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Research Report

# SK-PC-B70M alleviates neurologic symptoms in G93A-SOD1 amyotrophic lateral sclerosis mice

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ABSTRACT

SK-PC-B70M, an oleanolic-glycoside saponins fraction extracted from the root of *Pulsatilla koreana*, carries active ingredient(s) that protects the cytotoxicity induced by Aβ(1–42) in SK-N-SH cells. It was recently demonstrated that SK-PC-B70M improved scopolamine-induced deficits of memory consolidation and spatial working memory in rats, and reduced Aβ levels and plaque deposition in the brains of the Tg2576 mouse model of Alzheimer disease. In the present study, we investigated whether SK-PC-B70M produces helpful effects on the pathology of the G93A-SOD1 transgenic mouse model of amyotrophic lateral sclerosis (ALS). Administration of SK-PC-B70M (100 or 400 mg/kg/day) from 8 weeks to 16 weeks of age attenuated neurological deficits of G93A-SOD1 mice in several motor-function-related behavioral tests. SK-PC-B70M treatment significantly suppressed the accumulation of the by-products of lipid peroxidation, malonedialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE), in the spinal cord of G93A-SOD1 mice. Moreover, histologic analysis stained with cresyl violet or anti-choline acetyltransferase (ChAT) revealed that SK-PC-B70M suppressed neuronal loss in the ventral horn of the spinal cords of G93A-SOD1 mice. These results suggest that SK-PC-B70M affords a beneficial effect on neurologic deficits of G93A-SOD1 ALS mice.



## 1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease producing devastating neurologic deficits, including paralysis of voluntary muscles. Neurologic deficits begin focally and gradually spread over the body, leading to death, usually within 2–3 years of symptom onset (Mulder, 1982; Mitsumoto et al., 1998). ALS proceeds with selective neuronal death of lower motor neurons in the spinal cord and upper motor neurons in the absence of sensory symptoms (Nagai et al., 2001; Boillee et al., 2006; Pasinelli et al., 2000). Familial cases of ALS are known, but most cases of ALS (>90%) are sporadic and are likely caused by multi-factorial factors (Ferraiuolo et al., 2007; Wijesekera and Leigh, 2009), although the causes and underlying mechanisms are not completely understood.

Approximately 20%–25% of cases of familial ALS have mutations in the *SOD1* gene (Radunović et al., 1997; Piera and

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Robert, 2006). SOD1 (also called Cu/Zn SOD) is an enzyme with 153 amino acids that converts the superoxide anion to hydrogen peroxide. Transgenic G93A-SOD1 mice overexpressing a mutant form of human SOD1 display various neurologic deficits that resemble human ALS (Gurney et al., 1994), suggesting that failure in the cellular anti-oxidant system may be an important cause of ALS. Further evidence of the involvement of oxidative stress in ALS is supported by enhanced localization of biomarkers for lipid peroxidation, protein carbonyl levels, oxidized DNA, and peroxynitrite-mediated damage in ALS spinal cords (Barber and Shaw, 2010). Additional mechanisms, including mitochondrial dysfunction (Son et al., 2007; Barber and Shaw, 2010), glutamate excitotoxicity (Kosuge et al., 2009; Corona et al., 2007; Shaw and Ince, 1997), disrupted axonal transport (LaMonte et al., 2002; De Vos et al., 2007), and inflammation (Cleveland and Rothstein, 2001; Dupuis et al., 2004; Shaw, 2005; Browne et al., 2006, Ferraiuolo, 2007), have been proposed to contribute to the pathophysiologic mechanisms underlying ALS. Riluzole is the only drug available for ALS patients, but its therapeutic effects are modest because it only extends survival of ALS patients by a few months (Miller et al., 2003; Lacomblez et al., 2002; Bensimon et al., 2002; McGeer and McGeer, 2005). Therefore, new curative and/or additive treatments for ALS are needed.

SK-PC-B70M, an oleanolic-glycoside saponins fraction isolated from the root of *Pulsatilla koreana* (Ranunculaceae) using solvent-partitioning and subsequent chromatographic separation, inhibits the toxicity of Aβ(1–42) in SK-N-SH cells (Han et al., 2007a). Moreover, SK-PC-B70M improves scopolamine-induced deficits of memory consolidation and spatial working memory in rats (Han et al., 2007b). SK-PC-B70M reduces Aβ levels and plaque deposition in the brains of Tg2576 mice (Seo et al., 2009). In the present study, we determined whether SK-PC-B70M has a beneficial effect on the pathology of G93A-SOD1 transgenic mice.

