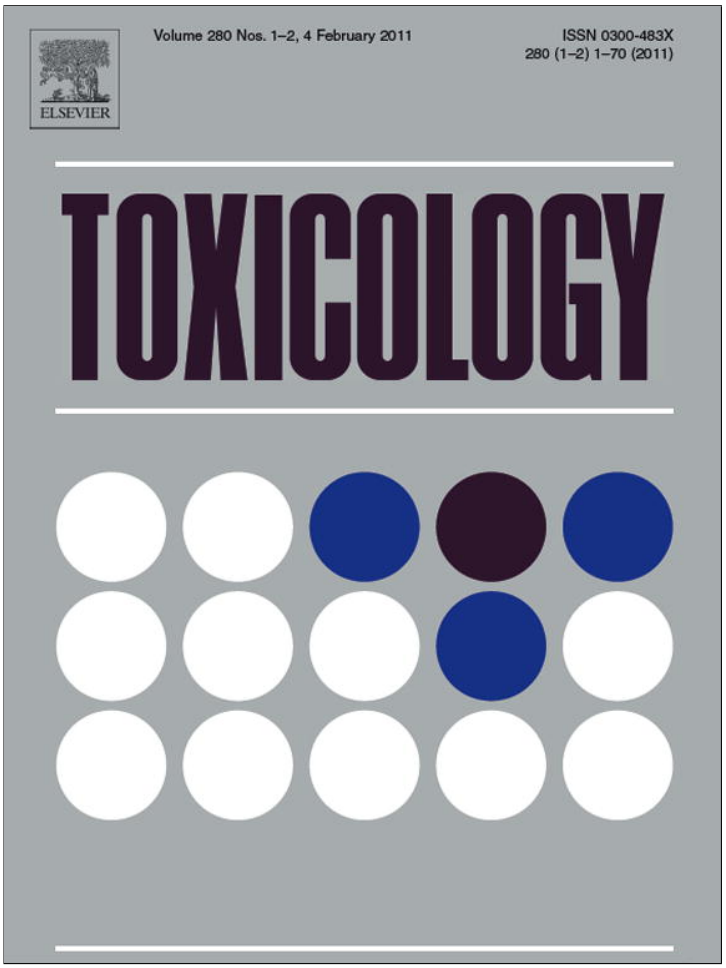

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Cellular glutathione content modulates the effect of andrographolide on β-naphthoflavone-induced CYP1A1 mRNA expression in mouse hepatocytes

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ABSTRACT

We previously reported that andrographolide (Andro), a major bioactive constituent of *Andrographis paniculata*, synergistically enhanced the inducible expression of CYP1A1 mRNA. In this study, although the synergism was confirmed at 24 h after the start of treatment with Andro and β-naphthoflavone (βNF), a CYP1A inducer, the expression was profoundly suppressed at an earlier phase, namely at 6–12 h, when the βNF-induced expression peaked. Although oxidized glutathione (GSSG) levels were higher in co-treated cells at 6 and 24 h, levels of reactive oxygen species varied depending on the treatment period and species, indicating no relation to the synergistic expression of CYP1A1 mRNA. Glutathione (GSH) and N-acetyl-L-cysteine (NAC) significantly enhanced the βNF-induced expression, and partly reversed the suppressive effect of Andro in the early phase. At 24 h, the addition of GSH or NAC had no effect on βNF-induced CYP1A1 mRNA expression, but significantly reduced the synergistic effect of Andro. The synergistic effect was enhanced by L-buthionine-(S,R)-sulfoximine, a GSH depleter. Furthermore, H_2O_2 and ascorbic acid further modified the profile of synergism of Andro on βNF-inducible CYP1A1 mRNA expression. These results suggest that GSH status might be involved in βNF-induced CYP1A1 mRNA expression, and the interaction of Andro with GSH might modulate the expression.

1. Introduction

Andrographolide (Andro: 3-[2-[decahydro-6-hydroxy-5-(hydroxyl methyl)-5,8a-dimethyl-2-methylene-1-naphthalenyl]ethylidene]dihydro-4-hydroxy-2(3H)-furanone) is a major diterpenoid constituent of the plant *Andrographis paniculata* Nees (Family Acanthaceae) (Singha et al., 2007), the extract of which is an important herbal medicine widely used in China, India and Southeastern Asian countries. The herb and Andro have been reported to have various biological activities (Jarukamjorn and Nemoto, 2008), including hepatoprotective (Singha et al., 2007), immunostimulatory (Xu et al., 2007), anti-thrombotic (Thisoda et al., 2006), anti-cancer (Sheeja and Kuttan, 2007), anti-diabetic (Reyes et al., 2006), anti-viral (Wiart et al., 2005) and anti-inflammatory (Shen et al., 2002) effects. Furthermore, the extracts of *Andrographis paniculata* and Andro have been used as health supplements in many countries.

