Effect of Glutathione on Synergistic Induction of CYP1A1 by Andrographolide

ผลของกลูตาไทโอนต่อการเหนี่ยวนำ CYP1A1 แบบเสริมฤทธิ์โดยสารแอนโดรกราโฟไลด์

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ABSTRACT

We previously reported that andrographolide (Andro), a major bioactive constituent of *Andrographis paniculata* or Fa-ta-lai-joan, synergistically enhanced the inducible expression of CYP1A1 mRNA. In this study, the synergism was confirmed at 24 h after treatment with Andro and β-naphthoflavone (BNF), a CYP1A inducer, in mouse hepatocytes. Glutathione (GSH) and N-acetyl-L-cysteine (NAC) significantly enhanced the BNF-induced expression in the early phase. At 24 h, the addition of GSH or NAC had no effect on BNF-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. These results suggest that GSH status might be involved in BNF-induced CYP1A1 mRNA expression, and the interaction of Andro with GSH might modulate the expression.

บทคัดย่อ

แอนโดรกราโฟไลด์ สารสำคัญในสมุนไพรฟ้าทะลายโจร ถูกรายงานว่ามีความสามารถเสริมฤทธิ์การเหนี่ยวนำการแสดงออกของ CYP1A1 mRNA การศึกษานี้ยืนยันว่าการเหนี่ยวนำแบบเสริมฤทธิ์นี้ แสดงออกในเซลล์ตับปฐมภูมิของหนูไมซ์ที่ 24 ชั่วโมง หลังจากได้รับแอนโดรกราโฟไลด์ ร่วมกับเบต้าแนฟโทฟลาโวน (BNF) ซึ่งมีคุณสมบัติเป็นสารเหนี่ยวนำ CYP1A1 กลูตาไทโอน (GSH) และ N-acetyl-L-cysteine (NAC) มีผลเพิ่มการแสดงออกของ CYP1A1ที่ถูกเหนี่ยวนำโดย BNF ในระยะเริ่มแรกของการทดสอบ ในขณะที่ GSH และ NAC ไม่ส่งผลใดๆ ต่อการเหนี่ยวนำ CYP1A1 โดย BNF ที่ระยะเวลา 24 ชั่วโมง แต่กลับลดการเสริมฤทธิ์เหนี่ยวนำ CYP1A1 ของแอนโดรกราโฟไลด์อย่างมีนัยสำคัญ L-buthionine-(S,R)-sulfoximine ซึ่งเป็นสารยับยั้งชีวสังเคราะห์ของกลูตาไทโอน แสดงผลเพิ่มการเสริมฤทธิ์การเหนี่ยวนำ CYP1A1 ของแอนโดรกราโฟไลด์ได้ ดังนั้นจึงสามารถสรุปได้ว่าระดับกลูตาไทโอนมีความสัมพันธ์กับการควบคุมกระบวนการเหนี่ยวนำการแสดงออกของ CYP1A1 โดย BNF และการเกิดปฏิกิริยาระหว่าง Andro และ GSH ส่งผลต่อการเปลี่ยนแปลงการเหนี่ยวนำดังกล่าว

Key Words : CYP1A1, andrographolide, glutathione

คำสำคัญ : ไซโตโครมพี 450 1 เอ 1 แอนโดรกราโฟไลด์ กลูตาไทโอน

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Introduction

Andrographolide (Fig. 1; Andro) is a major diterpenoid constituent of the plant *Andrographis paniculata* Nees or Fa-ta-lai-joan (Jarukamjorn and Nemoto, 2008), 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 extract of *A. paniculata* and Andro have been used as health supplements in many countries. Although numerous activities of *A. paniculata* and Andro have been established, little toxicological information is available, especially biotransformation and drug-herb interaction related to cytochrome P450 (P450).

