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Curcumin Attenuates Glial Cell Activation But Cannot Suppress Hippocampal CA3 Neuronal Cell Death in i.c.v. Kanic Acid Injection Model

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Kainic acid (KA) is a structural analogue of glutamate that interacts with specific presynaptic and postsynaptic receptors to potentiate the release and excitatory actions of glutamate. Systemic or intracerebroventricular (i.c.v.) administration of KA to experimental animals elicits multifocal seizures with a predominantly limbic localization, and results in neuronal death of cornu ammonia 1 (CA1), reactive gliosis and biochemical changes in the hippocampus and other limbic structures. Several lines of evidence suggest that reactive oxygen species (ROS) play a pivotal role in the pathogenesis of excitotoxic death by KA. Curcumin has been known to possess anti-oxidative and anti-inflammatory activities. In this study, the effects of curcumin on KA induced hippocampal cell death, reactive gliosis and biochemical changes in reactive glia were investigated by immunohistochemical methods. Our data demonstrated that curcumin attenuated KA-induced astroglial and microglial activation although it did not protect KA-induced hippocampal cell death.

Key Words: Curcumin, Kanic acid, Glial activation, Astrocyte, Microglia

INTRODUCTION

Flavonoids and monophenolic compounds have been well described over recent years as antioxidants and scavengers of reactive oxygen and nitrogen species. Curcumin is an active component of the food flavor that is widely used as a coloring agent in spice (Motterlini et al, 2000). It has been used for centuries in indigenous medicine for the treatment of a variety of inflammatory conditions and other disease (Lodha & Bagga, 2000). Curcumin is an antioxidant isolated from turmeric (*curcuma longa*) and has been known to have an anti-inflammatory activity (Ammon & Wahl, 1991; Surh et al, 2001). Recently, effects of curcumin on various cancers, inflammatory conditions, and oxidative stress are of great concerns of investigators. Although many studies support protective roles of curcumin, the effects of curcumin in kainic acid induced excitotoxicity have not been reported yet.

Kainic acid (KA), the analog of the excitatory amino acid L-glutamate, upon binding to non-NMDA glutamate receptors, causes depolarization of neurons followed by severe status epilepticus, neurodegeneration, plasticity, memory loss, and neuronal cell death (Izquierdo et al, 2000; Zagulska-Szymczak et al, 2001). The systemic or intracerebroventricular (i.c.v.) kainic acid injection produces pyramidal cell death in CA1 and CA3 areas (Sperk et al, 1983; Giusti et al, 1996) and cause reactive astro- and micro-gliosis over the hippocampus.

In this study, the effects of pre- and/or post-treatment of curcumin on kainic acid induced hippocampal excitotoxicity were investigated in the context of hippocampal CA3 cell death and reactive gliosis.

METHODS

Animals

Male ICR mice weighing 20 to 25 g at the beginning of experiments were used (MJC Inc., Seoul, Korea). The mice were housed 5 per cage in a room maintained at 18 to 22 ±1°C with an alternating 12 hour light-dark cycles. Food and water were available *ad libitum*. KA (Sigma, St Louis, MO) was prepared in saline (0.9%).

Intracerebroventricular (i.c.v.) injection of KA

The i.c.v. administrations of KA were performed following the procedure established by Laursen & Belknap (1986). Briefly, the mouse was injected at bregma with a 50 μl Hamilton microsyringe fitted with a 26-gauge needle that was inserted to a depth of 2.4 mm. The injection volume

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ABBREVIATIONS: KA, kainic acid; i.c.v., intracerebroventricular; ROS, reactive oxygen species; GFAP, glial fibrillary acidic protein; NOS, nitric oxide synthase.

was fitted to 5μl.

Administration of Curcumin

Curcumin was dissolved in 0.1 N NaOH with 0.1 M phosphate-buffered saline (PBS) and 200μg/kg was i.p injected daily for 3 days before kainic acid i.c.v. injection. After kainic acid i.c.v. injection, curcumin was further administered daily for 3 days with the same dose and the mice were sacrificed for immunohistochemical analysis.