Although several of the activities of *Andrographis paniculata* and Andro have been established, little toxicological information is available, especially on the drug–herb interaction related to cytochrome P450 (P450). P450 plays an important role in the pharmacology of drugs and toxicology of xenobiotics. Understanding how drugs or xenobiotics induce or inhibit P450 activity is biologically relevant and ultimately leads to better models for predicting the actions of these agents. After oral administration of the extract of *Andrographis paniculata*, levels of activities of the marker enzymes of CYP1A1 and CYP2B10 rose in mouse liver (Jarukamjorn et al., 2006). We observed that Andro by itself induced the expression of CYP1A1 mRNA in a concentration-dependent manner in mouse hepatocytes in primary culture, as did typical CYP1A inducers (Jaruchotikamol et al., 2007). Interestingly, Andro plus a typical CYP1A inducer synergistically induced CYP1A1 expression, and the synergism was inhibited by an aryl hydrocarbon receptor (AhR)-antagonist, resveratrol.

The gene expression of CYP1A1 is controlled by the activation of AhR upon the binding of a ligand. Several modifiers of the expression are known. For example, the mRNA expression is depressed

Abbreviations: AhR, aryl hydrocarbon receptor; Andro, andrographolide; APF, aminophenyl fluorescein; BHT, butylated hydroxytoluene; βNF, β-naphthoflavone; BSO, L-buthionine-(S,R)-sulfoximine; DAF-FM DA, diaminofluorescein-FM diacetate; DCF DA, 2′,7′-dichlorofluorescein diacetate; DMSO, dimethyl sulfoxide; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GSH, glutathione; GSSG, oxidized GSH; HPF, hydroxyphenyl fluorescein; NAC, N-acetyl-L-cysteine; P450, cytochrome P450; PI, propidium iodide; ROS, reactive oxygen species.

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by inflammatory cytokines and growth factors (Barker et al., 1992; Muntane-Relat et al., 1995) via a mechanism involving reactive oxygen species (ROS). Indeed, the expression of CYP1A1 mRNA was down-regulated by oxidative stress (Barker et al., 1994; Morel and Barouki, 1998). Park et al. (1996) suggested TCDD, a CYP1A1 inducer, to be an indirect genotoxicant, generating ROS in mouse hepatoma cells. Furthermore, Knerr et al. (2006) suggested CYP1 induction to be a major source of ROS in TCDD-treated hepatocytes.

Glutathione (GSH) is abundant in cells, especially in liver cells, and a major protective factor against oxidative stress. An important intracellular reductant, GSH is able to breakdown hydrogen peroxide (H_2O_2) and other oxidative stress inducers through a non-enzymatic process. Li et al. (2007) showed that Andro-treated cells over-produced H_2O_2 due to depletion of intracellular GSH, suggesting the depletion of GSH to be caused by the interaction between Andro and GSH (Zhang et al., 2008). Meanwhile, Andro increased intracellular GSH levels and protected cardiomyocytes against hypoxia/re-oxygenation injury (Woo et al., 2008), and also scavenged superoxide ($O_2^{\bullet -}$) as an anti-oxidant in HepG2 cells, a human hepatocellular carcinoma cell line (Li et al., 2007). Moreover, GSH levels and the activities of GSH reductase, GSH peroxidase, superoxide dismutase and catalase were significantly increased in Andro-supplemented mice (Trivedi et al., 2007).

Andro synergistically enhanced the expression of CYP1A1 mRNA induced by a ligand of AhR (Jaruchotikamol et al., 2007), however, the mechanism involved was unknown. Andro might regulate the intracellular redox status by altering GSH content or directly scavenging ROS. In the present study, we examined the effect of changes caused by Andro to intracellular GSH levels during the βNF-induced expression of CYP1A1 mRNA using mouse hepatocytes in primary culture.

2. Materials and methods

2.1. Materials

Materials for culturing hepatocytes were purchased from Gibco Invitrogen Corporation (Grand Island, NY), Wako Pure Chemical (Osaka, Japan) and Sigma Chemicals (St. Louis, MO). Percoll was obtained from GE Healthcare Bio-Sciences (Uppsala, Sweden). Andro, L-buthionine-(S,R)-sulfoximine (BSO), GSH, butylated hydroxytoluene (BHT), BES-H_2O_2-Ac, BES-So-AM, propidium iodide (PI) and LDH-cytotoxic Test Wako were from Wako Pure Chemical. β-Naphthoflavone (βNF), 2′,7′-dichlorofluorescein diacetate (DCF DA), and N-acetyl-L-cysteine (NAC) were obtained from Sigma Chemicals. Hydroxyphenyl fluorescein (HPF), aminophenyl fluorescein (APF) and diaminofluorescein-FM diacetate (DAF-FM DA) were obtained from Sekisui Medical (Tokyo, Japan). ReverTra Ace and G-Taq DNA polymerase were purchased from TOYOBO (Osaka, Japan) and Hokkaido System Science (Sapporo, Japan). The HT Glutathione Assay Kit and Accutase were obtained from Trevigen (Gaithersburg, MD) and Innovative Cell Technologies (San Diego, CA), respectively.

