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Research Article
Differential Impacts of Phenol Red on Benzo[*a*]pyrene and Dexamethasone-Modified Cytochrome P450s in Human Cancer Cells

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Abstract

Background and Objective: Phenol red, the pH indicator in cell culture media, influences the expression of cytochrome P450s (CYPs) in cell lines. This study aimed to examine how phenol red modified CYP induction by benzo[*a*]pyrene and dexamethasone in human hepatocarcinoma (HepG2), colorectal adenocarcinoma (Caco-2) and choriocarcinoma (BeWo) cells. **Materials and Methods:** The cells (1×10^5 cells/well in a 24-well plate) were incubated with benzo[*a*]pyrene (0.1, 1 and 10 μM) or dexamethasone (1, 5 and 10 μM) in either phenol red or phenol red-free media for 24 hrs. The mRNA expression of CYPs was determined by Real-Time Polymerase Chain Reaction (RT/qPCR). **Results:** Phenol red enhanced expression of benzo[*a*]pyrene-induced CYP1A2 inHepG2 and BeWo cells and suppressed benzo[*a*]pyrene-induced CYP2A6 expression in HepG2 and Caco-2 cells, benzo[*a*]pyrene induced CYP2B6 expression in HepG2 cells and benzo[*a*]pyrene- and dexamethasone-induced CYP3A4 expression in HepG2 and Caco-2 cells. The expression of CYP3A5 was affected differently in HepG2 and Caco-2 cell lines. Phenol red enhanced benzo[*a*]pyrene- and dexamethasone-induced CYP3A5 expression in Caco-2 cells but suppressed benzo[*a*]pyrene- and dexamethasone-induced CYP3A5 expression in HepG2 cells. **Conclusion:** Phenol red differentially influenced expression of benzo[*a*]pyrene- and dexamethasone-induced CYP1A2, CYP2A6, CYP2B6, CYP3A4 and CYP3A5 mRNAs in HepG2, Caco-2 and BeWo cells. Therefore, the inclusion of phenol red in cell culture media is of concern in studies of drug and xenobiotic metabolism via CYPs in human cell line models.

Key words: HepG2, Caco-2, BeWo, phenol red, metabolism, small intestinal enterocyte, CYP expression, mRNA expression

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Phenol red (Fig. 1a) is the pH indicator in cell culture media[1,2]. At pH 7.4 phenol red is bright red and it turns yellow in the presence of acidic cell-waste[1]. Due to its similar structure to the non steroidal hormone 17b-estradiol (Fig. 1b), phenol red possesses a partial estrogenic effect stimulating the Estrogen Receptor (ER) and influencing regulatory expression of cytochrome P450 (CYP) in estrogen-sensitive cell lines like the breast cancer cell line MCF-7[2]. Thus, to investigate CYP-associated metabolism, a phenol red-free medium is recommended for use during treatment periods to avoid interference with CYP activity[3]. CYP is a family of monooxygenase enzymes responsible for phase I metabolism[4]. CYPs metabolize drugs and xenobiotics to increase their polarity and facilitate their elimination from the body. However, CYP metabolism can also activate substrates and produce toxic metabolite(s)[4]. Benzo[*a*]pyrene (BaP; Fig. 1c) and dexamethasone (Dex; Fig. 1d) are CYP1, CYP2 and CYP3 inducers[4]. Exposure to CYP inducers can disturb the bioavailability and metabolism of CYP substrates leading to clinical outcomes that depend on the abundance and distribution of the affected CYP isoforms[5].

Human cell lines are *in vitro* models that represent human organs[6]. Although CYP expression in cell lines is not at the same level as that found in primary cells, the immortality and adaptability of cell lines can outweigh these limitations[6]. The human hepatocarcinoma (HepG2), colorectal adenocarcinoma (Caco-2) and choriocarcinoma (BeWo) cell lines are widely employed for metabolic studies representing liver, intestine and placenta, respectively[4,6]. The HepG2 cell line presents genotypic features that are similar to primary hepatocytes[7]. The Caco-2 cell line is superior to other colorectal carcinoma cell lines in that it can be spontaneously differentiated to have small intestinal enterocyte or colonocytes characteristics that are similar to the small intestine[8]. The BeWo cell line was developed from human embryonic stem cells and exhibits some characteristics of primary trophoblast cells such as microvillus projection and expression of marker enzymes and drug transporters[9]. Although the liver, intestine and placenta are major sites of CYP expression, evidence of the effect of phenol red on the expression of CYPs in the aforementioned human cell lines is limited. Therefore, this study aimed to examine how phenol red influences the expression of BaP- and Dex-induced CYP1A2, CYP2A6, CYP2B6, CYP3A4 and CYP3A5 in HepG2, Caco-2 and BeWo cells.

MATERIALS AND METHODS

Study area: The study was carried out at Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand from January-December, 2020.

