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MG-132 inhibits the TCDD-mediated induction of Cyp1a1 at the catalytic activity but not the mRNA or protein levels in Hepa 1c1c7 cells

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ABSTRACT

Previous studies have shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced degradation of aryl hydrocarbon receptor (AhR) is inhibited by MG-132, a potent inhibitor of the 26S proteasome. Therefore, the current study aims to address the effect of MG-132 on the AhR-regulated gene, cytochrome P450 1a1 (*Cyp1a1*), using murine hepatoma Hepa 1c1c7 cells. Our results showed that MG-132 at the highest concentration tested, 10 μM significantly increased the Cyp1a1 at mRNA, protein and catalytic activity levels through a transcriptional mechanism. On the other hand, MG-132 further potentiated the TCDD-mediated induction of Cyp1a1 at mRNA but not at protein level. In contrast, MG-132 significantly inhibited the TCDD-mediated induction of Cyp1a1 catalytic activity. In addition, we showed that the decrease in Cyp1a1 catalytic activity is not Cyp specific, as MG-132 significantly inhibited Cyp2b1 and total cytochrome P450 catalytic activities. These results prompted us to examine the effect of MG-132 on total cellular heme content and heme oxygenase-1 (HO-1) mRNA, a rate limiting enzyme of heme degradation. Our results showed that MG-132 significantly induced HO-1 mRNA in a concentration-dependent manner. Furthermore, MG-132 potentiated the induction of HO-1 mRNA by TCDD in a concentration-dependent manner. The induction of HO-1 mRNA level coincided with a decrease in total cellular heme content. In conclusion, the present study demonstrates for the first time that MG-132, despite of increasing Cyp1a1 mRNA expression, it decreases its activity probably through decreasing its heme content.

1. Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-activated basic helix–loop–helix (bHLH) protein that exists as cytoplasmic aggregates bound to two 90-kDa heat-shock proteins (HSP90), the 23-kDa heat-shock protein (p23), and hepatitis B virus X-associated protein 2 (XAP2). Upon ligand binding, the ligand–receptor complex dissociates from HSP90 and translocates to the nucleus where it associates with the aryl hydrocarbon receptor nuclear translocator (ARNT). The whole complex then acts as a transcription factor that binds to DNA promoter sequences termed xenobiotic responsive elements (XREs), thereby enhancing the transcription of an array of genes (Nebert and Duffy, 1997). Interest in the AhR rose with the discovery that some of these genes include members of the phase I and phase II drug metabolizing enzymes that are responsible for the metabolism of polycyclic aromatic hydrocarbons (PAHs) and other xenobiotics that also serve as ligands of the AhR. In essence, coordinate expression and activation of the AhR is a highly regulated process.

Cytochrome P450 1a1 (Cyp1a1) is an AhR-regulated gene with particular toxicological relevance. It metabolizes AhR ligands into highly reactive diol epoxide and quinone-containing compounds that result in the formation of covalent adducts when these genotoxic metabolites react with guanines in critical genes, potentially initiating tumorigenesis and other toxic responses (Spink et al., 2002).

Regulation of Cyp1a1 expression is primarily a transcriptional process that occurs through several XRE sequences which are located upstream of the transcription start site in all mammalian *Cyp1a1* genes (Hines et al., 1988). As would be postulated, inhibition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced degradation of the AhR by MG-132 superinduces Cyp1a1 mRNA expression (Ma et al., 2000).

Despite its reported induction of Cyp1a1 mRNA (Song and Pollenz, 2002), MG-132 has been reported to act as an antiproliferative agent. MG-132 caused cell cycle arrest and induced apoptosis in human prostate cancer cells (Shimada et al., 2006) and various hepatocellular carcinoma cell lines (Saarikoski et al., 2005). The mechanism of MG-132-induced apoptosis is not known.

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In addition, although the effect of MG-132 on the mRNA expression of Cyp1a1 is clearly established, there are no reports detailing its effect on Cyp1a1 activity. Herein, we show for the first time that despite causing superinduction of Cyp1a1 mRNA in the presence of TCDD, MG-132 significantly and effectively inhibited Cyp1a1 activity in murine Hepa 1c1c7 cells.

