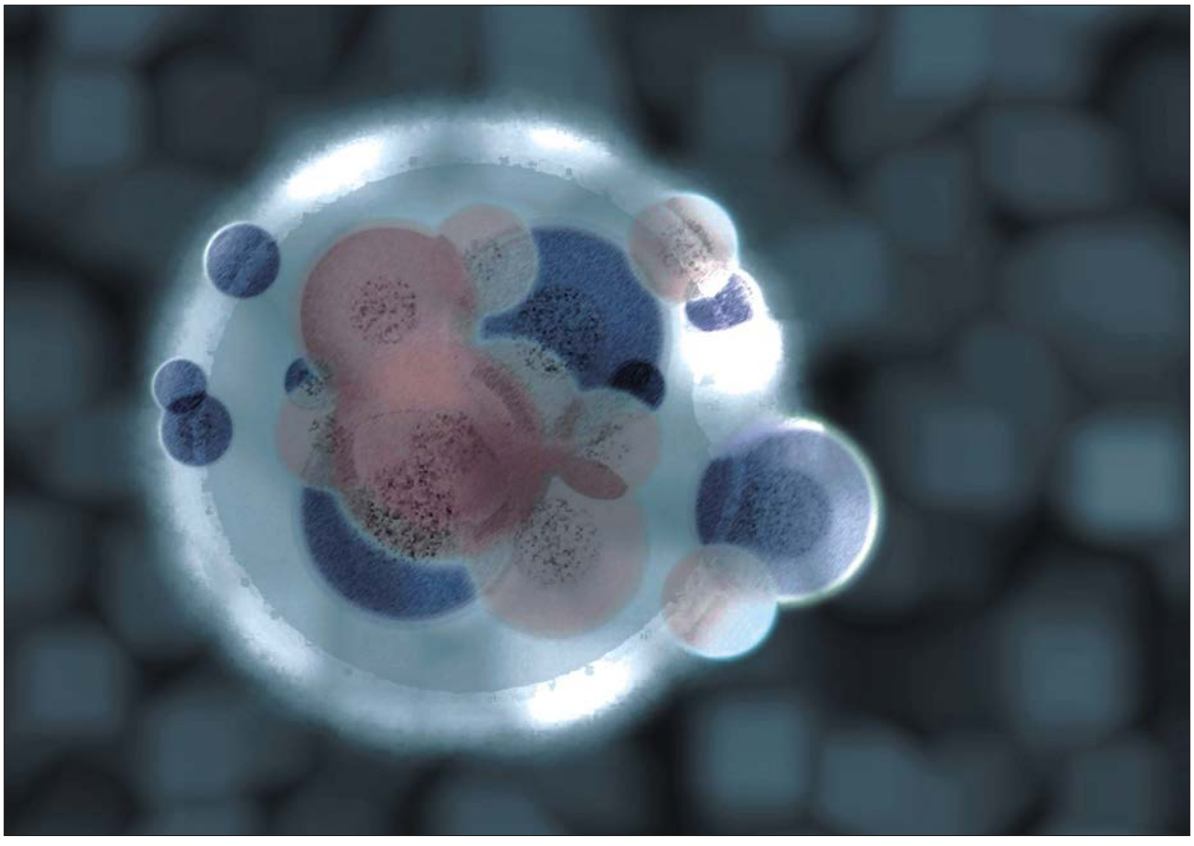

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 11 (2): 173-180, 2011
ISSN 1727-3048 / DOI: 10.3923/jbs.2011.173.180