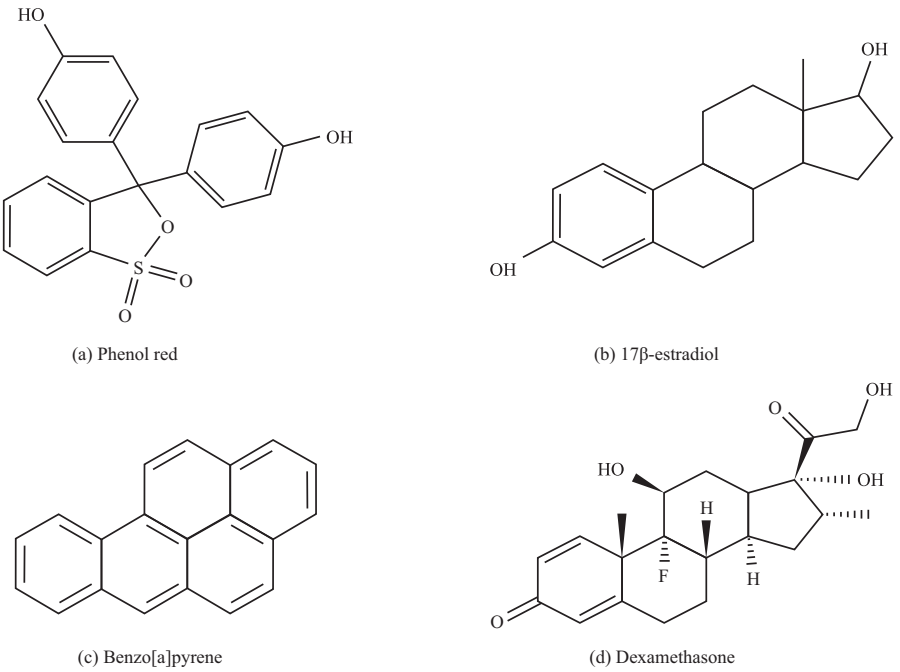

Fig. 1(a-d): Chemical structure of (a) Phenol red, (b) 17β-estradiol, (c) Benzo[*a*]pyrene and (d) Dexamethasone

Materials: Dulbecco's modified Eagle medium (+) phenol red (DMEM with phenol red, Cat. No. 11885-084), Dulbecco's modified Eagle (-) phenol red medium (DMEM phenol red-free medium, Cat. No. 31053-028), Fetal Bovine Serum (FBS), 1×Glutamax® and 1× penicillin, streptomycin and neomycin antibiotics (PSN) were purchased from Gibco® (New York, USA). BaP and Dex were products of Sigma-Aldrich Chemical (Missouri, USA). RedSafe™ nucleic acid staining solution was a product of iNtRON Biotechnology (Seongnam, South Korea). ReverTra Ace® was from Toyobo Co., Ltd. (Osaka, Japan). Taq DNA polymerase and dNTP mixture were products of Vivantis Technologies (Selangor Darul Ehsan, Malaysia). SYBR® Green I was supplied by Invitrogen Life Technologies (California, USA). Forward and reverse primers were synthesized by Bio Basic, Inc. (Ontario, Canada). All other chemicals were of the highest purity from commercial chemical suppliers.

Cell culture and experimental design: The HepG2 (ATCC HB-8065, Manassas, Virginia, USA), Caco-2 (RBRC-RBC0988 RIKEN cell bank, Wako, Saitama, Japan) and BeWo (provided by Dr. Isabella Ellinger, Medical University of Vienna, Vienna, Austria) cell lines were cultured in DMEM supplemented with 1 g L^{-1} D-glucose, L-glutamine, 110 mg mL^{-1} sodium pyruvate, 1×Glutamax®, 10 or 20% FBS and 1×PSN at 37°C with 5% CO_2 and 95% relative humidity. Each cell line was seeded into 24 well-plates (1×10^5 cells/well in 0.5 mL) for 72 hrs before incubation with tested compounds in phenol red or phenol red-free medium for 24 hrs. Each medium contained BaP at final concentrations of 0.1, 1 and 10 μM or Dex at final concentrations of 1, 5 and 10 μM. The control group was incubated with a medium that contained 0.1% dimethyl sulfoxide (DMSO). The medium was collected for the determination of cell viability and the cells were harvested for quantitative analysis of CYP mRNA.

Determination of cell viability: Cell viability was determined by resazurin assay based on the ability of viable cells to reduce non-fluorescent blue dye (resazurin) to fluorescent pink dye (resorufin). The medium was mixed with 1 mM resazurin (10:1) before incubation in 5% CO_2 at 37°C for 1 hr. The percentage of cell viability was calculated from an increasing rate of resorun measured at an excitation of 530 nm and emission of 580 nm as described by Chatuphonprasert *et al.*[10].

Quantitative analysis of mRNA expression by RT/qPCR: Total RNA was extracted by guanidinium thiocyanate-phenol-chloroform extraction method according to Chatuphonprasert *et al.*[11]. Concentration and purity of total RNA were measured by a NanoDrop 2000C (Thermo Fisher Scientific, Massachusetts, USA) at wave lengths of 260/280 and 260/230 nm, respectively. RedSafe™-stained total RNA (1 μg) in Tris-borate-EDTA buffer was separated under 1.25% agarose gel electrophoresis and RNA integrity was evaluated from qualities of 28S and 18S RNAs under a UV trans illuminator. Total RNA (500 ng) was reverse-transcribed using ReverTra Ace® under conditions recommended by the supplier (Toyobo Co., Ltd.) at 25°C for 10 min, 42°C for 60 min and 95°C for 5 min. Then cDNA was amplified by qPCR machine CFX96 Touch™ (Biorad, California, USA) with Bio-Rad-CFX manager version 3.1 program for quantitative analysis of CYP1A2, CYP2A6, CYP2B6, CYP3A4 and CYP3A5 mRNA expressions. The qPCR master mixture composed of 0.14 unit/μL Taq DNA polymerase, 1.67 mM $MgCl_2$, 0.12 mM dNTP mixture, 1:7,500 SYBR® Green I and 0.22 μM specific forward and reverse primers of each target gene (Table 1). The qPCR system includes denaturation at 95°C for 20 sec, annealing at an optional temperature for each gene (Table 1) for 20 sec and extension at 72°C for 20 sec. Primer specificity was evaluated by melting curve temperature. The results are shown as

