Toxicology and Applied Pharmacology 265 (2012) 43–50

Contents lists available at SciVerse ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

The role of DNA methylation in catechol-enhanced erythroid differentiation of K562 cells

Xiao-Fei Li, Xiao-Rong Wu, Ming Xue, Yan Wang, Jie Wang, Yang Li, Suriguga, Guang-Yao Zhang, Zong-Chun Yi *

Department of Biological Science and Biotechnology, School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China

ARTICLE INFO

Article history:
Received 3 May 2012
Revised 6 August 2012
Accepted 20 September 2012
Available online 27 September 2012

Keywords:
Catechol
DNA methylation
Erythroid differentiation
Benzene

ABSTRACT

Catechol is one of phenolic metabolites of benzene in vivo. Catechol is also widely used in pharmaceutical and chemical industries. In addition, fruits, vegetables and cigarette smoke also contain catechol. Our precious study showed that several benzene metabolites (phenol, hydroquinone, and 1,2,4-benzenetriol) inhibited erythroid differentiation of K562 cells. In present study, the effect of catechol on erythroid differentiation of K562 cells was investigated. Moreover, to address the role of DNA methylation in catechol-induced effect on erythroid differentiation in K562 cells, methylation levels of erythroid-specific genes were analyzed by Quantitative MassARRAY methylation analysis platform. Benzidine staining showed that exposure to catechol enhanced hemin-induced hemoglobin accumulation in K562 cells in concentration- and time-dependent manners. The mRNA expression of erythroid specific genes, including α-globin, β-globin, γ-globin, erythroid 5-aminolevulinate synthase, erythroid porphobilinogen deaminase, and transcription factor GATA-1 genes, showed a significant concentration-dependent increase in catechol-treated K562 cells. The exposure to catechol caused a decrease in DNA methylation levels at a few CpG sites in some erythroid specific genes including α-globin, β-globin and erythroid porphobilinogen deaminase genes. These results indicated that catechol improved erythroid differentiation potency of K562 cells at least partly via up-regulating transcription of some erythroid related genes, and suggested that inhibition of DNA methylation might be involved in up-regulated expression of some erythroid related genes.

Introduction

Catechol, also known as 1,2-dihydroxybenzene, is one of phenolic metabolites of benzene which is a general occupational hazard and a ubiquitous environmental air pollutant (EPA, 2002). Catechol is also widely used in pharmaceutical production, chemical industry and agriculture (Milligan and Häggblom, 1998). In addition, catechol occurs naturally in fruits and vegetables, and cigarette smoke contains catechol of which 200–400 μg is found in each cigarette (Stone et al., 1995).

A series of studies have revealed that catechol demonstrated cytotoxicity to various cells (de Oliveira et al., 2010; Hirose et al., 1999; Lima et al., 2008; Mansoor et al., 2010; Moran et al., 1996; Myburg et al., 2002) . Catechol is easily oxidized to generate reactive oxygen species (ROS), semiquinone radicals and quinines, which led to oxidative stress and finally caused cytotoxicity (Chouchane et al., 2006). It has been also found that catechol induced iron release from ferritin (Agrawal et al., 2001), inhibited basal mitochondria respiration (Barreto et al., 2005), and induced cell transformation, gene mutations, chromosome aberrations, aneuploidy, sister chromatid exchanges and unscheduled DNA synthesis (Fabiani et al., 2001; Tsutsui et al., 1997). Furthermore, catechol was found to increase invasion and metastasis of lung carcinoma cells by activation of Ca2+/PKC signal transduction (Gopalakrishna et al., 1994).

Erythropoietic depression is commonly observed in benzene-exposed human and animal individuals, but its precise mechanism is not fully understood (EPA, 2002). It is generally accepted that benzene metabolites are responsible for causing the toxicity of benzene (Snyder et al., 1989). Recently, the exposure to hydroquinone has been reported to significantly inhibit hemin-induced erythroid differentiation of HD3 chicken erythroblast cells (Badham and Winn, 2010). Our recent study also found that three phenolic metabolites of benzene, including phenol, hydroquinone, and 1,2,4-benzenetriol, inhibited the erythroid differentiation of