Change in mRNA Expression of Human Cytochrome P450 by Gold Nanoparticles

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Abstract: The effect of gold nanoparticles (AuNPs) on the mRNA expression of cytochrome P450 (CYP) enzymes as main metabolizing enzymes has been investigated. Citrate-stabilized AuNPs (AuCi) and polyethyleneimine-stabilized AuNPs (AuPEI) were synthesized and characterized for sizes and surface charges using Transmission Electron Microscopy (TEM) and zeta potential measurement, respectively. The induction effect of AuNPs on mRNA expression of CYPs 1A2, 2C9, 2E1 and 3A4 in human hepatocellular carcinoma (HepG2) cell line was measured with quantitative real time PCR method. The results showed that AuCi and AuPEI had average particle diameters of 15.00±2.77 and 5.02±1.81 nm and zeta potential of -40.60±1.22 and 8.10±1.59 mV, in orderly. After 48 h exposure to the AuCi (1 and 10 μM) and AuPEI (0.1 and 1 μM), the CYP1A2 mRNA level in HepG2 cells was over 2-fold induction. Moreover, an addition of 1 μM AuPEI to 10 μM α-naphthoflavone as CYP1A2 inducer caused the level of CYP1A2 mRNA to be significantly higher ($p \leq 0.05$) than that induced by α-naphthoflavone alone. The study shows that the mRNA expression change is likely to depend upon the size and charge of AuNPs. The smaller and positively charged AuPEI promote the cellular penetration, thus probably potentiating of CYP mRNA transcription.

Key words: Cytochrome P450, gold nanoparticles, mRNA expression, phase I metabolism, hepatocellular carcinoma cells

INTRODUCTION

Gold nanoparticles (AuNPs) are considered to be useful for biomedical and pharmaceutical applications due to their biocompatibility and small sizes (Murphy *et al*., 2008). However, the biological activities of AuNPs on the molecular or cellular function are not well established and still needed more investigation in order to fulfill the human use of AuNPs. The ability of AuNPs to interfere with the protein function was observed in the cell cycle of growth and proliferation (Bhattacharya *et al*., 2007). The AuNPs seem to molecularly interact with the cells by binding with cell integrities such as DNA, glycoprotiens and growth factor (Mukherjee *et al*., 2005; Goodman *et al*., 2006). Hence, the understanding of the AuNP effect on gene expression could be knowledgeable to the role of nanoparticles on cellular function. Recently, the expression of the genes involving in cell cycle, inflammation, metabolic process and apoptosis was found to be induced after intravenous administration of AuNPs to rodent (Cho *et al*., 2009). Recently, Balasubramanian *et al*. (2010) reported the attenuation of cytochrome P450 gene expression in rats by AuNPs. The metallic silver nanoparticles were found to barely induce the mRNA expression of cytochrome P450 in human cell lines (Lamb *et al*., 2010). Since cytochrome P450 (CYP or P450) enzymes, a superfamily of metabolizing enzymes involved in the metabolism of drug and xenobiotics, has not yet been investigated for gene expression in human cells with a challenge of AuNPs. Although the effect of AuNPs on CYP enzyme has been reported by our group, only the AuNP effect on CYP inhibition was conducted (Sereemaspun *et al*., 2008). In order to understand the role of AuNPs in transcriptomics of CYP genes as possibly related to their enzymatic activity, the gene expression induced by AuNPs is required to be explored. Moreover, the presence of AuNPs might change the inducibility on CYP gene

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expression of α-naphthoflavone (αNF) as CYP1A2 inducer, ethanol (EtOH) as CYP2E1 inducer and rifampicin (Rif) as inducer for CYP2C9 and CYP3A4 (Coleman, 2005). Therefore, in the present study, AuNPs stabilized with either anionic citrate or cationic polyethyleneimine, were synthesized and determined for their effect on mRNA induction of human cytochrome P450, namely, CYP1A2, CYP2C9, CYP2E1 and CYP3A4, in hepatocellular carcinoma (HepG2) cell line.

MATERIALS AND METHODS

Materials: For nanoparticle synthesis, hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4.3H_2O$), trisodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$) and polyethyleneimine (PEI,-$(CH_2CH_2NH)_n$-, MW = 750 KDa) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Glassware were cleansed with aqua regia ($HCl:HNO_3$ = 3:1, Carlo Erba, Milan, Italy) for the purpose of circumventing gold contamination, rinsed with ultrapure water and oven dried prior to use. For cell line experiment, hepatocellular carcinoma (HepG2) cells were obtained from the American Type Culture Collection (ATCC), Rockville, MD, USA. Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), L-glutamine, penicillin-streptomycin were purchased from Gibco (Invitrogen), Carlsbad, CA, USA. Dimethyl sulfoxide (DMSO), α-naphthoflavone (αNF) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Rifampicin (Rif) was kindly given by Siam Pharmaceutical, Bangkok, Thailand and ethanol was obtained from Labscan, Bangkok, Thailand.

