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

Kyung-Jin Jang¹, Kyou-Heung Lee¹, Sang-Lin Kim¹, Dong-Young Choi, Beom-Kyu Park, Doo-Hyun Im, Yong-Joon Cho, Wang-Kee Jhoo and Hyoung-Chun Kim

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

(Received February 14, 1997)

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, Polygala 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.

Rapid accumulation of 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
about 25 g were maintained on a 12:12 hr light:dark cycle and fed ad libitum. CMT was supplied by Bor-yung 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 semiquantitatively 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 dentate gyrus (DG) granular cell layer in the KA-treated animal. However, the FRA immunoreactivity was significantly reduced (p<0.01) in the KA-treated animal pre-exposed 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 cortex (data not shown).