2. Materials and methods

2.1. Materials

7-Ethoxyresorufin, fluorescamine, anti-goat IgG peroxidase secondary antibody, and protease inhibitor cocktail were purchased from Sigma Chemical Co. (St. Louis, MO). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, >99% pure, was purchased from Cambridge Isotope Laboratories (Woburn, MA). TRIzol reagent was purchased from Invitrogen (Grand Island, NY). Carbobenzoxy-l-leucyl-l-leucyl-leucinal (MG-132) was purchased from Calbiochem (San Diego, CA). Chemiluminescence Western blotting detection reagents were from GE Healthcare Life Sciences (Piscataway, NJ). Nitrocellulose membrane was purchased from Bio-Rad Laboratories (Hercules, CA). Cyp1a1 goat polyclonal and Gapdh rabbit polyclonal primary antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). All other chemicals were purchased from Fisher Scientific (Toronto, Ont.).

2.2. Biohazard precaution

TCDD is toxic and a likely human carcinogen. All personnel were instructed as to safe handling procedures. Lab coats, gloves, and masks were worn at all times, and contaminated materials were collected separately for disposal by the Office of Environmental Health and Safety at the University of Alberta.

2.3. Cell culture

Hepa 1c1c7 cells, generously provided by Dr. O. Hankinson at the University of California (Los Angeles, CA), were maintained in Dulbecco's Modified Eagle's Medium (DMEM), without phenol red, supplemented with 10% heat-inactivated fetal bovine serum, 20 μM l-glutamine, 50 μg/ml gentamicin sulfate, 100 IU/ml penicillin, 10 μg/ml streptomycin, 25 ng/ml amphotericin B, 0.1 mM non-essential amino acids, and vitamin supplement solution. Cells were grown in 75-cm^2 cell culture flasks at 37 °C in a 5% CO_2 humidified incubator.

2.4. Chemical treatments

Cells were treated in serum-free medium with 1 or 10 μM MG-132 in the presence and absence of 1 nM TCDD, as described in figure legends. TCDD and MG-132 were dissolved in dimethylsulfoxide (DMSO) and maintained in DMSO at −20 °C until use. In all treatments, the DMSO concentration did not exceed 0.05% (v/v).

2.5. RNA extraction and real-time PCR

After incubation with the test compounds for the specified time periods, total cellular RNA was isolated using TRIzol reagent, according to manufacturer's instructions (Invitrogen), and quantified by measuring the absorbance at 260 nm. For reverse transcription-polymerase chain reaction (RT-PCR), first-strand cDNA was synthesized from 1.0 μg of total RNA using the High-Capacity cDNA Reverse Transcription kit with random primers (Applied Biosystems). Real-time PCR reactions were performed on an ABI 7500 real-time PCR system (Applied Biosystems), using SYBR® Green PCR Master Mix (Applied Biosystems). The amplification reactions were performed as follows: 10 min at 95 °C, and 40 cycles of 94 °C for 15 s and 60 °C for 1 min. Primers and probes for mouse Cyp1a1 were: Forward primer 5′-GGT TAA CCA TGA CCG GGA ACT-3′, reverse primer 5′-TGC CCA AAC CAA AGA GAC TGA-3′. Heme oxygenase-1 (HO-1): forward primer 5′-GTG ATG GAG CGT CCA CAG C-3′, reverse primer 5′-TGG TGG CCT CCT TCA AGG-3′ and for β-actin: forward primer 5′-TAT TGG CAA CGA GCG GTT CC-3′, reverse primer 5′-GGC ATA GAG GTC TTT ACG GAT GTC-3′ were purchased from Integrated DNA Technologies (IDT, Coralville, IA). The fold change in the level of Cyp1a1 or HO-1 (target genes) between treated and untreated cells, corrected by the level of ß-actin, was determined using the following equation: Fold change = $2^{-\Delta(\Delta Ct)}$, where $\Delta Ct = Ct_{(target)} - Ct_{(ß\text{-}actin)}$ and $\Delta(\Delta Ct) = \Delta Ct_{(treated)} - \Delta Ct_{(untreated)}$.