Preparation and characterization of AuNPs: AuNPs at a concentration of 1,015 μM (200 ppm) were prepared using citrate and polyethyleneimine (PEI) as stabilizers. For synthesis of citrate-stabilized AuNPs (AuCi), 35 μL of 30% gold solution ($HAuCl_4.3H_2O$) was added to 49.5 mL ultrapure water. The sample was then heated up to 90°C in a water bath with stirring before addition of 0.5 mL of 0.4 M $Na_3C_6H_5O_7.2H_2O$. The solution was continued heating until it became dark red. The same procedure was used for synthesis of PEI-stabilized AuNPs (AuPEI) except for 0.5 mL of 0.36 M PEI solution was used instead of the citrate solution.

The Surface Plasmon Resonance (SPR) absorbance peak of synthesized AuNPs was characterized by using UV-visible spectroscopy (Biomate, Thermo Fischer, USA). Morphology and sizes of AuNPs were observed under Transmission Electron Microscopy (TEM) (JEM-2100, Joel, Japan) and the zeta potential measurement was performed using Zetasizer NanoZS (Malvern, UK) in order to determine total electrical surface charge of colloidal AuNPs.

Investigation of AuNP influence on CYP mRNA expression

Cell culture: The HepG2 were cultured in DMEM supplemented with 10% (v/v) FBS, 1% L-glutamine (200 mM) and 0.5% antibiotics (10,000 U mL^{-1} penicillin and 10,000 μg mL^{-1} streptomycin). Cultures were maintained in an atmosphere of 5% CO_2 at 37°C and medium was refreshed every three or four days with subculturing.

Cytotoxicity test of AuNPs in HepG2 cells: In order to obtain the concentration of AuNPs for gene experiment, evaluation of cytotoxicity of AuNPs on HepG2 cells was performed using the MTT assay. Briefly, HepG2 cells were seeded in 96-well tissue culture plate at a density of 2×10^4 cells/well in 100 μL of medium and incubated overnight at 37°C under 5% CO_2 atmosphere. The cells were then treated with 10 μL of AuNPs at concentrations of Au atom ranging from 0.1 to 90 μM (n = 8 for each concentration) and incubated for 48 h. The cells grown in only medium were used as a control. Then, the cells were washed with phosphate buffered saline (PBS) and refreshed with 100 μL of medium. Two microliters of 2% MTT in DMSO was added to each well and the plate was incubated at 37°C for 4 h. The dehydrogenase enzyme in the living cell results in a change of yellow tetrazolium MTT to purple formazan crystal. The formazan was dissolved in 100 μL of DMSO prior to measurement of UV absorbance at 570 nm using a microplate reader (Victor^3 V, PerkinElmer, USA). The percentage of cell viability was calculated. The concentrations yielded more than 90% cell viability were considered as non-toxic doses and were used for further study on CYP mRNA expression. It was noted that, from the result, the non-toxic concentrations obtained for AuCi and AuPEI were less than 10 μM and 1 μM, respectively.

Sample treatment for determination of HepG2 mRNA expression: The cells were seeded at approximately 1×10^6 cells per 25-cm^2 flask and grown in the medium under an atmosphere of 5% CO_2 at 37°C for 24 h. Thereafter, the cells were incubated for 48 h with medium containing AuNPs which were 1 and 10 μM AuCi, 0.1 and 1 μM AuPEI, the inducers which were 10 μM αNF (CYP1A2 inducer), 500 mM EtOH (CYP2E1 inducer) and 10 μM Rif (inducer for CYPs 2C9 and 3A4). In addition, the cells were treated with a mixture of each inducer and AuNPs. Since DMSO was used to dissolve αNF and Rif, cells incubated with the medium containing DMSO at a final

concentration of 0.01% was also studied while the cells incubated in the medium without any samples added were used as a control.

