Research Article

ตัวรับเอริลไฮโดรคาร์บอนและคอนสติตูทีฟแอนโดรสเตนเป็นปัจจัยกำหนดการเหนี่ยวนำการแสดงออกของไซโตโครมพี 450 1 เอ 2, 2 เอ 6 และ 2 เอ 13 โดยนิโคตินและเอ็นไนโตรโซไดเอธาโนลามีนในเซลล์มะเร็งตับ

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บทคัดย่อ

ตัวรับเอริลไฮโดรคาร์บอนและคอนสติตูทีฟแอนโดรสเตนเป็นปัจจัยกำหนดการเหนี่ยวนำการแสดงออกของไซโตโครมพี 450 1 เอ 2, 2 เอ 6 และ 2 เอ 13 โดยนิโคตินและเอ็นไนโตรโซไดเอธาโนลามีนในเซลล์มะเร็งตับ

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นิโคติน (nicotine) และ เอ็นไนโตรโซไดเอธาโนลามีน (*N*-nitrosodiethanolamine, NDELA) เป็นสารพิษต่อตับและเป็นสารตั้งต้นของสารก่อมะเร็งในตับของมนุษย์และสัตว์ ไซโตโครมพี 450 1 เอ (*CYP1A*) และ 2 เอ (*CYP2A*) เป็นเอนไซม์หลักในกระบวนการเมแทบอลิซึมของนิโคตินและไนโตรซามีนในตับ แต่ความรู้เกี่ยวกับผลกระทบของนิโคตินและไนโตรซามีนต่อไซโตโครมพี 450 อื่น ยังมีจำกัด การศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของนิโคตินและ NDELA ต่อการควบคุมการแสดงออกที่ระดับเอ็มอาร์เอ็นเอของ CYPs และตัวรับในนิวเคลียส ได้แก่ ตัวรับเอริลไฮโดรคาร์บอน (aryl hydrocarbon receptor, *AhR*) ตัวรับคอนสติตูทีฟแอนโดรสเตน (constitutive androstane receptor, *CAR*) และตัวรับเพรกเนนเอ็กซ์ (pregnane X receptor, *PXR*) ในเซลล์มะเร็งตับของมนุษย์ (HepG2) **วิธีการทดลอง:** เซลล์ HepG2 ($5x10^5$ เซลล์ต่อหลุม) เพาะเลี้ยงในอาหารเลี้ยง DMEM ที่เสริมด้วย 10% FBS และบ่มร่วมกับนิโคตินหรือ NDELA (1, 10, and 100 μM) เป็นเวลา 24 ชั่วโมงเปรียบเทียบกับกลุ่มควบคุม หรือ 0.1% เอธิลแอลกอฮอล์ (EtOH) ซึ่งเป็นตัวทำละลายของนิโคตินและ NDELA จากนั้นทำการสกัด total RNA และตรวจวัดการแสดงออกที่ระดับเอ็มอาร์เอ็นเอด้วยเทคนิค RT-qPCR **ผลการทดลอง:** ทั้งนิโคตินและ NDLEA ที่ความเข้มข้น 100 μM เพิ่มการแสดงออกที่ระดับเอ็มอาร์เอ็นเอของ *CYP1A2, CYP2A6* และ *CYP2A13* อย่างมีนัยสำคัญทางสถิติ ยิ่งไปกว่านี้นิโคตินและ NDELA ยังเพิ่มการแสดงออกที่ระดับเอ็มอาร์เอ็นเอของ *AhR* และ *CAR* อย่างมีนัยสำคัญทางสถิติ ที่น่าสนใจคือ นิโคตินเหนี่ยวนำเอ็มอาร์เอ็นเอของ *PXR* ในขณะที่ NDELA ไม่ส่งผลเปลี่ยนแปลง เอ็มอาร์เอ็นเอของ PXR **สรุป:** กลไกการควบคุมการแสดงออกของ *CYP1A2* และ *CYP2A6/13* โดยนิโคตินและ NDELA มี *AhR* และ *CAR* เป็นปัจจัยกำหนด อย่างไรก็ตามความแตกต่างในกลไกการควบคุมการแสดงออกของ PXR โดยนิโคตินและ NDELA เป็นประเด็นที่น่าสนใจในการศึกษาต่อไป

คำสำคัญ: นิโคติน, ไนโตรซามีน, ไซโตโครม พี450, *AhR, PXR, CAR*.

Aryl Hydrocarbon and Constitutive Androstane Receptors are Compulsory Determinants in the Induction of Cytochrome P450 1A2, 2A6, and 2A13 Expression by Nicotine and *N*-Nitrosodiethanolamine in HepG2 Cells

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Abstract

Aryl Hydrocarbon and Constitutive Androstane Receptors are Compulsory Determinants in the Induction of Cytochrome P450 1A2, 2A6, and 2A13 Expression by Nicotine and *N*-Nitrosodiethanolamine in HepG2 Cells

