Food and Chemical Toxicology 49 (2011) 1367–1372

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Inhibition of JAK1/STAT3 signaling mediates compound K-induced apoptosis in human multiple myeloma U266 cells

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ARTICLE INFO

Article history:
Received 26 October 2010
Accepted 14 March 2011
Available online 21 March 2011

Keywords:
Compound K
STAT3
JAK1
Apoptosis
Multiple myeloma

ABSTRACT

Signal transducer and activator of transcription 3 (STAT3) is an oncogenic transcription factor implicated in carcinogenesis. Here, the role of STAT3 pathway in the antitumor activity of an active ginseng saponin metabolite compound K (CK) was investigated in human multiple myeloma U266 cells. CK increased the cytotoxicity, accumulated the sub-G1 DNA population, cleaved poly (ADP-ribose) polymerase (PARP) and activated caspase-3 in U266 cells. Interestingly, CK inhibited phosphorylation of STAT3 and its upstream activators, the Janus activated kinase 1 (JAK1), but not JAK2. Furthermore, CK enhanced the expression of protein tyrosine phosphatase (PTP) SHP-1, but not PTEN. Additionally, CK down-regulated STAT3 target genes bcl-x_L, bcl-2, survivin, cyclin E and cyclin D1. Conversely, PTP inhibitor pervanadate reversed CK-mediated STAT3 inactivation and cleavages of caspase-3 and PARP. Overall, our findings demonstrate that JAK1/STAT3 signaling mediates CK-induced apoptosis in U266 cells and also suggest the chemopreventive potential of CK for treatment of multiple myeloma.

1. Introduction

Signal transducers and activators of transcription (STATs) is a family of transcriptional factors that involved in various cellular activities such as cell survival, proliferation, angiogenesis and immune responses (Levy and Darnell, 2002). Of the STAT family proteins, constitutive activation of STAT3 was found in many cancer cells including multiple myeloma (Hsiao et al., 2003), leukemia (Schuringa et al., 2000), prostate cancer (Mora et al., 2002), breast cancer (Dolled-Filhart et al., 2003), colon cancer (Lin et al., 2005) and so on. In particular, recent studies have reported the potential of many compounds from natural products in cancer treatment by targeting STAT3. For instances, curcumin, a diferuloylmethane, inhibited constitutive and IL-6-inducible STAT3 phosphorylation in human multiple myeloma cells (Bharti et al., 2003). Similarly, betulinic acid and ursolic acid suppressed STAT3 activation pathway through induction of SHP-1 in multiple myeloma cells (Pandey et al., 2010; Pathak et al., 2007).

Ginseng Radix, the root of *Panax ginseng* Meyer, has been utilized as a traditional medicinal herb in Asian countries. Ginsenosides, a class of steroid glycosides and triterpene saponins, are contained in *P. ginseng*. Compound K (CK; 20-*O*-β-$_D$-glucopyranosyl-20(*S*)-protopanaxadiol) (Fig. 1) is a metabolite of the ginsenosides that has anti-tumor and anti-angiogenic activities in cancer cells (Jeong et al., 2010; Kim do et al., 2009a; Oh et al., 2004; Yim et al., 2005). Thus, in the current study, the role of JAK/STAT3 pathway was elucidated in CK induced apoptosis in multiple myeloma U266 cells. Our study demonstrated that inactivation of JAK1/STAT3 pathway mediated CK-induced apoptosis in U266 cells.

2. Materials and methods

2.1. Chemical and reagents

Compound K (CK) was supplied by Korea Tobacco and Ginseng (KT & G) Central Research Institute. RPMI 1640 medium, fetal bovine serum (FBS), and antibiotic–antimycotic agent were purchased from WelGENE (Daegu, South Korea). 3-(4,5-

Abbreviations: AMPK, AMP-activated protein kinase; AP-1, activator protein-1; ATCC, American Type Culture Collection; bFGF, basic fibroblast growth factor; CK, compound K; ECL, enhanced chemiluminescence; FBS, fetal bovine serum; HRP, horseradish peroxidase; IL-6, interleukin-6; JAK, Janus activated kinase; MAPK, mitogen-activated protein kinase; MMP-9, metalloproteinase-9; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NF-κB, nuclear factor-kappa B; OD, optical density; PARP, poly (ADP-ribose) polymerase; PI, propidium iodide; PTEN, phosphatase and tensin homolog; PTP, protein tyrosine phosphatase; SD, standard deviation; SDS, sodium dodecyl sulfate; STAT3, signal transducer and activator of transcription 3.