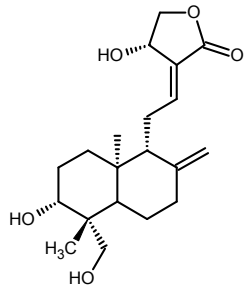

Fig. 1 Structure of Andrographolide

P450 plays an important role in the pharmacology of drugs and toxicology of xenobiotics (Guengerich, 2008). 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. Of these P450s, CYP1A plays a critical role in the metabolic activation of carcinogenic polycyclic aromatic hydrocarbons and heterocyclic aromatic amines/amides to reactive intermediates, respectively, leading to toxicity and cancer (Jacquet et al., 1996). CYP1A induction on carcinogenic and toxic potentials of environmental, occupational, dietary, and therapeutic chemicals has been a central focus on human risk evaluation and broadly influenced the fields of cancer research, toxicology, pharmacology, and risk assessment over the past half century (Stucker et al., 2000).

After oral administration of the extract of *A. paniculata*, activity-levels of the marker enzymes, CYP1A1 and CYP2B10, risen 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 by typical CYP1A inducers (Jaruchotikamol et al., 2007). Interestingly, Andro synergistically enhanced the expression of CYP1A1 mRNA induced by a ligand of AhR (Jaruchotikamol et al., 2007), however, the mechanism involved was unclear.

In the present study, we examined the effect of GSH and GSH modulators on BNF-induced expression of CYP1A1 mRNA in mouse hepatocytes in primary culture.

Materials and methods

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), and LDH-cytotoxic Test Wako were from Wako Pure Chemical. β-Naphthoflavone (BNF) and N-acetyl-L-cysteine (NAC) were obtained from Sigma Chemicals. ReverTraAce and G-Taq DNA polymerase were products of TOYOBO (Osaka, Japan) and Hokkaido System Science (Sapporo, Japan), respectively. The HT Glutathione Assay Kit and Accutase were obtained from Trevigen (Gaithersburg, MD) and Innovative Cell Technologies (San Diego, CA), respectively.

Animals

Seven-week-old male C57BL/6 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.

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 $5x10^5$ per dish on collagen-coated 35-mm Petri dishes and cultured at 37ºC 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. BNF 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 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.

Quantitative real-time RT-PCR

Mouse CYP1A1 and GAPDH mRNAs were quantified by real-time RT-PCR, as described previously (Jarukamjorn et al., 2010). 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.

Results and discussion

Effect of Andro on CYP1A1 mRNA expression

The expression of CYP1A1 mRNA in primary mouse hepatocytes after treatment with either 10 µM BNF, 25 µM Andro, or BNF+Andro were observed (Fig. 2). The effect of BNF reached a maximum level at around 9 h after which the expression decreased. Andro itself had a little effect, but BNF+Andro had a synergistic effect at 24 h, however, considerably suppressed BNF-induced CYP1A1 mRNA expression at 9 h. These results revealed a bimodal influence of Andro on BNF-inducible CYP1A1 mRNA expression, namely suppression early on and enhancement later.

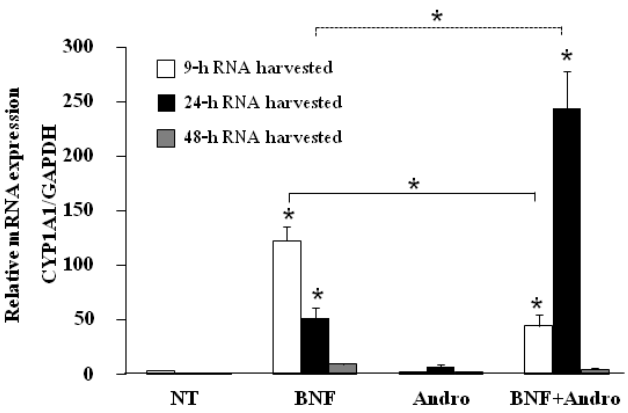

Fig. 2 Effect of Andrographolide on CYP1A1 mRNA expression at 9, 24, 48 h. Primary mouse hepatocytes were treated with 0.2% DMSO (non-treatment, NT), 10 μM BNF, 25 μM Andro, or BNF + Andro and total RNA was prepared at the indicated times. The expression of CYP1A1 mRNA was determined by quantitative RT-PCR after normalization with that of GAPDH mRNA. Each point represents the mean ± S.D (n = 4). * represents a significantly difference from the NT group at $p<0.05$ (one-way ANOVA, Tukey *post hoc* test).

