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Protection against phalloidin-induced liver injury by oleanolic acid involves Nrf2 activation and suppression of Oatp1b2

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Abstract

This study utilized pharmacological activation of Nrf2 with oleanolic acid (OA, 22.5 mg/kg, sc for 4d) and the genetic Nrf2 activation (Nrf2-null, wild-type, and Keap1-HKO mice) to examine the role of Nrf2 in protection against phalloidin hepatotoxicity. Mice were given phalloidin (1.5 mg/kg, ip for 8 h) to examine liver injury and the expression of toxicity-related genes. Phalloidin increased serum enzyme activities and caused extensive hepatic hemorrhage and necrosis in Nrf2-null and wild-type mice, but less injury was seen in Keap1-HKO mice and OA-pretreated mice. Phalloidin increased the expression of neutrophil-specific chemokine mKC and MIP-2 in Nrf2-null and WT mice, but such increases were attenuated in Keap1-HKO and OA-pretreated mice. Phalloidin increased, while Nrf2 activation attenuated, the expression of genes involved in acute-phase response (Ho-1) and DNA-damage response genes (Gadd45 and Chop10). Phalloidin is taken up by hepatocytes through Oatp1b2, but there was no difference in basal and phalloidin-induced Oatp1b2 expression among Nrf2-null, wild-type, and Keap1-HKO mice. In contrast, OA decreased phalloidin-induced Oatp1b2. Phalloidin activated MAPK signaling (p-JNK), which was attenuated by activation of Nrf2. In conclusion, this study demonstrates that protection against phalloidin hepatotoxicity by OA involves activation of Nrf2 and suppression of Oatp1b2.

Keywords

Phalloidin hepatotoxicity; Oleanolic acid; Nrf2-null mice; Keap1-HKO mice; inflammation; Oatp1b2

Introduction

Phalloidin is a toxin of the mushroom *Amanita phalloides* which produces severe liver damage characterized by marked hemorrhage, cholestasis, and necrosis (Frimmer 1987; Mengs and Trost 1981). Phalloidin is taken up by hepatocytes via the organic anion

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Conflict of interest

The authors declare that there are no conflicts of interest.

transporters (Meier-Abt et al. 2004), more specifically Oatp1b2 (Lu et al. 2008). Upon entry into hepatocytes, phalloidin binds to F-actin, which prevents trafficking along the cytoskeleton, and causes irreversible polymerization of actin filaments (Barriault et al. 1996; Herraez et al. 2009).

Oxidative stress is considered to play an important role in phalloidin-induced acute liver injury (Bouchard et al. 2000). In the early stages of cholestasis, phalloidin decreases bile flow, related mainly to a disruption of biliary glutathione (GSH) secretion, probably due to the rapid disruption of the hepatocanalicular transport of GSH (Bouchard et al. 2000). The cyclooxygenase inhibitor indomethacin was effective in protecting against phalloidin-induced acute liver injury (Barriault et al. 1994). These results suggest that oxidative stress and inflammation are involved in phalloidin-induced liver injury. Oxidative stress produced by tert-butylhydroquinone leads to translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) into the nucleus to activate cytoprotective genes, such as GSH S-transferase, but this event can be prevented by phalloidin (Kang et al. 2002).

Oleanolic acid (OA) is a triterpenoid that exists widely in fruits of Olea europaea and Ligustrum lucidum (Liu et al 1995; Guinda et al. 2010), in vegetables (Laszczyk 2009) and in many medicinal herbs (Pollier and Goossens 2012). OA is used as an over-the-counter Chinese medicine in the treatment of inflammatory diseases and cancer adjuvant therapies (Laszczyk 2009; Pollier and Goossens 2012). OA is an activator of Nrf2 (Reisman et al. 2009).

Nrf2 is a master transcription factor that protects against cellular injury such as the hepatotoxicity from acetaminophen, CCI4 and other hepatotoxicants (Klaassen and Reisman 2010). Recently, an Nrf2 “gene dose–response” model was generated using Nrf2-null mice, wild-type mice, Keap1-knockdown (Keap1-KD) mice with enhanced Nrf2 activation, and Keap1-hepatocyte knockout (Keap1-HKO) mice with maximum Nrf2 activation. Transcription profiling in the Nrf2 “gene dose–response” model by microarray analysis showed that these genes are constitutively expressed in a “gene dose–response” manner (Wu et al., 2011, 2012a, 2012b).

We have used this Nrf2 “gene-dose” model to examine the sensitivity towards 13 hepatotoxicants, and over-expression of Nrf2 protects against the hepatotoxicity produced by many hepatotoxicants including phalloidin (Liu et al. 2013a). However, this preliminary study needs to be confirmed, and whether OA protection against phalloidin is due to Nrf2-mediated anti-inflammatory effects and/or due to suppression of phalloidin transporter Oatp1b2 (Lu et al. 2008) needs to be defined. Thus, the purpose of the present study was to determine whether genetic constitutive over-expression of Nrf2 (Keap1-HKO mice) and/or pharmacological activation of Nrf2 (OA) protects against phalloidin hepatotoxicity and the mechanism(s) of protection.

Materials and Methods

Reagents

Phalloidin was purchased from Sigma-Aldrich (St. Louis, MO). Oleanolic acid was obtained from Guiyang Pharmaceutical Co (Guiyang, China) as described previously (Liu et al., 1995). All other chemicals were reagent grade.

Animals

Nrf2-null mice were obtained from Dr. Jefferson Chan (University of California, Irvine, CA) (Chan et al. 1996). Keap1-KD mice with Keap1 decreased throughout the body were supplied by Dr. Masayuki Yamamoto (Tohoku University, Sendai, Japan) (Okada et al. 2009). Nrf2-null and Keap1-KD mice were backcrossed into the C57BL/6 background, and > 99% congenicity was confirmed by Jackson Laboratories (Bar Harbor, ME). Keap1-HKO mice were generated by crossing Keap1-KD mice and AlbCre+ mice, which express Cre only in hepatocytes (Wu et al. 2011). The Nrf2 “gene dose–response” model has been characterized (Wu et al. 2011, 2012a, 2012b), and used in the initial hepatoprotection screening (Liu et al., 2013a). Mice were housed in AALAC accredited facilities at the University of Kansas Medical Center in a temperature-, light-, and humidity-controlled environment with free access to Teklad Rodent Diet #8604 (Harlan Laboratories, Madison, WI) and tap water. The animal treatment protocol was approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee.

Experimental design

The Nrf2 “gene-dose” model (Nrf2-null, wild-type, and Keap1-HKO mice) (Wu et al. 2011), and pharmacological Nrf2 activation (OA 22.5 mg/kg, sc for 3 days) were used in this study to determine the role of Nrf2 in protecting against phalloidin-induced liver injury. Mice were treated with either phalloidin (1.5 mg/kg, i.p.) or saline (10 ml/kg, i.p.). Eight hours after administration of phalloidin or saline, blood and liver samples were collected. Portions of livers were fixed in 10% neutral formalin for histological analysis, and the remainder was frozen in liquid nitrogen and stored at −80°C.

