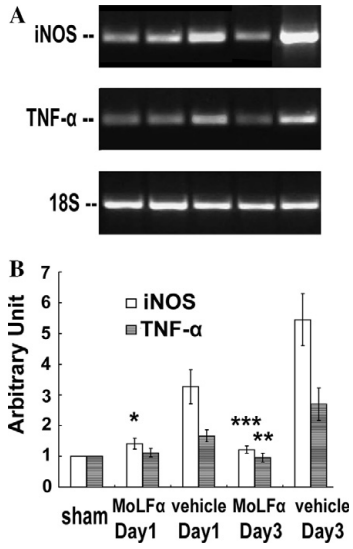

Fig. 6. Hepatic expressions of iNOS and TNF-α mRNA in mice subjected to TAA (200 mg/kg)-induced liver failure. Results were obtained using semiquantitative RT-PCR as described. Representative gels were depicted (A). Levels of iNOS and TNF-α mRNA had, respectively, increased by up to 5.4- and 2.7-fold in the vehicle group 3 days after TAA injection compared to those of the sham treatment group (B). There were statistically significant differences in iNOS (day 1, $^{*}p < 0.05$; day 3, $^{***}p < 0.001$) and TNF-α mRNA expressions (day 3, $^{**}p < 0.01$) between the MoLFα and vehicle groups. Data in the bar graphs are means ± SEM (three separate experiments).

area of the liver after a single injection of TAA. The protein expression of iNOS in sham group was scanty. However, it was estimated to be 2- and 4- to 5-fold higher in the day 1 and day 3 of vehicle group than in the day 3 of MoLFα group, respectively (Fig. 7). In brief, TAA-induced expressions of iNOS and TNF-α in the liver were effectively suppressed by α-MSH gene delivery.

Effect of α-MSH on α-SMA expression following acute TAA administration

The expression of α-smooth muscle actin (α-SMA), a marker of activated hepatic stellate cells (HSCs) [22], in the liver 1 and 3 days after TAA-administration was detected by immunohistochemical staining (Fig. 8). Intense specific staining for α-SMA was observed in the pericentral area of the liver after a single injection of TAA. The expression of α-SMA in sham group was scanty. It was estimated to be 2- and 3- to 4-fold higher in the vehicle group than in the MoLFα group (day 1 and day 3 after TAA, respectively). This indicated TAA-induced expression of α-SMA was attenuated by α-MSH gene transfection.

Discussion

We utilized a model of liver failure in which severe inflammation is induced with substantial mortality; in