2.6. Western blot analysis

Twenty-four hours after incubation with the test compounds, cells were collected in lysis buffer containing 50 mM HEPES, 0.5 M sodium chloride, 1.5 mM magnesium chloride, 1 mM EDTA, 10% (v/v) glycerol, 1% Triton X-100, and 5 μl/ml of protease inhibitor cocktail. The cell homogenates were obtained by incubating the cell lysates on ice for 1 h, with intermittent vortexing every 10 min, followed by centrifugation at 12,000 × *g* for 10 min at 4 °C. Proteins (25 μg) were resolved by denaturing electrophoresis, as described previously (Elbekai and El-Kadi, 2004). Briefly, the cell homogenates were dissolved in 1× sample buffer, boiled for 5 min, separated by 10% SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane. Protein blots were blocked for 24 h at 4 °C in blocking buffer containing 5% skim milk powder, 2% bovine serum albumin and 0.05% (v/v) Tween-20 in tris-buffered saline solution (TBS; 0.15 M sodium chloride, 3 mM potassium chloride, 25 mM Tris–base). After blocking, the blots were incubated with a primary polyclonal goat anti-mouse Cyp1a1 antibody for 2 h at room temperature or primary polyclonal rabbit anti-mouse Gapdh antibody for overnight at 4 °C in TBS containing 0.05% (v/v) Tween-20 and 0.02% sodium azide. Incubation with a peroxidase-conjugated rabbit anti-goat IgG secondary antibody for Cyp1a1 and Gapdh was carried out in blocking buffer for 1 h at room temperature. The bands were visualized with the enhanced chemiluminescence method according to manufacturer's instructions (Amersham, Arlington Heights, IL). The intensity of Cyp1a1 protein bands was quantified, relative to the signals obtained for Gapdh protein, using ImageJ software.

2.7. Determination of Cyp1a1 activity

The Cyp1a1-dependent 7-ethoxyresorufin-*O*-deethylase (EROD) activity was performed on intact, living cells as described previously (Elbekai and El-Kadi, 2004). Enzymatic activity was normalized for cellular protein content which was determined using a modified fluorescent assay (Lorenzen and Kennedy, 1993).

2.8. Transient transfection and luciferase assay

Hepa 1c1c7 cells were plated onto 6-well cell culture plates. Each well of cells was transfected with 4 μg of XRE-driven luciferase reporter plasmid pGudLuc1.1 using lipofectamine 2000 reagent according to the manufacturer's instructions (Invitrogen). Luciferase assay was performed according to the manufacturer's instructions (Promega) as described previously (Anwar-Mohamed and El-Kadi, 2008). Briefly, after incubation with test compounds for 24 h, cells were washed with PBS and a 200 μl of 1× lysis buffer (1× = 25 mM Tris-phosphate pH 7.8, 2 mM DTT, 2 mM EDTA, 10% glycerol, and 1% Triton X-100) was added into each well with continuous shaking for at least 20 min, then the content of each well was collected separately in 1.5 ml microcentrifuge tubes. The tubes were then centrifuged to precipitate cellular waste, 100 μl cell lysate was then incubated with 100 μl of stabilized luciferase reagent and luciferase activity was quantified using TD-20/20 luminometer (Turner BioSystems, Sunnyvale, CA).

2.9. Determination of Cyp2b1 and total cytochrome P450 activities

The Cyp2b1-dependent 7-pentoxyresorufin-*O*-deethylase (PROD) activity was performed on intact, living cells as described previously (Maheo et al., 1997). Enzymatic activity was normalized for cellular protein content which was determined using a modified fluorescent assay (Lorenzen and Kennedy, 1993).

Total cytochrome P450 activity was determined using a method described previously (Christou et al., 1990). In brief, Hepa 1c1c7 cells were plated onto 10 mm cell culture petridishes and treated MG-132 1 and 10 μM for 24 h. Thereafter, microsomal protein was isolated from pelleted Hepa 1c1c7 cells as described previously (Pottenger and Jefcoate, 1990). The protein concentration was measured using the method of Lowry (Lowry et al., 1951), and the total cytochrome P450 activity were measured using a modified method to measure the reduced-CO spectral absorbance.

