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Involvement of PPARγ in emodin-induced HK-2 cell apoptosis

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ABSTRACT

Emodin, a major compound in total rhubarb anthraquinones (TRAs), has exhibited nephrotoxicity in Sprague Dawley rats and cytotoxicity to HK-2 cells, a human proximal tubular epithelial cell line, in our previous study. However, the exact molecular mechanisms underlying emodin-induced cytotoxicity remain undefined. In this study, the exposure of HK-2 cells to emodin led to decreased cell viability, caspase 3 cleavage and activation, loss of mitochondrial membrane potential ($\Delta\Psi_m$), and cytochrome c release from mitochondria to cytosol. Meanwhile, the levels of peroxisome proliferator-activated receptor gamma (PPARγ) mRNA and protein expression were elevated. GW9662, an antagonist of PPARγ, dramatically ameliorated the release of cytochrome c, the activation of caspase 3, and the reduction of cell viability induced by emodin. Importantly, emodin at the concentration causing apoptosis enhanced the stability of PPARγ mRNA. Taken together, these findings suggest that PPARγ might mediate, at least in part, emodin-induced HK-2 cell apoptosis via mitochondrial pathway.

1. Introduction

Emodin (1, 3, 8-trihydroxy-6-methylanthraquinone) is a major active ingredient of total rhubarb anthraquinones (TRAs) isolated from the root of rhubarb (*R. palmatum L.* or *R. tanguticum Maxim*) (Wang et al., 2008). Although it is well known that emodin possesses a number of biological activities, such as anti-inflammatory, anti-virus, anti-bacteria, anti-tumor, and immunosuppressive properties (Kuo et al., 2001; Sato et al., 2000; Srinivas et al., 2003; Yu et al., 2013), its deleterious effects have been found in *in vivo* and *in vitro* investigations (National Toxicology Program, 2001; Muller et al., 1996; Westendorf et al., 1990). We have recently demonstrated that TRAs caused nephrotoxicity on Sprague Dawley rats, as evidenced by swelled and denatured renal tubule epithelial cells (Yan et al., 2006), and that emodin induced apoptosis in HK-2 cells (a human proximal tubular epithelial cell line) by cathpesin B and caspase 3 activation (Wang et al., 2008, 2007). However, the actual molecular mechanisms underlying emodin-induced cytotoxicity remains undefined and thereby effective prevention and therapy strategies against emodin-caused nephrotoxicity are still limited.

Apoptosis plays an important role in tissue homeostasis and embryonic development in all organisms (Hotchkiss et al., 2009). It is genetically controlled and regulated by a series of complex signaling pathways, which can be triggered by numerous stimuli, including antigens, carcinogens, ionizing radiation, growth factor withdrawal, and cytotoxic drugs, etc. (Bortner and Cidlowski, 2002). Cells undergoing apoptosis show characteristic morphological and molecular changes, including chromatin condensation, oligonucleosomal DNA fragmentation, and ultimately cell breakdown, companied by the activation of specific cysteine proteases known as caspases (Strasser et al., 2000). Caspase 3 plays a central role in the execution phase of cell apoptosis. Once activated by upstream caspases, such as caspase 8 and 9, caspase 3 cleaves several specific substrates including poly (ADP-ribosyl) polymerase and D4-GDI proteins, which are important for the occurrence of typical biochemical and morphological changes in apoptotic cells (Krieser and Eastman, 1999).

Peroxisome proliferator-activated receptor gamma (PPARγ), a hormone-activated nuclear receptor, acts as an important molecular in cell-fate determination. The activation of PPARγ by agonists inhibits proliferation and induces apoptosis in a variety of cell types (Zhang et al., 2012). Moreover, loss-of-function mutation of PPARγ has been found in some human cancers (Sarraf et al., 1999). Some studies have shown that emodin exerts anti-inflammation and anti-diabetics by activating PPARγ (Xue et al., 2010;

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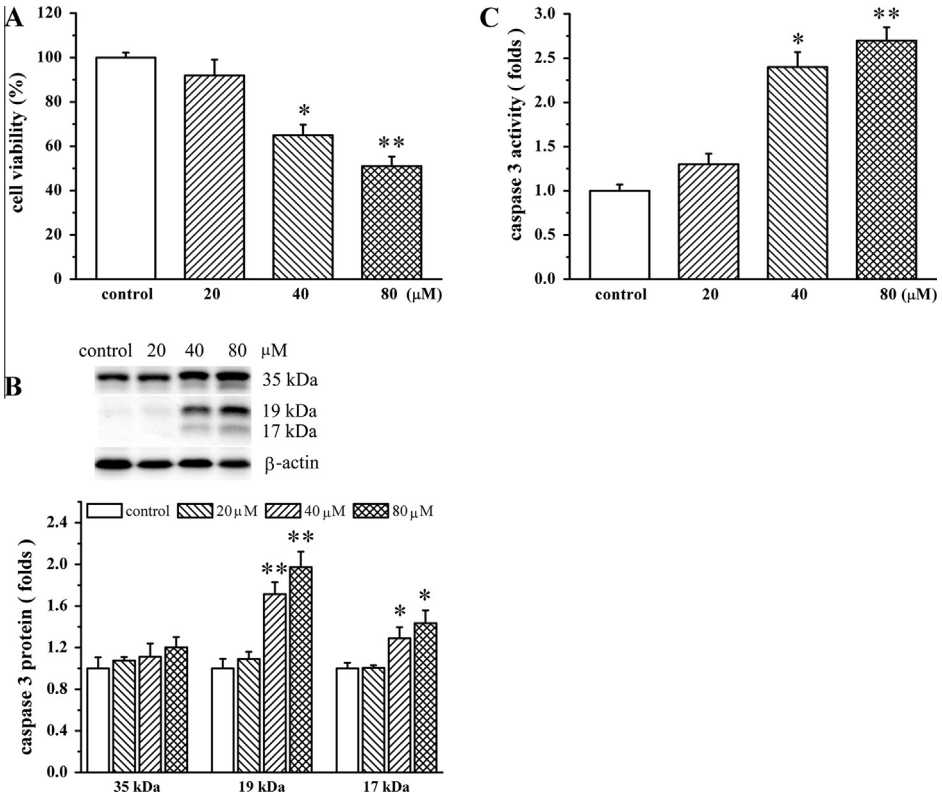