Table 1: Primer sequence used for determination of human genes

Genes		Primers (5'→3')	Annealing temperature (°C)	Product size (bp)	References
CYP1A2	F	ACA AGG CAC AAC GCT GAA	60.0	160	Chatuphonprasert *et al.*[10]
	R	AGG GCT TGT TAA TGG CAG TG			
CYP2A6	F	TCA AAG GCT ATG GCG TGG TA	60.0	284	Boonmaton *et al.*[13]
	R	CAT CAT GCG CAA CAG TGA CA			
CYP2B6	F	AGA CGC CTT CAA TCC TGA CC	60.0	105	Li *et al.*[14]
	R	CCT TCA CCA AGA CAA ATC CGC			
CYP3A4	F	GCC TGG TGC TCC TCT ATC TA	54.7	187	Chatuphonprasert *et al.*[12]
	R	GGC TGT TGA CCA TCA TAA AAG			
CYP3A5	F	CCT GAA CCT CTC AGG AGC ATT T	61.1	245	Suksawat *et al.*[7]
	R	AGA TCA GAG GGC TGG TGA GTT			
GAPDH	F	CAC CAT CTT CCA GGA GCG AG	61.1	72	Chatuphonprasert *et al.*[10]
	R	GAC TCC ACG ACG TAC TCA GC			

average fold-differences of CYP mRNA expression normalized with the reference gene (glyceraldehyde 3-phosphate dehydrogenase, GAPDH). The changes in cycle threshold between the gene of interest and the mean of the reference gene (ΔCt) and the changes in Ct between treatment and control (ΔΔCt) were calculated and extrapolated to $2^{-(\Delta\Delta Ct)}$ to find the fold-change of gene expression as described by Chatuphonprasert *et al*.[12].

Statistical analysis: The results are presented as mean±standard deviation (SD) (n = 4). A significant difference was analyzed by one-way analysis of variance (ANOVA) with Tukey's statistical *post hoc* test at $p<0.05$ using Statistical Package for the Social Science (SPSS, version 23.0, Armonk, New York, USA).

RESULTS

Effects of phenol red on cell viability of BaP- and Dex-treated HepG2, Caco-2 and BeWo cells: BaP (0.1 and 1 μM) increased viability of HepG2 cells in both phenol red and phenol red-free medium ($p<0.05$), while Dex did not (Fig. 2a). Viability of Caco-2 cells treated with either BaP or Dex in phenol red medium was comparable to the control, while in phenol red-free medium Caco-2 cell viability was increased by BaP (0.1 and 1 μM) and Dex (5 and 10 μM) ($p<0.05$) (Fig. 2b). Likewise, the viability of BeWo cells treated with BaP or Dex in phenol red medium was comparable to the control, while BaP (0.1 μM) increased the viability of BeWo cells in phenol red-free medium (Fig. 2c).