Effect of GSH modulators on BNF-induced CYP1A1 mRNA expression

Changes in intracellular GSH contents caused by either GSH, NAC, or BSO on BNF-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 (Fig. 3B) while GSH significantly increased BNF-induced CYP1A1 mRNA expression (Fig. 3A), and both increased the expression in BNF + Andro-treated cells. BSO decreased the expression in the BNF- or BNF + Andro-treated cells. In the late phase (24 h), BSO increased BNF-induced CYP1A1 mRNA expression, but GSH and NAC (Fig. 3C) 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.

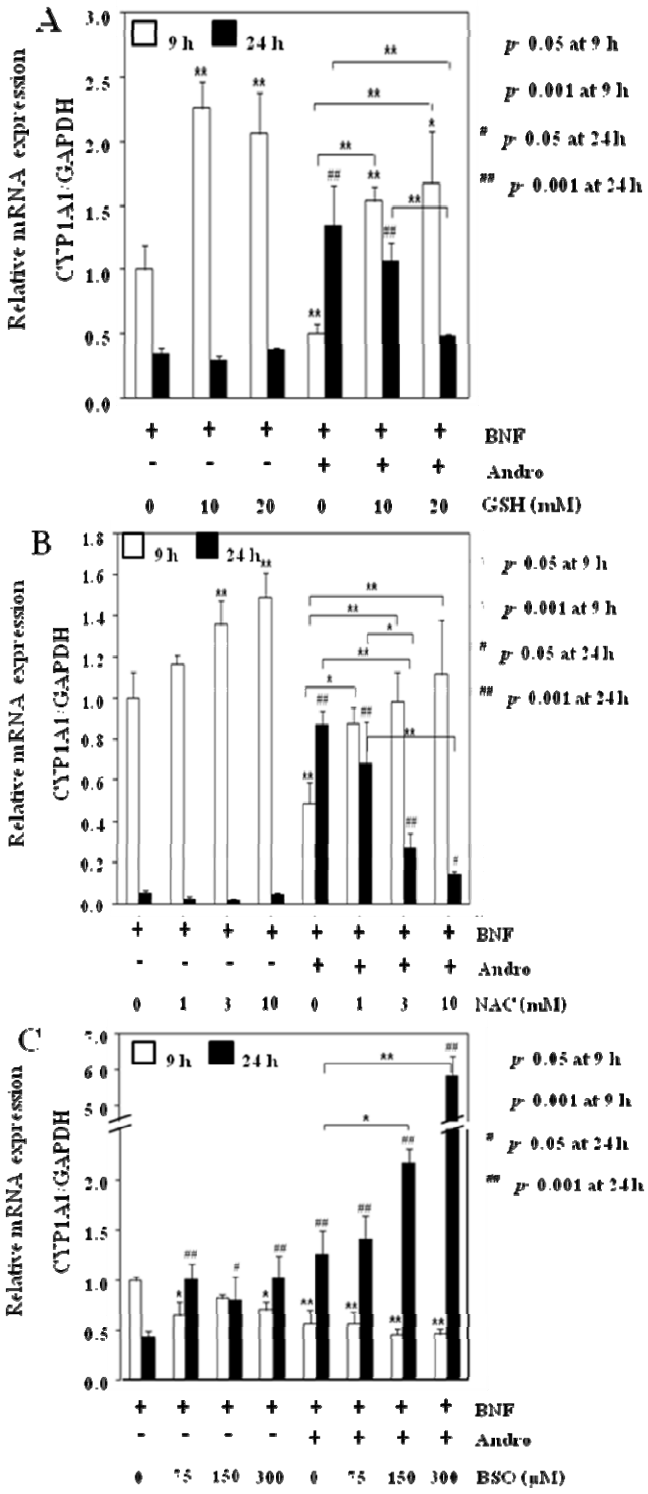

Fig. 3 Expression of CYP1A1 mRNA in the presence of GSH modulators. Primary mouse hepatocytes were treated with a combination of 10 μM BNF, 25 μM Andro, and GSH modulators. Total RNA was prepared 9 h (open column) or 24 h (black column) later and the expression of CYP1A1 mRNA was analyzed by real-time RT-PCR. Each value represents the mean ± S.D (n = 4). A) Effect of GSH. B) Effect of NAC. C) Effect of BSO.

