Journal of Trace Elements in Medicine and Biology 29 (2015) 275–283

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.de/jtemb

Toxicology

The role of mitogen-activated protein kinase in cadmium-induced primary rat cerebral cortical neurons apoptosis via a mitochondrial apoptotic pathway

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ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form 15 May 2014
Accepted 16 June 2014

Keywords:
Cadmium
Apoptosis
Mitochondrial apoptotic pathway
Mitogen-activated protein kinase
Primary rat cerebral cortical neurons

ABSTRACT

Cadmium (Cd) is an extremely toxic metal capable of severely damaging several organs, including the brain. Studies have shown that Cd induces neuronal apoptosis partially by activating the mitogen-activated protein kinase (MAPK) pathways. However, the underlying mechanism of MAPK involving the mitochondrial apoptotic pathway in neurons remains unclear. In this study, primary rat cerebral cortical neurons were exposed to Cd, which significantly decreased cell viability and the B-cell lymphoma 2/Bcl-2 associate X protein (Bcl-2/Bax) ratio and increased the percentage of apoptotic cells, release of cytochrome c, cleavages of caspase-3 and poly (ADP-ribose) polymerase (PARP), and nuclear translocation of apoptosis-inducing factor (AIF). In addition, Cd induced phosphorylation of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK. Inhibition of ERK and JNK, but not p38 MAPK, partially protected the cells from Cd-induced apoptosis. ERK and JNK inhibition also blocked alteration of the Bcl-2/Bax ratio, release of cytochrome c, cleavages of caspase-3 and PARP, and nuclear translocation of AIF. Taken together, these data suggest that the ERK- and JNK-mediated mitochondrial apoptotic pathways play important roles in Cd-induced neuronal apoptosis.

Introduction

Cadmium (Cd) is an environmentally and industrially pollutant, it can be accumulated in human body either through direct exposure to Cd-contaminated environment or by food chain [1]. It can enter the brain parenchyma and neurons causing neurological alterations in humans and animal models, leading to lower attention, olfactory dysfunction and memory deficits [2,3]. In addition, increasing evidences have demonstrated that Cd can induce neuronal apoptosis [4,5]. However, the exact mechanism through which Cd induces neuronal apoptosis is still unresolved.

Mitogen-activated protein kinase (MAPK) are important signal transduction enzymes that are unique to eukaryotes and are involved in many cellular processes, including development, differentiation, proliferation and apoptosis [6,7]. There are three main members of the MAPK family: c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) and p38 MAPK. These enzymes are regulated by a characteristic phosphorelay system, where a series of three protein kinases phosphorylate and activate one another [8]. Studies have shown that members of MAPK family may play a critical role in Cd-induced apoptosis of many cell types including neuronal cells [1,9–11]. However, it is less clear if Cd activates MAPK signaling pathways in primary cerebral cortical neurons.

The mitochondrial apoptotic pathway, which is thought to mediate Cd^{2+}-induced apoptosis [12,13], entails the release of death-promoting factors, such as cytochrome c and apoptosis-inducing factor (AIF), from the mitochondrial intermembrane space (IMS), which induce apoptosis in caspase-dependent and -independent manners respectively [14]. Caspases are cysteinyl proteases responsible for the cleavage of intracellular substrates, leading to apoptosis [15]. It was proven that Cd induces apoptosis through the mitochondrial apoptotic pathway in various cell types [16,17]. In our previous report [18] we have demonstrated that Cd-induced neuronal apoptosis is associated with activation of the mitochondrial apoptotic pathway by decreasing mitochondrial