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Nicotine and *N*-nitrosodiethanolamine (NDELA) are hepatotoxic and hepatocarcinogenic substances in human and animals. Cytochrome P450 (CYPs) 1A and 2A mainly metabolize nicotine and nitrosamines in the liver but knowledge regarding effects of nicotine and nitrosamines on other CYPs is still limited. The current study aimed to examine the effects of nicotine and NDELA on regulatory expressions of CYPs and nuclear receptors, aryl hydrocarbon receptor (*AhR*), pregnane X receptor (*PXR*), and constitutive androstane receptor (*CAR*) mRNA in human hepatocellular carcinoma cells (HepG2). **Materials and method:** HepG2 cells ($5x10^5$ cells per well) were cultured in DMEM supplemented with 10% FBS. The cells were treated with nicotine or NDELA (1, 10, and 100 μM) for 24 h, compared to control (non-treatment) or 0.1% ethanol (EtOH) which was used as the solvent of nicotine and NDELA. Total RNAs were extracted and the mRNA expression of each gene was determined by RT-qPCR. **Results:** Both of nicotine and NDLEA at 100 μM significantly up-regulated expression of *CYP1A2, CYP2A6*, and *CYP2A13* mRNA. Furthermore, nicotine and NDELA significantly induced the expression of *AhR* and *CAR* mRNA. Interestingly, nicotine induced *PXR* mRNA whereas NDELA did not. **Conclusion:** *AhR* and *CAR* might be compulsory determinants for regulation of *CYP1A2* and *CYP2A6/13* expression by nicotine and NDELA. However, different regulation of *PXR* by nicotine and NDELA is of interest for further study.

Keywords: Nicotine, Nitrosamine, Cytochrome P450, *AhR, PXR, CAR*.

Introduction

Nicotine (Figure 1A), a powerful alkaloid, is the major constituent of tobacco/cigarette and of its smoke. After inhaling smoke, nicotine enters through the bloodstream within few seconds and reaches the brain in 8-20 seconds, subsequently stimulating the receptors in central nervous system that reward essential activities like eating when hungry (Bergen and Caporaso, 1999).

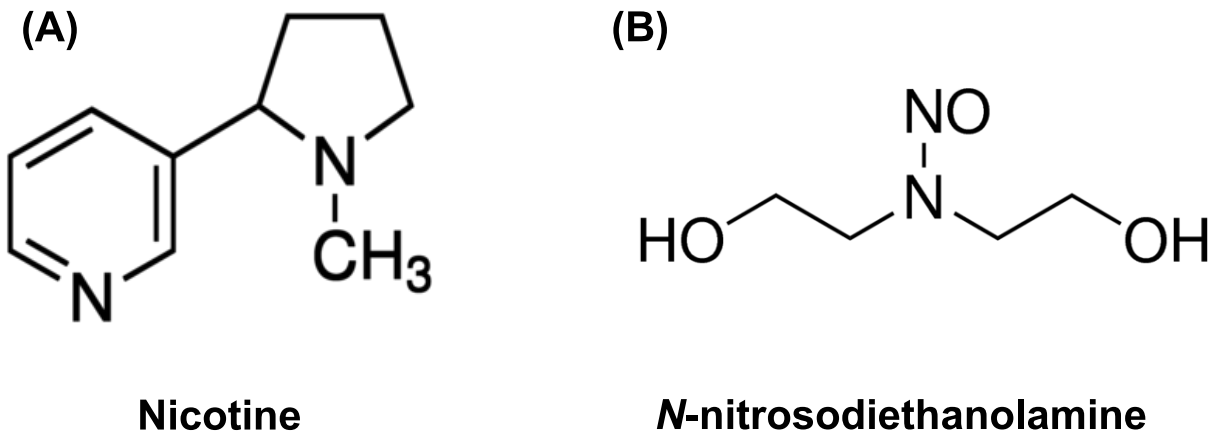

Figure 1. Structure of (A) nicotine and (B) *N*-nitrosodiethanolamine (NDELA)

Nitrosamines are *N*-nitroso compounds that have been detected in environment as contaminants of certain pesticides, tobacco, foods, and a broad range of consumer products, including cosmetics, lotions, and shampoos (Fan *et al.*, 1977). *N*-nitrosodiethanolamine (NDELA, Figure 1B) has been claimed as an impurity in a number of cosmetic products ranging from <1 to 48,000 ng/g (Fan *et al.*, 1977). NDELA is carcinogenic in rats and hamsters per oral and subcutaneous administration, respectively (Hilfrich *et al.*, 1978). The mechanism of NDELA formation is unknown but it might be a reaction of di- or tri-ethanolamine with a nitrosating agent in cosmetic formulations. NDELA has also been detected in cigarette smoke and smokeless tobacco product (Brunnemann and Hoffman, 1981).

Cytochrome P450s (CYPs) are hemoproteins located in the inner membrane of mitochondria and endoplasmic reticulum of cells. CYPs involve in synthesis and degradation of endogenous substances (endobiotics) to biotransformation of foreign compounds such as drugs, environmental pollutants, and carcinogens (xenobiotics) (Honkakoski and Negishi, 2000). Approximately 75% of known xenobiotics are metabolized by members of the CYP1, CYP2, and CYP3 families. The major constituent of tobacco, nicotine, is mainly metabolized in human liver by CYP2A subfamily (Bao, 2005). Nitrosamines become toxic when they are activated by CYPs. Phase I hydroxylation and alkylation yield nitrogen and carbonium ion. The extremely reactive carbonium ion attacks DNA bases, causing methylation. These specific DNA-adduct are most responsible for mutagenicity of *N*-nitrosamines (Jagerstad and Skog, 2005). Other cellular effects from exposure of *N*-nitrosamines include apoptosis, enabling DNA repair, and cell cycle blockage (Verghese *et al.*, 2006). Several studies have reported that nitrosamines cause necrosis of hepatocytes. CYPs activate xenobiotics through ligand-activated nuclear receptors, e.g. constitutive andostane receptor (*CAR*) and pregnane X receptor (*PXR*) to generate specific cellular responses to activating ligands (Pavek and Dvorak, 2008). Aryl hydrocarbon receptor (*AhR*) mainly regulates *CYP1A2* transcription in metabolism of polycyclic aromatic hydrocarbons (PAHs) (Nebert and Dalton, 2006; Pascussi *et al.*, 2008). Cigarette smoke activates *AhR* and

induces *CYP1A2* expression (Shimada *et al*., 2002). The induction of *CYP1A2* is mediated via binding of PAHs from the tobacco smoke to the AhR with consequent transcriptional activation of *CYP1A2* gene (Hukkanen *et al.*, 2011). However, the information of nicotine and NDELA against *PXR, CAR*, and *AhR* is very limited.