2.10. Determination of total cellular heme content

Cellular heme content was determined by a modification of the method of Ward (Ward et al., 1984). After a 24 h incubation period with 1 and 10 μM MG-132 in the presence and absence of 1 nM TCDD, cells were pelleted and boiled in 2.0 M oxalic acid for 30 min followed by resuspension in cold PBS and centrifugation at 14,000 × *g* for 15 min. The supernatant was then removed and the fluorescence of protoporphyrin IX was assayed using the Eclipse fluorescence spectrophotometer (Varian Australia PTY LTD., Australia) using an excitation wavelength of 405 nm and an emission wavelength of 600 nm. Background was determined by measuring the fluorescence in the absence of cells. Cellular heme content was calculated as a percent of serum-free medium treated cells following normalization of cellular heme content with cellular protein, which was determined using the method of Lowry (Lowry et al., 1951).

2.11. Statistical analysis

All results are presented as mean ± S.E. The comparison of the results from the various experimental groups and their corresponding controls was carried out by a one way analysis of variance (ANOVA) followed by Student–Newman–Keuls post hoc comparison or unpaired *t*-test, when applicable. The differences were considered significant when $P < 0.05$.

3. Results

3.1. Effect of MG-132 on Cyp1a1 mRNA and protein levels

To verify the effect of MG-132 on Cyp1a1 mRNA expression in Hepa1c1c7 cells, we analyzed Cyp1a1 expression in cells treated with MG-132 alone and in the presence of TCDD using quantitative real-time PCR. As expected, MG-132 at concentration of 10 μM, significantly increased Cyp1a1 at mRNA level (Fig. 1). Treatment with MG-132 also potentiated the induction of Cyp1a1 mRNA by TCDD at both the low (1 μM) and high (10 μM) concentrations of MG-132. The induction of Cyp1a1 mRNA is true to the finding that MG-132 prevents the degradation of the AhR in Hepa 1c1c7 cells. Next, we determined the effect of MG-132 on constitutive and TCDD-induced Cyp1a1 protein expression. MG-132 (10 μM) alone was able to significantly increase Cyp1a1 at protein level. Contrary to the mRNA expression results, MG-132 did not potentiate Cyp1a1 protein levels (Fig. 2). Rather, the lower and higher concentrations of MG-132 caused a slight decrease in Cyp1a1 protein levels, although the decrease did not reach statistical significance.

3.2. The inhibition of Cyp1a1 activity by MG-132

With the effect of MG-132 on Cyp1a1 mRNA and protein levels, it was of interest to determine how these effects translate to catalytic activity. In agreement with the mRNA expression data, MG-132 alone induced Cyp1a1 activity, but only at the higher concentration (10 μM, Fig. 3A). Surprisingly however, pretreatment of Hepa 1c1c7 cells with the lower and higher concentrations of MG-132 significantly decreased TCDD-mediated induction of Cyp1a1 activity (Fig. 3A).

3.3. Transcriptional induction of Cyp1a1 gene by MG-132

The fact that MG-132, at the highest concentration tested (10 μM), increased the Cyp1a1 mRNA, protein and catalytic activity levels raised the question whether this effect is mediated by an AhR-dependent mechanism. Therefore, we investigated the effect of MG-132 on the AhR-dependent luciferase activity using transiently transfected Hepa 1c1c7 cells with the XRE-driven luciferase reporter gene. The advantage of this bioassay is that luciferase induction can occur only through the AhR; therefore, other mechanisms, such as mRNA stability that might influence EROD activity are excluded (Sinal and Bend, 1997). Intriguingly, we found that MG-132 alone significantly increased the XRE-dependent luciferase

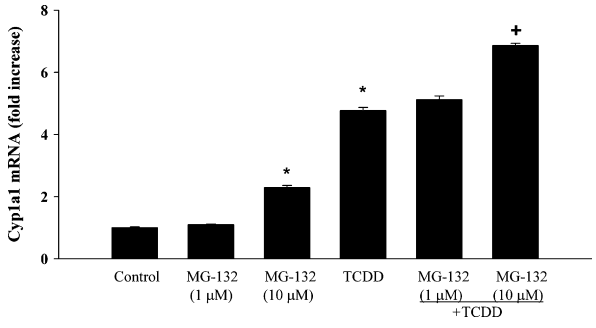

Fig. 1. Effect of MG-132 on Cyp1a1 mRNA levels. Hepa 1c1c7 cells were treated with MG-132 (1 and 10 μM) alone or in the presence of TCDD (1 nM) for 6 h. First-strand cDNA was synthesized from total RNA (1 μg) extracted from Hepa 1c1c7 cells. cDNA fragments were amplified and quantitated using ABI 7500 real-time PCR system as described under Section 2. Duplicate reactions were performed for each experiment, and the values presented are the means of three independent experiments. (*) $P < 0.05$, compared to control; (+) $P < 0.05$, compared to TCDD.