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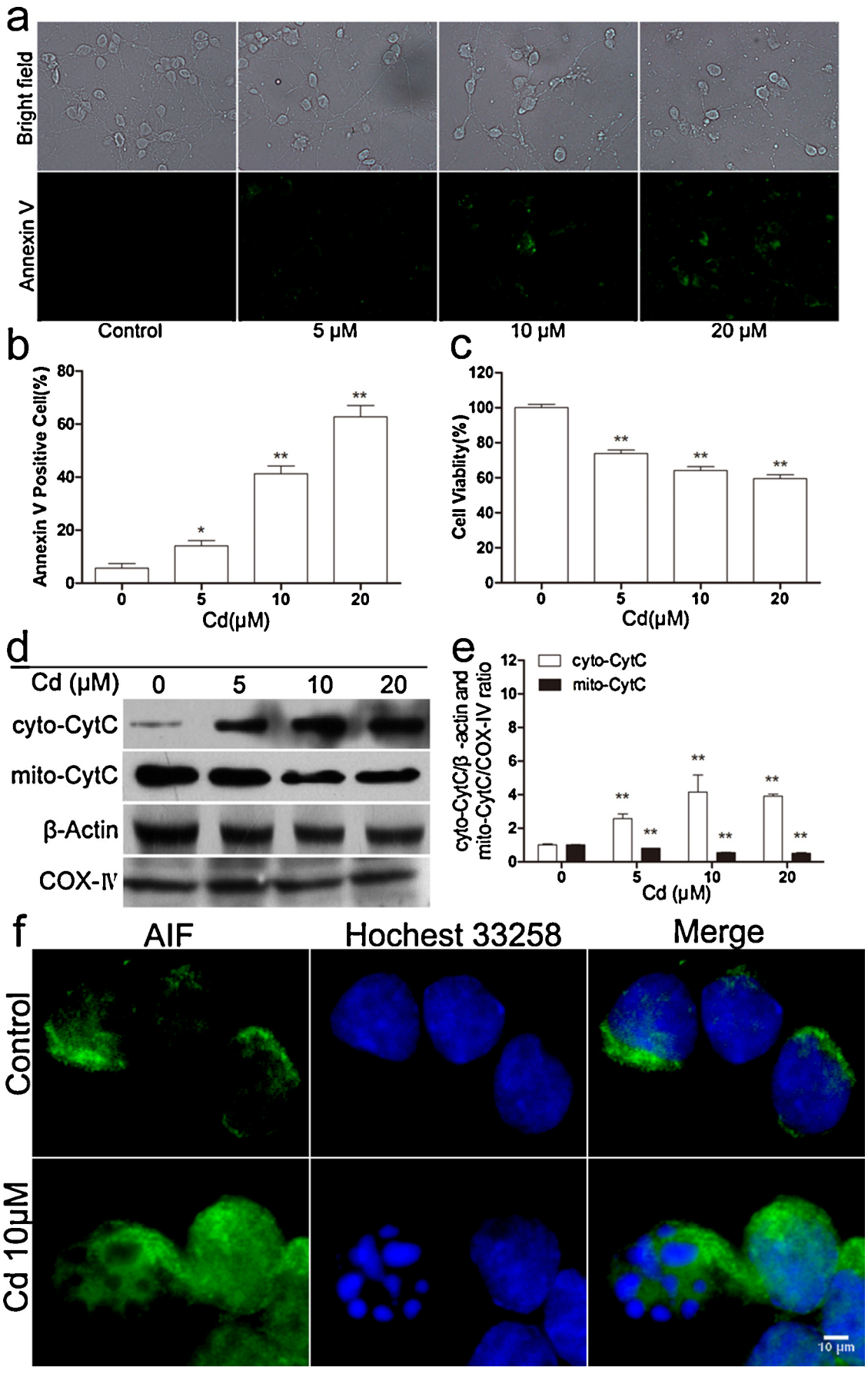
http://dx.doi.org/10.1016/j.jtemb.2014.06.006
0946-672X/


a
Bright field
Annexin V
Control
5 μM
10 μM
20 μM
b
Annexin V Positive Cell(%)
Cd(μM)
c
Cell Viability(%)
Cd(μM)
d
Cd (μM) 0 5 10 20
cyto-CytC
mito-CytC
β-Actin
COX-Ⅳ
e
cyto-CytC
mito-CytC
cyto-CytC/β -actin and mito-CytC/COX-IV ratio
Cd (μM)
f
AIF
Hochest 33258
Merge
Control
Cd 10μM
10 μm