Therefore, the study aimed to examine the impacts of nicotine and nitrosamine on the activities and mRNA expression of CYPs, namely *CYP1A2, CYP2A6*, and *CYP2A1*3, and nuclear receptors, namely *AhR*, *CAR*, and *PXR* in human hepatocarcinoma cells (HepG2).

Materials and methods

Chemicals - HepG2 cells (HB-8065™) was supplied by American Type Culture Collection (ATCC), Manassas, Virginia, USA. Nicotine, NDELA, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and resazurin were obtained from Sigma-Aldrich Corporation (St. Louis, Missouri, USA). Lactate dehydrogenase (LDH) assay kit was a product of Roche Diagnostics (Mannheim, Germany). Dulbecco's modified eagle medium (DMEM), 0.25% Trypsin, ethylenediaminetetraacetic acid (EDTA), and fetal bovine serum (FBS) were from Gibco Laboratories (Gaithersburg, MD, USA). Dulbecco's phosphate buffered saline (1×) w/o Ca & Mg w/o phenol red was purchased from Capricorn Scientific (Ebsdorfergrund, Germany). Trypan blue solution (0.4%) was a product of Corning Technology Company (Manassas, USA). Triton-X100 was obtained from PanReac AppliChem (Darmstadt, Germany). ReverTraAce®, 5× RT-buffer, 6× loading buffer were products of Toyobo® (Osaka, Japan). Random primers and SYBR green were purchased from Invitrogen™ Corporation (Carlsbad, California, USA). Redsafe™ nucleic acid staining solution (20,000×) was obtained from iNtRON Biotechnology (Burlington, MA, USA). dNTPs, RNase Inhibitor, and Taq DNA polymerase were obtained from Vivantis Technologies (Selangor, Malaysia). All other laboratory chemicals and solvents were of the highest purity from commercial suppliers.

Cell culture - HepG2 cells (HB-8065™) were cultured at the density of $5x10^5$ cells per well in a 6-well plate containing DMEM, 10% FBS, Glutamax® (1X), and penicillin, streptomycin, and neomycin (1×PSN) under sterile condition at 37 ºC with 5% CO_2. After seeding for 48 h, nicotine and NDELA (1, 10, and 100 µM) were added to the cells (n=4), compared to control (non-treatment) or 0.1% ethanol (EtOH) which was employed as the solvent of both nicotine and NDELA.

Cell viability testing – Resazurin solution (at a final concentration of 1 µM) was added to the cells after incubation with the tested compounds for 24 h. The Cells were incubated with resazurin for 1 h in the standard culture conditions. The relative fluorescence unit (RFU) was measured at an excitation wavelength of 530 nm and an emission wavelength of at 590 nm. % Cell viability was calculated compared to the non-treatment.

Total RNA preparation - Total RNA was extracted from the cells after incubation with the tested compounds for 24 h. The medium was discarded using an aspirator, and then 1× PBS was added to wash out medium in each well. Guanidinium thiocyanate-phenol-chloroform solution was added to each well. After that the mixture was transferred to a 1.5 mL-microfuge tube followed by vigorously shaking and centrifugation at 12,000 ×*g* at 4 ºC for 10 min. The clear supernatant was transferred to a new 1.5 mL-microfuge tube contained isopropanol and mixed well before subjected to centrifugation at 12,000 ×*g* at 4 ºC for 10 min to obtain RNA pellet. The pellet was washed by 80% ethanol and subjected to centrifugation at 12,000 ×*g* at 4 ºC for 10 min for 2 times. All RNA pellets were leaved for dryness at room temperature before reconstituted with sterile water. Finally, concentration and purity of RNA were determined and the stock at a concentration of 100 ng/µL was prepared for further analysis.

RNA integrity assessment – Each RNA sample (1 µg) was separated by 1.2% agarose gel electrophoresis in 1× Tris-borate-EDTA (TBE) buffer staining with RedSafe™ at 120 V for 20 min. Both 28 and 18S RNAs were considered for RNA integrity under an UV trans-illuminator coupled with Gene SNAP program.

Reverse transcription and qPCR - Reverse transcription (RT) was performed to generate cDNA. The RT reaction was consisted of ReverTraAce®, dNTP mixture, random primers, and ribonuclease inhibitor. Non template control (NTC) was set as a negative control using sterile water instead of RNA sample. Thermal cycler was set to generate cDNA at a multi-phase condition: 25 ºC (10 min), 42 ºC (60 min), 95 ºC (5 min), and 4 ºC (∞). The cDNA was kept at -20 ºC prior to qPCR. Gene expression was determined by qPCR. The qPCR reaction was consisted of Taq-DNA polymerase, $MgCl_2$, dNTP mixture, SYBR I dye, specific forward- and reverse-primers (Table 1), and 10 ng of cDNA. *Glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) was used as a reference gene. The amplification was operated in 4 steps including warming-up (95 ºC, 1 min), denaturation (95 ºC, 20 sec), annealing (optional indicated in Table 1, 20 sec), and extension (72 ºC, 20 sec). Bio-Rad CFX manager program was used to analyze data, melting point curve, and Cq of each gene.

