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The role of catechol-O-methyltransferase in catechol-enhanced erythroid differentiation of K562 cells

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ABSTRACT

Catechol is widely used in pharmaceutical and chemical industries. Catechol is also one of phenolic metabolites of benzene in vivo. Our previous study showed that catechol improved erythroid differentiation potency of K562 cells, which was associated with decreased DNA methylation in erythroid specific genes. Catechol is a substrate for the catechol-O-methyltransferase (COMT)-mediated methylation. In the present study, the role of COMT in catechol-enhanced erythroid differentiation of K562 cells was investigated. Benzidine staining showed that exposure to catechol enhanced hemin-induced hemoglobin accumulation and induced mRNA expression of erythroid specific genes in K562 cells. Treatment with catechol caused a time- and concentration-dependent increase in guaiacol concentration in the medium of cultured K562 cells. When COMT expression was knocked down by COMT shRNA expression in K562 cells, the production of guaiacol significantly reduced, and the sensitivity of K562 cells to cytotoxicity of catechol significantly increased. Knockdown of COMT expression by COMT shRNA expression also eliminated catechol-enhanced erythroid differentiation of K562 cells. In addition, the pre-treatment with methyl donor S-adenosyl-L-methionine or its demethylated product S-adenosyl-L-homocysteine induced a significant increase in hemin-induced Hb synthesis in K562 cells and the mRNA expression of erythroid specific genes. These findings indicated that O-methylation catalyzed by COMT acted as detoxication of catechol and involved in catechol-enhanced erythroid differentiation of K562 cells, and the production of S-adenosyl-L-homocysteine partly explained catechol-enhanced erythroid differentiation.

Introduction

Catechol (also known as 1,2-dihydroxybenzene) is widely used in pharmaceutical production, chemical industry and agriculture (Milligan and Hãggblom, 1998). It is also a metabolite of benzene which is a general occupational hazard and a ubiquitous environmental air pollutant (EPA, 2002). In addition, fruits, vegetables, and cigarette smoke contain catechol (Stone et al., 1995). Catechol is easily oxidized to generate reactive oxygen species (ROS), semiquinone radicals and quinones, then leading to cytotoxicity (Chouchane et al., 2006). Catechol-induced cytotoxicity has been found in 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; Wang et al., 2013). Catechol has also been found to induce iron release from ferritin (Agrawal et al., 2001), inhibit basal mitochondria respiration (Barreto et al., 2005), and induce genetic toxicity and cell transformation (Fabiani et al., 2001; Tsutsui et al., 1997). In addition, catechol increased invasion and metastasis of lung carcinoma cells by activation of Ca2 +/PKC signal transduction (Gopalakrishna et al., 1994).

We recently found that exposure to catechol enhanced hemin-induced erythroid differentiation via up-regulating transcription of some erythroid specific genes in K562 cells, which was accompanied by a decrease in DNA methylation levels at a few CpG sites in these genes (Li et al., 2012). Consistently, catechol-containing polyphenols, including tea catechins [catechin, epicatechin, and (−)-epigallocatechin-3-O-gallate (EGCG)], bioflavonoids (quercetin, fisetin, and myricetin), coffee polyphenols (caffeic acid and chlorogenic acid), have been found to inhibit the DNA methylation catalyzed by prokaryotic M.SssI DNA methyltransferase (DNMT) and human DNMT1, which is associated with the demethylation of the CpG islands in the promoters and the reactivation of methylation-silenced genes (Fang et al., 2003, 2007; Lee and Zhu, 2006; Lee et al., 2005). These findings suggested that inhibition of DNA methylation played a role in catechol-enhanced erythroid differentiation.

It has been well-known that various catechol-containing polyphenols are excellent substrates for the catechol-O-methyltransferase (COMT)-mediated methylation (Zhu and Liehr, 1996; Zhu et al., 1994, 2000, 2001). The COMT-mediated methylation of catechols not only reduced the cellular pools of S-adenosyl-L-methionine (SAM), but also generated an equimolar quantity of demethylated SAM,

Abbreviations: ALAS2, δ-aminolevulinate synthase 2; COMT, catechol-O-methyltransferase; COX, cyclooxygenase; DNMT, DNA methyltransferase; Hb, hemoglobin; HPLC, high performance liquid chromatography; NF-κB, nuclear factor κB; PBGD, porphobilinogen deaminase; PBS, phosphate buffered saline; ROS, reactive oxygen species; RT-PCR, reverse transcriptase-polymerase chain reaction; SAH, S-adenosyl-L-homocysteine; SAM, S-adenosyl-L-methionine; SD, standard deviation.

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S-adenosyl-L-homocysteine (SAH) which acted as a feedback potent inhibitor of various SAM-dependent methylation reactions (Guldberg and Marsden, 1975). Catechol polyphenols may indirectly inhibit DNMT through increased formation of SAH during their COMT-mediated O-methylation (Fang et al., 2007; Lee and Zhu, 2006; Lee et al., 2005). In the present study, we wanted to elucidate whether COMT played a role in catechol-enhanced erythroid differentiation of K562 cells.

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 passages 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 12 h-culture, the cells were treated with catechol (0, 20, 40, or 80 μM) for 24 h, 48 h or 72 h, then the cells were collected and stained by trypan blue, the living cells and dead (stained) cells were counted using a hemocytometer. The relative viability was calculated by the ratio of living cell density of each sample to that of control group. The cell mortality (the percentage of dead cells) was estimated by scoring 300–500 cells per sample.