Hepatotoxicity evaluation

Serum alanine aminotransferase (ALT) was determined as a biochemical indicator of hepatocellular necrosis using Pointe Scientific Liquid ALT Reagent (Canton, MI) according to the manufacturer’s protocol.

Histopathology

Liver samples were fixed in 10% formalin prior to routine processing and paraffin embedding. Liver sections (5 μm in thickness) were stained with hematoxylin and eosin and evaluated for liver injury.

Lipid peroxidation

Lipid peroxidation in livers was determined by quantifying thiobarbituric acid reactive substances (TBARS) using malondialdehyde (MDA) as the standard (Thermo Fisher Scientific Inc., Fair Lawn, NJ).

Total RNA isolation

Total RNA was isolated using RNAzol B reagent (Tel-Test, Inc., Friendswood, TX) according to the manufacturer's protocol. The concentration of total RNA in each sample was quantified spectrophotometrically at 260 nm. The integrity of each RNA sample was evaluated by formaldehyde–agarose gel electrophoresis before analysis.

Quantification of mRNA by RT-PCR

Total RNA in mouse livers was reverse-transcribed into cDNA by High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA), and the resulting cDNA was used for real-time PCR analysis using Power SYBR® Green PCR Master Mix in a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Oligonucleotide primers were designed with Primer3 software and are listed in Table 1. Relative expression of genes was calculated by the 2-ΔΔCt method and normalizing to the house-keeping gene G3PDH.

Western blot analysis

Liver protein was extracted with T-PER tissue protein extraction kit (Thermo Scientific, Rockford, IL) with freshly prepared proteinase inhibitors (Sigma, St. Louis, MO). Protein concentrations were determined using a BCA protein assay according to the manufacturer's instructions (Thermo Scientific, Rockford, IL). Approximately 40 μg of cytosolic protein was used for immunoblotting proteins of interest. The primary antibodies used in this study include Gclc (sc-27688) from Santa Cruz Biotechnology (Santa Cruz, CA); Nqo1 (Ab2346) and β-actin (Ab8227) from Abcam (Cambridge, MA); phosphorylated c-jun N-terminal kinase (p-JNK), and total JNK were from Cell Signaling (Cambridge, MA). Secondary antibodies were purchased from Sigma-Aldrich (St. Louis, MO). Protein-antibody complexes were detected using an enhanced chemiluminescent kit (Thermo Scientific, Rockford, IL) and exposed to HyBlot CL autoradiography film (Denville Scientific Inc., Metuchen, NJ).

Statistical analysis

Data were analyzed using a one-way ANOVA followed by Duncan's multiple range test ($p \leq 0.05$) utilizing SPSS 13 Software (SAS, NC).

Results

Phalloidin-induced acute hepatotoxicity

Phalloidin-induced hepatotoxicity was indicated by enzyme activities of alanine aminotranferase (ALT) in serum. In saline-treated mice, serum ALT activities in Nrf2-null, wild-type and Keap1-HKO mice were low with no differences among the genotypes. OA (22.5 mg/kg, sc) treatment alone also did not increase serum ALT (all below 50 U/L).

Phalloidin increased serum ALT activities to 5500 U/L in Nrf2-null mice, 4100 U/L in wild-type mice, 1250 U/L in Keap1-HKO mice, and 850 U/L in OA-pretreated mice (Fig. 1), indicating that phalloidin-induced liver injury was less in Keap1-HKO and OA-pretreated mice.

Liver histopathology

There were no observable abnormalities in livers of Nrf2-null, wild-type, Keap1-HKO, and OA-pretreated mice (data not shown). After phalloidin challenge, severe hemorrhage, inflammation and widespread necrosis (under low magnification) and extensive nuclear condensation (under high magnification) were seen in Nrf2-null mice. Similar lesions were also evident in phalloidin-treated wild-type mice, but to a lesser extent. In comparison, only swollen hepatocytes and foci of inflammation and necrosis were seen in Keap1-HKO mice and mice pretreated with oleanolic acid (Fig. 2).

Liver lipid peroxidation

Lipid peroxidation is a biomarker for phalloidin-induced liver injury (Barriault et al. 1996; Herraez et al. 2009). Fig. 3 shows the hepatic lipid peroxidation, as determined by the thiobarbiturate reactive substances produced by phalloidin in various Nrf2 genotypes of mice. Phalloidin increased hepatic MDA levels 117% in Nrf2-null mice, 70% in WT mice, but only 26% in the Keap1-HKO mice and 16% in OA-pretreated mice.

Expression of Nrf2 and Nqo1 mRNA in liver

To determine the role of Nrf2 activation in protecting against phalloidin-induced toxicity, the expression of Nrf2 and the Nrf2-target gene Nqo1 were quantified in livers of Nrf2-null, wild-type, Keap1-HKO, and OA-pretreated mice (Fig. 4). Basal expression of Nrf2 mRNA was low in Nrf2-null and wild-type mice, but was 5-fold higher in Keap1-HKO and OA-pretreated mice. Basal expression of Nqo1 mRNA was low in Nrf2-null and wild-type mice, but was 10-fold higher in Keap1-HKO and OA-pretreated mice. Phalloidin increased Nqo1 and Nrf2 mRNA levels, with the highest expression in Keap1-HKO and OA-pretreated animals. Western-blots also demonstrated that the Nrf2-target proteins Gclc and Nqo1 were higher in livers of Keap1-HKO mice and mice pretreated with OA (Fig. 5).

Expression of MAPK pathway in liver

Western-blots (Fig. 5) demonstrated that in Nrf2-null mice and wild-type mice challenged with phalloidin, p-JNK was markedly increased in Nrf2-null mice without apparent changes in total JNK. The p-JNK expression in livers of Keap1-HKO and OA-pretreated mice were much lower than that seen in Nrf2-null mice.

Expression of Oatp1b2 mRNA in liver

Because Oatp1b2 plays a key role in phalloidin uptake into hepatocytes (Lu et al. 2008), the expression of Oatp1b2 under basal and phalloidin challenge conditions was determined (Fig. 6). Basal expression of Oatp1b2 mRNA was similar in the three genotypes of mice and in the OA-pretreated mice. Phalloidin intoxication increased the expression of Oatp1b2 in

Nrf2-null (11-fold), wild-type (7-fold) and Keap1-HKO (8-fold) mice, respectively, but there was no increase in OA-pretreated mice.