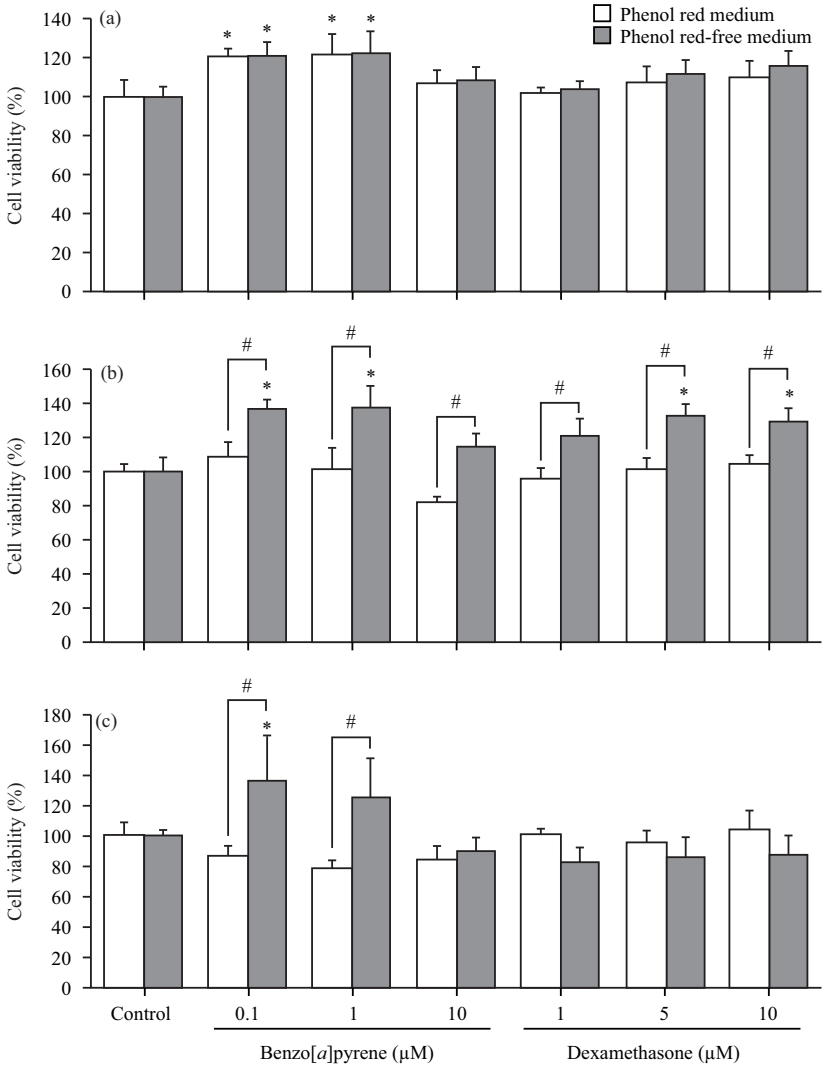

Fig. 2(a-c): Effects of phenol red on cell viability of BaP- and Dex-treated
(a) HepG2, (b) Caco-2 and (c) BeWocells. *$p<0.05$ vs. control, #$p<0.05$ vs. phenol red medium

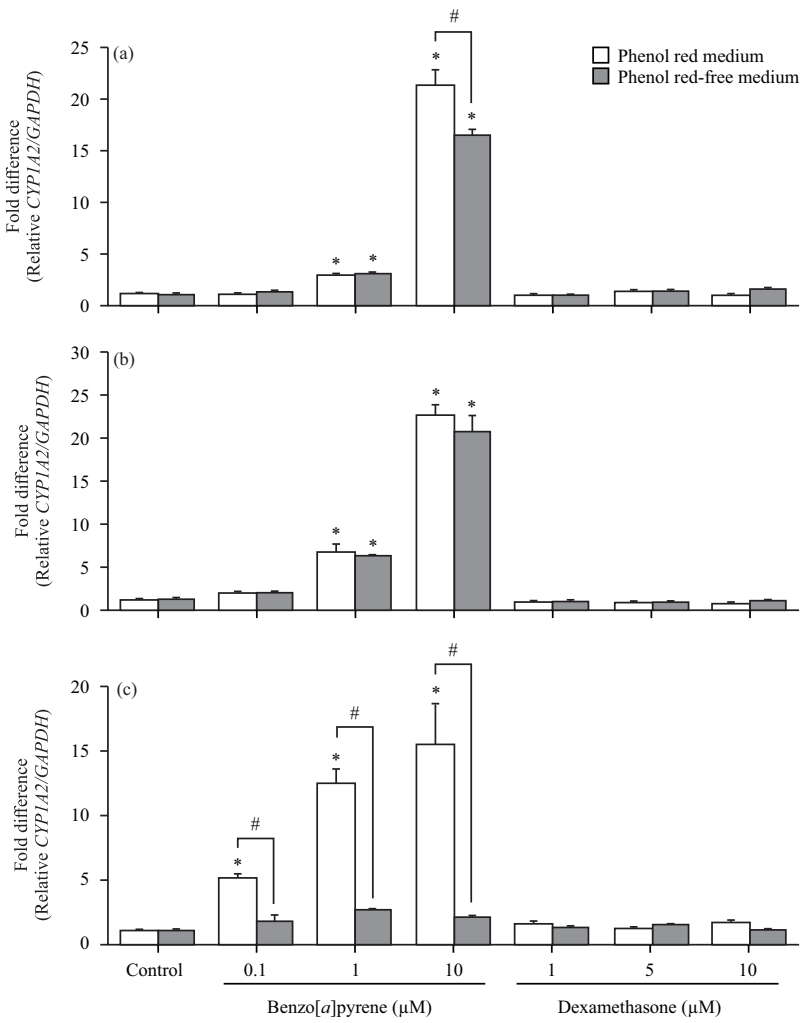

Fig. 3(a-c): Effects of phenol red on expression of CYP1A2 mRNA in BaP- and Dex-treated (a) HepG2, (b) Caco-2 and (c) BeWo cells. *p<0.05 vs. control, #p<0.05 vs. phenol red medium

Effects of phenol red on the expression of CYP1A2 mRNA in BaP- and Dex- treated HepG2, Caco-2 and BeWo cells: BaP (1 and 10 μM) induced expression of CYP1A2 in HepG2 (Fig. 3a) and Caco-2 cells (Fig. 3b) in both phenol red and phenol red-free medium ($p<0.05$). In contrast, BaP (0.1, 1 and 10 μM) up-regulated CYP1A2 expression in BeWo cells only in phenol red medium ($p<0.05$) with CYP1A2 expression in phenol red-free medium unchanged (Fig. 3c). Dex did not induce CYP1A2 expression in HepG2, Caco-2 or BeWo cells in both phenol red and phenol red-free medium.

Effects of phenol red on the expression of CYP2A6 mRNA in BaP- and Dex-treated HepG2, Caco-2 and BeWo cells: BaP (10 μM) induced expression of CYP2A6 in HepG2 cells in phenol red-free medium ($p<0.05$), but not in phenol red medium (Fig. 4a). Dex did not modify CYP2A6 expression in HepG2 cells in both media. In Caco-2 cells, 0.1 and 1 μM BaP increased CYP2A6 expression in phenol red-free medium ($p<0.05$) but only 1 μM BaP induced CYP2A6 expression in phenol red medium (Fig. 4b). Dex did not modify CYP2A6 expression in Caco-2 cells in both media. In BeWo cells,1 μM Dex increased expression of CYP2A6 in both phenol red and phenol red-free medium ($p<0.05$) (Fig. 4c).