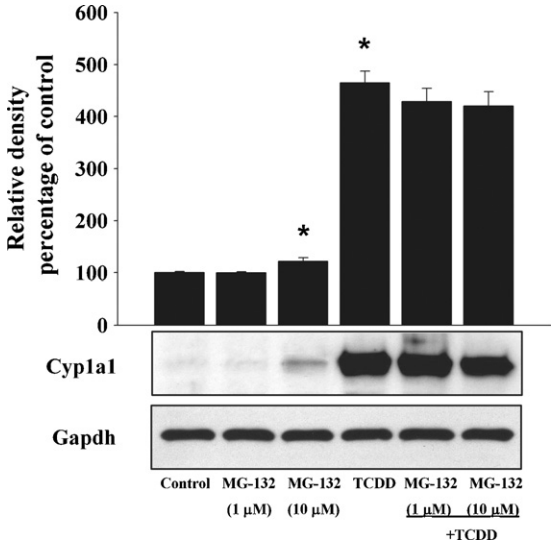

Fig. 2. Effect of MG-132 on Cyp1a1 protein levels. Hepa 1c1c7 cells were treated for 24 h with MG-132 (1 and 10 μM) in the presence of TCDD (1 nM). Protein (25 μg) was separated on a 10% SDS-PAGE and transferred to nitrocellulose membrane. Protein blots were then blocked overnight at 4 °C and then incubated with a primary Cyp1a1 antibody for 2 h at 4 °C, followed by 1 h incubation with secondary antibody at room temperature. Cyp1a1 protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to Gapdh signals, which was used as loading control. One of three representative experiments is shown. (*) $P < 0.05$, compared to control; (+) $P < 0.05$, compared to TCDD.

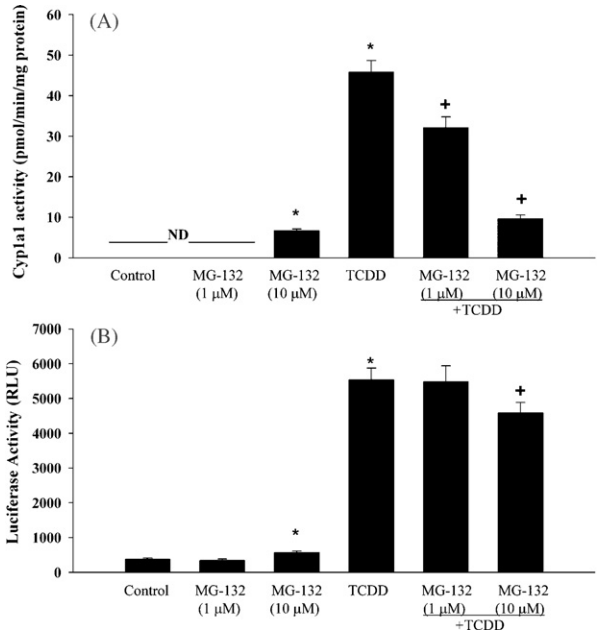

Fig. 3. Effect of MG-132 on Cyp1a1 catalytic activity, and luciferase activity. (A) EROD activity was measured in intact living cells treated with MG-132 (1 and 10 μM) in the presence and absence of TCDD (1 nM) for 24 h. Cyp1a1 activity was measured using 7-ethoxyresorufin as a substrate. Values are presented as mean ± S.E. ($n = 8$). (*) $P < 0.05$, compared to control; (+) $P < 0.05$, compared to TCDD. (B) Hepa 1c1c7 cells were transiently transfected with the XRE-luciferase transporter plasmid pGudLuc1.1. Cells were treated with MG-132 (1 and 10 μM) in the presence and absence of TCDD (1 nM) for 24 h. Cells were lysed and luciferase activity was measured according to manufacturer's instruction. Luciferase activity is reported as relative light unit. Values are presented as mean ± S.E. ($n = 4$). (*) $P < 0.05$, compared to control; (+) $P < 0.05$, compared to TCDD.