Expression of MIP-2 and mKC mRNA in liver

Neutrophil-specific chemokine macrophage inflammatory protein 2 (MIP-2) and mouse keratinocyte-derived chemokine (mKC) are biomarkers of inflammatory responses (Dorman et al. 2005). As shown in Figure 7, there was no difference in basal expression of MIP-2 (about 0.03 % of G3PDH) and mKC (around 1% of G3PDH) among the three genotypes of mice and the mice pretreated with OA. Eight hours after phalloidin administration, mRNA of MIP-2 increased 30-36 fold in Nrf2-null and WT mice, but only 3-4 fold in Keap1-HKO and OA-pretreated mice. Similarly, mRNA of mKC increased 10-14 fold in Nrf2-null and WT mice, but only 3-4 fold in Keap1-HKO and OA-pretreated mice, indicating that Nrf2 over-expression attenuated phalloidin-induced inflammatory responses.

Expression of Ho-1 and MT mRNA in liver

Ho-1 induction in rodent models of acute and chronic hepatic inflammation has been proposed as a biomarker of liver damage and inflammation (Sass et al. 2012). Figure 8 shows the marked induction of Ho-1 after phalloidin in Nrf2-null (31 fold) and wild-type mice (15 fold), but only 4 fold in Keap1-HKO and OA-pretreated mice, indicating Nrf2 deficiency makes animals susceptible to phalloidin induction of Ho-1. Like Ho-1, metallothionein (MT) is another cellular protein that protects against toxic stimuli (Klaassen et al. 1999). Keap-1-HKO mice had 5-fold higher MT mRNA than Nrf2-null mice (20.1 vs 4.2 %of G3PDH), and OA-pretreatment resulted in 8-fold induction of MT-1 (27.4 vs 4.2 %of G3PDH). Following the phalloidin challenge, MT mRNA increased over 100-fold in all groups (Fig. 8), and highest increases (1410 % of G3PDH) were seen in OA-treated mice which was significantly higher than that in Nrf2-null mice.

Expression of Gadd45 and Chop10 mRNA in liver

The endoplasmic reticulum (ER) plays an important role in liver injury by activation of signaling processes and pro-apoptotic events leading to hepatocellular damage (Ho et al. 2005; Nagy et al. 2007). The ER responsive genes Gadd45 and Chop10 (Gadd153) (Ho et al. 2005) were increased by phalloidin 70-100 fold in Nrf2-null mice, 30-45 fold in wild-type mice, and only 4-6 fold in Keap1-HKO and OA-pretreated mice, indicating less ER stress occurs when Nrf2 is activated prior to phalloidin administration (Fig. 9).

Discussion

In the present study, liver injury was the primary toxic effect observed after an acute dose of phalloidin (1.5 mg/kg). The acute hepatotoxicity of phalloidin was evident by both increases in serum enzyme activities and histopathology. Oxidative stress (TBARS), inflammatory responses (mKC, MIP-2) and hemorrhage were also evident leading to hepatocellular death. The present study shows that knockout of Nrf2 results in mice more sensitive to the acute hepatotoxicity produced by phalloidin, and activation of Nrf2, either by genetic engineering (Keap1-HKO) or by pharmacological activation (OA), protected against phalloidin-induced hepatotoxicity.

Phalloidin is rapidly taken up by hepatocytes through Oatp1b2 (mice) and OATP1B1 and OATP1B3 (human cells) (Lu et al. 2008; Meier-Abt et al. 2004). Oatp1b2-null mice transport less phalloidin into the liver and less liver injury is observed (Lu et al. 2008). In the present study, there was no significant difference in the basal expression of Oatp1b2 among the various Nrf2-genotypes and OA-pretreated mice. Phalloidin induced the expression of Oatp1b2 7-10 fold in Nrf2-null, wild-type, and Keap1-HKO mice, but not in OA-pretreated mice, suggesting that the protection of genetically graded Nrf2 activation against phalloidin toxicity is not due to diminished uptake of phalloidin into the hepatocytes. Although the dose of OA (22.5 mg/kg) used in the present study did not suppress Oatp1b2 prior to phalloidin challenge, OA at higher doses (90 and 135 mg/kg) did reduce Oatp1b2 expression (Liu et al., 2013b), and the inability of phalloidin to increase Oatp1b2 in OA-treated mice suggests that the protection of phalloidin hepatotoxicity by OA could also involve the reduced uptake of phalloidin into the liver.

Nrf2 is a master regulator that protects against reactive oxygen species, electrophiles and inflammation (Kensler et al. 2007; Klaassen and Reisman 2010), and as a result, the phalloidin-induced hepatic lipid peroxidation and inflammation-related gene expression, such as MIP-2 and mKC, was attenuated with both genetic Nfr2 activation, as in Keap1-HKO mice, and pharmacologic activation of Nrf2, as in OA-pretreated mice. It has been previously shown that the anti-inflammatory drug indomethacin could prevent phalloidin-induced lethality and liver injury (Barriault et al. 1994). The attenuation of acute inflammatory gene expression in mice with this Nrf2 “gene-dose” model has also been demonstrated for cadmium (Wu et al. 2012a), ethanol (Wu et al. 2012c), microcystin, carbon tetrachloride and acetaminophen (Liu et al. 2013a).

Ho-1 and MT-1 are two cellular protective proteins that respond to toxic stimuli (Klaassen et al. 1999; Sass et al. 2012). Induction of these acute phase proteins would be theoretically beneficial to reduce phalloidin toxicity. However, over-expression of these proteins can also be envisioned as a potential biomarker of increased tissue damage, and then progression of phalloidin acute hepatotoxicity. In the present study, both Ho-1 and MT-1 were induced dramatically by phalloidin. However, Ho-1 would more like a biomarker of tissue injury, as there was a decrease with Nrf2-mediated protection against phalloidin-induced liver injury. In contrast, MT-1 was increased more in mice with activation of Nrf2, and thus not a biomarker of phalloidin hepatotoxicity.

ER stress is a hallmark of phalloidin acute hepatotoxicity (Barriault et al. 1995). ER stress responsive genes Gadd45 and Gadd153 (Chop10) were markedly increased following phalloidin intoxication (Fig. 9). Gadd45 and Chop10 activation results in signaling processes and proapoptotic events leading to hepatocellular damage (Ho et al. 2005; Nagy et al. 2007). Pretreatment with compounds (methyl palmitate and praseodymium nitrate) that depress ER stress have been shown to decrease phalloidin-induced hepatotoxicity (Barriault et al. 1995). In the present study, increased Nrf2 signaling by genetic engineering or by pharmacological activation suppressed phalloidin-induced ER stress in the liver, indicating a potential mechanism of Nrf2 cellular protection.

MAP kinases are serine/threonine-specific protein kinases, which include JNK and ERK subfamily members. They are involved in directing cellular responses to a diverse array of toxic stimuli, including pro-inflammatory cytokines. They regulate proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis in various tissues (Pearson et al. 2001). In liver, MAPK activation is associated with the onset of apoptosis (Ghosh et al. 2011). The present data demonstrate that Nrf2 deficiency made phalloidin-challenged livers more susceptible to JNK activation, leading to hepatocellular death, which further suggests the importance of JNK in phalloidin-induced hepatocellular death.

