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Chongmyungtang Attenuates Kainic Acid-induced Seizure and Mortal Effect in the Mouse

Kyung-Jin Jang[1], Kyou-Heung Lee[1], Sang-Lin Kim[1], Dong-Young Choi, Beom-Kyu Park, Doo-Hyun Im, Yong-Joon Cho, Wang-Kee Jhoo and Hyoung-Chun Kim

[1]Boryung Pharmaceutical Central Research Institute, Kunpo 435-050, Korea and College of Pharmacy, Kangwon National University, Chunchon 200-701, Korea

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The *Chongmyungtang* (CMT; the combination of *Acorus gramineus, polygala tenuifolia* and *Poria cocos*) has been recognized to possess the preventive effect against several neurologic disorders in human. In this study, we examined the effect of CMT on the three parameters associated with kainic acid (KA)-induced neurotoxicities; seizure/mortality, increased fos-related antigen (FRA) and glial fibrillary acidic protein (GFAP) expression. KA induced vigorous convulsions lasting 4-6 hr. Pretreatments with CMT before KA injection significantly reduced the seizure intensity as well as the mortality. CMT pretreatments also attenuated the KA-induced increase in FRA/GFAP expression in the hippocampus. These results suggest that CMT has a neuroprotective effect against KA-induced neurotoxicities.

Key words : Chongmyungtang, Neuroprotective effect, Kainic acid-induced neurotoxicity

INTRODUCTION

The *Chongmyungtang* (CMT), a traditional Korean medicinal preparation (Huh, 1994), consists of *Acorus gramineus, Polyala tenuifolia* and *Poria cocos* as the ratio of 1:1:1 (dry weight). It has long been employed in the clinical treatment of mental disorder such as senile amnesia in Korea, although a very few studies on its pharmacological effect on the central nervous system has been performed.

Abundant neurochemical evidence suggests that glutamate is an excitatory neurotransmitter in the mammalian CNS. On the basis of radioligand binding studies, at least three excitatory amino acid receptor subtype have been defined: N-methyl-D-aspartate, quisqualate and kainic acid (KA) receptors (Greenmyre *et al.*, 1985). In particular, KA has been used as a tool in neuroscience to explore the mechanism of excitotoxicity in *in vivo* (Sperk, 1994). KA induces limbic seizures in rodents and can be used as a screening model for anticonvulsants for the treatment of epileptic seizures (Meldrome, 1985; Sperk, 1994; Kim *et al.*, 1996; Kim *et al.*, 1997). Since KA induces a significant amount of cell loss in the hippocampus, the hippocampal neuronal injury could result in functional impairment of learning and memory (Stores, 1971).

Correspondence to: Hyoung-Chun Kim, College of Pharmacy, Kangwon National University, Chunchon 200-701, Korea

Rapid accumulation of evidence suggests that various stimuli to neuronal circuits, including epileptic seizures (Morgan and Curran, 1991) as well as learning related-neuronal plasticity (Kaczmarek, 1993), increase the expression of immediate-early genes such as *c*-fos, *c*-jun and zif/268 in the limbic system with a specific pattern.

Considering that glial cells are involved in ion homeostasis, metabolism and neurotransmission in the normal central nervous system, it is therefore not unlikely that the KA-induced seizures might activate the glial cells. The activated astroglial reactions to KA-induced hippocampal lesions could be induced by dearrangements of brain homeostasis (Sperk, 1994). It is well-known that astroglial cells are identified by their immunoreactivity for glial fibrillary acidic protein (GFAP) (Tanaka *et al.*, 1992).

In order to clarify the effects of CMT on KA-induced neurotoxicity, the present study examined CMT-induced changes in the profile of seizure activity, mortality and fos-related antigen (FRA)/GFAP levels after KA treatment.