2.2. Animals

Seven-week-old male C57BL/6 or ddY mice were purchased from Sankyo Experimental Animals (Tokyo, Japan). Mice were housed in the Laboratory Animal Center of Toyama University under the supervision of certified laboratory animal veterinarians and treated according to a research protocol approved by the University's Institutional Animal Care and Use Committee.

2.3. Preparation of hepatocyte cultures and treatment

Mouse hepatocytes were prepared as described previously (Jaruchotikamol et al., 2007). Briefly, the cells were seeded at a density of 5×10^5 per dish on collagen-coated 35-mm Petri dishes and cultured at 37 °C and with 5% CO_2 in Waymouth MB 752/1 medium containing bovine serum albumin (2 g/l), insulin (0.5 mg/l), transferrin (0.5 mg/l), selenium (5 μg/l) and dexamethasone (10^{-9} M). The medium was renewed 3 h after seeding. βNF and Andro were dissolved in dimethylsulfoxide (DMSO), and BSO, NAC and GSH were dissolved in distilled water or medium. The treatment with the compounds (final concentration of DMSO: 0.2%) was performed 24 h after the seeding and total RNA was prepared at the time points indicated. The concentrations of compounds employed for the treatment were confirmed not to be toxic using the LDH-cytotoxic assay, according to the manufacturer's instructions.

2.4. Quantitative real-time RT-PCR

Mouse CYP1A1 and GAPDH mRNAs were quantified by real-time RT-PCR, as described previously (Jaruchotikamol et al., 2007). Briefly, hepatic total RNA was reverse-transcribed by ReverTraAce and cDNA was amplified by G-Taq DNA polymerase using specific TaqMan® Gene expression Detection Kits for *Cyp1a1*. Real-time PCR was performed using the ABI Prims 7000 Sequence Detection System (Applied Biosystems, Branchburg, NJ) with ABI SDS software. The amplified products of CYP1A1 and GAPDH were detected by monitoring the fluorescence of the reporter dye, FAM and SYBR Green, respectively. The values for the expression of CYP1A1 mRNA were normalized to those of GAPDH.

2.5. Staining of hepatocytes with DCF DA, BES-H_2O_2, BES-So, HPF, APF or DAF-FM DA, and PI, and analysis by flow cytometry

Mouse hepatocytes at 3 or 24 h after the start of treatment with βNF and/or Andro were washed with phosphate-buffered saline and stained with either DCF DA (5 μM), BES-H_2O_2-Ac (10 μM: H_2O_2 specific), BES-So-AM (10 μM: $O_2^{\bullet -}$ specific), HPF (5 μM: •OH/$ONOO^-$ specific), APF (5 μM: •OH/$ONOO^-$/OCl^- specific), or DAF-FM DA (5 μM: NO specific) for 30 min. After their detachment from the dish through Accutase treatment for 10–15 min at 37 °C, the cells were stained with propidium iodide (PI) for the determination of dead cells. Fluorescence was measured on the FACSCanto II and analyzed with FACSDiva software (Becton Dickinson Immunocytometry Systems, San Jose, CA). PI-negative cells were gated and the mean fluorescence intensity of the gated cells was calculated.

2.6. Measurement of intracellular GSH/GSSG content

Intracellular GSH/GSSG content was determined using the HT Glutathione Assay Kit, according to the manufacturer's instructions. Briefly, treated cells were scraped in 5% metaphosphoric acid and the suspension was sonicated. After centrifugation at 12,000 × *g* for 10 min, the supernatant was collected as cell lysate. Fifty microliter of each diluted lysate was added to a 96-well plate, followed by the further addition of 150 μl of the Reaction Mix. Coloring of the plate was immediately read at 405 nm in a microplate reader at 2-min intervals over a 10-min period. For the measurement of GSSG content, diluted cell lysate was treated with 4-vinylpiridine before addition of the reaction mix, and incubated for 1 h at room temperature. GSH or GSSG content was determined by comparing the net slopes of the samples with the slope of the standard GSSG curve.

2.7. Statistical analysis

Data are expressed as the mean ± SD. The comparison of the results from the various experimental groups was carried out with a one-way analysis of variance (ANOVA) followed by the Tukey *post hoc* test or the Student–Newman–Keuls method.