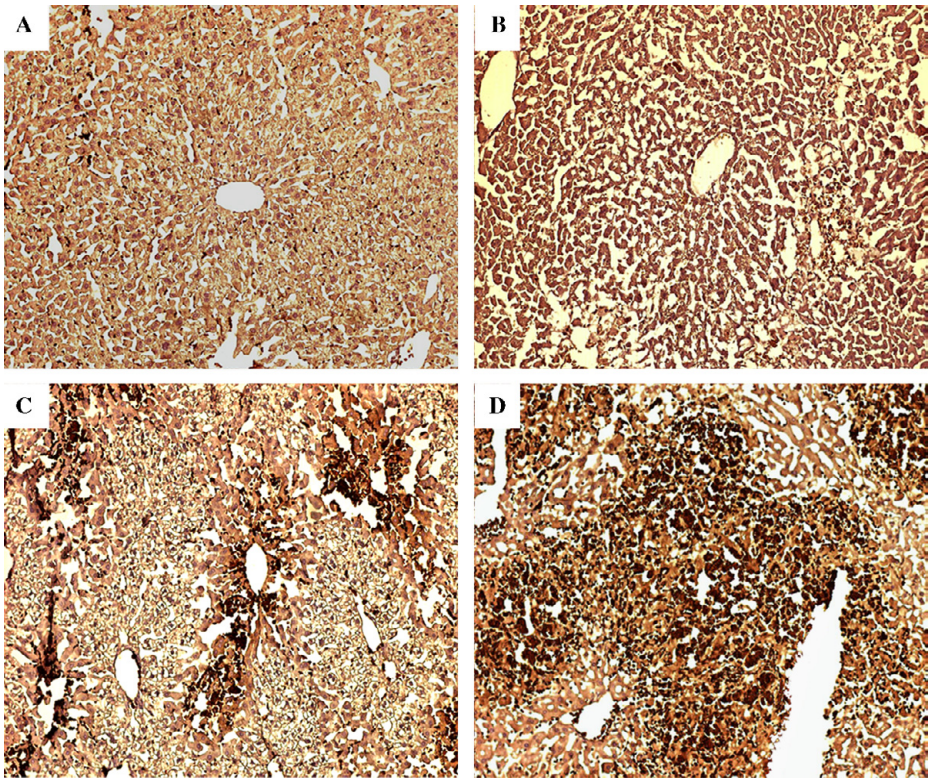

Fig. 7. Effect of α-MSH on iNOS expression in mice liver following TAA (200 mg/kg)-induced fulminant hepatic failure. The expression of iNOS was detected using immunohistochemical staining. Specific staining for iNOS was found mainly in the pericentral area of the liver 1 and 3 days after TAA administration (the deep brown color) in the vehicle group. The increase in iNOS was abolished in the MoLFα group. (A) Sham-treated; (B) MoLFα day 3; (C) vehicle day 1; and (D) vehicle day 3. Original magnification: 100×. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

such a model, therapeutic approaches that have the potential to significantly alter clinical outcomes can be defined. Our results showed that α-MSH gene therapy was effective against substantial lethality, and significantly decreased serum ALT levels in the TAA-induced acute inflammatory response. Histological changes, such as hemorrhaging and necrosis, in hepatic lobules were simultaneously improved by the treatment. These results are consistent with previous studies, which showed that α-MSH possesses strong anti-inflammatory activities [14,16].

TAA is a typical hepatotoxin and causes centrilobular necrosis by generation of ROS. Its injury is known to cause necrosis or apoptosis depending on the dose and duration of administration [5,23]. LD90 of TAA is 1000 mg/kg in mice [24]. However, low dose TAA (100 mg/kg) is known to cause 60% mortality [5]. The dosage (200 mg/kg) used in this article is according to our previous study [19]. Using TUNEL staining, we showed that TAA induces apoptosis in the peri-centrilobular area. α-MSH also significantly decreased the TUNEL-positive hepatocytes as compared to the vehicle group. Moreover, immunohistochemical analysis of α-SMA, a marker of activated HSCs, showed that α-MSH gene therapy decreased HSC activation, which is considered as the first step of liver fibrosis and propagates rapidly after CCl4-induced acute liver injury [22,25].

The α-MSH concentration ranges from 10 to 36 pg/ml which is known to increase (26–118 pg/ml) during hepatic encephalopathy in dogs [26]. No wonder the α-MSH ELISA kit (minimal detectable concentration: 0.15 ng/ml) failed to detect baseline and the levels in the vehicle group mice. It is estimated α-MSH gene delivery causes at least 10-fold increase as compared to the sham group (day 1, 1.58 ± 0.47 vs. 0.15 ng/ml at most). Recently, with hydrodynamics-based gene delivery, a simple and efficient in vivo transfection procedure, high level of gene expression can be obtained by intravenous injection of plasmid in a large-volume solution within a short time [18,27,28]. The hydrodynamic pressure generated by rapid injection of a large volume of naked plasmid into mouse tail veins resulted in efficient gene transfer to hepatic cells. However, a major concern with this technique is that it requires rapid injection of a large volume of solution; elevated levels of liver enzymes were noticed after the injection, and 3–10 days were required for recovery. In order to counter this effect, we used rapid plasmid injection followed by manual massage