Abbreviations: ALAS2, δ-aminolevulinate synthase 2; BFU-E, erythroid burst-forming unit; CFU-GM, colony-forming unit-granulocyte, macrophage; COMT, catechol-O-methyltransferase; DNMT, DNA methyltransferase; EGCG, (−)-epigallocatechin-3-O-gallate; GATA-1, GATA binding protein 1 (globin transcription factor 1); Hb, hemoglobin; HS, DNase I hypersensitive site; hMLH1, human mutL homologue 1; LCR, locus control region; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MGMT, O(6)-methylguanine methyltransferase; NF-E2, Nuclear Factor-Erythroid 2; PBGD, porphobilinogen deaminase; PBS, phosphate buffered saline; RARβ, retinoic acid receptor β; ROS, reactive oxygen species; RT-PCR, reverse transcriptase-polymerase chain reaction; SAH, S-adenosyl-L-homocysteine; SAM, S-adenosyl-L-methionine; SAP, Shrimp alkaline phosphatase.

* Corresponding author at: School of Biological Science and Medical Engineering, Beihang University, 37 Xueyuan Road, Beijing 100191, China. Fax: +86 10 8231 5554.
E-mail address: yizc@buaa.edu.cn (Z.-C. Yi).

0041-008X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.taap.2012.09.018

K562 cells (Wu et al., 2011). However, the effect of catechol on the erythroid differentiation of K562 cells and the role of catechol in benzene exposure-induced erythropoietic depression are still unclear.

DNA methylation, an epigenetic mechanism, is associated with condensed nuclease-resistant heterochromatin and silencing of gene expression, playing an important role in repressing the expression of tumor suppressor genes in cancer, X chromosome inactivation, and parental imprinting (Ballestar, 2011; Newell-Price et al., 2000). Substantial evidences implicated that DNA methylation were associated with erythroid genes switching (Bird et al., 1987; Kransdorf et al., 2006; Mabaera et al., 2007; Razin et al., 2000; Singal et al., 2002). *In vitro* experiments have demonstrated that chemically induced erythroid differentiation was associated with decreased DNA methylation levels in erythroid genes (Creusot et al., 1982; Fathallah et al., 2007; Tisdale, 1986). Recently, catechol-containing polyphenols have been proven to be strong inhibitors of DNA methylation (Fang et al., 2003, 2007; Lee and Zhu, 2006; Lee et al., 2005). We supposed that catechol could not inhibit erythroid differentiation, in contrast, might improve erythriod differentiation through reducing methylation level and subsequently up-regulated transcription activity of erythroid-specific genes. In the present study, using K562 cells as erythroid differentiation model, the effect of catechol on the erythroid differentiation and the DNA methylation levels at erythroid-specific genes were analyzed.

Materials and methods

Cell culture. K562 cells were maintained in RPMI-1640 culture medium (Invitrogen) supplemented with 10% (v/v) fetal bovine serum (HyClone), 100 units/ml penicillin (Sigma-Aldrich), and 100 μg/ml streptomycin (Sigma–Aldrich) at 37 °C in a humidified 5% CO_2 incubator. For the experiment, exponentially growing K562 cells at passage 4–8 after recovery were seeded and cultured in 24 well plates at an initial density of 3×10^5 cells/ml.

Detection of cell viability. Cell viability was determined with trypan blue dye exclusion. For measuring the effects of catechol (Sigma-Aldrich) on cell growth, K562 cells were seeded and cultured at an initial concentration of 3×10^5 cells/ml. After a 24-h culture, the cells were treated with catechol (0 μM, 20 μM, 40 μM, 80 μM, or 160 μM) for 24–72 h, then the cells were collected and stained by trypan blue, the living cells and dead cells were counted using a hemocytometer. The percentage of viable (unstained) cells was estimated by scoring 500 cells per sample.