## 2. Results

### 2.1. *Effect of SK-PC-B70M on mortality of G93A-SOD transgenic mice*

G93A-SOD1 transgenic mice showed an early onset of death starting from 11 weeks of age in our experimental conditions. G93A mice treated with SK-PC-B70M at 100 or 400 mg/kg/day from 7 weeks of age began to die at 12 s and 15 weeks of age, respectively (Fig. 1A). At 16 weeks of age, the survival rate in G93A mice had dropped to 25%, while in G93A mice treated with SK-PC-B70M at 100 or 400 mg/kg/day, the survival rate reached 57%. These results suggest that survival of G93A mice was moderately extended by treatment with SK-PC-B70M. During the SK-PC-B70M treatment period (7–16 weeks of age), the body weight of G93A-SOD1 mice measured at every week was slightly lower as time passed compared to the non-transgenic control, but SK-PC-B70M treatment did not change this relationship (Fig. 1B).

### 2.2. *SK-PC-B70M alleviated motor function deficits in G93A transgenic mice*

We determined whether administration of SK-PC-B70M is beneficial for motor function deficits in G93A mice using the Rotarod test, PaGE test, grip test, and limb extension reflex test beginning at 7 weeks of age, twice a week, sequentially in the order shown, as described in the Experimental Procedure section.

G93A control mice showed neurologic deficits of the hind limb extension reflex when suspended by the tail beginning from 8 weeks of age and the symptoms progressively worsened. Administration of SK-PC-B70M to G93A mice alleviated the symptoms compared with the G93A control mice by delaying

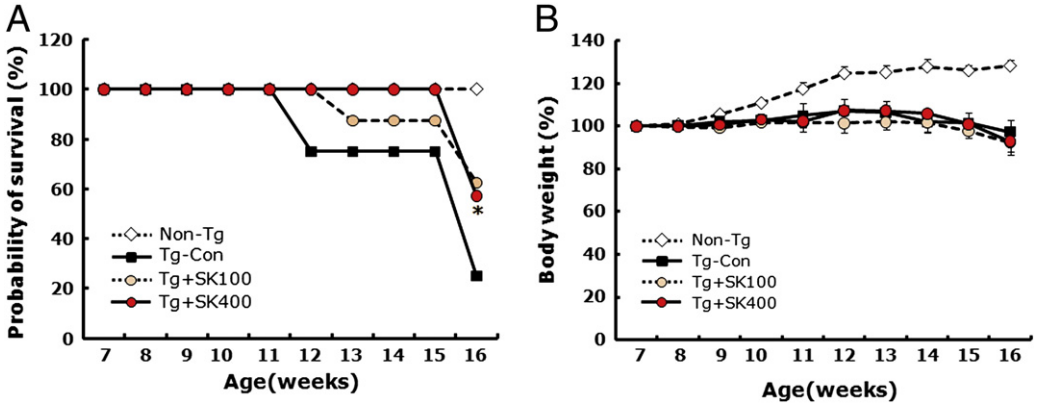


**Fig. 1 – Effects of SK-PC-B70M on the survival and body weight of G93A transgenic mice. (A) The cumulative probability of mortality for each group is shown. G93A transgenic mice were fed lab chow containing SK-PC-B70M at 100 or 400 mg/kg/day from 8 weeks of age. The number of animals examined for each group was 6–9. (B) Effect of SK-PC-B70M on the body weight changes of G93A transgenic mice. G93A transgenic mice were fed lab chow containing SK-PC-B70M at 100 or 400 mg/kg/day from 8 weeks of age. Body weights were monitored weekly and presented by percent values with respect to the body weight of each group at 7 weeks of age. Data are presented as the means ± SEM ($n = 6–9$). Non-Tg, non-transgenic control; Tg-Con, G93A transgenic control; Tg + SK100 and Tg + SK400, G93A transgenic mice treated with a low dose (100 mg/kg/day) or a high dose (400 mg/kg/day) of SK-PC-B70M, respectively. Asterisks; * denotes a significant difference between G93A transgenic mice group and the indicated group at $p < 0.05$ (two-way ANOVA, Bonferroni posttest to compare selected pairs of data).**