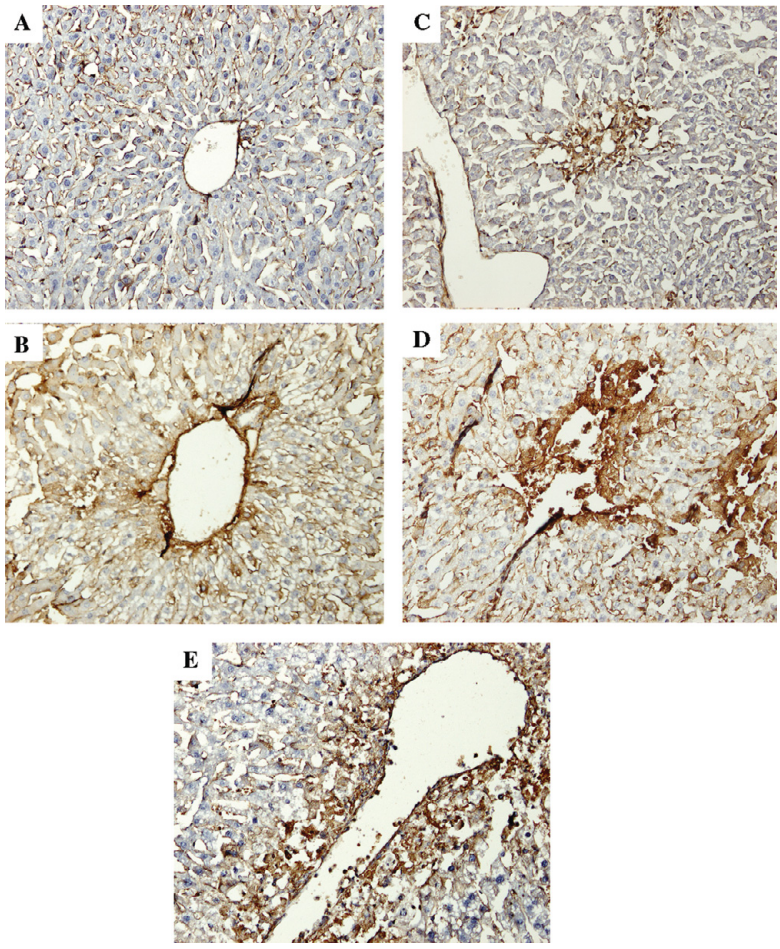

Fig. 8. Effect of α-MSH on the expression α-SMA in mice liver following TAA (200 mg/kg)-induced acute hepatic failure. The expression and localization of α-SMA, a marker of activated hepatic stellate cells, were detected by immunohistochemical staining. Intense specific staining for α-SMA was observed in the pericentral area of the liver 3 days after TAA administration. α-SMA-positive cells showed deep brown color stain. α-MSH gene therapy attenuated this upregulation effectively. (A) Sham-treated; (B) MoLFα day 1; (C) MoLFα day 3; (D) vehicle day 1; and (E) vehicle day 3. Original magnification: 100×. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

to minimize liver impairment as adapted from Liu and Huang [18].

Nuclear factor kappa B (NF-κB) is an inducible transcription factor that regulates numerous genes involved in proliferation, and the immediate-early steps of inflammatory responses [29]. Both pro- and antiapoptotic activities of NF-κB have been observed. However, pyrrolidine dithiocarbamate (inhibitor of NF-κB activator) protects against TAA-induced fulminant hepatic failure [30]. In its inactive form, NF-κB forms a complex in the cytosol with its endogenous inhibitor, IκBα, and related proteins. Degradation of IκBα is triggered by IκB kinase. Phosphorylated IκBα is ubiquitinated and then degraded by the proteasome. NF-κB is activated through degradation of IκB proteins that release the NF-κB complex for translocation to the nucleus where gene transcription is stimulated [29]. To investigate the possible hepatic protective mechanism of α-MSH, we further detected changes in IκBα after intoxication by immunoblot analysis. In the vehicle group, IκBα degradation was evident 1 day after TAA administration compared to the sham group. Interestingly, this degradation was prevented in the MoLFα group of mice indicating the inhibition of NF-κB activation by α-MSH.

We further analyzed the mRNA expressions of iNOS and TNF-α in TAA-stimulated hepatic tissues. Nitric oxide (NO) is a short-lived free radical that plays an important regulatory role in several biological processes [31]. The formation of NO from L-arginine and molecular oxygen is catalyzed by the enzyme, nitric oxide synthase (NOS). Whereas the neuronal and endothelial isoforms of NOS are constitutively expressed, inducible NOS (iNOS) is expressed after induction by thioacetamide [4,32]. Although the effects of iNOS on hepatocytes are still controversial, it is clear that excess NO is potentially harmful in some pathophysiological situations [33,34]. And proinflammatory cytokines, especially TNF-α, are thought to play a role in cell injury

associated with acute fulminant hepatitis [35]. α-MSH gene therapy could effectively attenuate the upregulation of iNOS and TNF-α mRNA after acute liver injury. Briefly, it is conceivable to suggest that α-MSH, by preventing the translocation of NF-κB to the nucleus, attenuates hepatic inflammatory response to TAA administration by reducing upregulation of a variety of proinflammatory mediators (TNF-α, iNOS, . . . , etc.).

In conclusion, α-MSH gene therapy can strikingly alter mortality after TAA-induced liver failure in mice. It also prevents a necroinflammatory response and hepatocyte apoptosis, inhibits IκBα degradation, and abrogates the increase in the proinflammatory mediator after acute liver injury. Therefore, it is postulated that modified hydrodynamics-based α-MSH gene therapy might be an effective prevention strategy for acute hepatic injury with potential future applications.

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