MATERIALS AND METHODS

Animals and treatment

All mice were handled in accordance with the NIH gulidelines for the humane care of laboratory animals. Male ICR mice (Korean FDA, Seoul, Korea) weighing

about 25 g were maintained on a 12:12 hr light:dark cycle and fed *ad libitum*. CMT was supplied by Boryung Pharmaceutical Central Research Institute (Kunpo, Korea). All other chemicals were of the first or special commercial grade. CMT (200 mg/kg) was dissolved in distilled water and orally administered by two times a day for one week. KA (25 mg/kg, ip) was injected 30 min after final administration of CMT. As described previously, seizure activity was rated during a 6-hr period following KA challenge (Bing *et al.*, 1996; Kim *et al.*, 1996). To understand the pathophysiological changes induced by the drug treatments, we observed the mortality of animals throughout three days after KA treatment.

Immunocytochemical analysis

Animals were anesthetized with 50 mg/kg of pentobarbital and then perfused with phosphate-buffered saline (PBS; pH 7.4, 150 ml) followed by 4% paraformaldehyde. The brains were then removed, stored in 4% paraformaldehyde overnight and then cut at 40 μm in the horizontal plane with a vibratome. Following overnight incubation in primary antibody, sections were then incubated with a secondary biotinylated antiserum (1:700 dilution) for 1 hr. Sections were always washed three times with PBS (pH 7.4) between each incubation step. 3,3'-Diaminobenzidine was used as the chromogen. Each dilution for the FRA antisera (courtesy of Dr. Idarola, M; Kim *et al*, 1996; Bing *et al*, 1996) or the GFAP antisera (Boehringer Mannheim, IN) was 1: 1,000.

FRA immunoreactivity was examined 6 hr (to understand the maximal induction) after KA administration (Pennypacker *et al.*, 1994). GFAP-like immunoreactivity was analyzed 3 days (to observe the reactive gliosis of the early stage) after KA injection (Sperk, 1994). The intensity for the immunoreactivity was semiquantitively graded as intense (grade 3), moderate (grade 2), weak (grade 1), very weak (grade 0.5) and not detectable (grade 0).

Statistical analysis

The significance of the change in seizure score was evaluated using by Student's t-test. The significance of the alteration in immunoreactivity was determined by the one way ANOVA with Duncan's multiple range test, and that in mortality was examined by *chi-square* test. Statistical significance was defined as $p<0.05$.

RESULTS

Neurotoxic signs

KA-induced epileptic seizure behavior lasted for 4-6 hr (seizure score was expressed as the mean±S.E.M.).

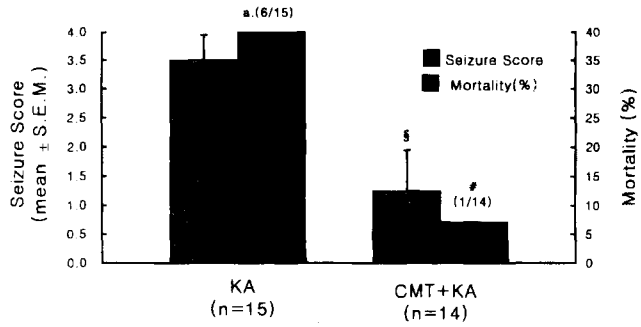

Fig. 1. The effects of CMT on seizure activity and mortality induced by KA. Seizures were scored according to the method by Kim *et al.* (1996). The significance of the changes in seizure score was determined by the Student's *t*-test. A *chi-square test* was used to test the significance of the changes in mortality. n=the number of mice receiving each treatment; a=died number/challenged number. #$p<0.05$ vs. KA; §$p<0.01$ vs. KA.

Pretreatments with CMT significantly reduced the seizure intensity ($p<0.01$) and the lethality ($p<0.05$) produced by KA (Fig. 1).

Immunocytochemical analysis

Fig. 2 shows FRA immunoreactivity in each group 6 hr after KA administration. Control animal exhibited a little induction in FRA immunoreactivity. Treatment with CMT alone, showed a basal level of FRA immunoreactivity as seen in control group. FRA immunoreactivity was significantly increased ($p<0.01$) in dentate gyrus (DG) granular cell layer in the KA-treated animal. However, the FRA immunoreactivity was dramatically decreased ($p<0.01$) in the KA-treated animal pre-exposed with CMT.