Statistical analysis - After raw data was obtained from Bio-Rad CFX manager program, fold difference was calculated in term of gene expression by subtraction of Cq between a target gene and *GADPH*. Average fold difference from each group was plot. Statistical analysis was done using one-way ANOVA with Turkey's *post hoc* (SPSS ver. 23).

Table 1. Primers for qPCR

Genes	Forward primers	Reverse primers	Product size (base pairs)	Annealing Temperature (ºC)
CYP1A2	5′-TCAAAGGCTATGGCGTGGTA-3′	5′-AGGGCTTGTTAATGGCAGTG-3′	160	60
CYP2A6	5′-ACAAGGGACACAACGCTGAA-3′	5′-CATCATGCGCAACAGTGACA--3′	284	60
CYP2A13	5′-CTTCAAGTCCCCTCAGTCG-3′	5′-TGTTCCCTCTAACCACCTCT-3′	238	64.5
AhR	5′-CAACAGCAACAGTCCTTGGC-3′	5′-GTTGCTGTGGCTCCACTACT-3′	112	60.5
CAR	5′-AGGACCAGATCTCCCTTCTCAAG-3′	5′-CGTGTTTGGAGACAGAAAGTGGTA-3′	82	58
PXR	5′-CAAGCGGAAGAAAAGTGAACG-3′	5′-CACAGATCTTTCCGGACCTG-3′	246	60.5
GAPDH	5′-CACCATCTTCCAGGAGCGAG-3′	5′-GACTCCACGACGTACTCAGC-3′	72	61.1

Results

Effects of nicotine and NDELA on the expression of *CYP1A2, CYP2A6*, and *CYP2A13* mRNAs

% Cell viability was higher than 80% for all treatments and not different from the control group (NT) (Figure 2). Hence, concentrations of nicotine and NDELA (1, 10, 100 µM) were non-toxic to metabolism of the cells. The expression of *CYP1A2* mRNA was significantly induced for 5 and 9 folds, respectively, by the highest dose (100 µM) of nicotine and NDELA (p<0.05, Figure 3). All doses of nicotine and 0.1% EtOH significantly increased the expression of *CYP2A6* mRNA for 2 to 5 folds (p<0.05) while the level of *CYP2A6* was up-regulated for 3 folds by NDELA at the concentration of 100 µM (Figure 4). *CYP2A13* was not induced by 0.1% EtOH (Figure 5). Nicotine (10 and 100 µM) and NDELA (all doses) significantly up-regulated the expression of *CYP2A13* mRNA for 20 to 100 folds (p<0.05).

Effects of nicotine and NDELA on the expression *AhR, CAR*, and *PXR* mRNAs

Only the highest dose (100 µM) of nicotine and NDELA markedly induced the expression of *AhR* mRNA for 4 and 2.5 folds, respectively (p<0.05, Figure 6). According to the *AhR* expression, level of *CAR* mRNA was significantly elevated (2-2.5-folds) by the highest dose of nicotine and NDELA (p<0.05, Figure 7). However, only nicotine at the highest dose significantly induced the expression of *PXR* mRNA (3 folds) while NDELA was not (Figure 8).

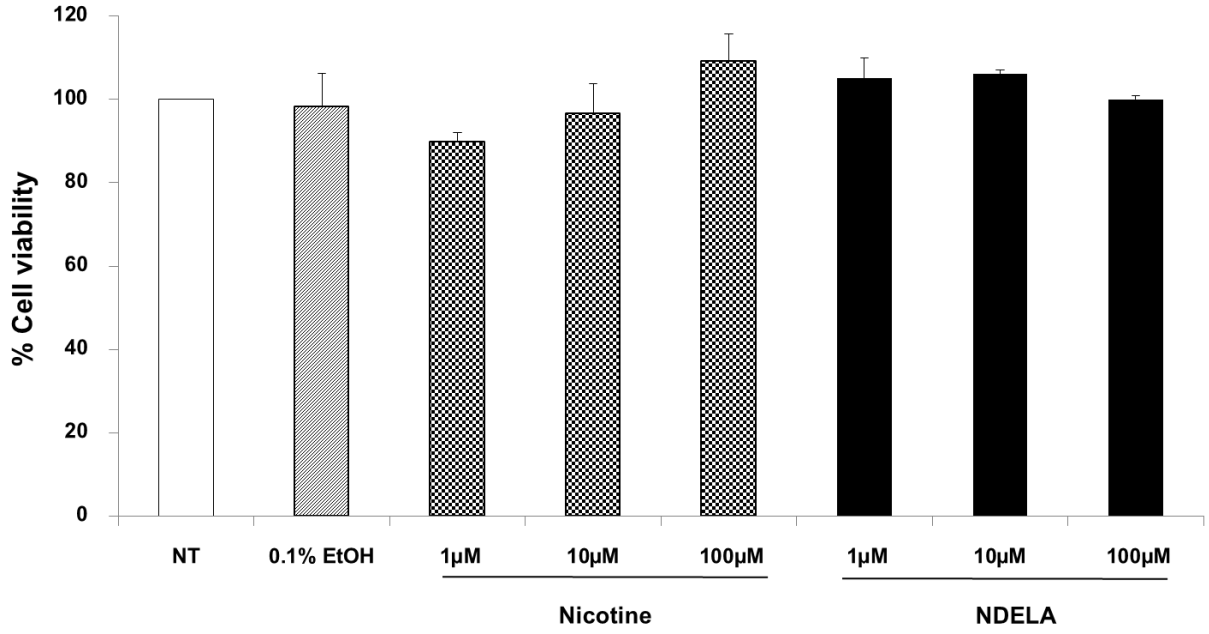

Figure 2. Effects of nicotine and *N*-nitrosodiethanolamine (NDELA) on HepG2 cell viability after 24 h-treatments

Data are presented as mean±SD (n=4). NT, non-treatment; EtOH, ethanol.

