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Benefit of a combined treatment with trientine and ascorbate in familial amyotrophic lateral sclerosis model mice

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Abstract

We previously reported that the common toxic gain-of-function in various mutant copper-zinc superoxide dismutases (SOD1) seen in patients with familial amyotrophic lateral sclerosis (ALS) was an abnormal copper release from the enzyme protein. In this study, trientine and ascorbate, known to have a beneficial effect in an animal model of Wilson disease, were administered to transgenic mice overexpressing a mutated human SOD1 (G93A). The onset of neurological signs in the treated group was significantly delayed compared with that in the control group, and the time to reach total paralysis in the treated group was delayed as well. Since the agents used in this study cause low toxicity in animals and humans, this treatment may be a good candidate for clinical application. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Familial amyotrophic lateral sclerosis; Copper-zinc superoxide dismutase; Copper; Transgenic mice; Trientine; Ascorbate

Amyotrophic lateral sclerosis (ALS) is a fatal progressive motor neuron disease with unknown etiology. About 10% of patients are familial (FALS), and the copper-zinc superoxide dismutase (SOD1) gene is mutated in approximately 20% of FALS cases [6,17]. Transgenic mice overexpressing a mutated human SOD1 develop neurological signs and pathological changes similar to FALS cases [10], although mice that overexpress a wild-type human SOD1 or mice with disrupted SOD1 gene have no change in motor neurons [10,16]. This indicates that the adversely acquired toxicity in mutant SOD1 contributes to the degeneration of motor neurons. In this regard, we previously reported that the release of copper ions from mutant SOD1 was a possible toxic gain-of-function [15].

Triethylenetetramine dihydrochloride (trientine), a chelating agent for copper, and ascorbate are known to have a beneficial effect in Long Evans Cinnamon (LEC) rats, an animal model for Wilson disease, in reducing copper toxicity [11,18]. Since we hypothesized that the copper toxicity in motor neurons would contribute to the pathogenesis of FALS, we examined the neuroprotective effect of a combined treatment with trientine and ascorbate in FALS transgenic mice. Part of the present study has been reported in abstract form [14].

Transgenic mice overexpressing a human SOD1 gene carrying Glycine 93 → Alanine mutation were obtained from The Jackson Laboratory (Bar Harbor, ME) (the strain designation B6SJL-TgN(SOD1-G93A)1Gurd1) and maintained as hemizygotes in the B6SJL background. The onset of neurological signs in this strain is delayed compared to the original strain [10] because of a reduction in transgenic copy number according to the information from The Jackson Laboratory. Transgenic progeny was identified by a polymerase chain reaction (PCR) on genomic DNA using ex4Pla 5'-CATCAGCCCTAATCCATCTGA-3' and ex4P2a 5'-TGGATCTTAGAATTCGCGAC-3', a pair of specific primers for the exon 4 of the human SOD1 gene. PCR was conducted with 32 cycles at 94°C for 1 min, 60°C for 1 min and 72°C for 1 min. Non-transgenic offspring were used as controls. All animals were handled in accordance with the Guideline for the Care and Use of Laboratory Animals at the Osaka University Graduate School of Medicine.

Trientine was kindly provided by Tsumura&Co. Eight mice were fed with L(+)-ascorbate (Wako Pure Chemical, Osaka, Japan) mixed in the standard diet AIN-93G (Oriental Yeast, Tokyo, Japan) at the concentration of 0.8% w/w and trientine in distilled water at the concentration of 0.2% w/v ad libitum beginning at 45 days of age. Seven mice fed with...
the diet AIN-93G and distilled water alone were used as the control group. The treatment was continued until mice reached the end-point. The diet was supplemented twice a week and distilled water was changed once a week.

The clinical signs and body weights of mice were monitored twice a week after entry into the protocol. We determined the onset of the neurological signs by subtle postural change of one limb and the end-point by the total paralysis of hindlimbs, when a mouse was lifted by the tail. The data were expressed as mean ± SD per group. Survival analysis was performed by Kaplan–Meier method and the statistical difference was determined by Mantel–Cox test using software StatView-5.0 for Macintosh (SAS Institute, Cary, NC). P < 0.05 was considered to be significant.

Fig. 1 indicates cumulative probabilities of the disease onset (Fig. 1A) and end-point (Fig. 1B) for mice in each group. Since two mice in the treated group died accidentally after the onset but before the end-point, they were censored in the analysis of the end-point. The untreated mice showed initial signs at a mean age of 195.4 ± 11.3 days, whereas the treated mice showed them at a mean age of 211.8 ± 12.2 days (Table 1). The combined treatment significantly delayed the disease onset (Fig. 1A, Mantel–Cox test, P = 0.030). The untreated and treated mice reached the end-point at a mean age of 244.3 ± 20.5 and 269.7 ± 19.3 days, respectively (Table 1). The end-point was also significantly delayed by the treatment (Fig. 1B, P = 0.045).