Hemoglobin (Hb) synthesis analysis. Benzidine staining was used to estimate the Hb accumulation in the K562 cells according to the procedure described previously (Yi et al., 2004). After K562 cells were treated with different concentration (0 μM, 20 μM, 40 μM or 80 μM) of catechol for 72 h or 40 μM catechol for 24 h, 48 h, or 72 h, the cells were collected and re-suspended in fresh culture medium, and then were induced to differentiate towards erythroid cells with 40 μM hemin (Sigma-Aldrich) for 48 h. After stimulation with hemin, the cells were harvested and washed twice with cold phosphate buffered saline (PBS), then 1 ml suspended cells were incubated with 0.2 ml fresh prepared benzidine solution (2 mg/ml in glacial acetic acid) and 5 μl 30% H_2O_2 for 10 min at room temperature. Ten minutes later, the cells were counted in a hemocytometer and blue-stained cells were considered positive for Hb accumulation, and then the percentage of Hb-positive cells was calculated.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis. Total RNA from K562 cells that had been treated with different concentrations (0 μM, 20 μM, 40 μM or 80 μM) of catechol for 72 h was extracted with Ribozol TM RNA Extraction Reagent (Amresco). Then RNA was converted to cDNA using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas). PCR amplification reaction was performed in a 10 μl reaction volume containing 2 μl cDNA, 5 μl PCR mix (containing 50 mM Tris HCl, 20 mM KCl, 4 mM $MgCl_2$, 0.1 U/μl Taq DNA polymerase, and 500 μM dNTPs), 0.5 μl forward primer (10 μM), 0.5 μl reverse primmer (10 μM) and 2 μl ddH_2O in a Tgradient Thermocycler (Biometra). The reactions were allowed to proceed under the following conditions: 4 min at 95 °C, 28 cycles of 95 °C for 1 min, indicated annealing temperature for 45 sec, and 72 °C for 45 sec. The PCR primer sequences and annealing temperatures for PCR are listed in Table 1. β-Actin was used as an internal control. The amplified DNA products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, visualized and photographed with ImageMaster Video Documentation System (Pharmacia Biotech).

DNA methylation analysis of K562 cell after catechol exposure. The Sequenom MassARRAY platform (CapitalBio, Beijing, China) was used to perform the quantitative methylation analysis of erythroid specific genes. This system uses matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry in combination with RNA base-specific cleavage (MassCLEAVE) (Ehrich et al., 2005). Using this system, DNA methylation was analyzed by gene-specific amplification of bisulfite-treated DNA followed by *in vitro* transcription and MALDI-TOF analysis. After K562 cells were treated with 40 μM catechol for 72 h, genomic DNA from K562 cells was extracted with the QIAamp DNA Mini kit (Qiagen) according to manufacturer's protocol. The concentration and purity of the DNA were determined by absorbance at 260 and 280 nm. The extracted genomic DNA was bisulfite-treated using the EpiTect Bisulfite kit (Qiagen) according to manufacturer's protocol. The concentration and purity of the bisulfite-converted DNA were determined by absorbance at 260 and 280 nm. Then, the bisulfite-modified DNA was amplified by PCR. The target regions were amplified using the primer pairs shown in Table 2. PCR primers were designed with epidesigner (http://www.epidesigner.com). Each forward primer was tagged with a 10 mer (5′-aggaagagag-3′) to balance the PCR by adjusting for melting temperature differences, and each reverse primer had a T7-promoter tag (5′-cagtaatacgactcactatagggagaaggct-3′) for *in vitro* transcription. After PCR reaction, unincorporated dNTPs were dephosphorylated by adding shrimp alkaline phosphatase (SAP), and SAP was then inactivated at 65 °C for 10 min. The PCR reaction products

Table 1
Sequences of primers used in reverse transcriptase PCR.

Gene	Sequence of primers	Annealing temperature(°C)	Size (bp)
α-globin	Forward: 5′-AAGGTCGGCGCGCACGC-3′ Reverse: 5′-CTCAGGTCGAAGTGCGGG-3′	58	101
β-globin	Forward: 5′-CTCATGGCAAGAAAGTGCTCG-3′ Reverse: 5′-AATTCTTTGCCAAAGTGATGGG-3′	60	181
γ-globin	Forward: 5′-ACAAGCCTGTGGGGCAA-3′ Reverse: 5′-GCCATGTGCCTTGACTTT-3′	58	158
erythroid PBGD	Forward: 5′-GGTCCTACTATCGCCTCCCTC-3′ Reverse: 5′-GAATCTTGTCCCCTGTGGTGG-3′	56	216
ALAS2	Forward: 5′-GCAGCACTCAACAGCAAG-3′ Reverse: 5′-ACAGGACGGCGACAGAAA-3′	56	239
NF-E2	Forward: 5′-ATTTGAGCCCCAAGCCCCAGC-3′ Reverse: 5′-GCTGGAGGGGACAGAGGCTGG-3′	62	315
GATA-1	Forward: 5′-CAGTCTTTCAGGGTGTACCC-3′ Reverse: 5′-GAGTGATGAAGGCAGTGCAG-3′	62	212
β-actin	Forward: 5′-TGGACTTCGAGCAAGAGATGG Reverse: 5′-ATCTCCTTCTGCATCCTGTCG	60	289