Effects of phenol red on the expression of CYP2B6 mRNA in BaP- and Dex-treated HepG2, Caco-2 and BeWo cells: BaP concentration-dependently up-regulated expression of CYP2B6 in HepG2 cells in phenol red-free medium ($p<0.05$), while expression of CYP2B6 in phenol red medium was not altered (Fig. 5a). Dex did not modify CYP2B6 expression in HepG2 cells. BaP and Dex did not affect CYP2B6 expression in Caco-2 and BeWo cells (Fig. 5b-c).

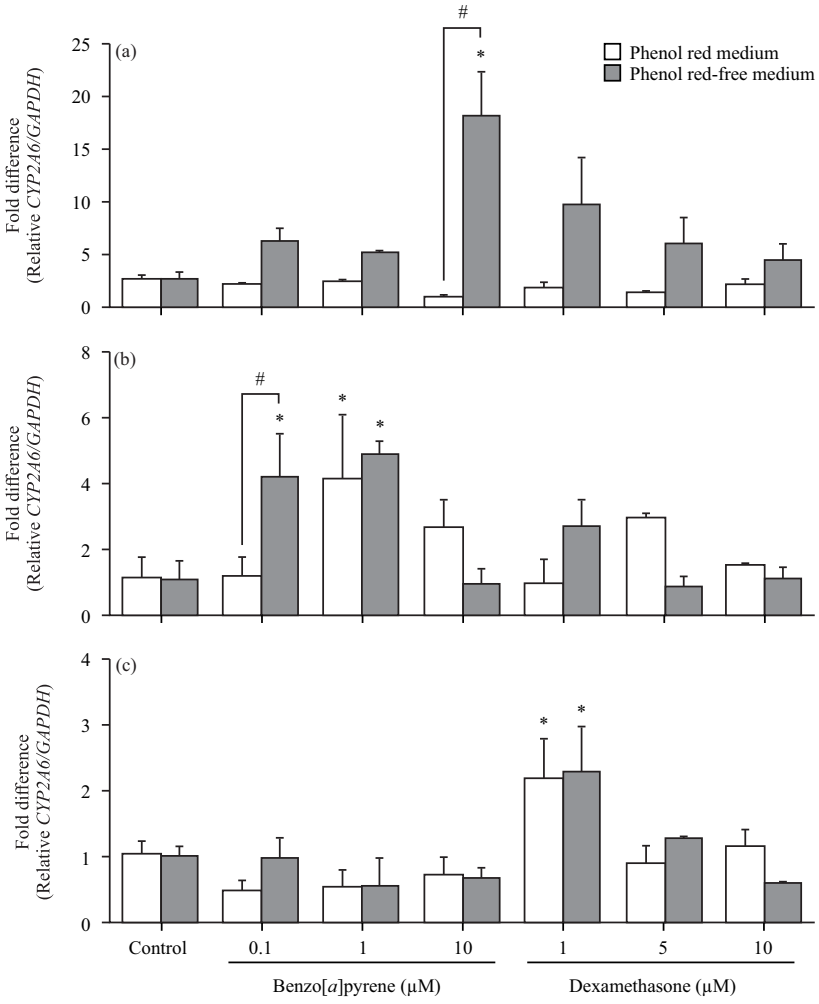

Fig. 4(a-c): Effects of phenol red on expression of CYP2A6 mRNA in BaP- and Dex-treated (a) HepG2, (b) Caco-2 and (c) BeWo cells.*p<0.05 vs. control, #p<0.05 vs. phenol red medium

Effects of phenol red on the expression of CYP3A4 mRNA in BaP- and Dex-treated HepG2, Caco-2 and BeWo cells: In HepG2 cells, BaP inversely up-regulated expression of CYP3A4 in a concentration-dependent manner in phenol red medium, while CYP3A4 expression in phenol red-free medium was induced by 0.1 and 1 μM BaP (p<0.05) (Fig. 6a). Correspondingly, Dex inversely up-regulated expression of CYP3A4 in a concentration-dependent manner in phenol red-free medium, while CYP3A4 expression in phenol red medium was induced by 5 μM Dex (p<0.05). The expression of CYP3A4 in Caco-2 cells in the phenol red medium was up-regulated by the highest concentration of BaP (10 μM), while CYP3A4 expression in the phenol red-free medium was induced by 1 μM BaP (p<0.05) (Fig. 6b). Dex up-regulated CYP3A4 expression in Caco-2 cells in both media, but the increase in CYP3A4 expression in phenol red-free medium was superior to that in phenol red medium. Expression of CYP3A4 mRNA was not detected in BeWo cells under any treatments (data not shown).

Effects of phenol red on the expression of CYP3A5 mRNA in BaP- and Dex-treated HepG2, Caco-2 and BeWo cells: BaP down-regulated expression of CYP3A5 in HepG2 cells in both phenol red and phenol red-free medium (p<0.05) (Fig. 7a). Likewise, in phenol red medium, Dex down-regulated CYP3A5 expression in HepG2 cells (p<0.05), but 1 uM Dex-treated HepG2 cells in phenol red-free medium showed induction of CYP3A5 (p<0.05). In Caco-2 cells, BaP and Dex induced CYP3A5 expression in phenol red medium, (p<0.05) (Fig. 7b). Expression of CYP3A5 mRNA was not detected in BeWo cells under any treatments (data not shown).