3. Results

3.1. Effect of Andro on the expression of CYP1A1 mRNA

Fig. 1 shows the expression of CYP1A1 mRNA in primary cultured hepatocytes of ddY mouse after treatment with either 10 μM βNF, 25 μM Andro, or βNF + Andro. The effect of βNF reached a maximum at around 9 h after which the expression decreased. Andro itself had a little effect, but βNF + Andro had a synergistic effect at 24 h as reported previously (Jaruchotikamol et al., 2007). Andro, however, considerably suppressed βNF-induced expression at 6–9 h. The bimodal effects of Andro on inducible CYP1A1 mRNA expression were also observed in primary culture of C57BL/6 mouse hepatocytes.

3.2. GSH/GSSG content after treatment with βNF, Andro, or βNF + Andro

After incubation for 24 h, levels of GSH were slightly higher and increased further in the presence of Andro (Fig. 2). GSSG was not detected in control cells at 6 and 24 h. Levels of both increased in the presence of βNF at 6 h, but had returned to control values at 24 h. Andro also increased GSSG levels and βNF + Andro synergistically enhanced the levels at both 6 and 24 h.

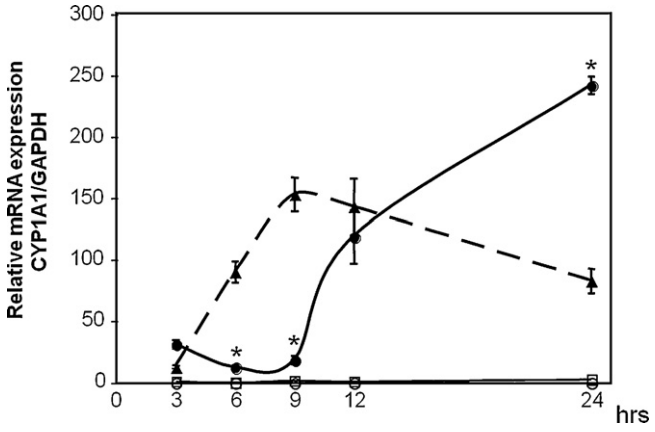

Fig. 1. Time course of CYP1A1 mRNA expression in mouse hepatocytes after combined treatment with βNF and Andro. ddY mouse hepatocytes were treated with DMSO (open circle), 10 μM βNF (closed triangle), 25 μM Andro (open square), or βNF + Andro (closed circle) and total RNA was prepared at the indicated times. The expression of CYP1A1 mRNA was determined by quantitative RT-PCR after normalization with that of GAPDH mRNA. Each point represents the mean ± SD ($n = 3$). * Represents a significant difference from the βNF group at $p < 0.01$ (one-way ANOVA, Tukey *post hoc* test).

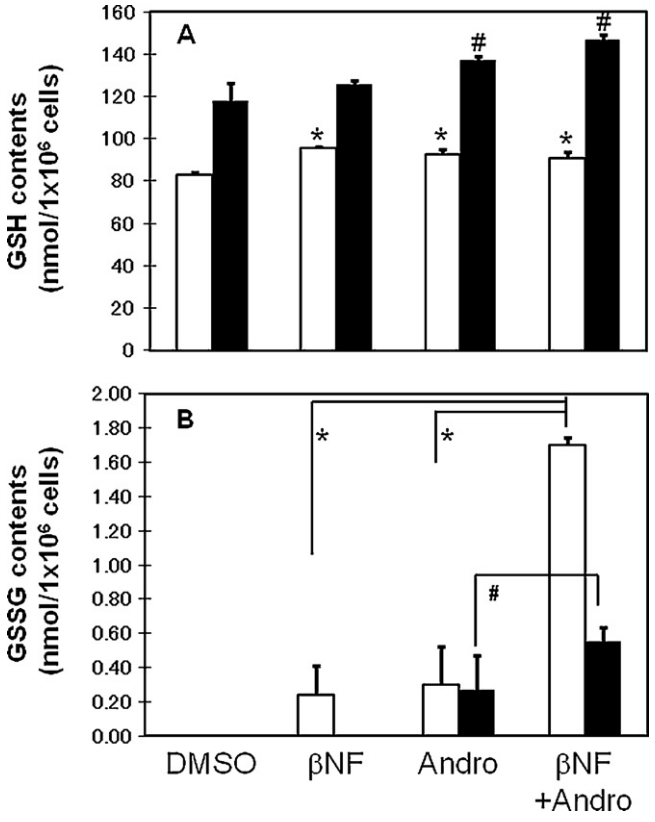

Fig. 2. GSH and GSSG levels in mouse hepatocytes treated with βNF and/or Andro. ddY mouse hepatocytes were treated with 10 μM βNF and/or 25 μM Andro and intracellular levels of GSH and GSSG at 6 h (open column) and 24 h (black column) were determined as described in Materials and methods. (A) Reduced GSH: calculated relative to that of DMSO-treated cells at 6 h (=1). (B) GSSG: calculated relative to that of βNF at 6 h (=1), but relative to that of Andro at 24 h (=1), because GSSG levels in DMSO-treated cells at 6 h or in DMSO- or βNF-treated cells at 24 h were too low to be detectable. Data are expressed as the mean ± SD ($n = 3$). * and #: Significant difference from the corresponding control at $p < 0.05$ at 6 and 24 h, respectively (one-way ANOVA, Tukey *post hoc* test).