RNA extraction: After sample treatment, total RNA was extracted from HepG2 cells using total RNA mini kit (Geneaid, Taiwan). According to the manufacturer's protocol, the 3×10^6 cells were transferred to a 1.5 mL microcentrifuge tube and the cells were centrifuged at 8,000 rpm for 20 sec. Then, the supernatant was completely removed and the cells were suspended in 100 μL of PBS before being lysed by adding 400 μL of lysis buffer (RB Buffer) and vortexed. The cell suspension was incubated at room temperature for 5 min. Then, 400 μL of 70% ethanol was added to the lysed cell and the suspension was mixed immediately by pipetting. The mixture was thereafter transferred to a column containing silica filter, placed in a 2-mL collection tube and centrifuged at 13,000 rpm for 2 min. Four hundred microliters of W1 Buffer was added into the column and centrifuged at 13,000 rpm for 1 min. Next, 600 μL of washing buffer was added into the column and centrifuged at the same speed for 1 min. Finally, the dried column was placed in a RNase-free 1.5-mL microcentrifuge tube with an addition of 50 μL of RNase-free water into the center of the column matrix. After 3 min, the tube was centrifuged at 13,000 rpm for 1 min to elude the purified RNA. Total RNA concentration was measured using UV-vis spectrophotometer at 260 nm and diluted to obtain RNA concentration of 0.5 μg μL^{-1} for further used as a template for synthesis of cDNA.

Synthesis of cDNA: RevertAid first strand cDNA synthesis kit (Fermentas, Lithuania) was used for synthesis of cDNA. According to the manufacturer's instruction, 1 μL (0.5 μg μL^{-1}) of oligomeric-dT nucleotide was added into 10 μL (0.5 μg μL^{-1}) of RNA and 1 μL of RNase-free water. The mixture was then incubated at 70°C for 5 min. Thereafter, the mixture was added with 4 μL of 5X reaction buffer, 1 μL of ribonuclease inhibitor (20 U μL^{-1}), 2 μL of 10 mM deoxyribonucleotide triphosphate (dNTP) and 1 μL of reverse transcriptase (200 U μL^{-1}), respectively. The mixture at a total volume of 20 μL was mixed, spun down and incubated at 42.0°C for 60 min and 70.0°C for next 10 min. The synthesized cDNA was used as a template for quantitative real-time polymerase chain reaction (PCR).

mRNA expression assay: For an assessment of AuNP effect on CYP mRNA expression, real-time PCR technique was performed using ABI PRISM 7700 sequence detection system (Applied Biosystem, USA). The designed mRNA sequences of CYP genes were selected and confirmed for specificity via NCBI (National Center for Biotechnology Information, USA) Blast search to ensure the correct targeted mRNA and avoidance of DNA contamination. Forward and reverse primer sequences for CYP genes and GAPDH gene (a house keeping gene) along with their PCR product lengths are shown in Table 1. The 200 μM stock solution of primers was diluted to 20 μM prior to use. A mixture of 20-μL PCR reaction composed of 1 μL of cDNA template (equivalent to total RNA of 250 ng), 0.1 μM forward primer, 0.1 μM reverse primer, 0.3X ROX (6-carboxyl-X-rhodamine) reference dye and 1X SYBR green PCR Master Mix (Finnzymes, Finland). The PCR condition was set according to the manufacturer's suggestion at 95°C for 10 min, followed by 50 cycles of 95°C for 15 sec, 60°C for 20 sec and 72°C for 40 sec. The relative amount of PCR product was analyzed using ABI PRISM sequence detector software based on the threshold cycle (C_T). The difference in C_T values of GAPDH gene and CYP mRNA was known as ΔC_T. The fold induction of CYP mRNA expression was calculated based on the formula of $2^{-\Delta\Delta CT}$ where $\Delta\Delta C_T = \Delta C_T$ of a control $-\Delta C_T$ of a treated sample.

Statistical analysis: The magnitude of mRNA expression was statistically compared using one-way analysis of variance (ANOVA) with Bonferroni post hoc test and the differences were considered significant at $p \leq 0.05$.

Table 1: Primer sequences for real time PCR analysis of CYP and GAPDH mRNA expression

Gene	Accession number	Sequence of primer (FP= forward, RP = reverse)	PCR product length (base pairs)
CYP1A2	NM-000761	FP: GGA GCA GGA TTT GAC ACA GTC A	94
		RP: GCT CCT TCT GGA TCT TCC TCT GTA	
CYP2C9	NM-000771	FP: TTC AGT CCT TTC TCA GCA GG	384
		RP: TTG CAC AGT GAA ACA TAG GA	
CYP2E1	NM-000773	FP: GTG GAG GCA GGT GCA CAG CA	120
		RP: TGG GCC AAC CGG GTG AAG GA	
CYP3A4	NM-017460	FP: CAG GAG GAA ATT GAT GCA GTT TT	78
		RP: GTC AAG ATA CTC CAT CTG TAG CAC AGT	
GAPDH	NM-002046	FP: CCA TGG CAC CGT CAA GGC TGA	151
		RP: CTC CAT GGT GGT GAA GAC GC	