In conclusion, the present study shows that Nrf2 activation prevents phalloidin-induced oxidative stress and liver injury. Nrf2 induces genes involved in antioxidant defense, and reduces ER stress and inflammatory responses as mechanisms of protection against phalloidin-induced hepatotoxicity. The protection against phalloidin hepatotoxicity by OA involves activation of Nrf2 and suppression of Oatp1b2.

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Abbreviations

ALT	alanine aminotransferase
Gadd45	growth arrest DNA damage response protein 45
OA	oleanolic acid
Nrf2	Nuclear factor erythroid-derived 2-like 2
NQO1	NAD(P)H quinone oxidoreductase 1
p-JNK	phosphorylated c-jun N-terminal kinase
TBARS	thiobarbiturate reactive substances

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Highlights

- Phalloidin induces inflammation and acute liver injury.
- Phalloidin induces ER stress and inflammatory gene expression.
- Activation of Nrf2 protects against phalloidin-induced inflammation.
- Oleanolic acid protects against phalloidin hepatotoxicity.
- Oleanolic acid activates Nrf2 and suppresses Oatp1b2.

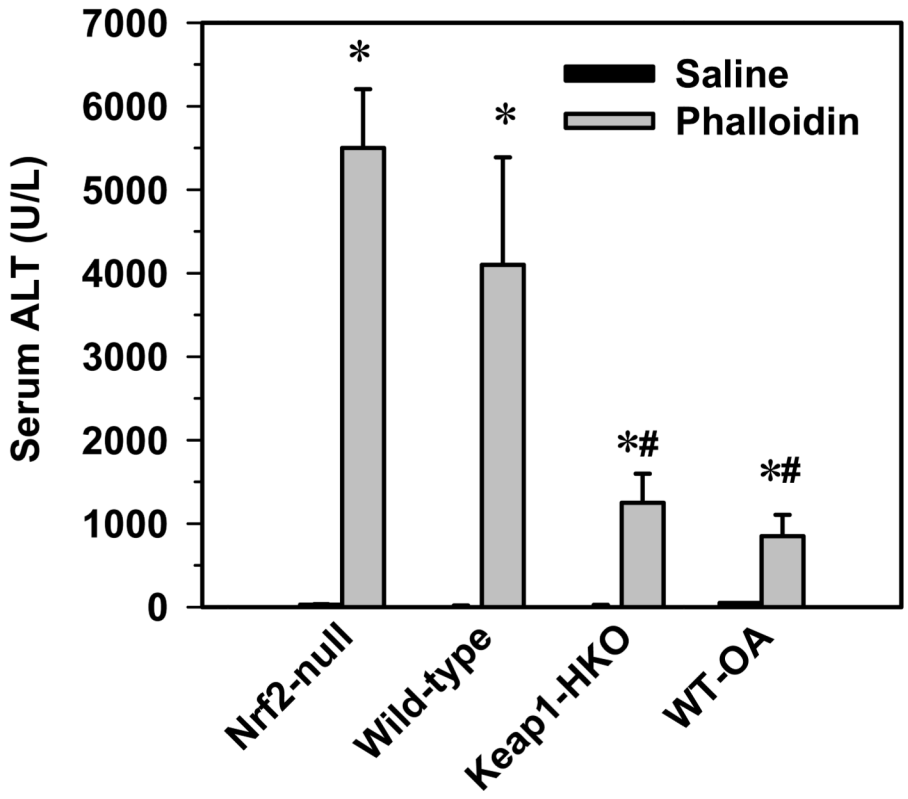

Fig. 1.

Serum activities of alanine aminotransferase (ALT). Nrf2 activation was achieved by genetic engineering (Keap1-HKO mice) and by pharmacological activation (oleanolic acid 22.5 mg/kg, sc for 3 days). Mice were challenged with phalloidin (1.5 mg/kg, i.p.) for 8 hrs. Values are expressed as mean ± S.E.M. (n=8-10). *Significantly different from the basal level of the same genotype ($p \leq 0.05$); #Significantly different from Nrf2-null mice ($p \leq 0.05$).

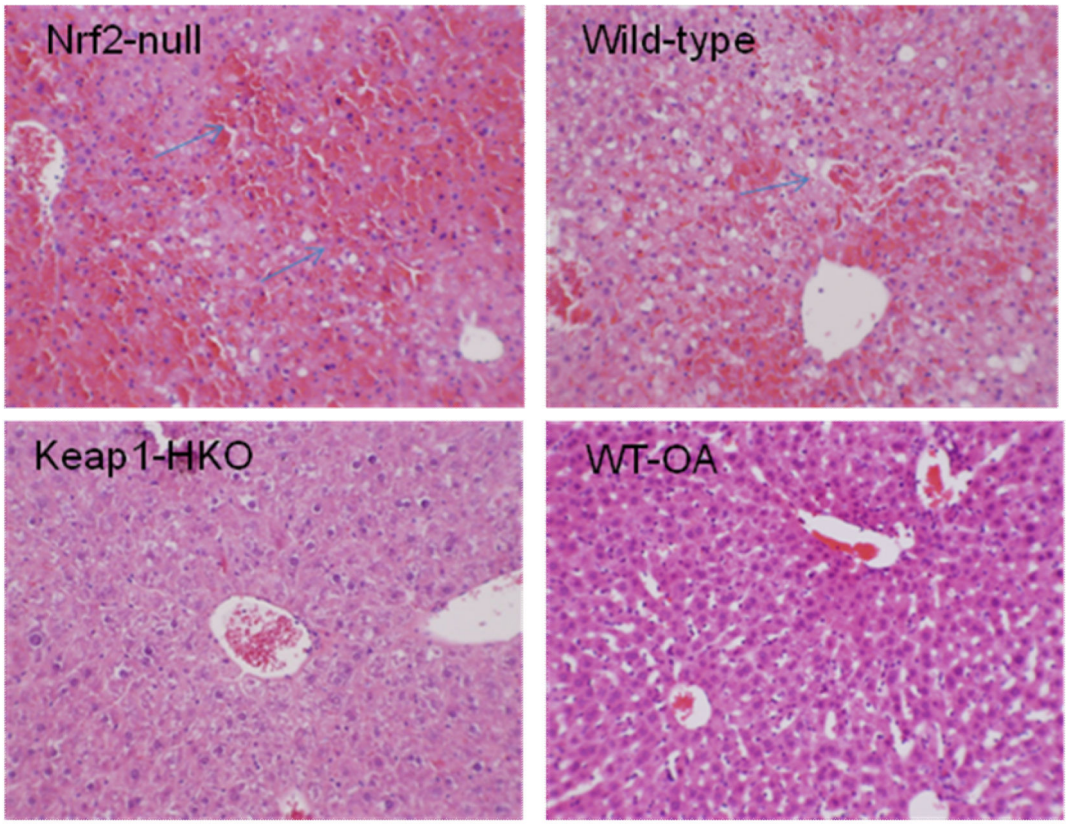

Fig. 2.

