Neurotrophic Effect of Magnolol in the Hippocampal CA1 Region of Senescence-Accelerated Mice (SAMP1)

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Magnolol has neurotrophic effects in primary cultured rat cortical neurons, which are expressed as the promotion of neurite outgrowth and neuronal survival. In this study, we investigated the protective effect of magnolol against age-related neuronal loss in the hippocampus using senescence-accelerated mouse (SAMP1). Magnolol (5, 10 mg/kg) was orally administered once a day for 14 d to 2- or 4-month-old mice, and evaluation was carried out when the mice were 4 or 6 months old. The density of neurofibrils decreased with aging in the stratum radiatum of the CA1 region in the hippocampus of SAMP1, not SAMR1. Treatment with magnolol significantly prevented the decrease of neurofibrils in the CA1, when it was administered in 2-month-olds. However, administration at 4 months of age did not result in a preventive effect. These findings suggest that the administration of magnolol before the initiation of neuronal loss may result in a protective effect in the hippocampus.

Key words magnolol; hippocampus; senescence accelerated mouse

Magnolol (5,5′-di-2-propenyl-1,1′-biphenyl-2,2′-diol), one of the main constituents in the stem bark of Magnolia obovata THUNB and Magnolia officinalis RHED,1,2 has been used as a traditional Chinese medicine and has a wide spectrum of pharmacological activities.3 In the central nervous system, magnolol has been reported to increase extracellular acetylcholine release in rat hippocampus,4 to decrease the release of serotonin, dopamine, and norepinephrine in rat hypothalamus,5 to reduce the increased production of prostaglandin E2 caused by chemical hypoxia in neurons,6 and to interact with γ-aminobutyric acid (GABA) receptors in rat brain.7 In addition, we reported that magnolol had neurotrophic properties, such as promotion of neurite outgrowth and neuronal survival under serum-deprived conditions in cultured rat cortical neurons.8 However, these actions have not been demonstrated in vivo experiments.

Senescence-accelerated mice (SAM) are selected by brother/sister mating of AKR/J mice, and are characterized by rapid accumulation of senile features and shortened life span (1—1.5 years compared to 2—2.5 years in control animals).9,10 SAM consist of nine inbred strains of accelerated senescence-prone mice (SAMP) and three of accelerated senescence-resistant mice (SAMR), the latter of which show normal aging. Until the age of 4 months, SAMP1 animals do not differ from SAMR1 either behaviorally or morphologically, but later they begin to rapidly accumulate senile changes. This results in disordering of their behavioral reactions and cognitive brain functions (orientation ability and learning capacity are particularly decreased) and appearance of age-specific morphological changes (lordokyphosis, loss of hair, frequent eye cataract, and mucosal inflammation).10 Kawaguchi et al. reported that SAMP1TA/Ngs, a substrain of SAMP1, manifests learning disturbance at 7 months of age on a step-down passive avoidance test.11 This study was undertaken to estimate the neurite outgrowth and neuronal survival effect of magnolol on the senescence-related morphological changes on the density of dendrite in the stratum radiatum of the hippocampal CA1 area, which plays an important role in learning and memory, in SAMP1 mice.

MATERIALS AND METHODS

Materials Magnolol (Fig. 1) was isolated from the cortex of M. officinalis RHED. The purity was determined by a high-performance liquid chromatography (single peak) and by nuclear magnetic resonance spectra.

Animal Experiments All experiments were conducted in accordance with the Guiding Principles for Care and Use of Laboratory Animals approved by the Japanese Pharmaceutical Society. The substrains of SAM, SAMP1, and SAMR1 were originally obtained from the Institute for Frontier Medical Sciences in Kyoto University and bred in the Faculty of Health and Welfare Science in Okayama Prefectural University. Mice were kept in an air conditioned room maintained at 25°C with humidity of 55%±5%. The animals were given food and water ad libitum. Two- to 4-month-old female SAMP1 and SAMR1 were used. SAMP1 mice were divided into three groups (Fig. 2). Since the many of neurodegradation was observed between 2 and 4 months of age in SAMP1 as shown in Fig. 3, two groups were designed; one is the group in which drug was administrated to the mouse that is followed by neurodegradation (Group I, II), and the other is the group in which drug was administrated to the mouse between on-going-neurodegradation (Group III). Magnolol was dispersed in distilled water, and orally admin-

Fig. 1. Structure of Magnolol

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istered in all groups. Group I mice were administrated magnolol at 5 and 10 mg/kg, once a day for 14 d from 2 months of age and were sacrificed at 4 months of age. Group II mice were administrated magnolol at 5 and 10 mg/kg, once a day for 14 d from 2 months of age, and were sacrificed at 6 months of age. Group III mice were administrated magnolol at 10 mg/kg once a day for 14 d from 4 months of age, and were sacrificed at 6 months of age.

**Histological Experiments** The hippocampus was isolated from the excised whole brain sample and fixed in a buffered 15% formalin solution (pH 7.4). The hippocampus was embedded in paraffin and thin-sectioned at 6 μm. These sections were stained with Bodian’s silver staining. The morphometric analysis of the sections was carried out by measuring the density of neurofibrils. Digital images of the hippocampus were obtained using a light microscope (BX51, Olympus, Tokyo, Japan) with a digital camera (DP12, Olympus, Tokyo, Japan). Three fields in the stratum radiatum at the hippocampal CA1 area were selected for measurement. The digitalized image was converted to a 256-gray-scale image and the density of neurofibrils without cell bodies was determined using Win ROOF software with an EVO D500 computer (Compaq, Tokyo, Japan).