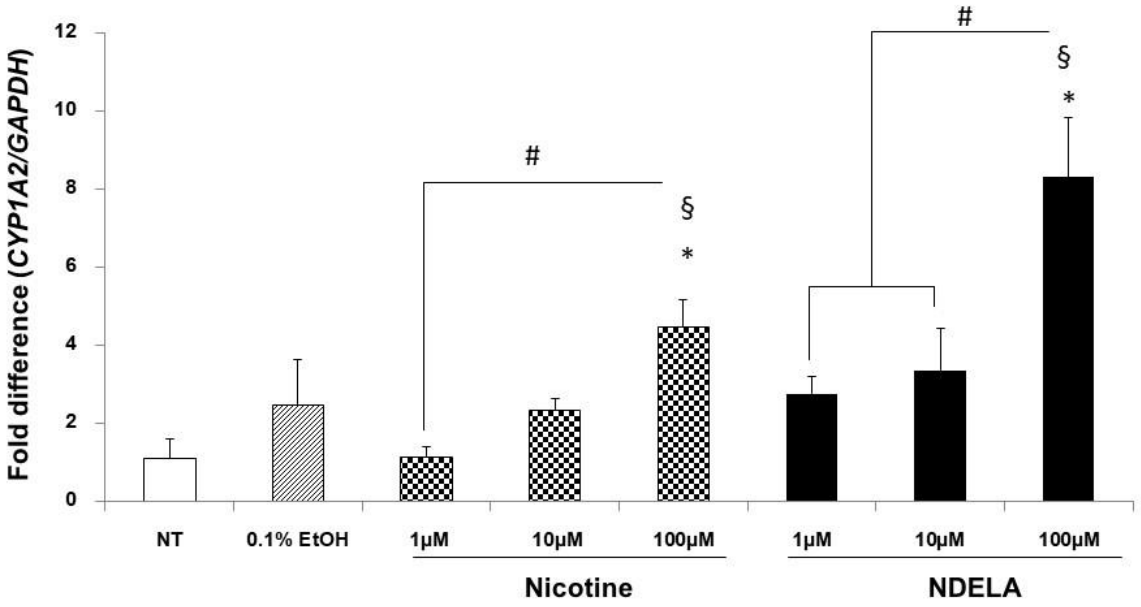

Figure 3. Effects of nicotine and *N*-nitrosodiethanolamine (NDELA) on relative expression of *CYP1A2* mRNA

Data are presented as mean±SD (n=4). NT, non-treatment; EtOH, ethanol. A significant difference was determined by ANOVA followed by Tukey's *post hoc* test. *p<0.05 vs NT; §p< 0.05 vs 0.1% EtOH; #p< 0.05 vs different concentrations in the same treatment.

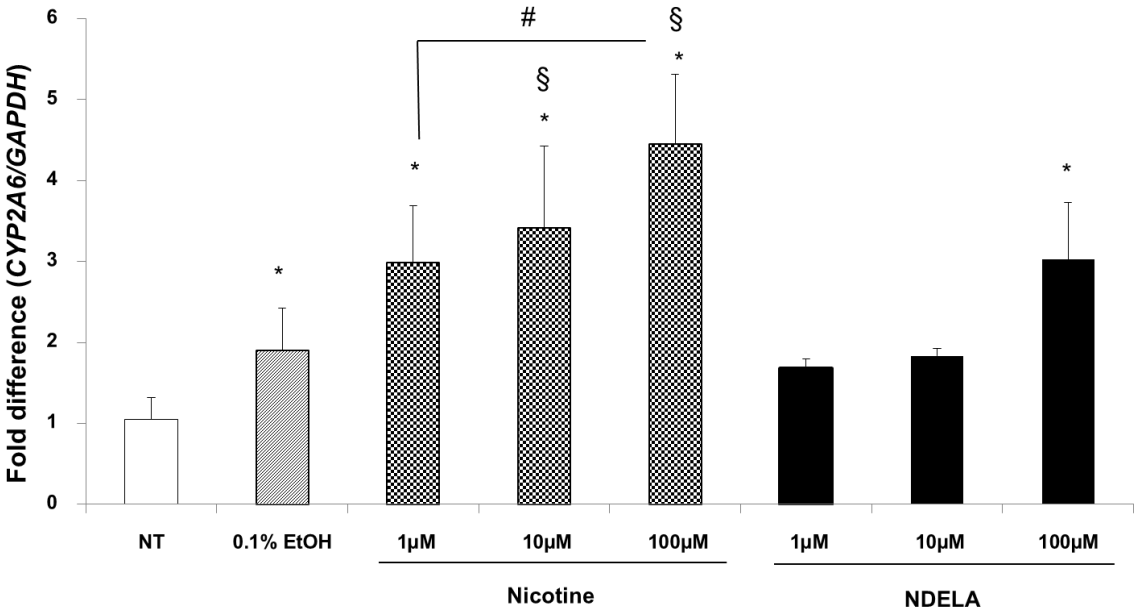

Figure 4. Effects of nicotine and *N*-nitrosodiethanolamine (NDELA) on relative expression of *CYP2A6* mRNA

Data are presented as mean±SD (n=4). NT, non-treatment; EtOH, ethanol. A significant difference was determined by ANOVA followed by Tukey's *post hoc* test. **p*<0.05 vs NT; §*p*< 0.05 vs 0.1% EtOH; #*p*< 0.05 vs different concentrations in the same treatment.