RESULTS

Synthesis and characterization of AuNPs: Both citrate stabilized gold nanoparticles (AuCi) and PEI-stabilized gold nanoparticles (AuPEI) showed the cherry-red solution with UV absorbance peaks at around 520 nm indicating the presence of AuNPs (Link and El-Sayed, 1999). The results from the zeta potential experiment indicated that AuCi were negatively charged nanoparticles while AuPEI were positively charged nanoparticles. The values of surface charges were -40.60±1.22 mV for AuCi and 8.10±1.59 mV for AuPEI. The TEM images of nanoparticles showed the spherical shape of AuCi and AuPEI (Fig. 1a, b). The averages particles sizes obtained from TEM analysis were 15.00±2.77 nm (n = 291) and 5.02±1.81 nm (n = 445) for AuCi and AuPEI, respectively.

Cytotoxicity test of AuNPs: The MTT assay was used to determine cytotoxicity of AuNPs on HepG2 cells. The results revealed that AuCi had no toxicity (approx. 90% viability) for a concentration range of 0.1-90 μM (Table 2). Contrarily, the dose-dependent toxicity was found for AuPEI. Hence, the non-toxic concentrations of AuPEI (0.1 and 1 μM) and of AuCi (1 and 10 μM) were selected for gene expression studies.

Effect of AuNPs on CYP mRNA expression: The mRNA expression of CYP enzyme of HepG2 cells exposed to the treated samples for 48 h was present in CYP fold induction (Fig. 2a-d). The levels of CYP mRNAs of the cells were significantly greater ($p \leq 0.05$) when exposed to their specific inducers, namely Rif (CYP2C9 and CYP3A4), αNF (CYP1A2) and EtOH (CYP2E1), compared to 1-fold induction of the uninduced control cells. It was notably that 0.01% DMSO used as drug solvent did not affect the CYP mRNA in HepG2 cells (data not shown). After cell incubation with either AuCi or AuPEI, the level of CYP1A2 mRNA was over 2-fold induction (Fig. 2a) and was greatest among the other CYP induction. The amount of AuNP-induced CYP mRNA was less than those induced by their own inducers; however, the exception was found for CYP2E1 mRNA expression in the presence of 0.1 μM AuPEI (Fig. 2c). From the result, an approximately 1.7-fold increase in CYP2E1 mRNA induction could be obtained from a fairly low concentration of AuPEI.

Combinations of each inducer and AuNPs were investigated for induction change of CYP mRNA in HepG2 cells compared to the corresponding inducer. The findings indicated that addition of AuNPs to the inducer (or so-called a mixture) caused less mRNA transcripts compared to those of sole inducer. Surprisingly, only a mixture of αNF and 1-μM AuPEI caused significant CYP1A2 mRNA induction to 12.70-fold compared to 8.08-fold induction by αNF (Fig. 2a). It was noted that the induction changes of CYP mRNA were compared statistically ($p \leq 0.05$) either between AuNPs and untreated control or between AuNP-inducer mixture and the corresponding inducer alone. When compared between

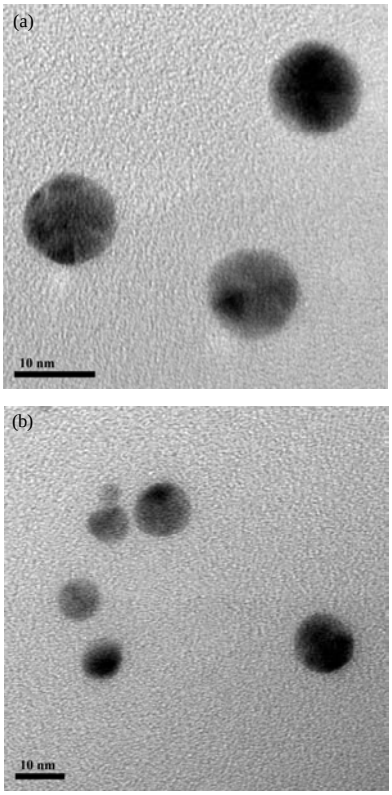

Fig. 1: TEM images of (a) citrate-stabilized gold nanoparticles and (b) PEI-stabilized gold nanoparticles (scale bar = 10 nm)

