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The effect of epigallocatechin gallate on suppressing disease progression of ALS model mice

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Abstract

Epigallocatechin gallate (EGCG) is a constituent of green tea, and increasing evidence suggests that EGCG has neuroprotective effects on oxidative stress-injured neuronal cells, especially motoneurons. Although the neuroprotective effects of EGCG have been demonstrated in Parkinson's and Alzheimer's diseases and ischemic stroke models, there has been no report on the effect of EGCG on an in vivo model of amyotrophic lateral sclerosis (ALS). This study was undertaken to evaluate the effect of EGCG on ALS model mice with the human G93A mutated Cu/Zn-superoxide dismutase (SOD1) gene. We treated each group of 11 ALS model mice with EGCG (1.5, 2.9, and 5.8 μg/g body weight), dissolved in 0.5 ml of 0.9% sterile NaCl, and one group of 11 with 0.5 ml of 0.9% sterile NaCl (control group) intraorally every day after 60 days of age (presymptomatic treatment). The treatment of more than 2.9 μg EGCG/g body weight significantly prolonged the symptom onset and life span, preserved more survival signals, and attenuated death signals. These data suggest that EGCG could be a potential therapeutic candidate for ALS as a disease-modifying agent.

Keywords: ALS; EGCG; Transgenic mouse; Neuronal cell death

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder caused by selective degeneration of motor neurons. Recent studies using ALS models or the spinal cord of ALS patients have emphasized the role of oxidative stress, excitotoxicity, calcium mediated toxicity, genetic defects, autoimmunity and accumulation of abnormal proteins as its pathogenic mechanisms [3,5,7,12,14,24]. In the case of oxidative stress, antioxidant therapy has been tried to prevent neuronal cell death and/or delay the progression of ALS [2,5,17,25].

Epigallocatechin gallate (EGCG), one of the major constituents of green tea, has been known to have anti-apoptotic, anti-cancer, anti-mutagenic, and anti-neurodegenerative effects. Our previous study showed that EGCG prevented oxidative stress-induced apoptosis of motor neurons transfected with the human G93A mutated Cu/Zn-superoxide dismutase (SOD1) gene, by up-regulating survival signals, including phosphatidylinositol 3-kinase (PI3-K), phospho-Akt (pAkt), and phospho-glycogen synthase kinase-3β (pGSK-3β) and by down-regulating of death signals, such as mitochondrial damage, activated caspase-3, and cleaved poly(ADP-ribose) polymerase (PARP) [17]. Recently, we demonstrated that increased activity of GSK-3β was one of the important pathogenic mechanisms of an in vitro model of familial ALS [19], and EGCG had an inhibitory effect on GSK-3β activity [17]. Considering these neuroprotective effects of EGCG in the in vitro model of ALS, we can hypothesize that treatment with EGCG may delay symptom onset and prolong survival time of an animal model of familial ALS.

In this present study, we examined whether treatment with EGCG could delay the outbreak and/or progression of ALS and what would be its effect on the PI3-K/Akt and GSK-3β pathway as well as on the caspase-3 pathway in transgenic mice carry-

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ing the human G93A mutated SOD1 gene, causing autosomal dominant familial ALS in human.

All procedures on animals were performed in accordance with the National Institute of Toxicological Research of Korean Food and Drug Administration guideline for the care and use of laboratory animals. Behavioral experiments were conducted in a single-blind fashion in order to avoid the effect of subjectivity.

Forty-four transgenic mice out of F1 generations [B6SJL-Tg(SOD1-G93A)1Gur/J; The Jackson Laboratory, Bar Harbor, MA, USA] and four wild mice (only for western blotting) were used after evaluation of genotype using polymerase chain reaction on tail DNA. To examine the effect of EGCG (Sigma, Saint Louis, MO, USA) on ALS model mice, three different doses of EGCG (1.5, 2.9, and 5.8 μg/g body weight), dissolved in 0.5 ml of 0.9% sterile NaCl, were intraorally injected into three 11 animal groups [EGCG group (1.5; 2.9; 5.8 μg/g body weight)] once a day from 60 days after birth. These concentrations were obtained, based on daily consumption of EGCG by human (70 kg) having 1, 2, and 4 cups of green tea a day, respectively [13]. And an equivalent volume of the vehicle was injected into the remaining 11 animals (control group) by the same procedure.

The clinical condition of the ALS model mice was evaluated three times per week from 50 days after birth. When shaking of the limbs of the ALS model mouse, which may be due to clinical involvement of upper motor neuron system, was observed on suspension of the mouse in the air by its tail, it was defined as symptom onset [8,25]. At this stage, clonus, hyperreflexia, and crossed spread of spinal reflexes were also detectable in most mice. When ALS model mice could not right themselves within 30 s when placed on their sides on a flat surface, it was defined as endpoint and they were sacrificed [10,25].