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doi:10.1016/j.fct.2011.03.021

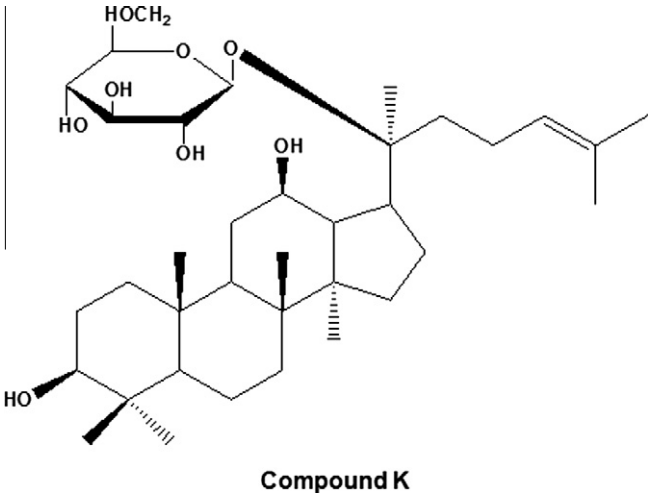

Fig. 1. Chemical structure of compound K (CK). Compound K (CK, 20-*O*-$_{\beta\text{-D}}$-glucopyranosyl-20(*S*)-protopanaxadiol), $C_{36}H_{62}O_8$, molecular weight = 623.

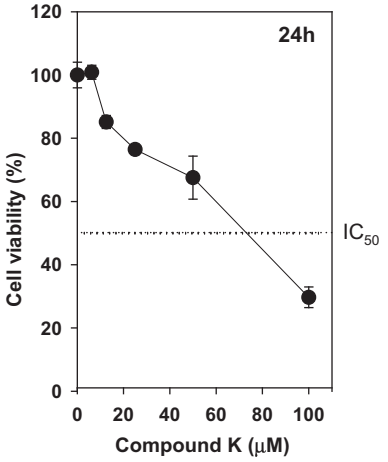

Fig. 2. Compound K (CK) exerts cytotoxic effect against U266 cells. Cells were plated onto 96-well microplate at a density of 2×10^4 cells/well and treated with various concentrations of CK (0, 6.25, 12.5, 25, 50 or 100 μM) for 24 h. Cell viability was evaluated by MTT assay. Data represent mean ± S.D from three independent experiments.

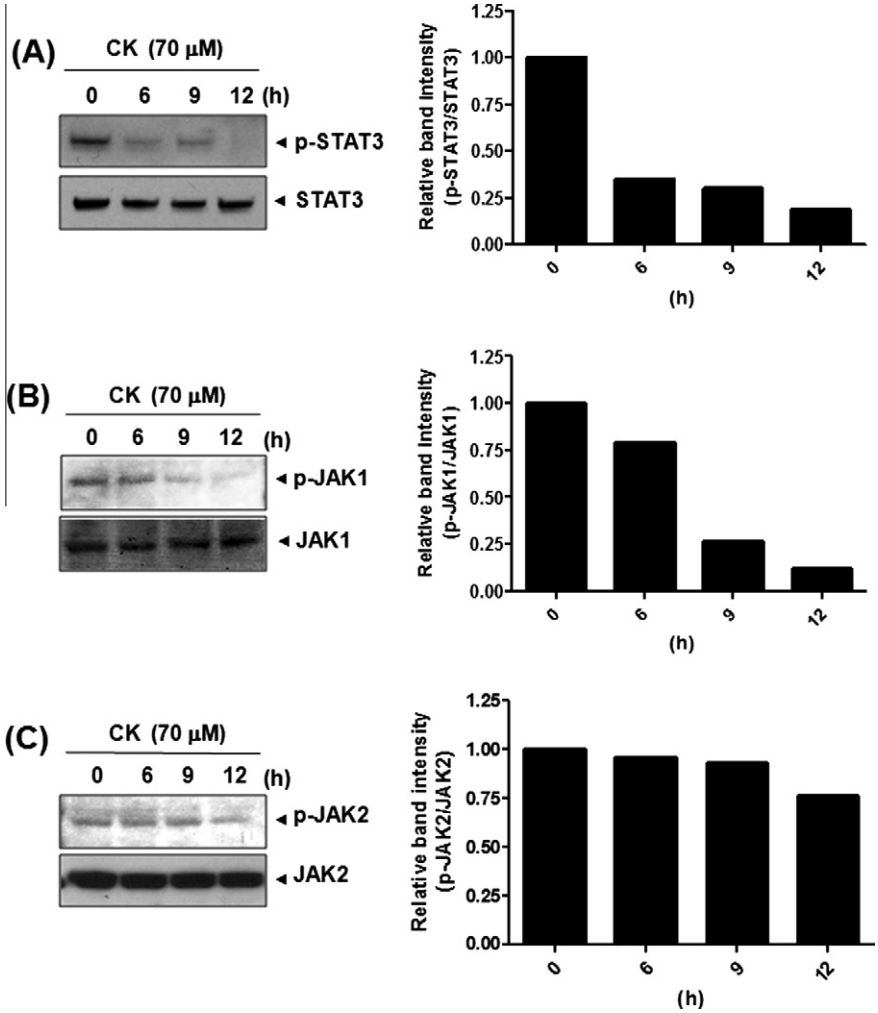

Fig. 3. Compound K (CK) suppresses STAT3 signaling in U266 cells. U266 Cells (1×10^6 cells/ml) were treated with 70 μM CK for 0, 6, 9 or 12 h. Cell lysates were prepared and subjected to Western blotting for (A) phospho-STAT3 and STAT3, (B) phospho-JAK1 and JAK1, and (C) phospho-JAK2 and JAK2. Bar graphs represent fold change of protein expression.