Table 2: Cell viability of HepG2 cell line after 48 h incubation with citrate-stabilized gold nanoparticles (AuCi) and polyethyleneimine-stabilized gold nanoparticles (AuPEI)

Concentration of AuNPs (μM)	Viability (%) AuCi	AuPEI
0.1	87.34±10.44	97.89±2.59
1	89.85±14.47	92.74±10.15
2	92.23±9.13	94.50±10.44
5	91.46±9.47	82.92±7.11
10	95.35±3.99	80.44±6.65
20	84.96±5.35	80.84±10.27
50	88.18±9.34	52.03±8.65
90	89.96±8.54	13.15±3.93

Values are mean±SD, n = 8

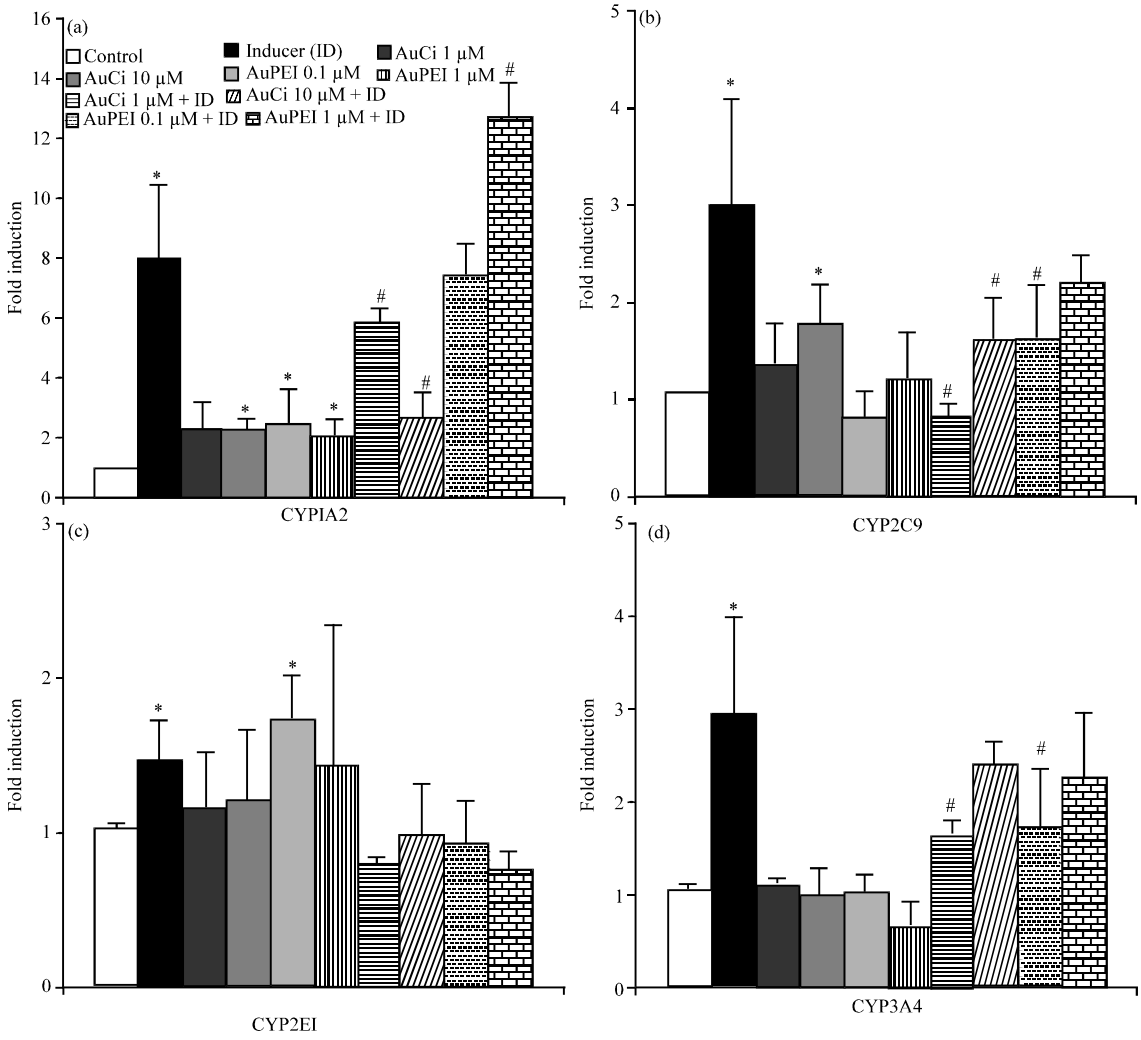

Fig. 2: Expression levels of (a) CYP1A2, (b) CYP2C9, (c) CYP2E1 and (d) CYP3A4 in HepG2 cells after incubation with a CYP inducer (α-naphthoflavone for CYP1A2, rifampicin for CYPs 2C9 and 3A4, ethanol for CYP2E1), 1 and 10 μM AuCi, 0.1 and 1 μM AuPEI, a mixture of AuCi (1 and 10 μM) and each inducer, and a mixture of AuPEI (0.1 and 1 μM) and each inducer. The data represent the mean±SD (n = 4) and (*), (#) represent data that are statistically different ($p \leq 0.05$) from the untreated control and sole inducer, respectively

the inducibility of AuCi and AuPEI at the same concentration (1 μM), AuPEI at 1 μM showed an ability to up-regulate the CYP1A2 mRNA with significant differences regardless of the presence of the inducer. In contrast, 1 μM AuCi could significantly down-regulate CYPs 1A2, 2C9 and 3A4 mRNA when inducers were presented. The similar trend in mRNA expression change was observed when 10-times higher dose (10 μM) of AuCi was used (Fig. 2a-b, d). For 0.1 μM AuPEI, they could significantly induce CYP1A2 and CYP2E1 mRNA, but when mixed with inducers they could reduce the extent of mRNA induction for all CYPs with statistical difference ($p \leq 0.05$) found in CYPs 2C9 and 3A4.

DISCUSSION

In order to determine the effect of AuNPs on the CYP induction of HepG2 cells, the cytotoxicity of AuNPs was necessary to ensure no any cell damage at the concentrations used in PCR assay. The results showed that cationic charged AuPEI caused more toxicity than the anionic charged AuCi as compared at the same concentrations. The positive surface charges of AuPEI were responsible for more interaction between nanoparticles and the cell membrane comprising negatively charged phospholipid bilayer. Many reports revealed the same findings in that the positively charged