The rotarod (Ugo Basile, Comerio, Italy) turned around its longitudinal axis at a rate of 1 rpm acceleration/8 sec from 4 rpm/min. The training room for the rotarod test was maintained at 22–24 °C, and noise was kept to a minimum. The rotarod test consisted of acclimation sessions and training sessions. For each acclimation session, the mice were gently moved to the training room once a day, placed into a white waiting box, taken out of the box in turns, and given the previously mentioned handling [28], which consisted of rubbing their head and back for about 10 min. Acclimation sessions were performed over three consecutive days. The training sessions were started 1 day after the last acclimation session. Mice were given four training trials per day with an inter-trial interval for 10 min. Four training trials were considered as one session. The training sessions were performed over five consecutive days. For each training trial, mice were gently placed on the rod in the orientation opposite to that of the already rotating rod, so that they could acquire the necessary skilled behavior on the rotating rod to prevent a fall. The time spent walking on the rotating rod was measured, and the time evaluated through three repeats was averaged. When the time was less than 10 s, the trial was called a rotarod failure [28].

Based on the results of the effects of EGCG on symptom onset, rotarod failure and endpoint, four mice in the EGCG (2.9 μg/g body weight) treated group, the control group and the wild mouse group were anesthetized with pentobarbital sodium at 124 days from birth, which was around endpoint of control mice, and the intracardiac perfusion of phosphate-buffered saline (PBS) was then carried out. Subsequently, the spinal cord was swiftly removed, cooled in ice-cold artificial CSF for 5 min, and stored in a −80 °C freezer. Western blotting was performed by using the same procedure used in our previous study [18,20]. Antibodies for p85*a* PI3-K (1:1000, Sigma, Saint Louis, MO, USA), pAkt (1:1000, Cell signaling, Beverly, MA, USA), pGSK-3β (Ser 9) (1:1000, Santa Cruz Biotech, Delaware, CA, USA), cytochrome *c* (1:500, Santa Cruz Biotech, Delaware, CA, USA), caspase-3 (1:1000, Santa Cruz Biotech, Delaware, CA, USA), cleaved caspase-3 (Asp 175) (1:1000, Cell signaling, Beverly, MA, USA), and PARP (1:500, Pharmingen, San Diego, CA, USA) were used as a primary antibodies. The results of several Western blots were quantified with an image analyzer (Bio-Rad, Quantity One-4,2,0).

Data were expressed as mean ± standard error. The data were analyzed with Duncan's Multiple Range Test (SAS 9.0), and differences with *p*-value of less than 0.05 were considered statistically significant. Cumulative probabilities were depicted with the Kaplan–Meier survival analysis (SPSS12.0; Chicago, IL) [28].

In order to evaluate the effect of EGCG on symptom onset, motor activity, disease progression, and endpoint of ALS model mice, several doses of EGCG (1.5, 2.9, and 5.8 μg/g body weight), dissolved in 0.5 ml of 0.9% sterile NaCl, were intraorally injected into three 11 animal groups [EGCG group (1.5 μg/g body weight; 2.9 μg/g body weight; 5.8 μg/g body weight)] once a day from 60 days after birth. Compared with the control group, the treatment of 2.9 and 5.8 μg EGCG/g body weight showed significant improvements in all evaluated parameters, but the treatment of 1.5 μg EGCG/g body weight did not. Also, there was no significant difference between 2.9 and 5.8 μg EGCG/g body weight.

Describing in detail, symptom onset (115.00 ± 2.28 days in the control group) and the time of rotarod failure (120.27 ± 2.64) were significantly delayed in mice treated with 2.9 μg EGCG/g [128.91 ± 2.48 ($p < 0.01$) and 139.45 ± 2.62 ($p < 0.01$), respectively] and 5.8 μg EGCG/g [128.17 ± 2.74 ($p < 0.01$) and 140.38 ± 2.81 ($p < 0.01$), respectively] body weight, but not in mice treated with 1.5 μg EGCG/g body weight (119.09 ± 3.09 and 124.01 ± 2.73, respectively) (Fig. 1A and Table 1). Endpoint (123.55 ± 2.10 in the control group) was retarded about 19.27 and 20.40 days in mice treated with 2.9 μg EGCG/g (142.82 ± 2.47, $p < 0.01$) and 5.8 μg EGCG/g (143.95 ± 2.58, $p < 0.01$) body weight, respectively, but not in mice treated with 1.5 μg EGCG/g body weight (128.37 ± 2.51) (Fig. 1B and Table 1), and symptom duration (8.5 ± 0.45 in the control group) was significantly prolonged in mice treated with 2.9 μg EGCG/g (13.91 ± 0.56, $p < 0.05$) and 5.8 μg EGCG/g (15.78 ± 1.46, $p < 0.05$) body weight, respectively, but not in mice treated with 1.5 μg EGCG/g body weight (9.28 ± 0.52) (Table 1). These findings indicate that EGCG has protective effects on ALS model mice with G93A mutant human SOD1, by delaying symptom onset, rotarod failure and endpoint, and by prolonging symptom duration.