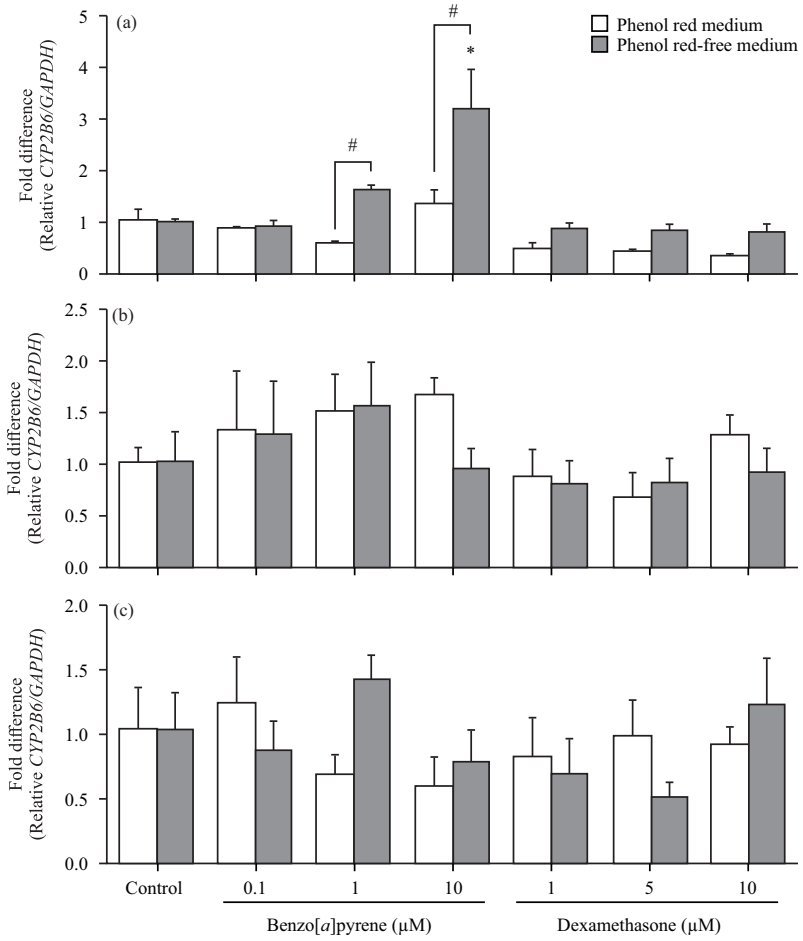

Fig. 5(a-c): Effects of phenol red on expression of CYP2B6 mRNA in BaP- and Dex-treated (a) HepG2, (b) Caco-2 and (c) BeWo cells. *p<0.05 vs. control, #p<0.05 vs. phenol red medium

DISCUSSION

In the present study, BaP at concentrations of 0.1, 1 and 10 μM and Dex at concentrations of 1, 5 and 10 μM were selected to examine the effects of phenol red on the expression profiles of CYPs in HepG2, Caco-2 and BeWo cells. BaP is an environmental contaminant encountered via cigarette smoke, gasoline combustion and charcoal-grilled food[13]. It is a CYP1 inducer[4], but CYP2A6, CYP2C19 and CYP3A4 have also been reported to play a role in BaP metabolism[13,15]. The induction of CYP1A2 and CYP2A6 in HepG2 cells was observed by BaP in the concentration range of 2-64 μM with nocytotoxicity[13,16]. Besides, Dex is an inducer for CYP1A, CYP2B and CYP3A, which causes an increased risk of medication error and therapeutic failure[4,17]. Dex exhibits biphasic action on expression of CYP3A4 in human hepatocytes; at low concentrations (nM) it is partially induced through the Glucocorticoid Receptor (GR) pathway, while at high concentrations (>10 μM) up-regulation of CYP3A4 expression occurs via the Dex-mediated Pregnane X Receptor (PXR) pathway[17]. A prior study reported EC_{50} and Emax concentrations of Dex in primary human hepatocytes at 9.21-19.90 and 6.54-19.20 μM, respectively[18]. Commercially available media contain phenol red as an indicator in the range of 3-45 μM. The phenol red media used in this study contained phenol red at a concentration of 39.85 μM.

Two BaP-like structure-procarcinogens, benzo[*a*] anthracene (36.09-250 μM) and benzo[*k*]fluoranthene (22.56-160 μM) induce proliferation of HepG2 cells via the mitogen-activated protein kinase signalling activation pathway[19] and BaP plus fluoranthene has been shown to increase the number of colon adenocarcinoma cells (HT-29)[20]. Dex inhibited HepG2 cell proliferation at high concentration (127 μM) but the inhibitory effect was not observed at concentrations less than 64 μM[21]. Dex was reported to induce colon carcinoma cell (T84) proliferation via activation of the

