Beneficial effect of ginseng root in SOD-1 (G93A) transgenic mice

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Abstract

Many patients with amyotrophic lateral sclerosis (ALS; motor neuron disease) use natural or traditional therapies of unproven benefit. One such therapy is ginseng root. However, in some other disease models, ginseng has proven efficacious. Ginseng improves learning and memory in rats, and reduces neuronal death following transient cerebral ischemia. These effects of ginseng have been related to increases in the expression of nerve growth factor and its high affinity receptor in the rat brain, and antioxidant actions, inter alia. Since such actions could be beneficial in ALS as well, we studied the effect of ginseng (Panax quinquefolium), 40 and 80 mg/Kg, in B6SJL-TgN(SOD1-G93A)1Gur transgenic mice. The ginseng was given in drinking water, from age 30d onwards. We measured the time to onset of signs of motor impairment, and survival. There was no difference between the two ginseng groups (n=6, 6) in either measure. However, compared to controls (n=13), there was a prolongation in onset of signs (116d vs. 94d, P<0.001), and survival (139d vs. 132d, P<0.05). These experiments lend support to the use of ginseng root in ALS. Future experiments using this model could examine for symptomatic effects of ginseng, measure the effect of specific ginsenosides (which differ between ginseng species), and elucidate their mechanisms of action.

Keywords: Amyotrophic lateral sclerosis; Transgenic mice; Ginseng

1. Introduction

Many ALS patients use unconventional or alternative therapies, of uncertain efficacy or toxicity. One example is ginseng root, long used in Chinese medicine for a variety of ailments.

The active ingredients of ginseng are contained in the roots of members of the family Araliaceae, particularly the genera Panax and Eleutherococcus [1]. The chief species are Panax ginseng (Asian ginseng), Panax quinquefolium (American ginseng), Eleutherococcus senticosus (Siberian ginseng), and less frequently, Panax notoginseng and Panax japonicus. Roots of plants 5–7 years old are air dried (white ginseng) or steamed (red ginseng). The major active ingredients are glycosides termed ginseng saponins or ‘ginsenosides’ (Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, etc) that vary quantitatively and qualitatively between species, which may account for postulated interspecies differences in therapeutic potential.

Several mechanisms are proposed to explain the effect of ginsenosides in different disease models studied to date. The beneficial effects of ginseng on learning and memory [14,15] may be mediated through the observed upregulation of ChAT, NGF, and trkA mRNA expression in rat brain [2]. The protective effect in transient forebrain ischemia may be mediated through antioxidant and hydroxyl radical scavenging activity [3–5], and may involve altered NO release or synthesis [1]. The levels of serotonin and acetylcholine in the brain are increased [6,7], calcium influx into neurons reduced [8], and synapse formation increased [4].

Based on our present understanding, several of these effects could be beneficial in ALS. As such, we decided to test the effect of ginseng in a transgenic mouse model of ALS. These mice over-express a human SOD-1 gene carrying a mutation (G93A) responsible for some familial ALS.

2. Methods

Transgenic mice (B6SJL-TgN(SOD-1G93A)1Gur were obtained from Jackson Laboratories (Bar Harbour, Maine), as breeding pairs. Offspring bearing the human transgene...
were identified from blood samples (retro-orbital venous sampling), using PCR amplification of a fragment of the transgene, and primers and conditions suggested by Jackson laboratories (www.Jax.org). After weaning, animals were individually housed, with 12 h light/dark cycle. Affected animals were randomly assigned to one of 3 groups, receiving 0 (n=13), 100 (n=6), 200 (n=6) ug/ml crude ginseng powder (P quinquefolium, Sigma) dissolved in the drinking water, beginning at age 30d. Aqueous solutions were given ad libitum, and were changed three times per week. Daily intake per animal did not vary between groups, and averaged 6–7 ml/day over the experiment. The calculated average daily intake was 0, 40 mg/kg or 80 mg/kg crude ginseng powder.