3.3. Generation of ROS after treatment with βNF, Andro, or βNF + Andro

To investigate ROS status, mouse hepatocytes were stained with either DCF DA, BES-H_2O_2-Ac, BES-So-AM, HPF, APF, or DAF-FM DA at 3 and 24 h after the treatment and analyzed by flow cytometry (Fig. 3A–F) respectively. Marked production of ROS was observed in control hepatocytes, possibly in response to the preparation of primary culture. Mouse hepatocytes in primary culture were fragile, and most died during the process of detaching from collagen-coated dishes on treatment with trypsin and/or EDTA. However, Accutase was useful for detaching a high percentage (60–70%) of viable mouse hepatocytes. The staining reagents can permeate the cell membrane and are digested by esterase. Their digested forms react with ROS, resulting in the emission of fluorescence. Accordingly, ROS can be detected by measuring the fluorescence in living cells. Therefore, after staining with the above compounds on dishes and detachment using Accutase, the cells collected were further stained with propidium iodide (PI). DCF is widely used to detect various kinds of ROS due to its high sensitivity, but its specificity for individual ROS species is not so high. By contrast, BES-H_2O_2-Ac, BES-So-AM, HPF, APF and DAF-FM DA are relatively specific, targeting H_2O_2, $O_2^{\bullet -}$, $^{\bullet}OH + ONOO^-$, $^{\bullet}OH + ONOO^- + OCl^-$, and NO, respectively. At 3 h, levels detected by DCF were reduced in the cells treated with either βNF, Andro, or βNF + Andro, whereas BES-H_2O_2-Ac, BES-So-AM, HPF, APF and DAF-FM revealed no reduction. At 24 h, ROS levels detected either staining reagent showed a tendency of reduction in the treated cells. Although the patterns of change in ROS levels differed with the reagents used, no correlation with the effect of Andro on βNF-induced CYP1A1 mRNA expression dependent on the treatment period was found.

3.4. Effects of H_2O_2 and antioxidants on the βNF-induced expression of CYP1A1 mRNA modified by Andro

To observe the influence of oxidative stress, the hepatocytes were treated with either H_2O_2, or antioxidants, butylated hydroxytoluene (BHT) or ascorbic acid, together with βNF and/or Andro. H_2O_2 significantly increased βNF-induced and Andro-enhanced CYP1A1 mRNA expression at 24 h, but did not modify it at 9 h (Fig. 4A). Both antioxidants suppressed CYP1A1 expression at 9 h, but ascorbic acid at high concentration significantly increased the synergism of Andro at 24 h (Fig. 4B and C). Addition of BHT also showed an increasing tendency of the synergism, but the change was not significant.

3.5. Modification by GSH of the expression of CYP1A1 mRNA

The effects of changes in intracellular GSH contents caused by either GSH, NAC, or BSO on βNF-induced CYP1A1 mRNA expression were observed at early (9 h) and late (24 h) phases. NAC itself acts as an antioxidant and is a precursor of GSH. BSO is a potent and specific inhibitor of γ-glutamylcysteine synthetase, resulting in a reduction in intracellular GSH levels. In the early phase, NAC increased while GSH significantly increased βNF-induced CYP1A1 mRNA expression, and both increased the expression in βNF + Andro-treated cells. BSO decreased the expression in the βNF- or βNF + Andro-treated cells. In the late phase, BSO increased βNF-induced CYP1A1 mRNA expression, but GSH and NAC had no effect. In the presence of Andro, BSO further increased the expression, and GSH or NAC significantly attenuated the synergistic effect in a concentration-dependent pattern.