**Statistical Analysis** Each value is expressed as the mean ± standard error of the mean (S.E.M.). Statistical significance was evaluated by one-way ANOVA with Bonferroni correction. *p*-value less than 0.05 was considered significant.

**RESULTS**

**Age-Related Change of Density of Neurofibrils in the Hippocampal CA1 Area** The density of neurofibrils at the stratum radiatum in the hippocampal CA1 area age-related decreased in SAMP1 mice. That of SAMR1 mice showed no change from 2 to 10 months of age (Fig. 3). In SAMP1 mice, the mean value of % area of neurofibrils at stratum radiatum in the CA1 was 7.21 ± 0.51% in the 2-month-old group, 5.60 ± 1.01% in 4-month-old group, 5.12 ± 0.56% in the 6-month-old group, 4.52 ± 0.35% in the 8-month-old group and 4.44 ± 0.31% in the 10-month-old group. Figs. 5A and B show the typical photographs of the stratum radiatum in the hippocampus at 2 and 6 months of age SAMP1, respectively. In addition, the density of neurofibrils in hippocampal CA3 area were age-related decreased similarly in either SAMP1 and SAMR1. (data not shown).

**Effect of Magnolol on Age-Related Decrease of Density of Neurofibrils in the Hippocampal CA1 Area** In group I, the oral treatment with magnolol from 2 months of age significantly prevented, in a dose-dependent manner, age-related decreased density of neurofibrils in 4-month-old SAMP1 (Fig. 4). In group II, oral treatment with magnolol from 2 months of age significantly, prevented in a dose-dependent manner, age-related decrease of density of neurofibrils in 6-month-old SAMP1 (Figs. 4, 5C). In group III, oral treatment with magnolol from 4 months of age had no effect on age-related decrease of density of neurofibrils at 6 months of age (Figs. 4, 5D).

**DISCUSSION**

SAMP8 and SAMP10 mice are known to develop a learning and memory disturbance and are used as possible animal
models for the early onset of Alzheimer’s disease.\textsuperscript{12,13} On the other hand, SAMP1, which are separate from several SAMP strains, is characterized by senile amyloidosis and age-related decline in antibody-forming capacity.\textsuperscript{14,15} However, SAMP1TA/Ngs, a substrain of SAMP1, has been found to exhibit learning disturbance at 7-months of age.\textsuperscript{11} Some preliminary experiments also showed that SAMP1 developed age-related learning and memory disorders.

It is well known that the hippocampus plays an important role in learning and memory. The CA1 area has become the focus of particularly intense research because it is presumed to play an important role in memory function.\textsuperscript{16} The hippocampal pyramidal neurons receive one set of inputs from various cortical layers through its apical dendrites and a second set of inputs, representing a certain constellation of activity in neighboring pyramidal neurons, through its basal dendrites, which extend horizontally.\textsuperscript{17} In this study, a density of neurofibrils in hippocampal CA1 area indicates the number of apical dendrites.

The oral administration of magnolol prevented against the age-related decrease of the density of neurofibrils in the stratum radiatum at the hippocampal CA1 region at 4 and 6 months of age by administration from the 2-month-old group (group I, II). However, magnolol had no effect at 6 months of age by administration from the 4-month-old group (group III). We considered that these effects of magnolol are based on the neurite outgrowth effect and/or the nerve protection effect.

We previously reported that magnolol had neurotrophic properties, such as promotion of nerve growth factor-like neurite outgrowth in cultured rat cortical neurons.\textsuperscript{8} Sasaki \textit{et al.} reported that acidic fibroblast growth factor fragment-treated SAMP8 mice showed an increase of medial septum neuron density in the basal forebrain and improvement of learning and memory disturbance.\textsuperscript{18} We reported that magnolol induced a characteristically delayed increase in intracellular free Ca\textsuperscript{2+} after drug perfusion in human neuroblastoma SH-SY5Y cells.\textsuperscript{19} In addition, this delayed Ca\textsuperscript{2+} mobilization has been observed in some growth factors.\textsuperscript{20–22} Thus, it is likely that the growth factor-like action of magnolol may be involved in the mechanism of dendrite preservation in the present study.

Another possible mechanism for effect of magnolol is neuroprotection. Magnolol have antioxidant and free radical scavenging activities.\textsuperscript{23,24} In the SAMP1 brain, the lower Cu/Zn-SOD activity and endogenous antioxidants contents\textsuperscript{25} and higher carbonyl content, an index of oxidative stress marker,\textsuperscript{26} demonstrated compared with those of SAMR1. Moreover, some studies using exogenous antioxidant treatment have demonstrated that the increased oxidative stress contribute to age-related neurodegradation in the brain.\textsuperscript{27,28} In this study, it is suggested that magnolol may prevent oxidative stress-induced neuronal loss in the hippocampus through its antioxidant and free radical scavenging activities. Brain lipid hydroperoxide levels were greater at 2 months of age than that of 1 month of age in SAMP8 but not SAMR1.\textsuperscript{29} It is suggested that oxidative stress from an early age may be a cause of the impairments and degeneration in the brain seen in these strains. It will be able to explain the result that magnolol treatment from a young age (Group I, II) was more effective on neuroprotection in present study.

In conclusion, these findings suggest that the administration of magnolol before the initiation of neuronal loss may exhibit a protective effect in the hippocampus. These findings
emphasize magnolol as a growth factor-like low-molecular chemical compound.

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