Neuroscience Letters 453 (2009) 186–189

Contents lists available at ScienceDirect

Neuroscience Letters

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

# Curcumin attenuates ethanol-induced toxicity in HT22 hippocampal cells by activating mitogen-activated protein kinase phosphatase-1

Hyun-Ock Pae[a], Sun-Oh Jeong[a,b], Min Zheng[a], Hun-Yong Ha[a], Kang-Min Lee[b], Eun-Cheol Kim[c], Dong-Hwan Kim[d], Sung-Yeon Hwang[e], Hun-Taeg Chung[a,*]

[a] Department of Microbiology and Immunology, Wonkwang University School of Medicine, 344-2 Shinyong-dong, Iksan, Jeonbuk 570-749, Republic of Korea
[b] Division of Biological Sciences, College of Natural Science, Chonbuk National University, Jeonju, Republic of Korea
[c] Department of Oral and Maxillofacial Pathology, College of Dentistry, Wonkwang University, Iksan, Republic of Korea
[d] Haneul Hyang Gi Clinical Center, Iksan, Republic of Korea
[e] Department of Herbology, Wonkwang University School of Oriental Medicine, Iksan, Republic of Korea

ARTICLE INFO

*Article history:*
Received 4 December 2008
Received in revised form 4 February 2009
Accepted 12 February 2009

*Keywords:*
Curcumin
Ethanol
Mitogen-activated protein kinase
Mitogen-activated protein kinase phosphatase-1

ABSTRACT

Ethanol causes neurotoxicity through formation of reactive oxygen species and activation of mitogen-activated protein kinase (MAPK) pathways. MAPK phosphatase-1 (MKP-1) is one of the phosphatases responsible for dephosphorylation/deactivation of MAPKs. In this report, we examined the potential involvement of MKP-1 in cytoprotective effects of the well-known antioxidant curcumin. In HT22 hippocampal cells, ethanol caused cell death and activation of p38 MAPK and other two kinases. Blockage of p38 MAPK by its inhibitor protected HT22 cells against ethanol-induced toxicity. Curcumin attenuated ethanol-induced cell death, inhibited activation of p38 MAPK, and activated MKP-1. In HT22 cells transiently transfected with small interfering RNA against MKP-1, curcumin failed to inhibit ethanol-induced activation of p38 MAPK and to protect HT22 cells from ethanol-induced toxicity. Our results suggest that curcumin can attenuate ethanol-induced neurotoxicity by activating MKP-1 which acts as the negative regulator of p38 MAPK. This novel pathway may contribute to and explain at least one of the cytoprotective actions of curcumin.



Curcumin, a biologically active component of turmeric (*Curcuma longa*), has been discovered to have a variety of pharmacological activities, including anti-inflammatory, antioxidant, and anti-proliferative effects [12]. Recently, several lines of investigations have shown that curcumin reduces cytotoxicity induced by a variety of neurotoxins, including β-amyloid peptide [16], kainic acid [15], 3-nitropropionic acid [11], 1-methyl-4-phenylpyridinium ion [4], glutamate [18] and ethanol [1]. However, the actual cytoprotective mechanisms of curcumin are still not fully understood.

Ethanol, a powerful neurotoxin that is particularly harmful to the developing nervous system, causes neuronal damage, probably through abnormal induction of neuronal cell death [17]. It is well established that ethanol-induced cell death is related to oxidative stress from the metabolic enzymes activated during ethanol intoxication [8] and potential activation of the mitogen-activated protein kinase (MAPK) pathways [10]. There are three well-defined MAPK subfamilies: the extracellular signal-regulated kinase-1/2 (ERK-1/2), the c-Jun N-terminal kinase-1/2 (JNK-1/2), and the p38 MAPK. The MAPK pathway is activated through a cascade of sequential phosphorylation events, and dephosphorylation of MAPKs mediated by phosphatases represents a highly efficient mode of kinase deactivation. A number of protein phosphatases are known to deactivate MAPKs, including tyrosine, serine/threonine, and dual-specificity phosphatases. In mammalian cells, the dual-specificity protein phosphatases, which are often referred to as MAPK phosphatases (MKPs), are the primary phosphatases responsible for dephosphorylation/deactivation of MAPKs [3]. To date, at least 10 MKPs have been identified in mammalian cells, with MKP-1 being the archetype [19]. Although MKP-1 was initially thought to be a phosphatase specific for the ERK-1/2, recent studies demonstrated that MKP-1 also efficiently inactivated JNK-1/2 and p38 MAPK [3,19].

In this study, we report that curcumin attenuates ethanol-induced toxicity in HT22 hippocampal cells through its activation of MKP-1. We have found that curcumin induces MKP-1 activation, which is correlated with inactivation of p38 MAPK that mediates ethanol-induced toxicity.

The hippocampal cell line (HT22 cells) used in this study is a sub-line cloned from the parent HT4 cells that were immortalized from primary mouse hippocampal neurons using a temperature-sensitive small virus-40 T antigen [7]. Curcumin was isolated from the rhizomes of turmeric (*Curcuma longa*), as previously

* Corresponding author. Tel.: +82 63 850 6762; fax: +82 63 851 5066.
*E-mail address:* htchung@wku.ac.kr (H.-T. Chung).