Fig. 3 manifests GFAP-like immunoreactivity in each group 3 days after KA treatment. Either control or CMT alone shows a basal level of GFAP-like immunoreactivity. The GFAP-like immunoreactivity was significantly enhanced ($p<0.01$) in the KA-treated animal. The reactive gliosis caused by KA was significantly reduced ($p<0.05$) by the pretreatment with CMT.

DISCUSSION

In the present study, pretreatments with CMT effectively prevented seizure activity and mortality induced by KA. Moreover, CMT clearly reduced hippocampal FRA and GFAP protein induced by KA. In addition, KA-initiated FRA immunostaining increased not only in the hippocampus but also in the limbic structure such as entorhinal cortex. Pretreatment with CMT also appeared to attenuate KA-induced increase of FRA immunoreactivity in the entorhinal coretx (data not shown).

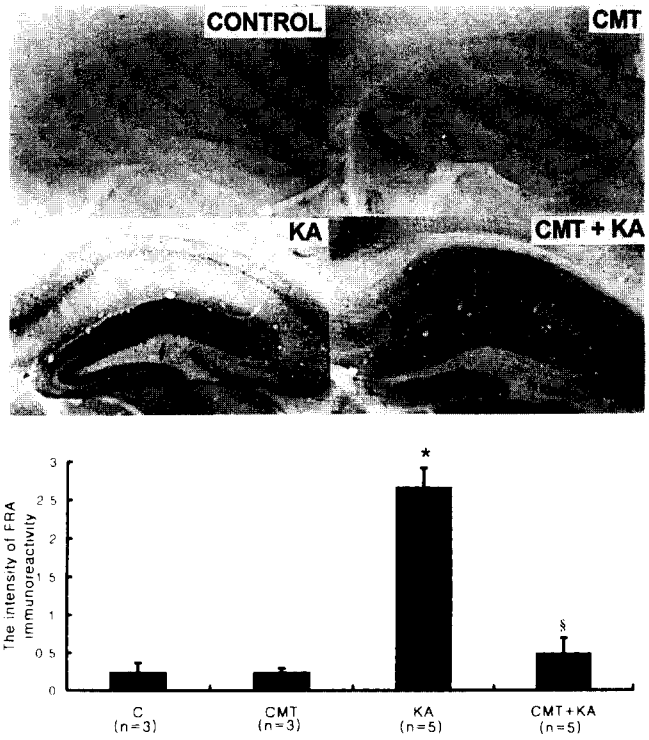

Fig. 2. Representative photomicrographs of immunostained sections against FRA 6 hr after KA treatment. Hippocampal sections from control and CMT-treated mice revealed very little FRA immunostaining. Dense immunostaining for FRA was visualized in the DG (note the arrow mark) after KA injection. The KA-treated mouse pretreated CMT showed dramatically decreased FRA immunostaining in the DG. Scale bar=150 μm. The intensity of immunoreactivity for FRA was semiquantitively graded as intense (grade 3), moderate (grade 2), weak (grade 1), very weak (grade 0.5) and not detectable (grade 0). n=the number of mice receiving each treatment. Each value is the mean±S.E.M.. *p<0.01 vs. control or CMT. §p<0.01 vs. KA (ANOVA with Duncan's multiple range test).