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.

Conclusions

In conclusion, 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 suggested 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 *vice vesa* can metabolize these compounds to be reactive metabolites. Therefore, caution is assessed when using Andro or extract of *A. paniculata* as a health supplement.

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References

Guengerich, FP. 2008. Cytochrome P450 and chemical toxicology. Chem. Res. Toxicol. 21: 70-83.

Jacquet, M., Lambert, V., Baudoux, E., Muller, M., Kremers, P., Gielen, J. 1996. Correlation between P450 CYP1A1 inducibility, Msp1 genotype and lung cancer incidence. Eur. J. Cancer. 32A: 1701-1706.

Jaruchotikamol, A., Jarukamjorn, K., Sirisangtrakul, W., Sakuma, T., Kawasaki, Y., and Nemoto, N. 2007. Strong synergistic induction of CYP1A1 expression by andrographolide plus typical CYP1A inducers in mouse hepatocytes. Toxicol. Appl. Pharmacol. 224: 156-162.

Jarukamjorn, K., and Nemoto, N. 2008. Pharmacological aspects of *Andrographis paniculata* on health and its major diterpenoid constituent andrographolide. J. Health Sci. 54: 370-381.

Jarukamjorn, K., Don-in, K., Pearaksa, P., and Sripanidkulchai, B. 2006. Impact of *Andrographis paniculata* crude extract on mouse hepatic cytochrome P450 enzymes. J. Ethnopharmacol. 105: 464-467.

Jarukamjorn, K., Kondo, S., Chatuphonprasert, W., Sakuma, T., Kawasaki, Y., Nemoto, N. 2010. Gender-associated modulation of inducible CYP1A1 expression by andrographolide in mouse liver. Eur. J. Pharm. Sci. 39: 394-401.

Reyes, BA., Bautiata, ND., Tanquilut, .C., Anunciado, RV., Leung, AB., Sanchez, GC., Magtoto, RL., Castronuevo, P., Tsukamura, H., and Maeda, KI. 2006. Anti-diabetic potentials of *Momordica charantia* and *Andrographis paniculata* and their

effects on estrous cyclicity of alloxan-induced diabetic rats. J. Ethnopharmacol. 105: 196-200.

Sheeja, K., and Kuttan, G. 2007. Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth in vivo by *Andrographis paniculata* extract and andrographolide. Immunopharmacol. Immunotoxicol. 29: 81-93.

Shen, YC., Chen, CF., and Chiou, WF. 2002. Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti-inflammatory effect. Br. J. Pharmacol. 135: 399-406.

Singha, PK., Roy, S., and Dey, S. 2007. Protective activity of andrographolide and arabinogalactan proteins from *Andrographis paniculata* Nees. against ethanol-induced toxicity in mice. J. Ethnopharmacol. 111: 13-21.

Stucker, I., Jacquet, M., de Waziers, I., Cenee, S., Beaune, P., Kremers, P., Hemon, D. 2000. Relation between inducibility of CYP1A1, GSTM1 and lung cancer in a French population. Pharmacogenetics. 10: 617-627.

Thisoda, P., Rangkadilok, N., Pholphana, N., Worasuttayangkurn, L., Ruchirawat, S., and Satayavivad, J. 2006. Inhibitory effect of *Andrographis paniculata* extract and its active diterpenoids on platelet aggregation. Eur. J. Pharmacol. 553: 39-45.

Wiart, C., Kumar, K., Yusof, M.Y., Hamimah, H., Fauzi, Z.M., and Sulaiman, M. 2005. Antiviral properties of ent-labdene diterpenes of *Andrographis paniculata* Nees, inhibitors of herpes simplex virus type I. Phytother. Res. 19: 1069-1070.