Biomedicine & Pharmacotherapy 64 (2010) 264–270

Available online at ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

Original article

Ellagic acid protects hepatocytes from damage by inhibiting mitochondrial production of reactive oxygen species

Jung Me Hwang [a], Jin Sook Cho [b], Tae Hyeon Kim [c], Young Ik Lee [a,*,b]

[a] *Liver cell Signal Transduction Lab., Metabolic Syndrome Research Division, Korea Research Institute of Bioscience and Biotechnology, PO Box 115, 52, Eoundong, Yusong 305-606, Republic of Korea*
[b] *Molecular Cancer Research Division, Lee's Biotech Co., BVC #202, Yusong, Taejon 305-600, Republic of Korea*
[c] *Department of Gastroenterology, Wonkwang University School of Medicine and Hospital, IkSan, Chonbuk, Republic of Korea 500-711*

ARTICLE INFO

Article history:
Received 22 April 2009
Accepted 7 June 2009
Available online 23 October 2009

Keywords:
Reactive oxygen species
Ellagic acid
Mitochondrial depolarization
Apoptosis
Concanavalin A
Cytoprotective effect

ABSTRACT

The aim of this experiment is to investigate the antioxidative and antiapoptotic roles of ellagic (EA) acid in in vitro and in in vivo experiment. We measured protective properties of EA against oxidative stress-induced hepatocyte damage in vitro and Concanavalin (ConA)-induced liver damage in vivo. EA, a potent antioxidant, exhibited protective properties against oxidative stress-induced hepatocyte damage by preventing vitamin k3 (VK3)-induced reactive oxygen species (ROS) productions, apoptotic and necrotic cellular damage and mitochondrial depolarization, which is a main cause of ROS production. EA also protects against cell death and elevation of glutathione (GSH), alanine transaminase (ALT) and asparatate transaminase (AST) in Con A-induced fulminant liver damage in mice. These results show that antioxidant and cytoprotective properties of EA prevent liver damage induced by various type of oxidative stress.

1. Introduction

Alterations of the cellular reduction/oxidation state through the production of ROS plays a crucial role in the steps that initiate and regulate the progression of liver damage. Furthermore, ROS are involved in liver damage induced by many different causes, including alcohol, virus infection, alterations of lipid and carbohydrate metabolism, and xenobiotics [1]. Increases in ROS are related to mitochondrial dysfunction, because the impairment of mitochondrial function is a key event in ROS-signaling and subsequent activation of apoptosis [2]. In addition to being the sites of electron transfer, oxidative phosphorylation, and adenosine triphosphate (ATP) synthesis, mitochondria also function as major players in the regulation of cell death [3]. Mitochondrial dysfunction, such as the loss of the inner transmembrane potential, results in excessive secondary ROS production and release of inner membrane proteins, including caspase activators and the caspase-independent death effector (AIF) [4]. For these reasons, cellular antioxidants such as GSH and thioredoxin are suggested to downregulate apoptosis by reducing ROS levels [5]. Loss of the mitochondrial membrane potential (MMP) and mitochondria dysfunction are early events that activate cellular necrosis and apoptosis. The MMP is an electrochemical gradient formed by pumping protons across the inner mitochondrial membrane, using the energy generated from the electron transfer chain. Damage to the mitochondria initiates mitochondrial membrane pore transition (MMPT), which is a rapid increase in the permeability of the inner mitochondrial membrane that results in collapse of the MMP, uncoupling of oxidative phosphorylation, and osmotic swelling of mitochondria [6]. Ellagic acid (EA), a plant polyphenol, exerts strong antioxidative effects. EA also possesses broad chemoprotective properties against a variety of different carcinogens, including nitrosamines, azoxymethane, mycotoxins, and polycyclic aromatic hydrocarbons [7]. In this study, we evaluated the protective effects of EA on VK3-induced hepatocyte damage in vitro and ConA-induced animal in vivo. VK3 induces apoptosis by elevating peroxide and superoxide radicals, and was used to test the ability of EA to protect cultured cells from ROS-mediated cell death. ConA is a pan-T cell activator, which causes sustained tumor necrosis factor α (TNF-α)-mediated c-jun N-terminal kinase (JNK) activation, massive cell death *via* both apoptosis and necrosis, induces fulminant liver failure in mice and humans [8]. This study evaluated the ability of EA to protect cultured cells from ROS production, cellular damage (apoptosis and necrosis), and mitochondrial depolarization after VK3-induced apoptosis. Further, we tested the ability of EA to prevent liver damage in vivo by measuring cell death, GSH, ALT, and AST levels in Balb/C mice after ConA-induced fulminant liver failure. The results indicate that EA protected against hepatocyte damage both in vitro and in vivo, suggesting that EA has therapeutic potential to prevent liver failure mediated by ROS.