Immunohistochemistry

After the injection of KA, all mice were transcardially perfused and post-fixed for 4 hours in 4% paraformaldehyde. Brains were cryoproteced in 30% sucrose, sectioned coronally (45μm) on a freezing microtome and collected in cryoprotectant for storage at −20°C until processed. Floating sections of hippocampus were processed Baker's published methods (Baker & Farbman, 1993). Sections were performed cresyl violet stainings. Sections were rinsed with PBS three times to remove cryoprotectant. And pre-incubated for 30 min in 0.1 M PBS with 1% bovine serum albumin and 0.2% Triton X-100 and incubated overnight with the following primary antisera: anti-goat GFAP (1 : 1000, Santa Cruz, CA, USA), anti-mouse OX-42 (1 : 500, Accurate Chemical & Scientific Corp, NY, USA), anti-rabbit eNOS (1 : 1000, BD Bioscience, USA), and anti-mouse iNOS (1 : 1000, BD Bioscience, USA). On the following day, sections were incubated for 1 hour in biotinylated mouse secondary antibody obtained from Vector laboratories. After incubation with the ABC kit (Vectastain Elite Kit, Vector Laboratories, CA, USA), antigens were detected with 3,3-diaminobenzidine tetrahydrochloride (DAB) as the chromogen. Sections were mounted, air-dried and dehydrated through graded ethanols, cleared in histoclear, and coverslipped using Permount (Fisher).

RESULTS

The effects of curcumin on KA-induced hippocampal cell death and reactive gliosis were investigated in mouse hippocampus. Curcumin itself (200 μg/kg, i.p.) did not cause any significant effects in cell viability and/or glial activations (Fig. 1A, 2A and 3A). In order to elucidate whether curcumin has a protective effect against KA-induced hippocampal CA3 cell death, cresyl violet staining was performed. KA i.c.v. injection resulted in significant CA3 hippocampal neuronal death (Fig. 1B), but curcumin did not block KA-induced CA3 hippocampal neuronal death irrespective of the administration protocol (Fig. 1C~E).

KA i.c.v. injection also induced considerable astroglial and microglial activations in the region that neuronal death occurred (Fig. 2B~B) determined by immunohistochemical studies with astrocyte and microglial markers such as GFAP and OX-42, respectively. Treatment of curcumin clearly attenuated KA-induced GFAP immunoreativity (Fig. 2C~E). This effect was obvious when curcumin was administered prior to kainic acid treatment (Fig. 2C and E). Although post-treatment of curcumin itself did not seem to suppress KA-induced astrocyte activation (Fig. 2D), it potentiated suppression mediated by pre-treatment of

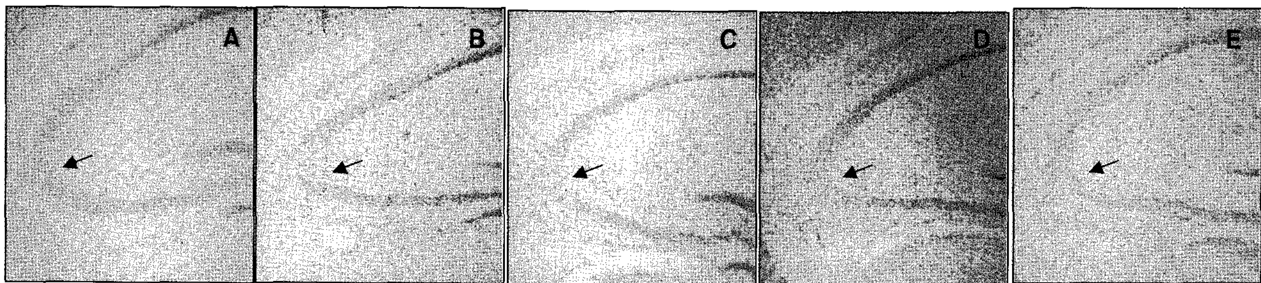

Fig. 1. Determination of cell death by cresyl violet immunostainings of hippocampal CA3 area. Curcumin only (A), KA i.c.v. only (B), pre-treatment of curcumin before KA injection (C), post-treatment of curcumin after KA injection (D), treatment of curcumin before and after KA injection (E). Arrows indicate CA3 region.