Histological analysis of phalloidin-treated livers (1.5 mg/kg, ip) from Nrf2-null, wild-type, Keap1-HKO, and WT mice pretreated with oleanolic acid (22.5 mg/kg, sc for 3 days). Left panel, low magnification (100 ×). Right panel, high magnification (200 ×). Arrows indicates hemorrhage and inflammation and arrowheads indicate hepatocellular necrosis. Magnitude is 200 ×.

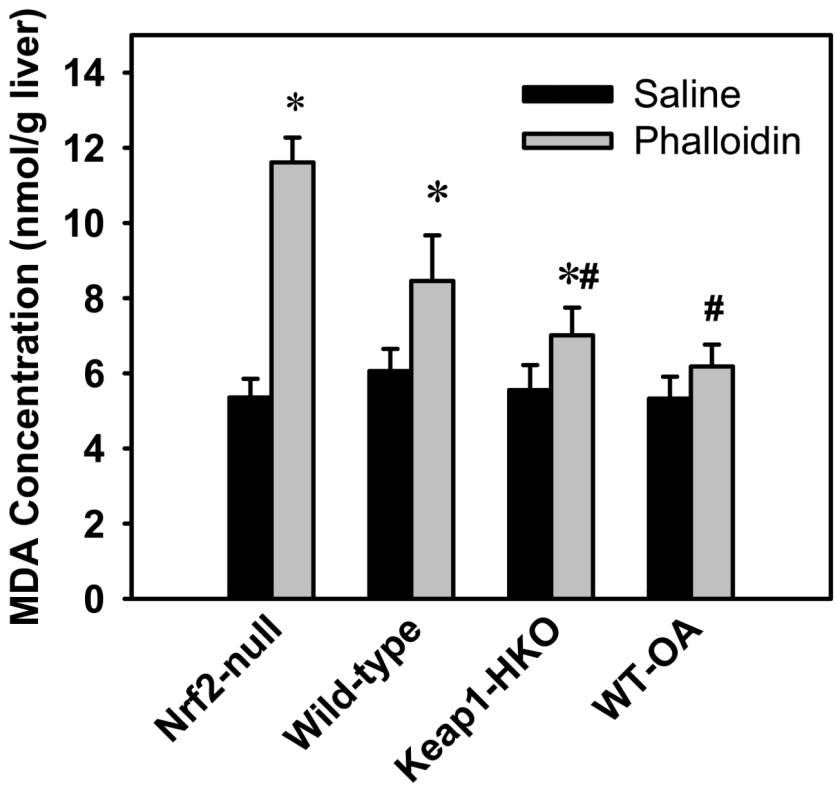

Fig. 3.

Lipid peroxidation in livers of Nrf2-null, wild-type, Keap1-HKO, and WT mice pretreated with oleanolic acid (22.5 mg/kg, sc for 3 days), as determined by thiobarbiturate reactive substances, using malondialdehyde (MDA) as the standard. Values are expressed as mean ± S.E.M. (n=8-10). *Significantly different from the basal level of the same genotype ($p \leq 0.05$); #Significantly different from Nrf2-null mice ($p \leq 0.05$).

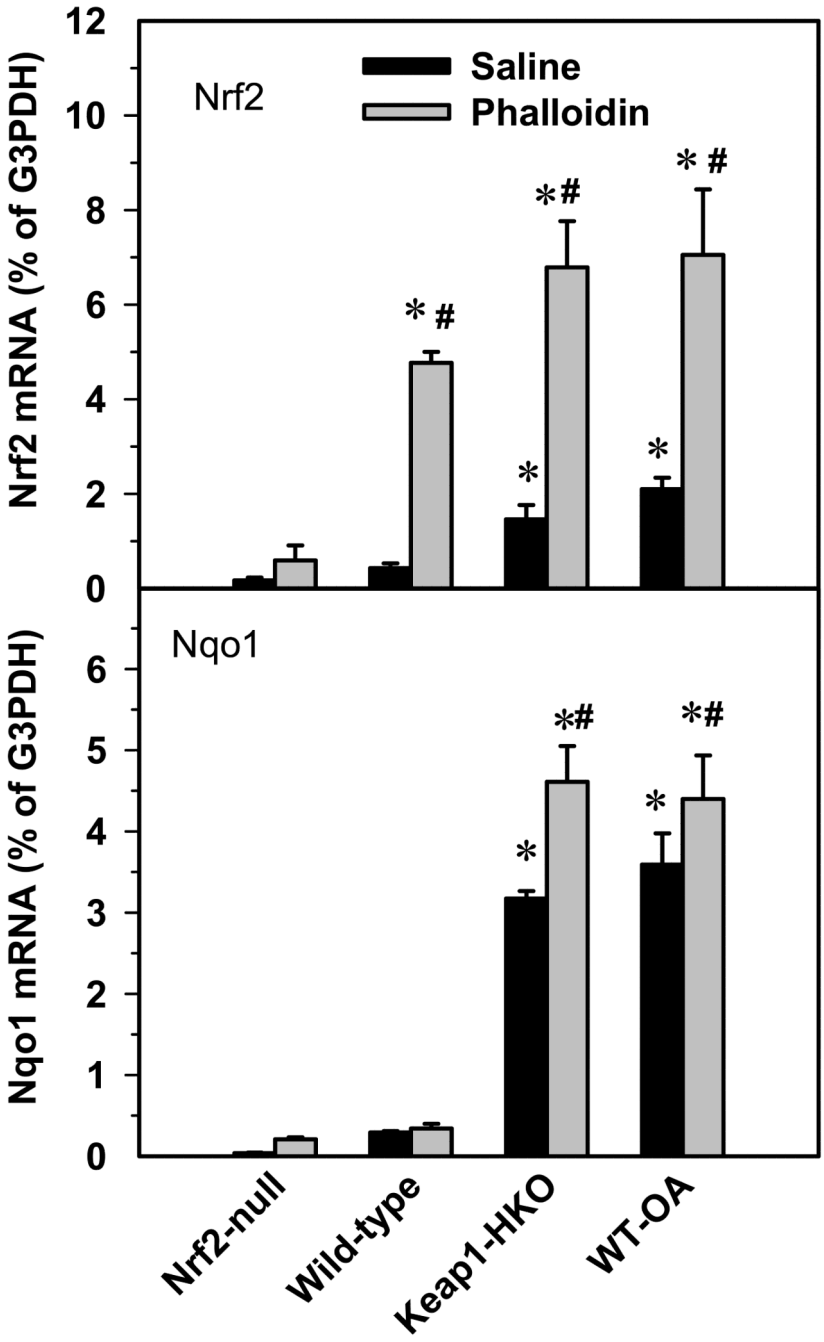

Fig. 4.

Relative transcript levels of NAD(P)H quinone oxidoreductase 1 (Nqo1) and Nrf2 in Nrf2-null, wild-type, Keap1-HKO and OA-pretreated mice. Mice were administered saline (10 ml/kg, i.p.) or phalloidin (1.5 mg/kg, i.p.) for 8 hrs. Values are expressed as mean ± S.E.M. (n=8-10). *Significantly different from the basal level of the same genotype ($p \leq 0.05$); #Significantly different from Nrf2-null mice ($p \leq 0.05$).