nanoparticles more effectively disturbed the cell membrane or fewer amounts of them required to cause cell damage as compared to the anionic nanoparticles (Goodman *et al*., 2004; Osaka *et al*., 2009). Moreover, the smaller size particles were able to easier uptake into the cells (Foged *et al*., 2005; Aillon *et al*., 2009). Correspondingly, AuPEI which had the 3 times smaller in size than AuCi could exhibit greater penetration into the cell membrane. From the results, the non-toxic concentrations of AuNPs (more than 90% viability) used for mRNA expression study of HepG2 cells were 1 and 10 μM for AuCi and lower concentrations, 0.1 and 1 μM, for AuPEI. The change in expression of CYP mRNA by either AuNPs or the mixture of CYP inducer and AuNPs was interpreted in fold induction pattern. The inducing effect of AuNP-containing samples for CYPs 2C9, 2E1 and 3A4 mRNA seemed to be fairly low in that almost all of the fold induction was in the range of only 1-to 2-fold even though their own inducers were added, hence the AuNPs were unlikely to potentiate the CYP induction of the inducers. For CYP1A2, it was the only gene that could be greater induced by AuNPs to about 2-to 3-fold when the sole AuNPs were used and to about 2-to 13-fold if the sample contained both inducer and AuNPs. According to the result, more obvious change in CYP mRNA expression was seen in AuPEI-containing samples compared to AuCi-containing samples especially at the same or lower concentrations. Since the findings of cellular CYP induction were similar to the cytotoxicity results, it was implied that the cationic AuPEI were able to pass thorough the cell membrane and enter into the cell nuclei which eventually interacted with the mRNA transcription process of CYP enzyme.