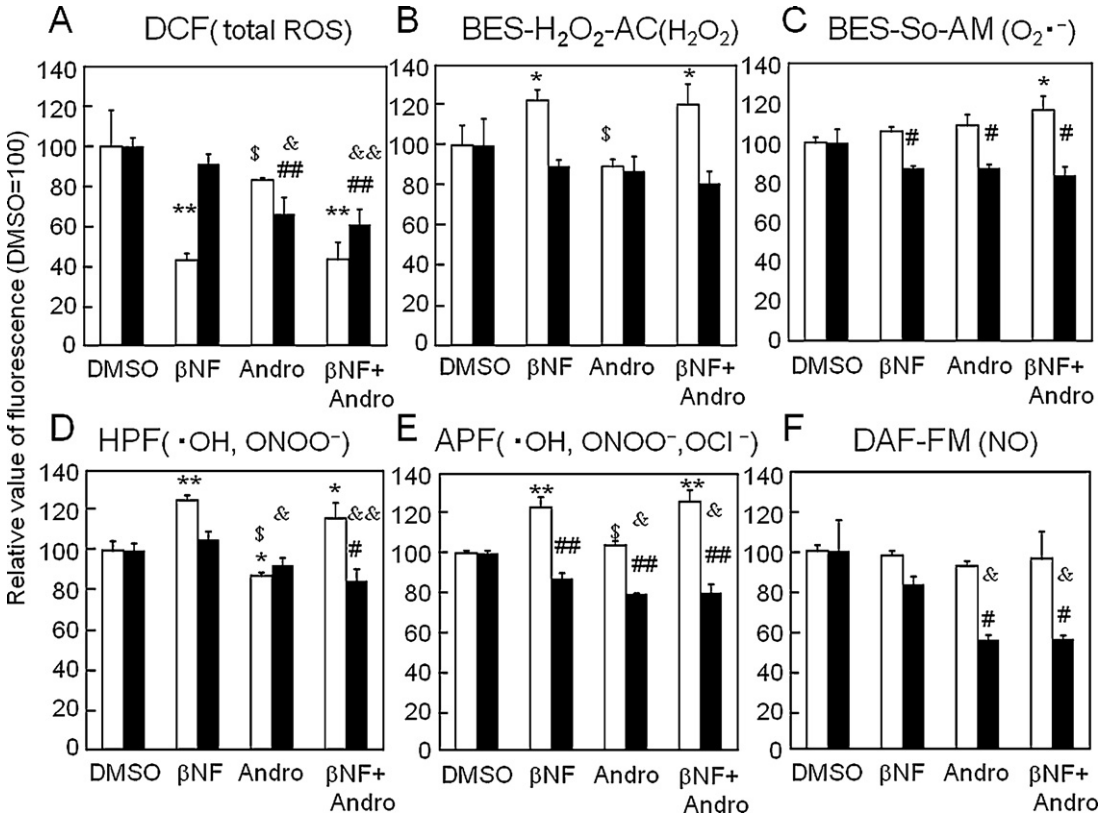

Fig. 3. Generation of ROS in mouse hepatocytes treated with βNF and/or Andro. ddY mouse hepatocytes treated with 10 μM βNF and/or 25 μM Andro for 3 h (open column) or 24 h (black column) were treated with ROS staining reagent on culture dishes and then with PI after having detached. The fluorescence was detected using FACSCant II and analyzed with FACS Diva software. PI-negative and green fluorescence-positive cells were gated, and the mean fluorescent intensity in gated cells was calculated. Values are given relative to that of the DMSO-treated group (100). (A) DCF DA (5 μM), (B) BES-H_2O_2-Ac (10 μM), (C) BES-So-AM (10 μM), (D) HPF (5 μM), (E) APF (5 μM), (F) DAF-FM DA (5 μM). Each value represents the mean ± SD ($n = 3$). Significance was calculated using the one-way ANOVA followed by Tukey *post hoc* test (A–E) or the Student–Newman–Kuels method (*F*). * and **: Represent significantly differences from the DMSO group at 3 h at $p < 0.05$ and $p < 0.01$, $ between the βNF and Andro groups at 3 h at $p < 0.01$, # and ## from the DMSO group at 24 h at $p < 0.05$ and $p < 0.01$, and & and && from the βNF group at 24 h at $p < 0.05$ and $p < 0.01$, respectively.

4. Discussion

The present study revealed a bimodal influence of Andro on βNF-inducible CYP1A1 mRNA expression, namely suppression early on and enhancement later. These modifications inversely reflected the change in intracellular GSH content. Although GSSG was detected in the presence of Andro, no ROS related to the modification of CYP1A1 mRNA expression was found.

We have previously observed a synergistic enhancement of CYP1A1 expression by Andro *in vivo* and *in vitro* (Jaruchotikamol et al., 2007; Jarukamjorn et al., 2010). However, the present investigation demonstrated that Andro suppressed βNF-inducible CYP1A1 mRNA expression at around its peak, and that the synergism was most prominent during the decreasing phase. Therefore, it is necessary to reveal the mechanism of the opposing effects of Andro on CYP1A1 mRNA expression.