Fig. 2. Effects of curcumin on KA-induced astrocyte activation determined by GFAP immunostainings of hippocampal CA3 area. Curcumin only (A), KA i.c.v. only (B), pre-treatment of curcumin before KA injection (C), post-treatment of curcumin after KA injection (D), treatment of curcumin before and after KA injection (E).

Fig. 3. Effects of curcumin on KA-induced microglial activation determined by OX-42 immunostainings of hippocampal CA3 area. Curcumin only (A), KA i.c.v. only (B), pre-treatment of curcumin before KA injection (C), post-treatment of curcumin after KA injection (D), treatment of curcumin before and after KA injection (E).

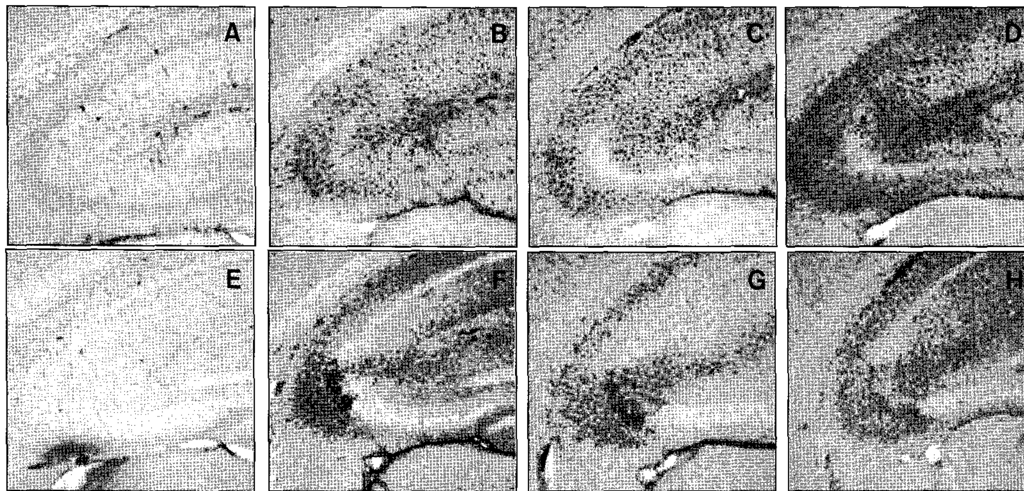

Fig. 4. Effects of curcumin on KA-induced nitric oxide synthases (NOSs) expressions. A, B, C, and D: eNOS immunostainings. E, F, G, and H: iNOS immunostainings. Curcumin only (A & E), KA i.c.v. only (B & F), pre-treatment of curcumin before KA injection (C & G), treatment of curcumin before and after KA injection (D & H).

curcumin (Fig. 2E).

Microglial activation was also checked using OX-42 immunohistochemistry. OX-42 immunoreactivity was increased by KA treatment (Fig. 3B). Curcumin showed suppression of KA-induced reactive gliosis in microglia as similar as in astrocytes (Fig. 3). Pre-treatment of curcumin mainly attenuated KA-induced microglial activation while post-treatment contributed partially to the attenuation of KA-induced microglial activation (Fig. 3C ~ E).

Given the fact that nitric oxide (NO) originated from glial cells plays roles in the modulation of stress response (Iwase et al, 2000; Sola et al, 2002), iNOS (inducible NOS) and eNOS (endothelial NOS) immunoreactivities during the curcumin-mediated attenuation of KA-induced glial cell activation were determined. Curcumin itself did not affect expression of NOSs (Fig. 4A ~ E). KA injection increased both eNOS and iNOS (Fig. 4B ~ F) immunoreactivities. Quite intriguingly, pre- and post-treatment showed an opposite effect on the immunoreactivities of iNOS and eNOS. It slightly increased KA-induced eNOS expression (Fig. 4D), whereas it slightly decreased KA-induced iNOS expression (Fig. 4H).