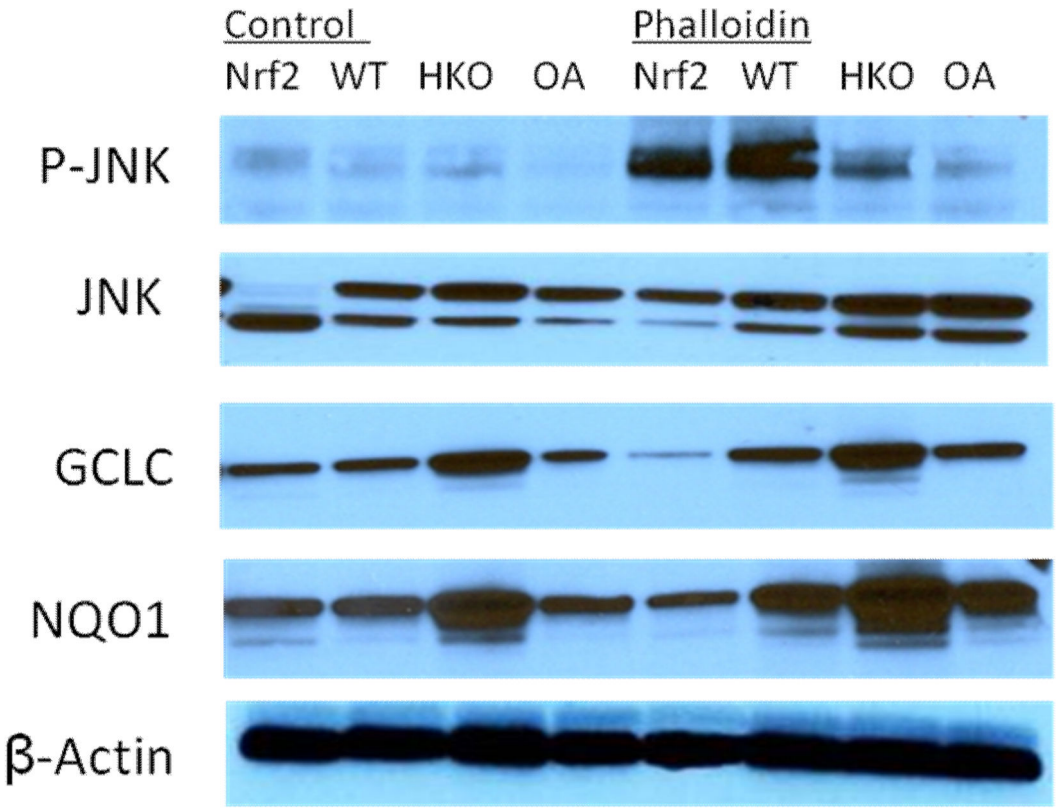

Fig. 5.

Representative Western-blot analysis. Mice were administered saline (10 ml/kg, i.p.) or phalloidin (1.5 mg/kg, i.p.). Liver proteins were subjected to western-blot analysis. The expression of Nrf2-targeted protein Nqo1 and Gclc were higher in Keap1-HKO mice and in mice pretreated with OA at both basal levels and after phalloidin. Phalloidin activated p-JNK in Nrf2-null and wild-type mice but not in Keap1-HKO and OA-pretreated mice.

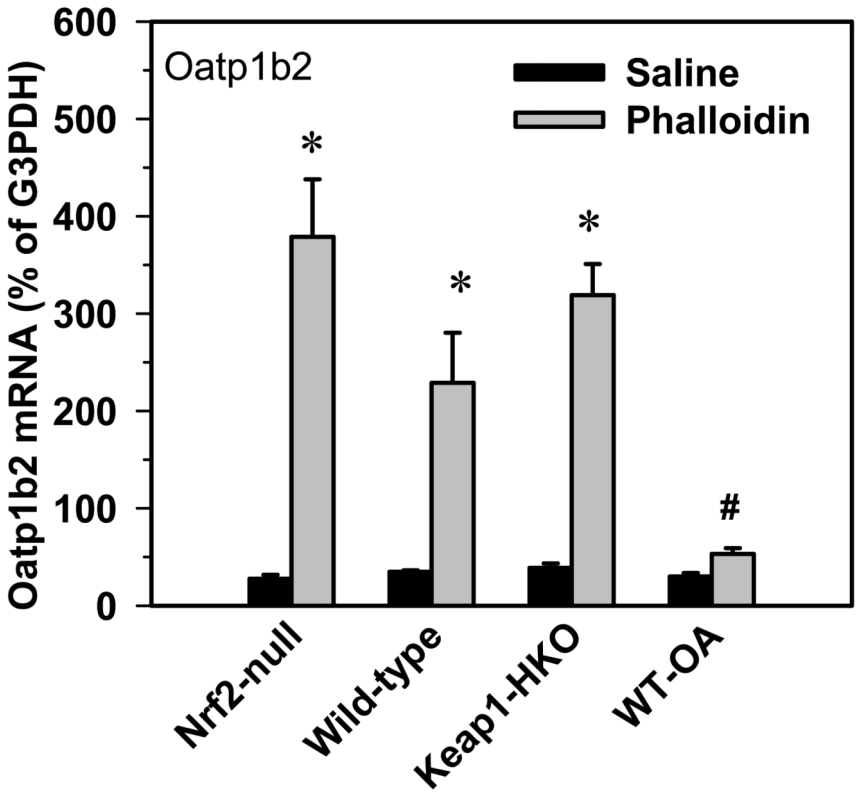

Fig. 6.

Relative transcript levels of organic anion transporting peptide 1b2 (Oatp1b2) in Nrf2-null, wild-type, Keap1-HKO, and OA-pretreated mice. Mice were administered saline (10 ml/kg, i.p.) or phalloidin (1.5 mg/kg, i.p.). Values are expressed as mean ± S.E.M. (n=8-10). *Significantly different from the basal level of the same genotype ($p \leq 0.05$); #Significantly different from Nrf2-null mice ($p \leq 0.05$).