* Corresponding author. Tel.: +82 42 860 4150; fax: +82 42 860 4597.
E-mail address: yilee@mail.kribb.re.kr (Y.I. Lee).

0753-3322/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biopha.2009.06.013

2. Materials and methods

2.1. Cell line, animals and materials

Chang human liver (CHL) cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, and 100 mg/ml streptomycin. Male Balb/c mice were obtained from Damul Science (Seoul, Korea) and maintained with free access to water and food prior to the experiment. The DeadEnd™ in situ terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) kit was used to detect cell death in liver sections (Promega, Madison, WI). Tetramethylrhodamine methylester (TMRM), 2',7'-dichloro fluorescein diacetate (DCFH-DA), and hydroethidine (HE) were obtained from Molecular Probes, Inc. (Eugene, OR). Carbonyl cyanide p-chlorophenyl hydrazone (CCCP), GSH, VK3, ConA, and rotenone, an inhibitor of election transport chain, were purchased from Sigma-Aldrich (Missouri, USA). The DNA fragmentation detection ELISAplus kit was obtained from Roche (Mannheim, Germany).

2.2. Mitochondrial membrane potential assay

The MMP of intact cells was measured by flow cytometry using the lipophilic cationic probe, 5,5',6,6'-tetrachloro-1,1',3,3'-iodide (JC-1) (Molecular probe, Eugene, OR). JC-1 is a ratiometric, dual-emission fluorescent dye that stains living mitochondria in a MMP dependent manner. In healthy cells, JC-1 binds to the mitochondrial membrane with MMPs of 80-100 mV and forms the J-aggregate, which fluoresces at 590 nm (red) in response to 488 nm excitation. Upon mitochondrial injury, the MMP is lost causing the J-aggregate to disperse as monomers into the cytoplasm and fluorescing at 527 nm (green) in response to 488 nm excitation. Quantitation of red and green fluorescent signals determines the extent of damage to the mitochondria. To measure MMP, CHL cells were trypsinized, pelleted by centrifugation, and the cell pellets were resuspended in 500 ul of phosphate buffered saline (PBS), incubated with 10 uM JC-1 for 20 min at 37 °C. The cells were subsequently washed once with cold PBS, suspended to a final volume of 350 ul with PBS, and analyzed using a FACScan flow cytometer.