Fig. 1. Effects of emodin on HK-2 cell viability and caspase 3 activity. HK-2 cells were treated without or with emodin (20, 40, 80 μM) for 24 h, cell viability (**A**), and caspase 3 cleavage (**B**) and activation (**C**) were measured as described in Section 2. Data are presented as mean ± SEM ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* control.

Yang et al., 2014). However, it is still unknown whether PPARγ is involved in emodin-induced HK-2 cell apoptosis. The present study was aimed to evaluate the role of PPARγ in emodin-induced HK-2 cell apoptosis.

2. Materials and methods

2.1. Cell culture and chemicals

HK-2 human proximal tubular epithelial cells were obtained from American Type Culture Collection (CRL-2190; ATCC, Manassas, VA, USA). HK-2 cells were cultured in DF12 medium (DMEM/F12 1: 1 mixture) supplemented with 10% fetal bovine serum (FBS, GIBCO, Grand Island, NY, USA), 100 units/ml penicillin G, and 100 μg/ml streptomycin (Hyclone Labs, Logan, UT, USA), in a 37 °C incubator containing an atmosphere of 5% CO_2 in air. Cells were treated without or with various concentrations of emodin (dissolved in dimethyl sulfoxide (DMSO)) for the indicated time periods. The final concentration of DMSO in cell culture medium was less than 0.1%.

Emodin, 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5–diphenyl-tetrazolium bromide (MTT), Ac-DEVD-pNA, GW9662 (a potent antagonist of PPARγ), and Actinomycin D were purchased from Sigma-Aldrich (St Louis, MO, USA). 5, 5′, 6, 6′-Tetrachloro-1, 1′3, 3′-tetraethylbenzimidazolo-carbocyanine iodide (JC-1) was obtained from Calbiochem (La Jolla, CA, USA). PCR primers, TRIzol, MMLV reverse transcriptase were obtained from Invitrogen (Carlsbad, CA, USA). SYBR Green PCR Core Reagent Kit was obtained from Applied Biosystems (Foster City, CA, USA). Mitochondrial/Cytosol Fractionation Kit was from BioVision (Milpitas, CA, USA). Antibodies against human caspase 3 (full length) and cleaved caspase 3, horseradish peroxidase-linked anti-biotin antibody and horseradish peroxidase-linked goat anti-rabbit IgG, and biotinylated protein ladder were from Cell Signaling Technology (Danvers, MA, USA). Antibodies against human PPARγ, cytochrome c, cytochrome c oxidase IV (COX IV) and β-actin were from Santa Cruz Biotechnologies (Dallas, Texas, USA). ECL Western blotting detection reagents were from GE Healthcare Life Sciences (Pittsburgh, PA, USA). Formaldehyde was from Polyscience (Warrington, PA, USA). Other chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Determination of cell viability and caspase 3 activity

Cell viability was measured by MTT assay as previously described (Wang et al., 2007). Briefly, after 24 h of treatment with or without emodin, MTT was added into each well and the purple formazan crystals were dissolved in dimethylsulfoxide (DMSO, 0.5%). The optical density was determined with a microplate reader (Bio-Rad, USA) at a wavelength of 510 nm. Caspase 3 activity was measured using a caspase 3 colorimetric substrate, Ac-DEVD-pNA, as described earlier (Wang et al., 2008). In brief, equal amount of cytosolic proteins were mixed in a microplate with assay buffer and Ac-DEVD-pNA. After 2 h of incubation at 37 °C, the colorimetric release of *p*-NA from Ac-DEVD-pNA was determined at 405 nm.

2.3. Measurement of mitochondrial membrane potential ($\Delta\Psi_m$)

$\Delta\Psi_m$ was measured by fluorescent dye JC-1 as described by Lv et al. (2012). JC-1 is a cationic dye that exhibits potential-dependent accumulation in the mitochondrial membrane. The normal potential (polarization) enables JC-1 to enter and accumulate in the membrane, where aggregate JC-1 produces a red signal. The loss of membrane potential (mitochondrial depolarization) prevents JC-1 entry into mitochondria, thus JC-1 remains in the cytosol in a green fluorescent monomeric form. Therefore, mitochondrial depolarization can be indicated by a decrease in the red/green fluorescent intensity ratio. For JC-1 staining, HK-2