activity (Fig. 3B). On the other hand, pretreatment of Hepa 1c1c7 cells with the higher concentration of MG-132 slightly but significant decreased the TCDD-induced luciferase activity. This result is in concordance to the findings that MG-132 increases Cyp1a1 mRNA, protein, and catalytic activity levels, and further provides a novel mechanism by which MG-132 increases the *Cyp1a1* gene expression.

3.4. The inhibition of Cyp2b1 and total cytochrome P450 activities by MG-132

In order to examine whether the observed effect of MG-132 on the Cyp1a1 activity is Cyp specific, we determined the effect of MG-132 on the Cyp2b1 and total cytochrome P450 activities in Hepa 1c1c7 cells. Our results showed that MG-132 alone significantly decreased the constitutive Cyp2b1 catalytic activity (Fig. 4A). TCDD alone significantly increased the Cyp2b1 catalytic activity. In addition, MG-132 at the concentration of 1 and 10 μM significantly decreased the TCDD-mediated induction of Cyp2b1 catalytic activity (Fig. 4A). Furthermore, MG-132 significantly decreased the total cytochrome P450 activity in a dose-dependent manner (Fig. 4B). These results demonstrate that the inhibitory effect of MG-132 on Cyp enzymes is not Cyp specific.

3.5. Effect of MG-132 on heme metabolism

In an attempt to explain the decrease in Cyp1a1, Cyp2b1, and total cytochrome P450 catalytic activities by MG-132, we sought to determine its effect on heme metabolism. Cyp enzymes are heme containing proteins and the absence of which results in loss of protein function. We first measured the expression of heme

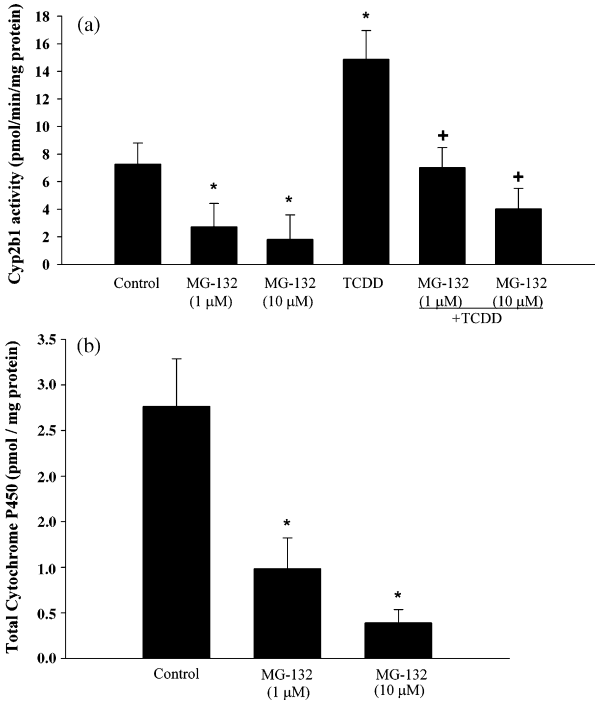

Fig. 4. Effect of MG-132 on Cyp2b1 and total cytochrome P450 activities. (A) PROD activity was measured in intact living cells treated with MG-132 (1 and 10 μM) in the presence and absence of TCDD (1 nM) for 24 h. Cyp2b1 activity was measured using 7-pentoxyresorufin as a substrate. Values are presented as mean ± S.E. ($n = 8$). (*) $P < 0.05$, compared to control; (+) $P < 0.05$, compared to TCDD. (B) Cells were treated with MG-132 (1 and 10 μM) alone for 24 h. The total cytochrome P450 activity was measured spectrophotometrically as described under Section 2. (*) $P < 0.05$, compared to control.