For the well-established CYP inducers (Rif, αNF, EtOH), the mechanism of induction is via the receptor-dependent mRNA transcription. The inducers act as the ligand binding to specific receptor for CYP expression. Induction of CYP1A2 is activated through aryl hydrocarbon receptor (AhR) and induction of CYP2C9 is regulated by pregnane X receptor (PXR) and constitutive androstane receptor (CAR) (Chen *et al*., 2004; Al-Dosari *et al*., 2006). CYP3A4 induction is known to be activated through PXR (Gibson *et al*., 2002; Quattrochi and Guzelian, 2001). For CYP2E1, the induction is believed to corroborate by the post-transcription or translation levels (Kim *et al*., 1990; Kim *et al*., 2001; Abdelmegeed *et al*., 2005) and stabilization of CYP2E1 mRNA (Tsutsumi *et al*., 1993). The mechanistic action of AuNPs on the CYP expression has not been reported and no any conclusion has been made so far. The uptake of nanoparticles into the cells is critical for cellular activities. The smaller and cationic AuNPs like AuPEI are more readily enter into the cell nuclei and interfere with the process of gene transcription. However in some cases of uninducibility of AuNPs and/or the less induction of AuNP-inducer mixture obtained in the study might be explained from the ability of nanoparticles to stimulate the cell secretion of inflammatory cytokines such as interleukin-6 and/or nitric oxide. Correspondingly, an increment of inflammatory cytokines in the cells incubated with titanium dioxide nanoparticles (Park *et al*., 2008) and chitosan nanoparticles (Pattani *et al*., 2009) was reported. The occurrence of inflammatory cytokines and nitric oxide could inhibit CYP mRNA transcription or destabilize CYP mRNA, thus leading to down-regulation of CYP mRNA expression (Pascussi *et al*., 2000; Aitken and Morgan, 2007; Aitken *et al*., 2008). In contrary, for greater fold induction as seen in CYP1A2, it implied that the AuNPs could pass into the nuclear membrane and become the ligand binding to the nuclear receptor, AHR. The results obtained were in agreement with some previous findings; the luciferase reporter-engineered HepG2 cells incubated with silver nanoparticles showed the higher induction of CYP1A2 mRNA than CYP3A and CYP2C mRNAs (Lamb *et al*., 2010). Similarly, the expression of cyp1a1 gene in mice was induced to 28-fold by AuNPs compared to about 2-fold induction of cyp4a and cyp3a genes (Balasubramanian *et al*., 2010). In fact, the induction of CYP1A mRNA by heavy metal (arsenite, cadmium, chromium) was reported and it seemed that the metal might probably increase the rate of mRNA transcription (Tully *et al*., 2000; Elbekai and El-Kadi, 2005; Elbekai and El-Kadi, 2007). However, from the molecular basis, neither the nanoparticles nor the metal would possibly act as ligands for AHR (Denison and Nagy, 2003). Hence, the AuNPs might not activate the AHR directly for the CYP mRNA expression but the indirect way or the multiple mechanisms. The binding of some metals to the metal responsive transcription factor (MTF) has been proposed (Muller and Kerten, 2003), so it is plausible for activation through the MTF by AuNPs and thus potentiating the AHR activities. Lastly, according to the limited data on the concentrations of AuNPs used for the induction study, an attempt to draw any conclusion on concentration-dependent effect could not be clearly made. However, from the present study it could be estimated that the higher concentration of AuNPs resulted in more induction of mRNA expression. It was in agreement with the dose-dependency of some flavonoids which behaved like AHR agonist and antagonist at the high and low amount of flavonoids, consecutively (Lu *et al*., 1996; Ashida *et al*., 2000).

In conclusion, from this study the AuNPs are subjected to the significant mRNA induction of CYP1A2 in HepG2 cells. The mechanism of how the AuNPs affect the mRNA transcription is still not fully understood and is possibly via the indirect pathway. However, the surface charges of the nanoparticles is likely to be relative with cellular uptake and CYP transcription in which the higher level of mRNA expression is more pronounced on positively charged AuNPs. If the assumption from the current study also occurs *in vivo*, the surface modification of the nanoparticles may be predictive of CYP-dependent metabolic activities.

ACKNOWLEDGMENTS

This study supported in part by grant from the Faculty of Graduate Studies, Chulalongkorn University, Thailand and the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (project code: HR-1166-I). The authors wish to thank Professor Apiwat Mutirangura (M.D.), Faculty of Medicine, Chulalongkorn University for his guidance.

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