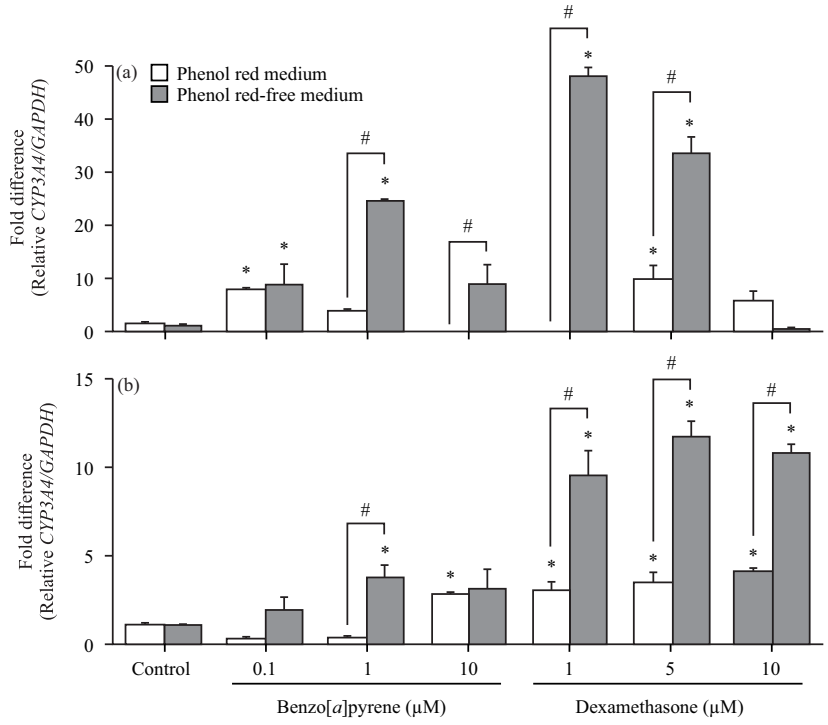

Fig. 6(a-b): Effects of phenol red on expression of CYP3A4 mRNA in BaP- and Dex-treated
(a) HepG2 and (b) Caco-2. *p<0.05 vs. control, #p<0.05 vs. phenol red medium

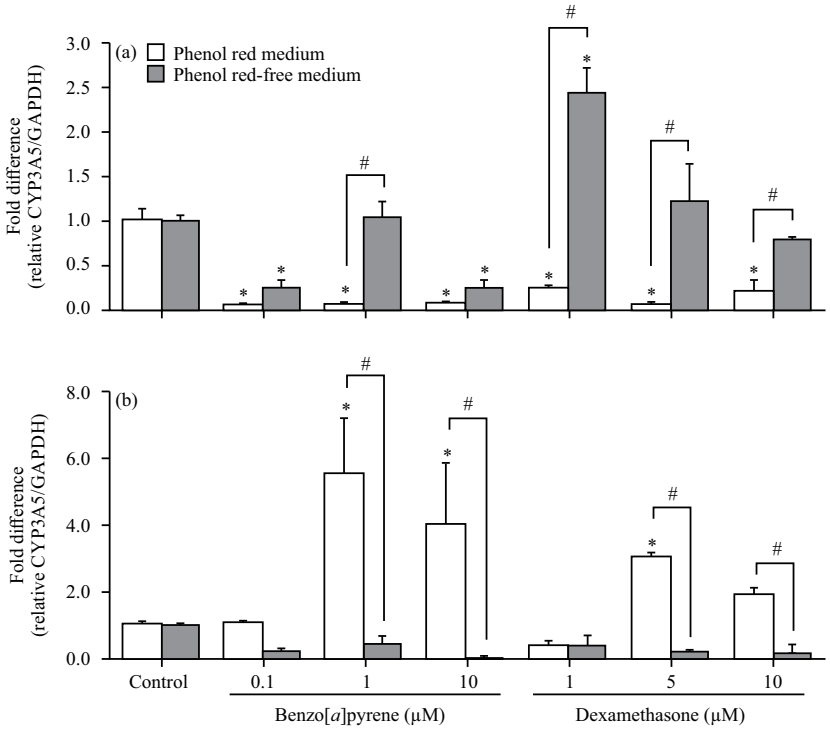

Fig. 7(a-b): Effects of phenol red on expression of CYP3A5 mRNA in BaP- and Dex-treated
(a) HepG2 and (b) Caco-2. *p<0.05 vs. control, #p<0.05 vs. phenol red medium

cyclin-dependent kinase 1 pathway[22]. In the present study, BaP increased HepG2 cell viability either with or without phenol red, Dex did not affect HepG2 cell viability and BaP and Dex increased Caco-2 cell proliferation only without phenol red. This might be explained by the estrogenic activity of phenol red mediating interaction between the phenol red/Estrogen Receptor (ER) complex and the Aryl hydrocarbon Receptor/Aryl hydrocarbon receptor Nuclear Translocator (AhR/ANRT) complex, resulting in suppression of transcription and a decrease in cell proliferation[23].Co-treatment of MCF-7 cells with 17b-estradiol (0.001 μM) and Dex (0.1 μM) suppressed proliferation[24]. BaP (0.1-10 μM) has been shown to increase BeWo cell proliferation and differentiation[25] and in the current study BeWo cell viability was consistently greater in phenol red-free than phenol red medium. These observations support the partial estrogenic activity of phenol red stimulating negative feedback in the ER-AhR/ANRT complex leading to down-regulation of transcription of b-human chorionic gonadotropin[23,25].

CYP1A2 is abundant in the liver and is also found in extra hepatic tissues[4]. CYP1A2 plays an important role in the metabolism of polycyclic aromatic hydrocarbons such as BaP[4]. BaP undergoes oxidative metabolism via CYP1A2, producing reactive carcinogens that trigger DNA damage and promote cancer[4]. BaP increased expression of CYP1A2 in HepG2 cells at 10 μM[13]. The present study demonstrated a significant induction of CYP1A2 expression by BaP in HepG2 and BeWo cells in both phenol red and phenol red-free medium, with superior induction in phenol red medium. This phenomenon supports the estrogenic action of phenol red activating CYP1A2 to produce excessive amounts of free radicals and an active benzo[*a*]pyrene diol epoxide metabolite, which is a potent CYP1A inducer[4,26].