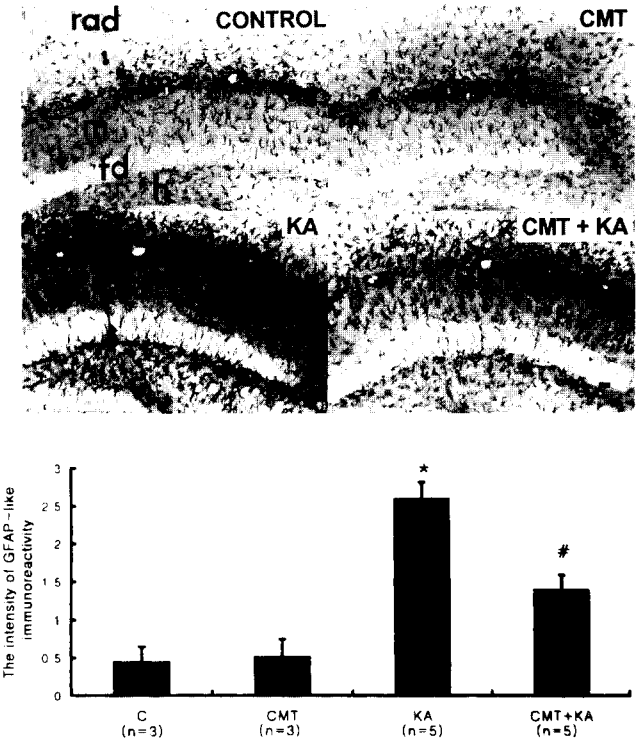

Fig. 3. Representative photomicrographs of immunostained sections with GFAP 3 days after KA administration. Hippocampal sections from control and CMT-treated mice manifested a little GFAP immunostaining with a homogenous distribution. The reactive astrogliosis was noted in the dentate molecular layer after KA injection. The KA-induced reactive gliosis was attenuated by the pretreatment with CMT. **rad**; stratum radiatum of CA1, **lm**; lacunosum-moleculare of CA1, **m**; dentate molecular layer, **fd**; fascia dentata, **h**; dentate hilus. Scale bar=150 μm. The intensity of GFAP-like immunoreactivity was semiquantitively graded as intense (grade 3), moderate (grade 2), weak (grade 1), very weak (grade 0.5) and not detectable (grade 0). n=the number of mice receiving each treatment. Each value is the mean±S.E.M.. *p<0.01 vs. control or CMT. #p<0.05 vs. KA (ANOVA with Duncan's multiple range test).

The FRA is a family of inducible transcription factors (Morgan and Curran 1991). The FRA recognizes activator protein-1(AP-1) DNA response elements in the promoter regions of target genes to regulate gene transcription (Mogan and Curran 1991). The conditions associated with neuronal stimulation/plasticity can induce the expression of AP-1 transcription factors (Morgan and Curran 1991; Kaczmarek 1993); systemic administration of KA also induces *c*-fos and FRA immunoreactivity in the rodent brain (Pennypacker., 1994; Bing *et al.*, 1996; Kim *et al.*, 1996). Consistently, the increase in FRA immunoreactivity in the DG has been reported during various types of neuronal plasticities including learning/memory and neuronal degeneration following neuronal stimulation (Kaczmarek 1993; Dash *et al.*, 1995)

There are AP-1-like sequences in the promotor regions of the most target genes including prodynorphin, proenkephalin (Kim *et al.*, 1997) and GFAP (Pennypacker *et al.*, 1994). The DNA binding activity for the GFAP AP-1 sequence increased during reactive gliosis induced by KA insults (Pennypacker *et al.*, 1994). Therefore, CMT may prevent the stimulation of neuronal activity after KA.

We observed that the barbiturate-like sedative effect produced by CMT is similar to the behavioral effect induced by NMDA receptor antagonist (Kim *et al.*, 1996) or GABA-mimic drug (Pennypacker *et al.*, 1993). However, the physiological significance of this phenomenon is still unresolved.

It was demonstrated that KA administered to rats resulted in significant cognitive deficits in water maze performance, increased activity in the open field test and spontaneous recurrent seizures (Stafstrom *et al.*, 1989). However, the precise reason/mechanism for

the cognitive deficits after KA injection is also remains to be explored.

One of the problems we have in interpreting neuroprotective effects with the CMT that has anticonvulsant properties is to determine whether the protection is secondary to a reduction in seizure severity or is a true neuroprotective effect, although we observed a strong relationship between the extent of reduction of FRA protein in the DG and the reduction of seizures and mortality (Kim *et al.*, 1996).