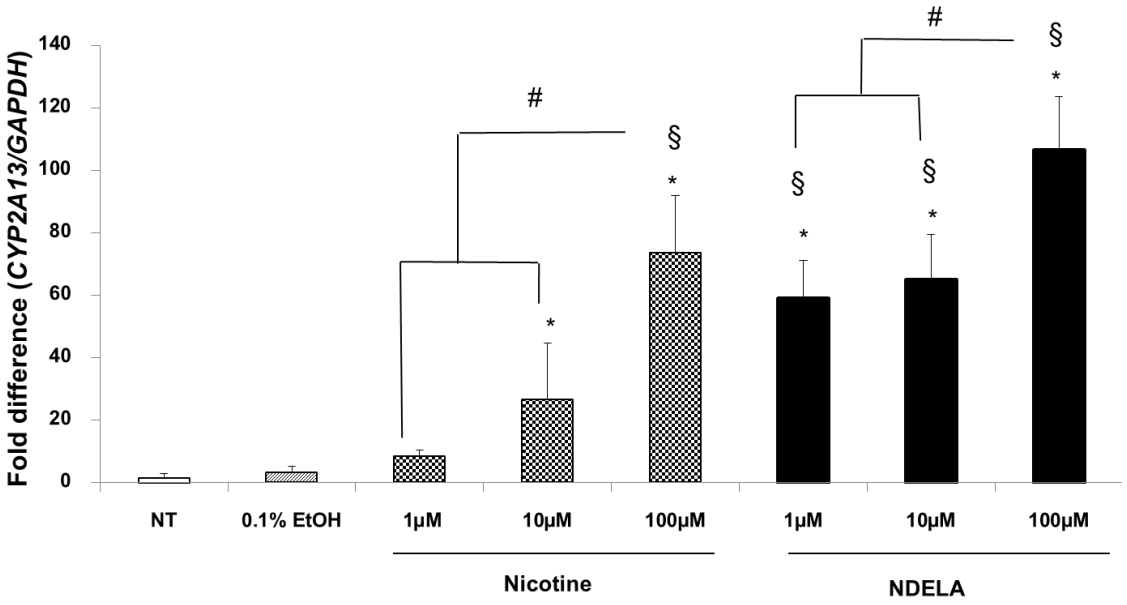

Figure 5. Effects of nicotine and *N*-nitrosodiethanolamine (NDELA) on relative expression of *CYP2A13* mRNA

Data are presented as mean±SD (n=4). NT, non-treatment; EtOH, ethanol. A significant difference was determined by ANOVA followed by Tukey's *post hoc* test. **p*<0.05 vs NT; §*p*< 0.05 vs 0.1% EtOH; #*p*< 0.05 vs different concentrations in the same treatment.

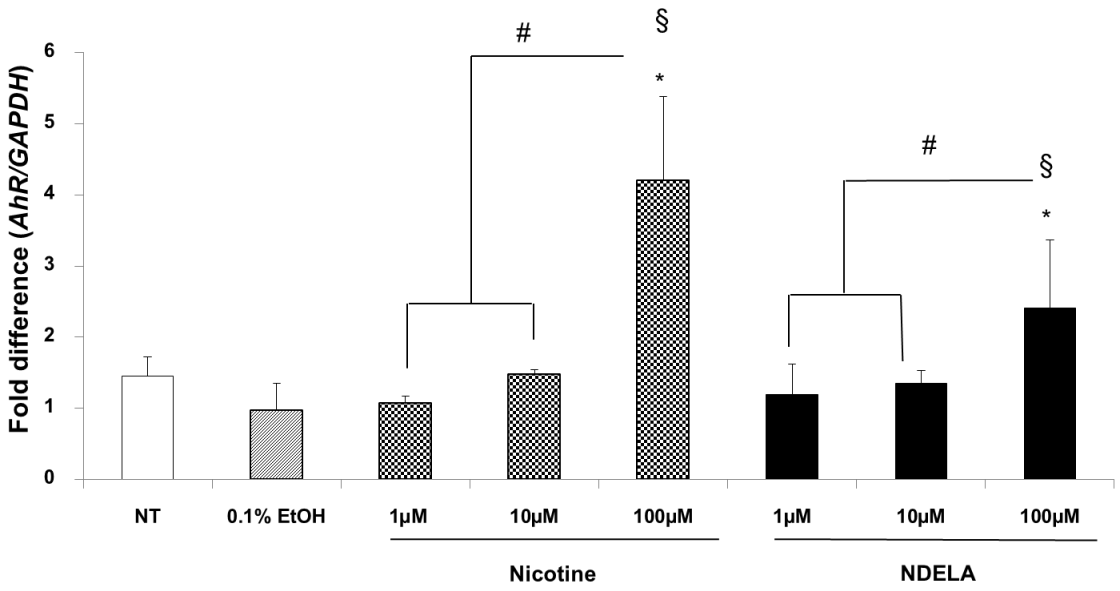

Figure 6. Effects of nicotine and *N*-nitrosodiethanolamine (NDELA) on relative expression of *AhR* mRNA

Data are presented as mean±SD (n=4). NT, non-treatment; EtOH, ethanol. A significant difference was determined by ANOVA followed by Tukey's *post hoc* test. **p*<0.05 vs NT; §*p*< 0.05 vs 0.1% EtOH; #*p*< 0.05 vs different concentrations in the same treatment.