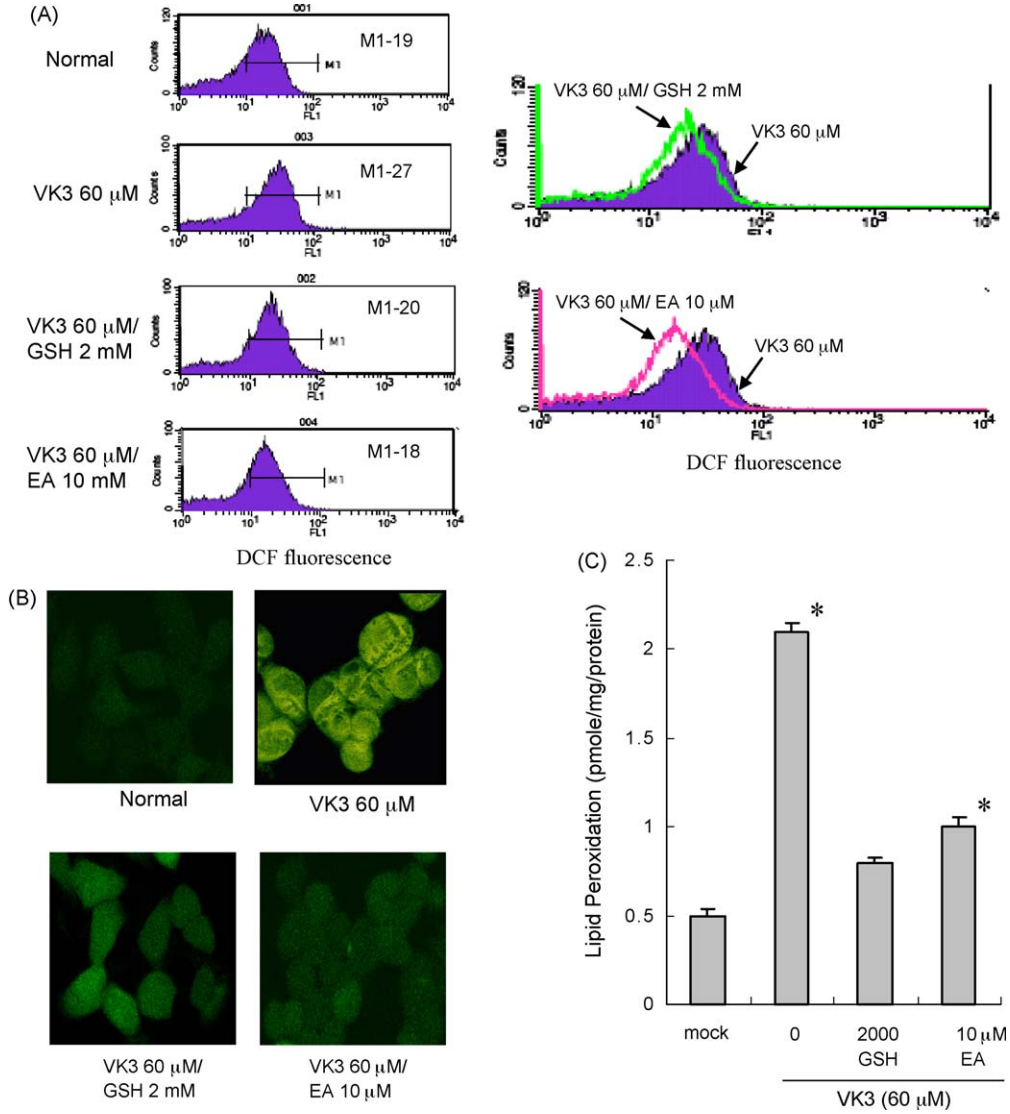

Fig. 1. EA inhibits VK3 induced ROS production in CHL cells. A. DCF fluorescence of a CHL cell line treated with 60 μM VK3, VK3 with GSH (2 mM) or VK3 with EA (10 μM) measured with a FACscan flow cytometer with an excitation and emission setting of 488 nm and 530 nm, respectively. B. DCF fluorescence of CHL cell lines treated with VK3 (60 μM), VK3 with GSH (2 mM) or VK3 with EA (10 μM) measured with confocal laser scanning microscopy. C. Total cellular lipid peroxidation product, 4-hydroxyalkenals, and MDA were determined by a colorimetric lipid peroxidation assay of cell extracts. * indicates reduction over VK3 (60 μM) treated CHL cells ($P < 0.05$). * indicates reduction over VK3 (60 μM) treated CHL cells ($P < 0.05$).