0304-3940/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2009.02.025

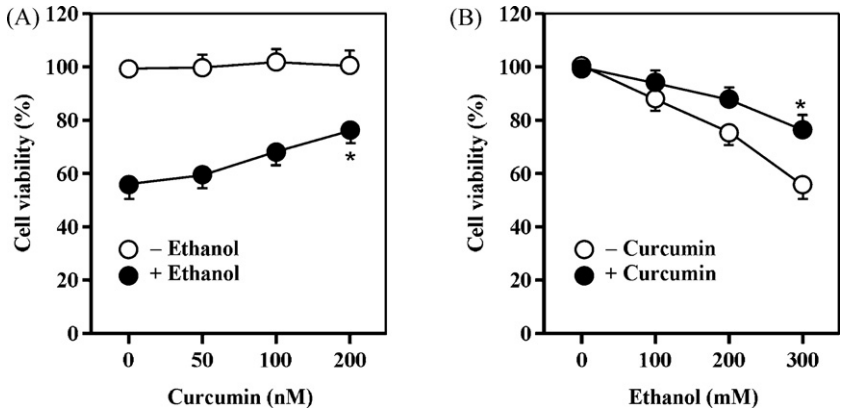


**Fig. 1.** Effects of curcumin on ethanol-induced cell death. Cells were pre-incubated for 2 h with curcumin at indicated concentrations (A) or 200 nM (B), and then exposed for 24 h to ethanol at 300 mM (A) or indicated concentrations (B). Cell viability was determined by MTT assay and the percentage of cell survival was calculated as compared with control untreated cells. Data are mean ± S.E. ($n = 6$). *$P < 0.05$ with respect to ethanol alone.

described [13]. This compound was prepared as 10 mM stock solution in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the culture medium was less than 0.1%. Ethanol, DMSO, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and Dulbecco's-modified Eagle's medium (DMEM, without L-glutamine) were purchased from Sigma (St. Louis, MO). U0126 and SB203580 were obtained from Tocris (Ellisville, MO). SP600125 was purchased from Calbiochem (San Diego, CA). HT22 cells were maintained in DMEM, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Gibco BRL, Gaithersburg, MD) at 37 °C in a 5% $CO_2$ atmosphere. The cells were pre-incubated for 2 h with curcumin (50–200 nM), and then exposed for 24 h to ethanol (100–300 mM) to measure cell viability that was determined by MTT assay. Inhibitors of ERK-1/2 (U0126), p38 MAPK (SB203580), and JNK-1/2 (SP600125) were added 30 min before ethanol treatment, and then in combination with ethanol. MKP-1 small interfering (si) RNA pooled oligonucleotides were purchased from Santa Crutz Biotechnology (Santa Cruz, CA). HT22 cells were plated at $10^5$ cells per well in 6-well plates and transfected with MKP-1 siRNA using Oligofectamine reagent (Invitrogen

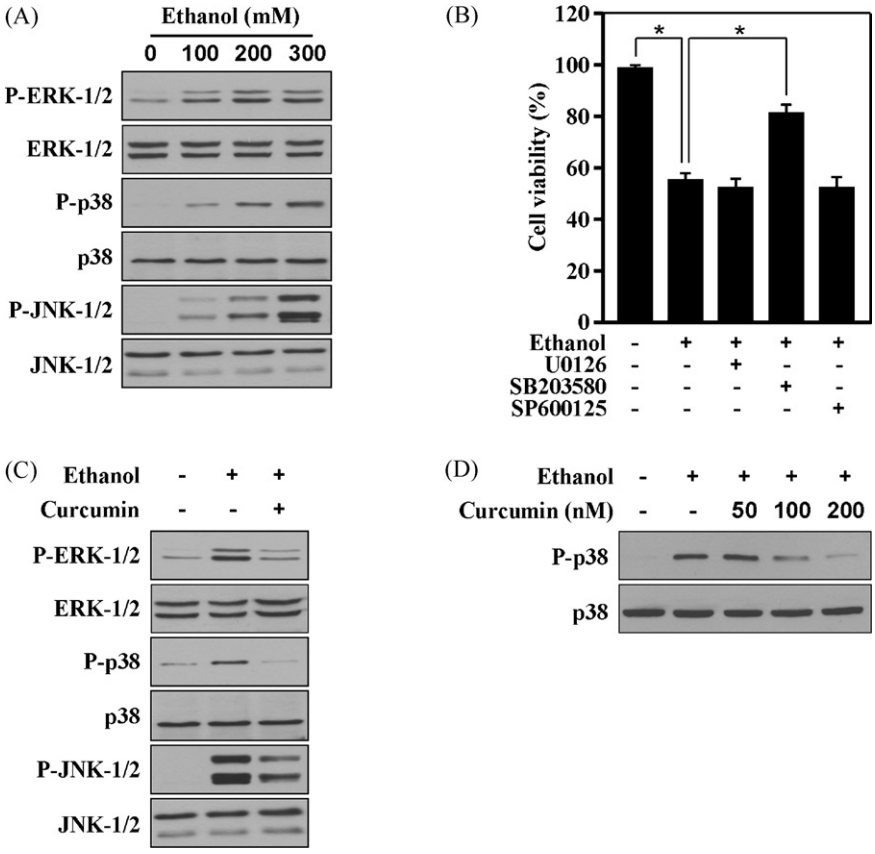


**Fig. 2.** Effects of curcumin on ethanol-induced MAPK activation. Cells were exposed for 6 h to ethanol at indicated concentrations (A), or pre-incubated for 10 min with U0126 (ERK-1/2 inhibitor; 10 μM), SB203580 (p38 MAPK inhibitor; 10 μM), or SP600125 (JNK-1/2 inhibitor; 20 μM), and then exposed for 24 h to 300 mM of ethanol (B). Cells were pre-incubated for 2 h with 200 nM of curcumin (C) or indicated concentrations of curcumin (D), and then exposed for 6 h to 300 mM of ethanol. Isolated protein (10 μg) was subjected to SDS-PAGE and Western blot analysis using phosphor-specific and protein-specific MAPK antibodies (A, C and D). Cell viability was determined by MTT assay (B) and the percentage of cell survival was calculated as compared with control untreated cells. Data are mean ± S.E. ($n = 6$). *$P < 0.05$ with respect to each group.