DISCUSSION

Glial cells exert many active roles in brain homeostasis through redox regulation (Schipper et al, 1999). They possess a substantial aptitude for plasticity and, indeed, functional and phenotypic changes are frequently encountered in reactive gliosis observed in brain injuries (Arvanitis et al, 2001; Gutierrez et al, 2001). However, neither the process by which glial cells become reactive nor the functional consequences of this reactive phenotype are well understood. The significance of reactive gliosis is still poorly defined, but it is clear that these cells are an important source of cytokines in inflamed brain (Yoshida et al, 2001; Kong et al, 2002). In contrast with resting astrocytes, reactive astrocytes produce proinflammatory cytokines, participate in antigen presentation in certain conditions, and may also limit inflammation and preserve neurons by secretion of endogenous materials (Ben-Hur et al, 2001). These data suggest that reactive astrocytes play roles in inflammatory responses in the brain.

Microglia, the major immunocompetant cells in the CNS, is believed to play an important role in inflammatory process in the brain. Many epidemiological studies suggested that nonsteroidal anti-inflammatory drugs can reduce the incidence and slow down the progress of

Alzheimer's disease (McGeer & McGeer, 1999). Considering the inflammatory reactions around the senile plaques, the target of nonsteroidal anti-inflammatory drugs might be microglia. Further, NO produced by microglia has been reported to be involved in the death of dopaminergic neurons in the MPTP model of Parkinson's disease (Dehmer et al, 2000).

Intracerebroventricular KA administration (0.1 μg/kg) induces reactive gliosis in addition to the neuronal cell death. Soluble factors released from activated glial cells as a result of KA administration may be involved in neurotoxic or/and neuroprotective processes. Thorough examination of the spatiotemporal patterns of various genes and proteins may help us understand molecular events leading to hippocampal cell death. Therefore, it is widely assumed that careful analysis of the glial responses may provide means to uncover the molecular events leading to such long-lasting phenomena as neural plasticity and neurodegeneration.

In this study, curcumin suppressed astro- and microgliosis induced by KA administration. But this suppressive property of curcumin was effective only when curcumin was applied prior to KA injection. This result might suggest that curcumin could modify CA3 hippocampal neuronal death but could not block cell death, presumably due to the severity of KA excitotoxicity.

NO plays roles in the modulation of stress response (Iwase et al, 2000; Sola et al, 2002). It has been previously demonstrated that iNOS is only expressed in microglia (Sola et al, 2002). Accordingly, the immunostaining pattern of iNOS was in accordance with that of microglia. However, eNOS immunoreactivity was not matched with that of iNOS. Curcumin increased KA-induced eNOS expression albeit the increase was not considerable. Further studies are necessary to understand a possible role of each NOS in the production of NO in terms of spatiotemporal pattern and intensity.

Glial activation might contribute to the secondary damage following the primary insults by producing many neurotoxic factors such as proinflammatory cytokines or NO. For example, chromogranin A-induced microglial activation causes neuronal cell death of cerebellar granule cells by inducing caspase-3-dependant apoptosis (Kingham et al, 1999). Given the data that curcumin suppressed glial activation caused by kainic acid, our study supports that the activated astrocytes and microglia contribute to KA-induced neuronal death presumably by a secondary neurotoxicity and that curcumin exerts protective effects by suppressing activated glial cells.

REFERENCES

Ammon HP, Wahl MA. Pharmacology of Curcuma longa. *Planta Med* 57: 1−7, 1991

Arvanitis DL, Stavridou AI, Mori de Moro G, Szuchet S. Reactive astrocytes upregulate one or more gene products that are recognized by monoclonal antibody H. *Cell Tissue Res* 304: 11−19, 2001

Baker H, Farbman AI. Olfactory afferent regulation of the dopamine phenotype in the fetal rat olfactory system. *Neuroscience* 52: 115−134, 1993