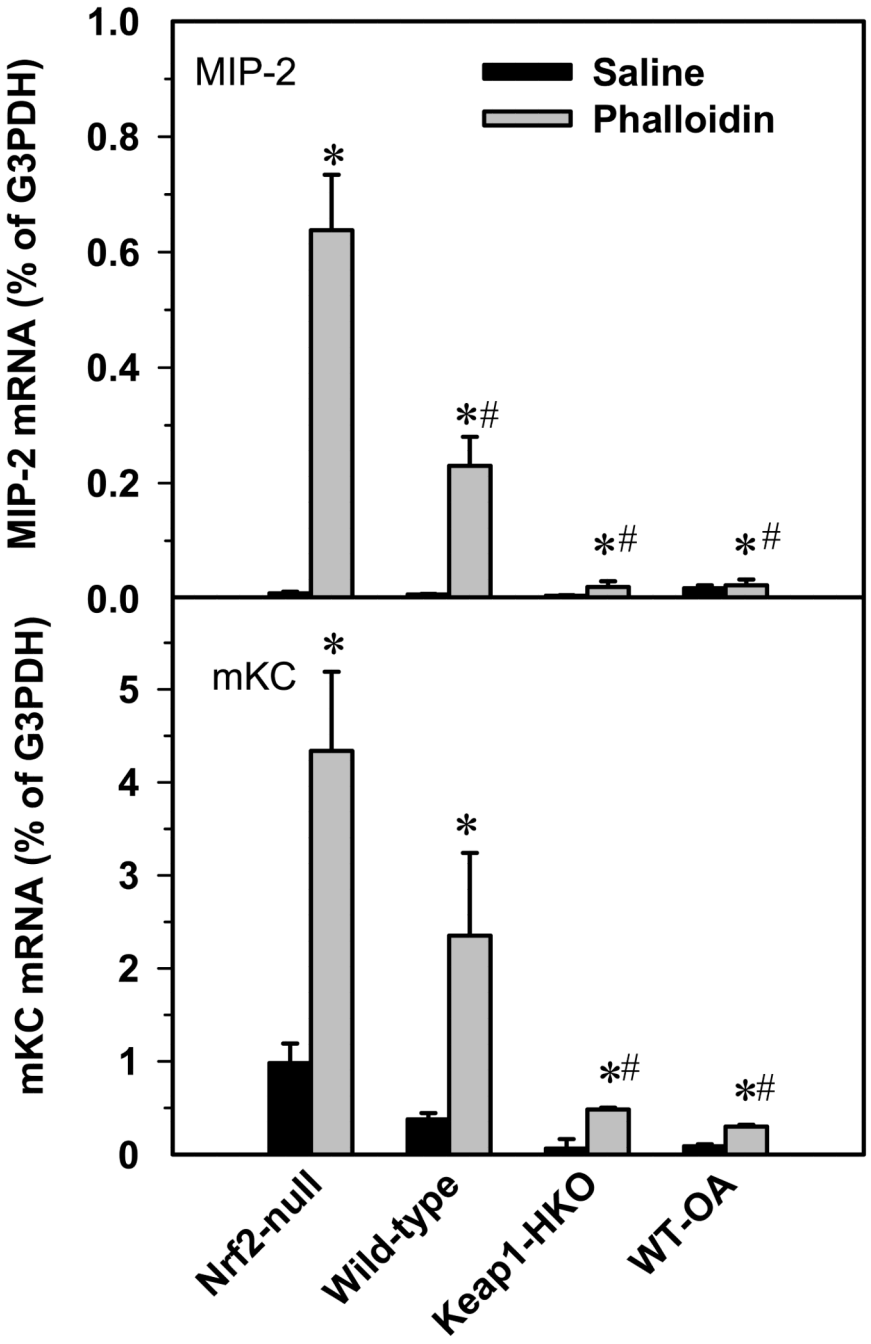

Fig. 7.

Relative transcript levels of neutrophil-specific chemokine macrophage inflammatory protein 2 (MIP-2) and mouse keratinocyte-derived chemokine (mKC) in Nrf2-null, wild-type, Keap1-HKO, and OA-pretreated mice. Mice were administered saline (10 ml/kg, i.p.) or phalloidin (1.5 mg/kg, i.p.). Values are expressed as mean ± S.E.M. (n=8-10). *Significantly different from the basal level of the same genotype ($p \leq 0.05$); #Significantly different from Nrf2-null mice ($p \leq 0.05$).

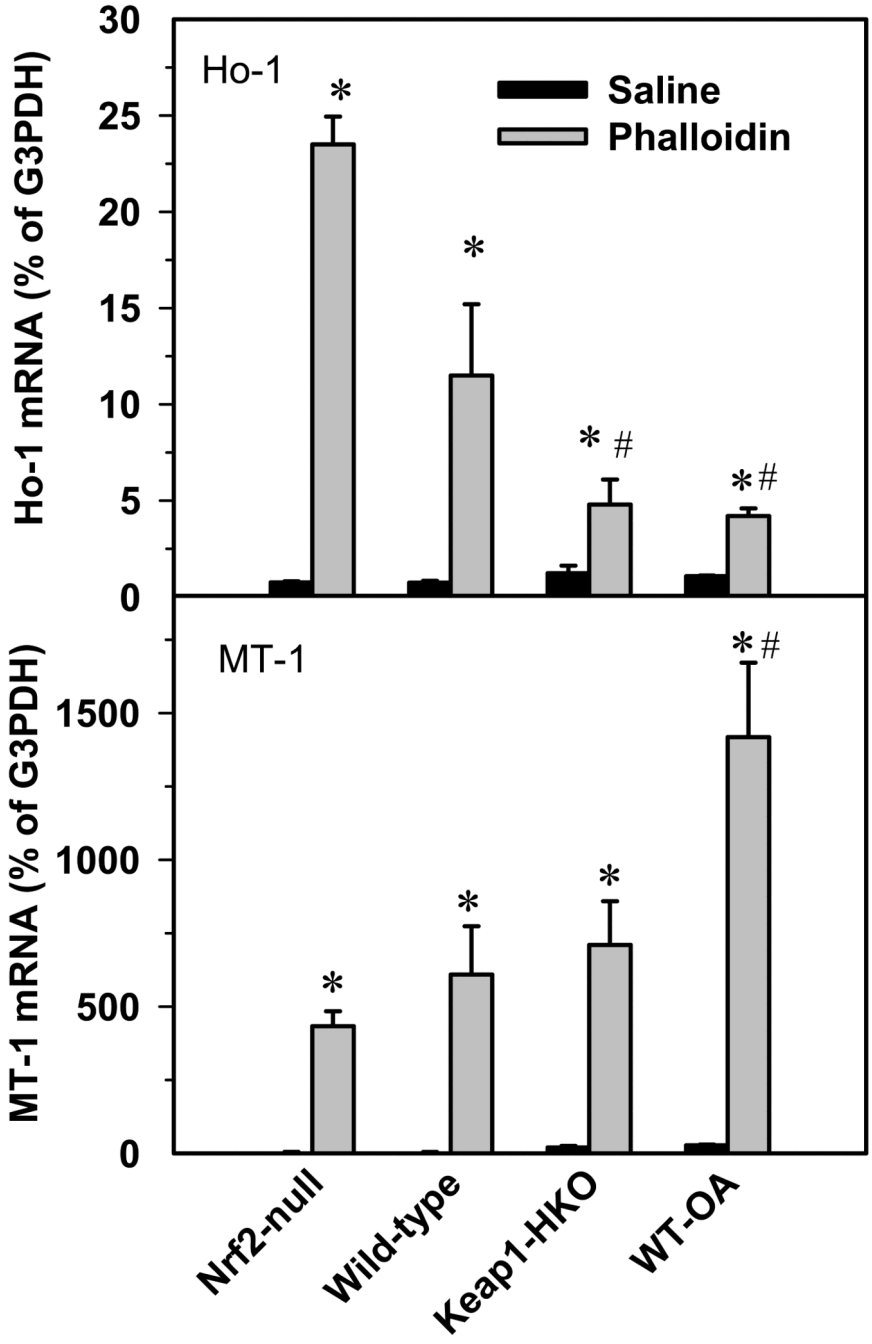

Fig. 8.