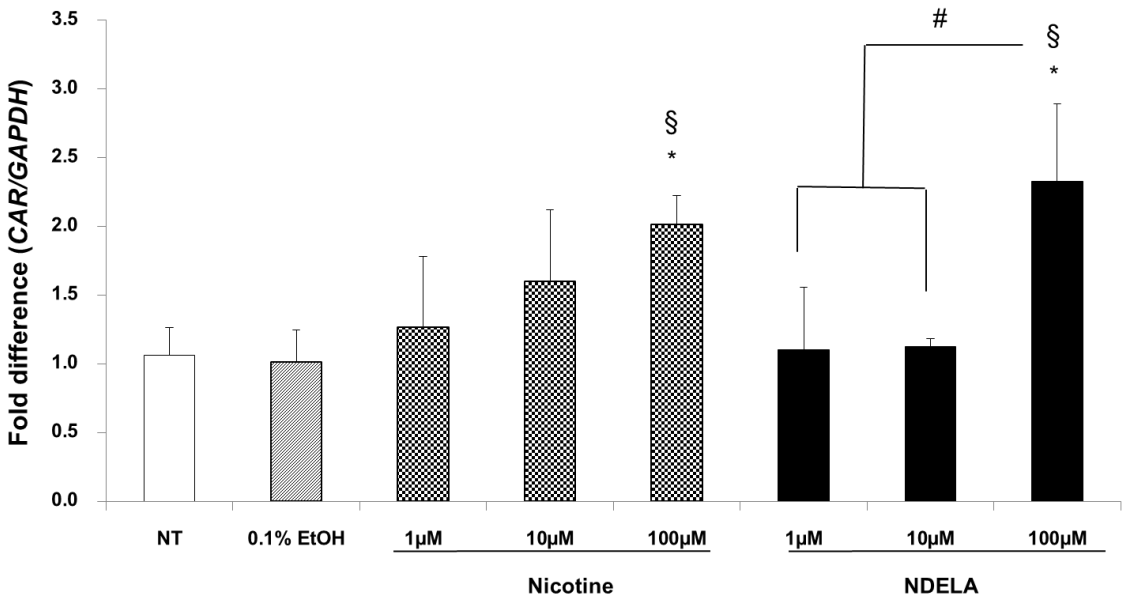

Figure 7. Effects of nicotine and *N*-nitrosodiethanolamine (NDELA) on relative expression of *CAR* mRNA

Data are presented as mean±SD (n=4). NT, non-treatment; EtOH, ethanol. A significant difference was determined by ANOVA followed by Tukey's *post hoc* test. **p*<0.05 vs NT; §*p*< 0.05 vs 0.1% EtOH; #*p*< 0.05 vs different concentrations in the same treatment.

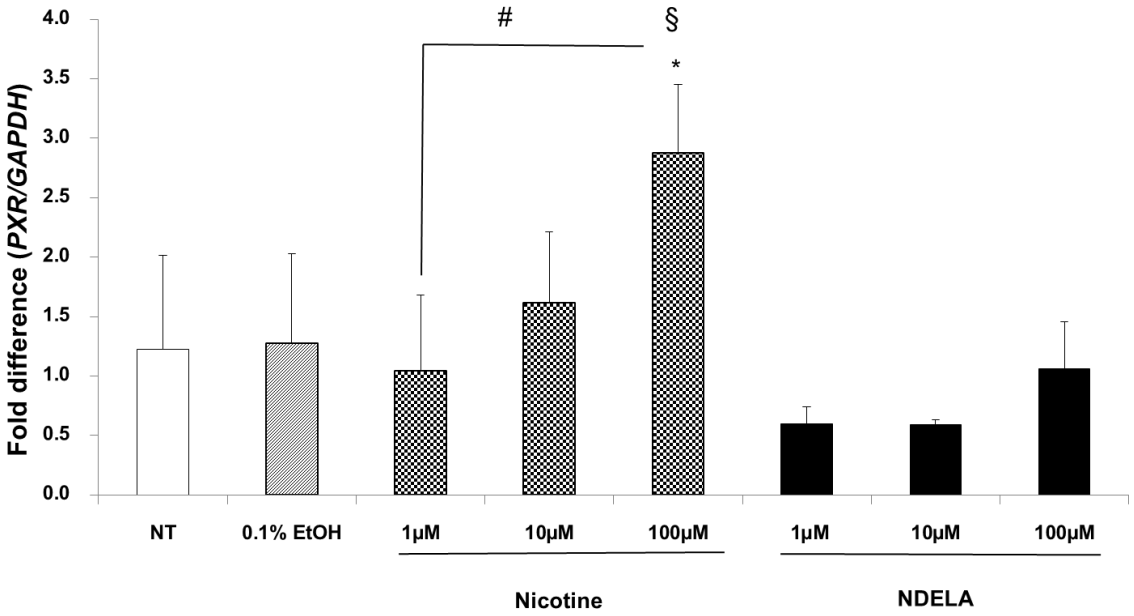

Figure 8. Effects of nicotine and *N*-nitrosodiethanolamine (NDELA) on relative expression of *PXR* mRNA

Data are presented as mean±SD (n=4). NT, non-treatment; EtOH, ethanol. A significant difference was determined by ANOVA followed by Tukey's *post hoc* test. **p*<0.05 vs NT; §*p*< 0.05 vs 0.1% EtOH; #*p*< 0.05 vs different concentrations in the same treatment.