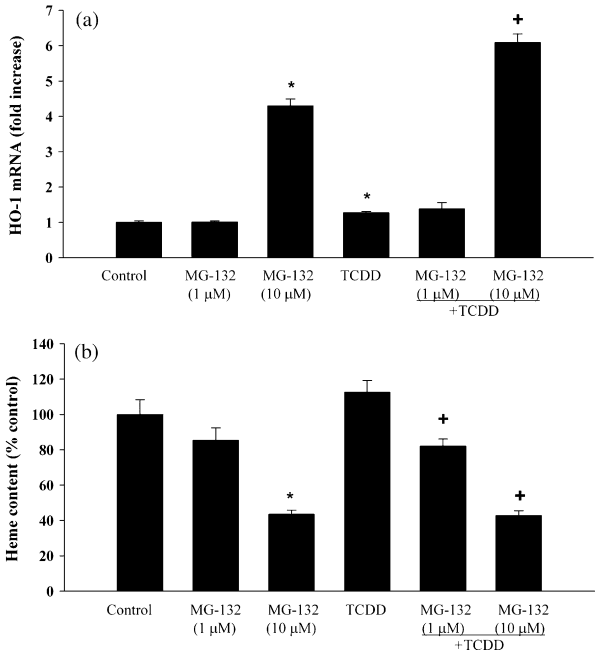

Fig. 5. Effect of MG-132 on HO-1 mRNA and cellular heme content. (A) The effect of MG-132 on HO-1 mRNA levels in Hepa 1c1c7 cells. Cells were treated with MG-132 (1 and 10 μM) alone or in the presence of TCDD (1 nM) for 6 h. First-strand cDNA was synthesized from total RNA (1 μg) extracted from Hepa1c1c7 cells. cDNA fragments were amplified and quantitated using ABI 7500 real-time PCR system as described under Section 2. Duplicate reactions were performed for each experiment, and the values presented are the means of three independent experiments. (*) $P < 0.05$, compared to control; (+) $P < 0.05$, compared to TCDD. (B) Total heme content in Hepa 1c1c7 cells treated with 1 and 10 μM MG-132 in the presence and absence of 1 nM TCDD for 24 h. The conversion of heme to protoporphyrin IX by oxalic acid was measured fluorometrically. Cellular heme content was calculated as a percent of serum-free medium treated cells following normalization with cellular protein. (*) $P < 0.05$, compared to control; (+) $P < 0.05$, compared to TCDD.

oxygenase-1 mRNA levels in Hepa 1c1c7 cells exposed to MG-132 in the presence and absence of TCDD. When cells were treated with MG-132 alone, the higher concentration caused a significant induction in HO-1 mRNA levels (Fig. 5A). In TCDD-only treated cells, HO-1 mRNA levels were slightly elevated, but were significantly potentiated when the cells were pretreated with 10 μM MG-132 (Fig. 5A). To assess the functional significance of an increase in HO-1 expression, we measured the effect of MG-132 on cellular heme content. In reflection with the HO-1 mRNA results, 10 μM MG-132 caused a significant decrease in heme content. Also, in the presence of TCDD, the 1 and 10 μM concentrations of MG-132 significantly decreased cellular heme content (Fig. 5B).

4. Discussion

In this study, we report for the first time an inhibition of Cyp1a1 activity by the proteasome inhibitor, MG-132 despite an increase in mRNA levels. The finding is significant to understanding the mechanisms underlying the antiproliferative and anticancer effects of MG-132 (Pajonk et al., 2005).

Undoubtedly, the increase in Cyp1a1 mRNA by MG-132 is a consequence of the inhibition of AhR degradation and subsequent increase in the AhR protein half-life (Davarinos and Pollenz, 1999). AhR ligands act as negative regulators of AhR expression to inhibit detrimental effects of prolonged AhR activation. Western blot analysis has demonstrated that the amount of the AhR is maximal 1 h after treatment with the potent AhR ligand, TCDD, decreasing significantly thereafter (Davarinos and Pollenz, 1999). In liver and non-liver cell culture models, TCDD decreased AhR protein levels by

80% to >95% within 4 h of treatment (Pollenz, 2002). Furthermore, the AhR protein is decreased in multiple organs of rats following exposure to TCDD (Pollenz et al., 1998).