To detect the cytotoxicity of catechol to COMT shRNA-expressed K562 cells, the cells were treated with catechol (0, 20, 40, or 80 μM) for 48 h, and then the relative viability and the cell mortality were estimated using trypan blue dye exclusion method.

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 concentrations (0, 20, 40, or 80 μM) of catechol for 24 h or 48 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 was harvested and washed twice with cold phosphate buffered saline (PBS), and 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.

For COMT shRNA-expressed K562 cells, after treatment with catechol (0, 20, or 40 μM) for 48 h, the cells were re-suspended in fresh culture medium and induced to differentiate with 40 μM hemin for 48 h, and then Hb synthesis was estimated by benzidine staining.

In the experiments for the effects of SAM (Sigma-Aldrich) and SAH (Sigma-Aldrich) on hemin-induced hemoglobin synthesis, K562 cells were treated with catechol (40 μM), SAM (40 μM), SAH (40 μM), catechol (40 μM) together with SAM(40 μM), or catechol (40 μM) together with SAH (40 μM) for 48 h, and then the cells were re-suspended in fresh culture medium and were induced to differentiate with 40 μM hemin for 48 h. After the stimulation of hemin, Hb synthesis in K562 cells was estimated by benzidine staining.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis. After K562 cells were treated with catechol (0, 20, 40 or 80 μM), SAM (40 μM), SAH (40 μM), or catechol (40 μM) together with SAM (40 μM), or catechol (40 μM) together with SAH (40 μM) for 48 h, and COMT shRNA-expressed K562 cells were treated with 40 μM catechol for 48 h, the expression of erythroid specific genes was estimated by RT-PCR. In addition, after K562 cells were transfected with COMT shRNA-expressed plasmid (pGPU6/GFP/Neo-COMT-homo-723) or control plasmid, and selected with G418 for 30 days, COMT expression was also determined by RT-PCR. Briefly, total RNA from these cells was extracted with Ribozol TM RNA Extraction Reagent (Amresco). Then RNA was converted to cDNA using the RevertAidTM 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 condition: 4 min at 95 °C, 28 cycles of 95 °C for 1 min, indicated annealing temperature for 45 s, and 72 °C for 45 s. 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).

Plasmid constructs and cell transfection. COMT shRNA-expressed plasmid pGPU6/GFP/Neo-COMT-homo-723 was designed and constructed by GenePharma (Shanghai). The plasmid can express COMT shRNA: 5′-GAAGGACAAGGTCACCCTTGTTTCAAGAGAACAAGG GTGACCTTGTCCTTCTT-3′. Its specific targeting sequence was 5′-GAAGGACAAGGTCACCCTTGT-3′. A scramble sequence was inserted in pGPU6/GFP/Neo plasmid used as control. COMT shRNA-expressed plasmid (PGPU6/GFP/Neo-COMT-homo-723) or control plasmid was transfected into K562 cells with Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's instructions. After transfection for 24 h, the transfected cells were selected with G418 for 30 days.

Detection of guaiacol concentration. The guaiacol (2-methoxyphenol) concentration was determined using high performance liquid chromatography (HPLC). After K562 cells and COMT shRNA-expressed K562 cells were exposed to catechol for 48 h, the cultures were collected and centrifuged at 3000 g for 20 min, and then the supernatant was transferred into a 1.5 ml eppendorf tube. 200 μl of supernatant per sample was added to 200 μl of acetonitrile, which was then vortexed for 4 min, centrifuged at 12, 000 g for 5 min, and then the separated supernatant was set in an eppendorf tube and used for HPLC analysis.

Waters 600 HPLC system with a Waters 2487 UV/Vis detector was used to determine guaiacol concentration in each sample. The filtered extract was injected into the HPLC fitted with a Hypersil ODS2 silica C18 column (Thermo) (200 mm × 4.6 mm) with particle size of 5 μm. The mobile phase consisted of 66.7% (v/v) aqueous solution of triethylamine

Table 1
Sequences of primers used in reverse transcriptase-PCR.

Gene	Sequence of primers (5′ → 3′)	Annealing temperature (°C)	Size of PCR product (bp)
α-Globin	Forward: AAGGTCGGCGCGCACGC Reverse: CTCAGGTCGAAGTGCGGG	58	101
β-Globin	Forward: CTCATGGCAAGAAAGTGCTCG Reverse: AATTCTTTGCCAAAGTGATGGG	60	181
γ-Globin	Forward: ACAAGCCTGTGGGGCAA Reverse: GCCATGTGCCTTGACTTT	58	158
PBGD	Forward: GGTCCTACTATCGCCTCCCTC Reverse: GAATCTTGTCCCCTGTGGTGG	56	216
ALAS2	Forward: GCAGCACTCAACAGCAAG Reverse: ACAGGACGGCGACAGAAA	56	239
COMT	Forward: TACTGCGAGCAGAAGGAGTG Reverse: CCAGCGAAATCCACCATCC	55	227
β-Actin	Forward: TGGACTTCGAGCAAGAGATGG Reverse: ATCTCCTTCTGCATCCTGTCG	60	289