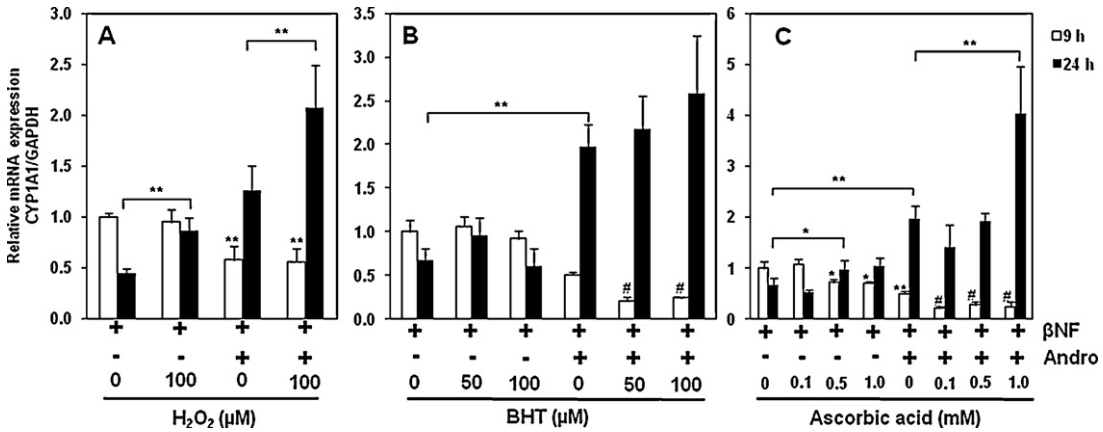

Fig. 4. Expression of CYP1A1 mRNA in mouse hepatocytes in the presence of H_2O_2 or antioxidants. C57BL/6 mouse hepatocytes were treated with a combination of 10 μM βNF, 25 μM Andro, and H_2O_2, or BHT, or ascorbic acid at indicated concentrations. Total RNA was prepared 9 h (open column) or 24 h (black column) later and the expression of CYP1A1 mRNA was analyzed by real-time PCR. Each value represents the mean ± SD ($n = 4$). (A) Effect of H_2O_2, (B) effect of BHT, and (C) effect of ascorbic acid. * and **, and # and ## represent significantly differences from the βNF and βNF + Andro group at 9 and 24 h, at $p < 0.05$ and $p < 0.001$, respectively (one-way ANOVA, Tukey *post hoc* test).

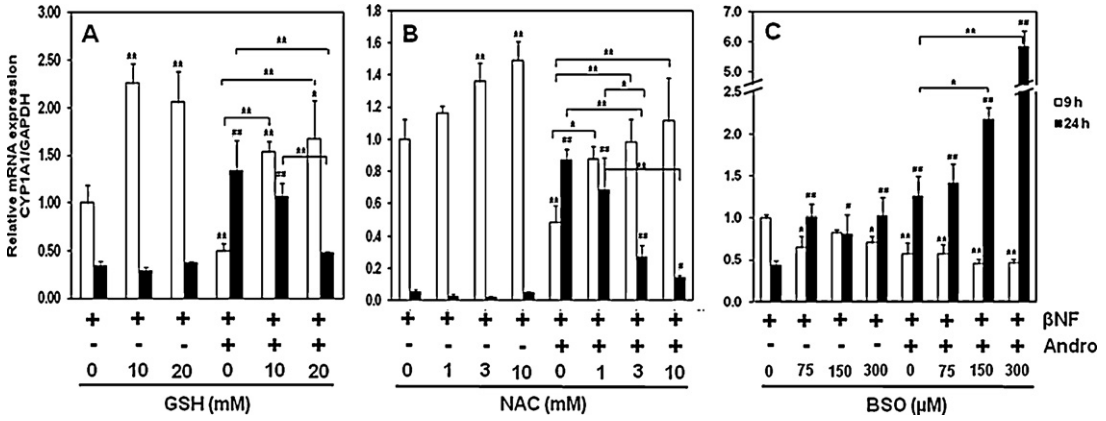

Fig. 5. Expression of CYP1A1 mRNA in mouse hepatocytes in the presence of GSH modulators. C57BL/6 mouse hepatocytes were treated with a combination of 10 μM βNF, 25 μM Andro, and GSH modulators. Total RNA was prepared 9 h (open column) or 24 h (black column) later and the expression of CYP1A1 mRNA was analyzed by real-time RT-PCR. Each value represents the mean ± SD ($n = 4$). (A) Effect of GSH, (B) Effect of NAC, and (C) Effect of BSO. * and **, and # and ## represent significantly differences from the βNF or βNF + Andro group at 9 and 24 h, at $p < 0.05$ and $p < 0.001$, respectively (one-way ANOVA, Tukey *post hoc* test).

Since expression of CYP1A1 mRNA induction by AhR ligands was down-regulated by oxidative stress (Barouki and Morel, 2001; Morel and Barouki, 1998) and Andro caused oxidative stress (Li et al., 2007; Zhang et al., 2008), Andro and/or its metabolites might affect intracellular redox status, resulting in a reduction in CYP1A1 mRNA expression early on. However, on an entirely opposite effect of Andro on oxidative stress, namely over-production of H_2O_2 (Li et al., 2007) on depletion of GSH (Zhang et al., 2008) or an increase in GSH (Woo et al., 2008), was reported, suggesting that Andro exerts different effects on oxidative stress depending on the cell lines employed. As described above, since we observed a synergistic effect of Andro on the expression of CYP1A1 mRNA by Andro *in vivo* and *in vitro*, primary cultures of mouse hepatocytes would be appropriate to reveal the effect of oxidative stress on CYP1A1 expression in the presence of Andro.