The regulated ligand-induced degradation of the AhR is accomplished by the ubiquitin-mediated 26S proteasome degradation pathway following cytoplasmic protein complex dissociation and nuclear translocation. In fact, MG-132 prevented the disappearance of the AhR protein in whole cell and nuclear extracts after treatment with TCDD and significantly increased AhR/ARNT complex binding to the XRE (Davarinos and Pollenz, 1999).

In the current study we demonstrated that MG-132 increased Cyp1a1 mRNA, protein, and catalytic activity levels. Furthermore, MG-132 did not alter the TCDD-mediated induction of Cyp1a1 at protein levels whereas; the catalytic activity was significantly decreased. The fact that MG-132 alone increased Cyp1a1 mRNA, protein, and catalytic activity levels prompted us to investigate whether, this induction is AhR-dependent or not. Therefore, we examined the effect of MG-132 on Cyp1a1 promoter activity. Our results demonstrated that MG-132 induced the activation of the AhR-driven luciferase activity. In agreement with our results, it has been previously shown that MG-132 is able to increase the cytosolic transformation of AhR and the subsequent activation of the XRE (Santiago-Josefat et al., 2001). Thus, we conclude that MG-132 increases the *Cyp1a1* gene expression through a transcriptional mechanism. The fact that MG-132 significantly decreased the TCDD-mediated induction of the XRE-dependent luciferase activity suggests that both compounds compete for the AhR and provide further evidence for the AhR-dependent mechanism for the MG-132 mediated effect.

Since MG-132 was able to decrease the Cyp1a1 catalytic activity, we investigated whether MG-132-mediated effect is Cyp specific. Therefore, we examined the effect of MG-132 on the Cyp2b1 and total cytochrome P450 activities. Our results show that MG-132 decreased both Cyp2b1 and total cytochrome P450 activities suggesting that MG-132 decreased all Cyp probably through affecting their active sites.

The decrease in Cyp1a1 and Cyp2b1 activities by MG-132 is also a consequence of proteasome inhibition. MG-132 increased HO-1 expression and significantly decreased heme content that resulted in the formation of hollow Cyp1a1 and Cyp2b1 proteins. HO, an enzyme of 32 kDa, catalyzes the oxidative conversion of heme into biliverdin which serves an important role in protecting cells from oxidative damage, such as free radicals (Marilena, 1997). The HO-1 isozyme, the major isoform, is inducible in response to oxidative stimuli and offers protection against further cellular injury in the presence of ROS (Takahashi et al., 2004). HO-1 expression is regulated by several transcription factors, including the nuclear factor erythroid 2-related factor 2 (Nrf2) protein. Nrf2 is a member of the Cap'n'Collar family of basic region-leucine zipper (bZIP) transcription factors whose activity is tightly controlled by the negative regulator Kelch-like ECH associating protein 1 (Keap1) (Jaiswal, 2000; Nioi et al., 2003). Keap1 is a cytoplasmic factor that interacts with the actin cytoskeleton and Nrf2, thus retaining Nrf2 in the cytoplasm. In addition, Keap1 targets Nrf2 to proteasomal degradation through the ubiquitin pathway (Nioi et al., 2003). Thus, inhibition of proteasome degradation by MG-132 results in increased levels of Nrf2 and subsequent activation of HO-1 transcription.

Although the correlation between the inhibition of Cyp1a1 activity and the anti-cancer effects of MG-132 is not clear, the link between these two effects is quite evident. Apart from its role in metabolizing xenobiotics into carcinogenic by-products, the level of Cyp1a1 activity has been correlated to cancer pathogenesis. The suppression of inducible Cyp1a1 has been associated with the up-regulation of Bax expression and apoptosis of alveolar cells (Ghanem et al., 2006), decrease in cell proliferation, and reduction of tumor formation in animal models (Roos et al., 1998).

In conclusion, we have shown that MG-132 induces Cyp1a1 mRNA but inhibits its activity by increasing HO-1 levels and decreasing heme content. The decrease in Cyp1a1 activity may provide a mechanistic explanation for the observed pro-apoptotic and anti-proliferative effects of MG-132.

Conflict of interest statement

There are no conflicts of interest.

Acknowledgments

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