Relative transcript levels of stress protein gene heme oxygenase-1 (Ho-1) and metallothionein (MT-1) in Nrf2-null, wild-type, Keap1-HKO, and OA-pretreated mice. Mice were administered saline (10 ml/kg, i.p.) or phalloidin (1.5 mg/kg, i.p.) for 8 hrs. Values are expressed as mean ± S.E.M. (n=8-10). *Significantly different from the basal level of the same genotype ($p \leq 0.05$); #Significantly different from Nrf2-null mice ($p \leq 0.05$).

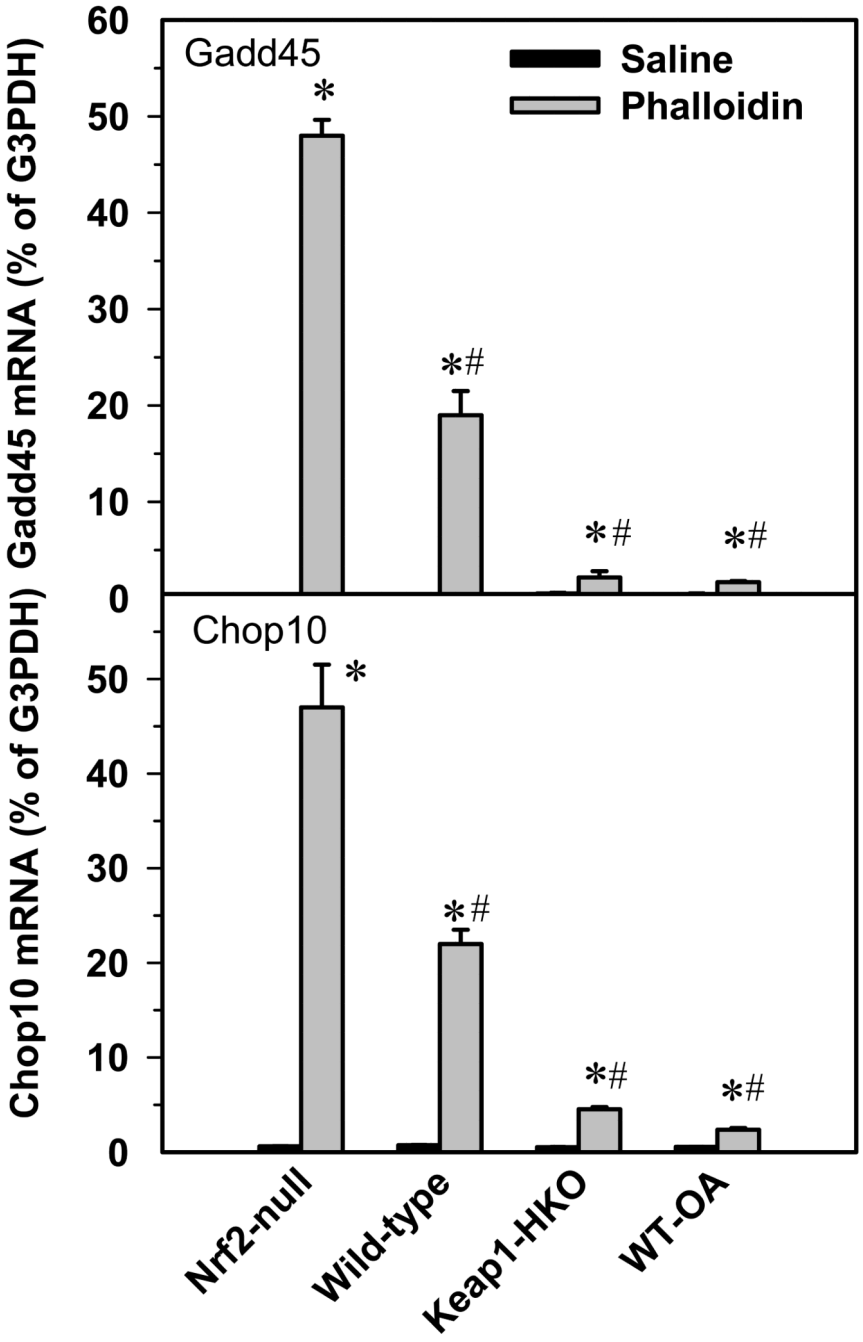

Fig. 9.

Relative transcript levels of ER stress responsive gene Gadd45 and Chop10 (Gadd153) in Nrf2-null, wild-type, Keap1-HKO, and OA-pretreated mice. Mice were administered saline (10 ml/kg, i.p.) or phalloidin (1.5 mg/kg, i.p.). Values are expressed as mean ± S.E.M. (n=8-10). *Significantly different from the basal level of the same genotype ($p \leq 0.05$); #Significantly different from Nrf2-null mice ($p \leq 0.05$).

Table 1

Primer Sequences

	GenBank		
Gene	**Number**	**Forward**	**Reverse**
Chop10	X67083	CTGCACCAAGCATGAACAGT	CTACCCTCAGTCCCCTCCTC
G3PDH	M32599	AACTTTGGCATTGTGGAAGG	GGATGCAGGGATGATGTTCT
Gadd45	NM_007836	TGAGCTGCTGCTACTGGAGA	TCCCGGCAAAAACAAATAAG
Gclc	BC019374	TGGCCACTATCTGCCCAATT	GTCTGACACGTAGCCTCGGTAA
HO-1	M33203	CCTCACTGGCAGGAAATCATC	CCTCGTGGAGACGCTTTACATA
IL-6	J03783	GCCCACCAAGAACGATAGTCA	GAAGGCAACTGGATGGAAGTCT
MIP2	NM_009140	CCTCAACGGAAGAACCAAAGAG	CTCAGACAGCGAGGCACATC
mKC	NM_008176	TGGCTGGGATTCACCTCAAG	GTGGCTATGACTTCGGTTTGG
MT-1	NM_013602	CTCCGTAGCTCCAGCTTCAC	AGGAGCAGCAGCTCTTCTTG
NQO1	BC004579	TATCCTTCCGAGTCATCTCTAGCA	TCTGCAGCTTCCAGCTTCTTG
Nrf2	BC026943	CGAGATATACGCAGGAGAGGTAAGA	GCTCGACAATGTTCTCCAGCTT
Oatp1b2	NM_020495	CAAACTCAGCATCCAAGCAA	GGCTGCCAAAAATATCCTGA