Animals were examined daily. For each animal, the age of onset of clinical signs (abnormal splaying of the hindlimbs when suspended by the tail [9,10] was recorded). The age at which each animal was unable to right itself within 30 s after being placed on its side was accepted as a surrogate endpoint for death [9,10], and it was euthanized at this point.

Disease onset and survival were tabulated by the method of Kaplan and Meier, with log-rank test of significance for comparisons between treatment groups (ginseng 40 mg/kg vs. ginseng 80 mg/kg; and vs. control).

3. Results

The control animals (n=13) developed signs of disease at 94±10.7 days (mean±standard deviation), and succumbed at 132±8.3 days. This compares with values previously reported for this strain of 94±12.7 (n=60) and 133±11.8 (n=57) [9,10].

There was no significant difference in either onset (115.7±11.71 vs. 117.2±14.0), or survival (137.0±8.53 vs. 141.2±10.27), between animals receiving 40 or 80 mg/kg Panax quinquefolium respectively. Since there was no difference in either measure, we combined the two groups for greater statistical power.

Compared to control, ginseng significantly prolonged onset to clinical signs (116d vs. 94d mean; P<0.001), Fig. 1. Compared to control, ginseng also prolonged surrogate death. (139d vs. 132d; P<0.05), Fig. 2.

4. Discussion

Crude ginseng powder added to the drinking water significantly delayed onset, and significantly prolonged survival, in this mouse model of ALS. The effect was most significant for onset. However, if outliers beyond three standard deviations from the group mean are excluded from analysis, (since there is occasional spontaneous reduction in transgene overexpression during breeding, or death from unrelated causes), two animals would be excluded, from the control group only, (one of precocious death and one of delayed death). Although the mean survival would be unchanged in the control group (132±4.6), tests of significance comparing control with ginseng survival would become more significant. (The significance value would increase from P<0.05 to P<0.005, by virtue of reduced variance in the data.)

Several mechanisms would be possible to explain the beneficial effect seen. As previously noted, an increase in NGF action could explain the beneficial effects seen. Equally, there could be enhancement of other trophic factor effects of possible relevance to ALS (such as BDNF, CNTF, IGF). Antioxidant effects, or altered nitric oxide action, could be beneficial, separately or in addition.
Interestingly, one report suggests that ginseng may alter transcriptional activity of the SOD-1 gene [11]. The results are encouraging, and compare favorably with other compounds tested in this animal model [9]. As such, there may be some interest in determining the mechanism(s) of action of ginseng in this model, and altering experimental conditions to optimize the therapeutic effect. For example, although there was no significant difference between the two ginseng groups in either onset or survival, there was a slight trend in favor of accentuated survival with the higher (80 mg/kg) dose. Perhaps higher doses could be more effective.

There are two caveats in the interpretation of our data. First, although it seems most plausible that the beneficial effects seen relate to a neuroprotective action of ginseng, no experiments were undertaken to rule out a symptomatic effect (which might act beneficially on the clinical endpoints chosen, without fundamentally changing the underlying pathophysiological process). Second, the results are strictly applicable only to Panax quinquefolium (American ginseng). Traditionally, different actions are ascribed to different ginseng species. These differences may be explained by quantitative and qualitative differences in ginsenosides. Future experiments could examine the effect of other ginseng species or specific ginsenosides in this model.

Finally, these results do lend credence to the current empirical use of ginseng in ALS. The side effects of ginseng seem minor. The reported LD50 in rodents is 10–30 g/Kg [12], and chronic treatment in several animal species shows no or little evidence of toxicity. Side effects in humans are apparently minor at low doses. Nervousness, insomnia, and gastrointestinal upset have been reported with prolonged high doses (15 g/day for 2 years) [13]. Nonetheless, in the absence of controlled studies, the presence or absence of side effects should be interpreted with caution.

References