Ben-Hur T, Cialic R, Itzik A, Barak O, Yirmiya R, Weidenfeld J. A novel permissive role for glucocorticoids in induction of febrile and behavioral signs of experimental herpes simplex virus encephalitis. *Neuroscience* 108: 119−127, 2001

Dehmer T, Lindenau J, Haid S, Dichgans J, Schulz JB. Deficiency of inducible nitric oxide synthase protects against MPTP toxicity in vivo. *J Neurochem* 74: 2213−2216, 2000

Giusti P, Lipartiti M, Franceschini D, Schiavo N, Floreani M, Manev H. Neuroprotection by melatonin from kainate-induced excitotoxicity in rats. *FASEB J* 10: 891−896, 1996

Gutierrez E, Huang Y, Haglid K, Bao F, Hansson HA, Hamberger A, Viano D. A new model for diffuse brain injury by rotational acceleration: I model, gross appearance, and astrocytosis. *J Neurotrauma* 18: 247−257, 2001

Izquierdo LA, Barros DM, Ardenghi PG, Pereira P, Rodrigues C, Choi H, Medina JH, Izquierdo I. Different hippocampal molecular requirements for short- and long-term retrieval of one-trial avoidance learning. *Behav Brain Res* 111: 93−98, 2000

Iwase K, Miyanaka K, Shimizu A, Nagasaki A, Gotoh T, Mori M, Takiguchi M. Induction of endothelial nitric-oxide synthase in rat brain astrocytes by systemic lipopolysaccharide treatment. *J Biol Chem* 275: 11929−11933, 2000

Kingham PJ, Cuzner ML, Pocock JM. Apoptotic pathways mobilized in microglia and neurones as a consequence of chromogranin A-induced microglial activation. *J Neurochem* 73: 538−547, 1999

Kong GY, Kristensson K, Bentivoglio M. Reaction of mouse brain oligodendrocytes and their precursors, astrocytes and microglia, to proinflammatory mediators circulating in the cerebrospinal fluid. *Glia* 37: 191−205, 2002

Laursen SE, Belknap JK. Intracerebroventricular injections in mice. Some methodological refinements. *J Pharmacol Methods* 16: 355−357, 1986

Lodha R, Bagga A. Traditional Indian systems of medicine. *Ann Acad Med Singapore* 29:37−41, 2000

McGeer EG, McGeer PL. Brain inflammation in Alzheimer disease and the therapeutic implications. *Curr Pharm Des* 5: 821−836, 1999

Motterlini R, Foresti R, Bassi R, Green CJ. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. *Free Radic Biol Med* 28: 1303−1312, 2000

Schipper HM, Bernier L, Mehindate K, Frankel D. Mitochondrial iron sequestration in dopamine-challenged astroglia: role of heme oxygenase-1 and the permeability transition pore. *J Neurochem* 72: 1802−1811, 1999

Sola C, Casal C, Tusell JM, Serratosa J. Astrocytes enhance lipopolysaccharide-induced nitric oxide production by microglial cells. *Eur J Neurosci* 16: 1275−1283, 2002

Sperk G, Lassmann H, Baran H, Kish SJ, Seitelberger F, Hornykiewicz O. Kainic acid induced seizures: neurochemical and histopathological changes. *Neuroscience* 10: 1301−1315, 1983

Surh YJ, Chun KS, Cha HH, Han SS, Keum YS, Park KK, Lee SS. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. *Mutat Res* 480−481: 243−268, 2001

Yoshida H, Imaizumi T, Kumagai M, Kimura K, Satoh C, Hanada N, Fujimoto K, Nishi N, Tanji K, Matsumiya T, Mori F, Cui XF, Tamo W, Shibata T, Takanashi S, Okumura K, Nakamura T, Wakabayashi K, Hirashima M, Sato Y, Satoh K. Interleukin-1beta stimulates galectin-9 expression in human astrocytes. *Neuroreport* 12: 3755−3758, 2001

Zagulska-Szymczak S, Filipkowski RK, Kaczmarek L. Kainate-induced genes in the hippocampus: lessons from expression patterns. *Neurochem Int* 38: 485−501, 2001