Discussion and conclusion

CYP1A2 enzyme involves in detoxification and bioactivation of common environmental pollutants. Many evidences demonstrated induction of CYP1A2 enzymes by nicotine *in vitro* in rat lungs (Price *et al.*, 2004) and human pulmonary explant cultures (Wei *et al.*, 2002), and *in vivo* in kidneys, livers, lungs, placenta, and brains of both mice and rats (Anandatheerthavarada *et al.*, 1993; Iba and Fung, 1999; Wang *et al.*, 2009; Singha *et al.*, 2009). There is no animal and clinical research on the effect of NDELA on *CYP1A2* expression. According to this study, exposure to nicotine or NDELA for 24 h significantly increased the expression of *CYP1A2* mRNA (Figure 3). *AhR* is a key nuclear receptor which regulates transcriptional activation of *CYP1A2* mainly in metabolism of procarcinogen such as PAHs (Nebert and Dalton, 2006; Pascussi *et al.*, 2008). Cigarette smoke activates *AhR* and induces *CYP1A2* expression (Shimada *et al.*, 2002). The induction of *CYP1A2* is mediated via binding of PAHs from tobacco smoke to *AhR* with subsequent transcriptional activation of *CYP1A2* gene (Hukkanen *et al.*, 2011). Therefore, induction of *CYP1A2* in HepG2 cells by nicotine and NDELA might involve in regulatory mechanism of AhR.

CYP2A6 and *CYP2A13* play major roles in metabolism of nicotine, nitrosamines, coumarin, and other clinical drugs, and *vice versa* they are induced by several xenobiotics (Cupp and Tracy, 1998). A significant induction of *CYP2A6* and *CYP2A13* expression was observed following treatment of nicotine to human temporal lobe isolated cerebral endothelial derived hCMEC/D3 cells (Zuikova *et al.*, 2018). Hepatic *CYP2A6* mRNA was down-regulated in nicotine treated African Green monkeys (Ferguson *et al.*, 2012). *CYP2A13* was more active than *CYP2A6* in metabolic activation of *N,N*-dimethylaniline and *N*-nitrosomethylphenylamine which demonstrated that human *CYP2A13* was functional toward many toxic chemicals previously shown to be substrates of *CYP2A6* (Su *et al.*, 2000), corresponding to our results that *CYP2A13* was more inducible than *CYP2A6* by nicotine and NDELA (Figure 4 and 5). These findings supported several previous reports the induction of hepatic *CYP2A6* and *CYP2A13* expression by nicotine and nitrosamines (Miyazaki *et al.*, 2005; Chaing *et al.*, 2011). CYP2A protein was increased in alcohol-fed castrated micro pig, but not in non-castrated

group (Niemelä *et al.*, 1999). CYP2A6 mRNA and protein were unaffected by ethanol in African Green monkeys (Ferguson *et al.*, 2012). In human, CYP2A protein was co-localized with aldehyde adducts in human alcoholic liver disease, indicated that CYP2A expression was linked to processes which led to production of reactive oxygen radicals and eventual liver damage by alcohol (Niemelä *et al.*, 2000). In our case, 0.1% ethanol induced CYP2A6 mRNA in HepG2 might be explained by the effect of ethanol metabolism via generation of acetaldehyde. Expression of *CYP2A* is altered by a variety of substrates via activation/deactivation of various nuclear receptors and transcriptional factors (Itoh *et al.* (2006) described that level of *CYP2A6* expression is correlated with that of *PXR*. However, in the present study nicotine induced the expression of *PXR* while NDELA did not (Figure 8). Therefore, NDELA might induce *CYP2A6* expression via other nuclear receptor-mediated pathways.

PXR and *CAR* associate in regulatory pathways of *CYPs* (Itoh *et al.*, 2006). *CAR* is an orphan nuclear receptor that greatly influences transcription of several *CYPs*. Although *CAR* has structural and functional similarities with *PXR*, it binds to fewer chemicals than *PXR* due to its smaller ligand-binding pocket (Chai *et al.*, 2016). *CAR* gives transcriptional control to its target genes in a similar way to *PXR*. The function of *CAR* was first noticed when it modulated transcriptional pathway of *CYP2B* (Honkakoski *et al.*, 1998). *CAR* binds to nuclear receptor interaction motifs 1 and 2 in phenobarbital responsive enhancer module (PBREM) found in promoter region of *CYP2B*. Whereas human *CAR* especially activates *CYP2B6*, mouse *CAR* activates *CYP2B10*. Besides *CYP2B*, *CAR* can induce other *CYPs*. Both human and mouse *CAR* induced *CYP3A4* expression by binding to xenobiotic responsive enhancer module (XREM) in promotor region of *CYP3A* (Goodwin *et al.*, 2001). Moreover, *CYP1A1* and *CYP1A2*, a couple of typical target genes of *AhR*, were activated by *CAR* via an *AhR*-independent pathway (Yoshinari *et al.*, 2010). Cigarette smoke extract induced expression of *CYP2B6* mRNA in *CAR*-expressed HepG2 cells (Washio *et al.*, 2011). According to the present observations, relationship among expressions of *CYP1A2, CYP2A6, CYP2A13*, *AhR*, and *CAR* were demonstrated.

Nebert and Dalton (2006) reported roles of CYP1A, CYP2A, and AhR on the pathway of environmental carcinogenesis. CYP1 and CYP2A metabolize xenobiotics (environmental pollutants, heavy metal, drugs, and food stuffs) to reactive-oxygenated metabolites which are carcinogenetic and mutagenic via binding to DNA or protein, and resulting in oxidative stress, mutation and cancer (Nebert and Dalton, 2006). Therefore, exposure to nicotine or NDELA at high dose and/or for long period might increase the risk of cancer.

In conclusion, regulatory mechanism of *CYP1A2, CYP2A6*, and *CYP2A13* expression by nicotine and NDELA in HepG2 cells might involve with *AhR* and *CAR*. A different regulation of *PXR* by nicotine and NDELA is worth for further study.

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