The mean body weight of the treated mice increased more than that of the untreated mice, and the body weight of the treated mice still kept increasing even after that of the untreated mice reached the peak (Fig. 2).

There was no difference in the amount of water intake

Fig. 1. Cumulative probability of the disease onset (A) and survival (B) in control or treated FALS mice. The onset of the disease and end-point were significantly delayed in the treated group.

Table 1

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<th>Control group</th>
<th>Treated group</th>
<th>P-value</th>
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<td>Onset (days)</td>
<td>195.4 ± 11.3</td>
<td>211.8 ± 12.2</td>
<td>0.030</td>
</tr>
<tr>
<td>End-point (days)</td>
<td>244.3 ± 20.5</td>
<td>269.7 ± 19.3</td>
<td>0.045</td>
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* The data were expressed as mean ± SD. The statistical difference was determined by Mantel–Cox test.

Fig. 2. Change in the body weight of control and treated FALS mice. The body weight of the treated mice increased more than that of the control mice, and still increased even after the body weight of the control mice reached the peak.
between the two groups (2.5 ml/day). The expected dose of trientine taken by the treated group was 125–170 mg/kg weight/day. There was no clinical untoward effect of the agents among the treated animals.

We have demonstrated that the treatment with trientine and ascorbate has a protective effect on the onset of the disease and the end-point in FALS transgenic mice. The body weight in the treated mice peaked later, possibly by the treatment, because the decline of body weight has been correlated with the disease advance [4]. Thus, chelating of copper ions and protection from oxidative stress appear to be beneficial in this FALS model. We previously found that mutant SOD1 from erythrocytes of FALS patients possessed abnormal copper peaks in ion exchange chromatographic fractions without relation to SOD1 activities [15], suggesting that free copper ions released from rapidly-degraded or loosely-folded mutants might be involved in the pathogenesis of FALS. Corson et al. [5] also suggested that the SOD1 mutant-mediated disease was caused by aberrant copper-mediated chemistry catalyzed by less tightly folded mutant enzymes. Taken together, the copper-mediated oxidative stress seems to be very attractive as the common nature of toxic gain-of-function in FALS with SOD1 mutation. Although copper ions are essential for activation of many cuproproteins such as SOD1, the excessive concentration of copper ions is toxic to cells. Free cupric ion (Cu^{2+}) can be reduced by superoxide to cuprous ion (Cu^{+}), which then reacts with hydrogen peroxide to generate hydroxyl radical (Fenton or Haber–Weiss reaction). Hydroxyl radical oxidizes intracellular components including lipid membranes, proteins and nucleic acids, and cause cellular damage [1].

Wilson disease is an autosomal recessive disease with a defect in the cellular export system of copper ions, and manifests with cellular damage in the liver and the central nervous system [3]. In LEC rats that possess the gene defect homologous to that in Wilson disease [20], free radicals produced by accumulated copper in the liver are thought to cause oxidative injury to hepatocytes [1], where copper chelating agents including trientine and antioxidants including ascorbate have been reported to delay the onset of hepatitis [11,18]. Trientine is also known to decrease the formation of hydroxyl radicals from cuprous ions in vitro [8]. Ascorbate works as an antioxidant by scavenging free radicals, although it can also have a pro-oxidant property in the presence of copper ions by reducing them for Fenton reaction [2]. In general, ascorbate at a high concentration or in the presence of low level of copper ions acts as an antioxidant rather than a pro-oxidant [2]. Since we used ascorbate in combination with trientine at the same concentration as used in the study of LEC rats [11], we believe that ascorbate functioned as an antioxidant.

The present study suggested that the elimination of the oxidative stress mediated by copper ions from mutant SOD1 would suppress the degeneration of motor neurons in FALS. Gurney et al. [9] showed the efficacy of vitamin E in delaying the clinical onset but not extending the survival period in FALS mice. They suggested that peroxidation of lipid membranes inhibited by vitamin E was related to delay in the disease initiation, but other factors such as glutamate excitotoxicity were related to the disease progression. Thus, a lack of beneficial effect of vitamin E on survival might be explained by the fact that it inhibited lipid peroxidation but not oxidation of other intracellular components [13]. After we had initiated the present study, Hottinger et al. [12] reported that d-penicillamine, another copper chelating agent, had a significant benefit on the disease onset and survival of FALS mice when it was administered orally by force. Taken together, removal of copper ions may become a novel therapeutic approach to FALS patients. D-penicillamine at a high dose can cause many adverse effects. Trientine is regarded as an alternative agent for penicillamine-intolerant patients with Wilson disease because of its less frequent adverse effects [19]. It has been reported that normal B6C3F1 mice administered trientine at 3000 ppm in water for 90 days showed no adverse clinical sign [7]. Thus, this treatment may be a good candidate for clinical application. It is now necessary to determine whether the administration of trientine alone or ascorbate alone before onset or after onset has a beneficial effect on FALS mice, and whether a combined therapy with other agents effective on FALS mice such as riluzole [9] can extend the survival period.

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