CYP2A6 plays a role in the metabolism of nonplanar small molecules such as ketones and nitrosamines, which include therapeutic drugs such as valproic acid, pilocarpine and tegafur[4]. BaP (10 μM) increased the expression of CYP2A6 in HepG2 cells[13]. Correspondingly, in the current study, BaP induced CYP2A6 expression in HepG2 and Caco-2 cells in phenol red medium. These observations suggest that the regulatory mechanism of CYP2A6 expression is the same in the liver and intestine[27]. The observation that CYP2A6 induction was higher in phenol red-free than phenol red medium might be explained by the hypothetical interaction of phenol red with the ER and AhR/ARNT complex to change the transcriptional binding region and/or the promoter and down-regulate transcription[23]. In contrast, Dex (1 μM) induced expression of CYP2A6 in BeWo cells phenol red-independently. This suggests that Dex, at low concentration, regulates CYP2A6 in the placenta via the PXR-activated pathway, not the GR-associatedpathway[17].

CYP2B6 plays a role in the metabolism of several groups of therapeutic drugs including anaesthetic (propofol and ketamine), smoking cessation (bupropion), anti-retroviral (efavirenz) and anti-cancer (cyclophosphamide) drugs and toxic substances like aflatoxin B1 and pesticides (organo phosphorus, N,N-diethyl-m-toluamide)[4]. Benzo[*a*] pyrene-3-ol, a BaP metabolite, was found in human hepatic CYP2B6-transfected insect cells treated with BaP (50 μM)[15]. The present study found that BaP (10 μM) induced expression of CYP2B6 in HepG2 cells in phenol red medium and the higher induction of CYP2B6 by BaP in the presence of phenol red describes the negative regulation of CYP2B6 transcription by phenol red-bound ER and AhR/ARNT protein interaction[23]. The lack of a change in CYP2B6 expression by all treatments in Caco-2 and BeWo cells might be caused by the very low abundance of CYP2B6 in these two cell types.

CYP3A4 and CYP3A5 are highly expressed in the liver and intestine and are also found in extra hepatic organs[4]. Dex (10 μM) induced CYP3A expression in HepG2 cells 2.7-fold[28]. The present study found that Dex up-regulated expression of CYP3A4 and CYP3A5 in HepG2 cells in phenol red-free medium with less induction in phenol red medium. This is likely to be related to the estrogenic effect of phenol red suppressing Dex-induced CYP3A via ER activation. Dex (0.1 μM) activated the GR and caused a protein-protein interaction and competitive binding to the DNA binding site with the estrogen/ER complex, which resulted in slight inhibition of estrogen-mediated ER activation in MCF-7 cells[24]. The observation that expression of CYP3A5 was lower than CYP3A4 in HepG2 cells is supported by compensatory regulation of CYP3A4 and CYP3A5; over expression of CYP3A4, the major CYP3A iso form might down-regulate CYP3A5 expression[29]. The complete suppression of CYP3A4 expression in BaP-treated HepG2 cells in phenol red medium might be due to phenol red potentiating the major pathway of BaP metabolism (via CYP1A2), thereby down-regulating CYP3A4 expression[29]. The observation that induction of CYP3A4 expression by BaP and Dex was at nearly comparable levels could be supported by the existence of common regulatory mechanisms in the liver and intestine[27]. In Caco-2 cells, expression of Dex-induced CYP3A4 mRNA was greater in phenol red-free medium. This suggests that Dex up-regulates CYP3A4 expression via the GR regulatory pathway[17]. Phenol red estrogenic activity might activate the ER leading to GR/ER interaction preventing activation of the CYP3A4 transcription

pathway, resulting in suppression of CYP3A4 expression[24]. The finding that expression of CYP3A5 was higher in the presence of phenol red might be due to a compensatory mechanism to up-regulate CYP3A5, a minor CYP3A isoform, in place of CYP3A4[29]. In the present study, CYP3A4 and CYP3A5 mRNAs were not detected in BeWo cells. This is presumably because these two isoforms do not function in the placenta. During pregnancy, CYP3A7 is mainly expressed in the fetal liver and the placenta and plays an important role in drug and xenobiotic metabolism[30].

This study raised a strong concern on phenol red in a cell culture medium which might affect the expression profiles of CYP induction. Therefore, phenol red-free culture medium might be the optimal system to study drug metabolism and interaction via expression of CYPs in human cell lines. Nonetheless, the impact of phenol red against CYP inducers in cell lines is still limited and worth further study, including examining how nuclear receptors associated with regulation of CYP induction by phenol red.

CONCLUSION

Phenol red differentially influenced CYP expression depending on the inducer, the abundance of CYPs and the type of cell. Therefore, the inclusion of phenol red in cell culture media is an important consideration for studies of drug and xenobiotic metabolism by CYPs in human cell line models.

SIGNIFICANCE STATEMENT

This study discovers the differential impacts of phenol red on modulatory effects of benzo[*a*]pyrene and dexamethasone against profiles of CYPs in HepG2, Caco-2 and BeWo cells which can be beneficial for researchers to choose the appropriate cell culture medium depending on cell type and/or purpose of study, particularly drug and xenobiotic metabolism study.

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