Despite the uncertainties in the mechanisms of action of CMT, our results raise a promising possibility that CMT may work as a safe neuroprotective agent or a cognition enhancer, since the neuroprotective effect of CMT in the KA model is recognized without causing severe side effects in contrast to choline esterase inhibitors (Lee and Benfield 1994). Recently, we observed that the CMT affects the learning and memory process in the central nervous system; CMT attenuated the memory registration impairment induced by scopolamine in the Step-Down test (data not published). Thus, the present finding supports the idea that the neuroprotective action may, at least partially, play a positive role in procognitive action of CMT to mouse. The favorable results with CMT in animal study may encourage further trials to evaluate its full therapeutic potential in the age-related amnesia, neurodegenerative disorders associated with Alzheimer's disease and ischemic disorder.

REFERENCES CITED

Bing, G., McMillian, M., Kim, H. C., Pennypacker, K., Feng, Z., Qi, Q., Kong, L. Y., Iadarola, M. and Hong, J. S., Long-term expression of the 35000 mol wt. Fos-related antigen in rat brain after kainic acid treatment. *Neuroscience,* 73,1159-1174 (1996).

Dash, P. K., Moore, A. N. and Dixon, E., Spatial memory deficits, increased phosphorylation of the transcription factor CREB, and induction of the AP-1 complex following experimental brain injury. *J. Neurosci.*, 15, 2030-2039 (1995).

Greenamyre, J.T., Olson, J.M.M., Penny, J.B. and Young, A.B., Autoradiographic characterization of N-methyl-D-aspartate-quisqualate, and kainate-sensitive glutamate binding sites. *J. Pharmac. Exp. Ther.*, 233, 254-263 (1985).

Huh, J., *Dongeuybokam* (1st ed)., Yuhkam Press, Seoul, 1994.

Meldrome, B., Possible therapeutic applications of antagonists of excitatory amino acid neurotransmitter. *Clin. Sci.*, 68, 113-122 (1985).

Morgan, J. I. and Curran, T., Proto-oncogene transcription factors and epilepsy. *Trends Pharmacol. Sci.*, 12, 343-349 (1991).

Kaczmark, L., Molecular bilogy of vertebrate learning: Is c-fos a new beginning ? *J. Neurosci. Res.*, 34, 377-381(1993).

Kim, H. C., Pennypacker, K., Bing, G., Bronstein, D., McMillian, M. K. and Hong, J. S. The effect of dextromethorphan on kainic acid-induced seizures in the rat. *Neurotoxicology*, 17, 375-386 (1996).

Kim, H. C., Suh, H. W., Bronstein, D., Bing, G., Wilson, B. and Hong, J. S. , Dextromethorphan blocks opioid peptide gene expression in the rat hippocampus induced by kainic acid. *Neuropeptides,* 31, 105-112 (1997).

Lee, C. R. and Benfield, P., Antiracetam: an overview of its pharmacodynamic and pharmacokinetic properties and a review of its therapeutic potential in senile cognitive disorders. *Drugs and Aging*, 3, 257-273 (1994).

Pennypacker, K. R., Walczak, D., Thai, L., Mason, F. E., Douglass, J. and Hong, J. S. Kainic acid induced changes in opioid peptides genes and AP-1 protein expression in the rat hippocampus. *J. Neurochem.*, 60, 204-211 (1993).

Pennypacker, K. R., Thai, L., Hong, J. S. and McMillian, M. K. Prolonged expression of AP-1 transcription factors in the rat hippocampus after systemic kainate treatment. *J. Neurosci.*, 14, 3998-4006 (1994).

Sperk, G., Kainic acid seizures in the rat. *Prog. Neurobiol.*, 42, 1-32 (1994).

Stafstrom, C., Edwards, M.. and Holmes, G., Status epilepticus following kainic acid administration: effect of age. *Epilepsia,* 30, 673-679 (1989).

Stores, G., Cognition function in children with epilepsy. *Dev. Med. Child. Neurol.*, 13, 390-393 (1971).

Tanaka, H., Araki, M. and Masuzawa, T., Reaction of astrocytes in the gebril hippocampus following transient ischemia: Immunohistochemical observations with antibodies against glial fibrillary acidic protein, glutamine synthetase, and S-100 protein. *Exp. Neurol.*, 116, 264-274 (1992).