In the present study, an increase in GSSG after treatment with Andro and/or βNF suggested oxidative stress. However, although the treatment changed the proportion of the population with high-intensity fluorescence among cells stained with various reagents for detecting ROS, the patterns differed depending on the reagent; DCF-staining showed a decrease in βNF- and βNF + Andro-treated cells, but BES-H_2O_2-AC, HPF and APF-staining showed an increase at 3 h, and most of the reagents revealed a decrease at 24 h. The decrease in ROS levels was neither additive nor synergistic in the presence of the two compounds. Therefore, the decrease in DCF-stained cells is not explained by the observations for other stained cells, indicating some other DCF-reactive ROS to be involved, or that the generation of ROS does not reflect the formation of GSSG in the present culture system.

Intracellular GSH levels greatly altered the effects of Andro on CYP1A1 expression. When the levels were increased with the addition of GSH or NAC, the βNF-induced mRNA expression at 9 h was increased in the absence or presence of Andro. However, the synergistic effect of Andro at 24 h was reduced in the presence of GSH and NAC, and the expression was further enhanced in the presence of BSO. Therefore, an increase in GSH content competed with the suppressive effect of Andro early on, and a decrease supported the synergism. If GSH eliminates ROS in the present experimental system, our results are not in consistent with a report by Barker et al. (1994), that the expression of CYP1A1 was inhibited by ROS and recovered with the addition of NAC. One possible explanation of the different effects of GSH on CYP1A1 expression in the presence of Andro is that Andro can form a complex with GSH (Zhang et al., 2008). We observed an increase in the expression of several glutathione S-transferases in the presence of Andro in our culture system (Chatuphonprasert et al., 2009). These results suggest that, although Andro and a complex of Andro-GSH exist in certain proportion in the cell, Andro itself exerts the synergistic effect, which might be increased after reduced production of the complex through a reduction in GSH content and *vice versa,* indicating that GSH is a key factor associated the synergism of inducible CYP1A1 expression by Andro. Furthermore, the evidence that synergism of CYP1A1 mRNA at 24 h was enhanced in the presence of H_2O_2 (Fig. 4A) might support above idea of no relationship between oxidative stress and synergism of CYP1A1 expression. Addition of H_2O_2 may decrease free GSH level by interaction between them, resulting in the elevated expression of CYP1A1 mRNA. Furthermore, since GSH-related modifications were entirely opposite between early and late phase of the CYP1A1 induction, our observations suggest that time-dependently different regulation mechanisms might be involved in the induction (see Fig. 5).

BHT and ascorbic acid further suppressed the Andro-supppressed inducible expression of CYP1A1 mRNA at 9 h significantly. At 24 h, on the contrary, both compounds demonstrated additive effect on the synergistic expression of CYP1A1 mRNA. Previous reports of the effect of these antioxidants on CYP1A1 expression are limited. BHT itself induced CYP1A1 mRNA and EROD activity in rat and mouse liver (Price et al., 2004; Sun and Fukuhara, 1997), while combination of BHT and flavones or flavanone did not modify the EROD activity (Sun and Fukuhara, 1997). However, there is no description on mechanism of BHT about CYP1A1 expression. Ascorbic acid was reported to suppress CYP1A1 expression in human HepG2 cells (Chang et al., 2009) and in sepsis rats (Kim and Lee, 2006). However, Ueta et al. (2001) observed that ascorbic acid at high dosage maintained CYP1A1 mRNA expression in the lung of rats inhaling cigarette smoke. The reason for the different action of ascorbic acid has not been revealed yet. Paolini et al. (1999) suggested that ascorbic acid may behave as a potential pro-oxidant, depending on experimental conditions. Further investigation is required to reveal a mechanism of the additive modification of the modulation of Andro on inducible CYP1A1 mRNA expression by antioxidants.

In conclusion, we observed that the modification of inducible CYP1A1 mRNA expression by Andro was bimodal depending on treatment period and the modulation was retrieved by changing the intracellular GSH content. These results suggest that GSH status might be involved in the regulatory mechanism of CYP1A1 induction, and interaction of Andro with GSH might modulate the expression. Further investigation of a mechanism of the modulation of Andro on inducible CYP1A1 mRNA expression is of interest, since many environmental carcinogens can induce CYP1A1, which can metabolize these compounds to be reactive metabolites to bind

to DNA. Thus, caution is assessed when using Andro or extract of *Andrographis paniculata* as a health supplement.

Conflict of interest statement

None declared.

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