



Evaluation of longevity enhancing compounds against transactive response DNA-binding protein-43 neuronal toxicity

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ABSTRACT

In simple systems, lifespan can be extended by various methods including dietary restriction, mutations in the insulin/insulin-like growth factor (IGF) pathway or mitochondria among other processes. It is widely held that the mechanisms that extend lifespan may be adapted for diminishing age-associated pathologies. We tested whether a number of compounds reported to extend lifespan in *C. elegans* could reduce age-dependent toxicity caused by mutant TAR DNA-binding protein-43 in *C. elegans* motor neurons. Only half of the compounds tested show protective properties against neurodegeneration, suggesting that extended lifespan is not a strong predictor for neuroprotective properties. We report here that resveratrol, rolipram, reserpine, trolox, propyl gallate, and ethosuximide protect against mutant TAR DNA-binding protein-43 neuronal toxicity. Finally, of all the compounds tested, only resveratrol required *daf-16* and *sir-2.1* for protection, and ethosuximide showed dependence on *daf-16* for its activity.

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1. Introduction

For more than 75 years, people have been fascinated by the discovery that rats living on a restricted diet (dietary restriction) showed increased lifespan (McCay et al., 1989), a phenomenon that is under investigation in primates (Colman et al., 2009; Mattison et al., 2012). Of great interest is the fact that not only do many organisms show increased lifespan under dietary restriction conditions but they also show decreased incidences of age-related pathologies (Anderson and Weindruch, 2012). Additional mechanisms that regulate longevity have been discovered including mitochondrial function and the insulin/insulin-like growth factor (IGF) signaling pathway. Molecular and genetic approaches have begun to decipher the cellular mechanisms of lifespan extension and this has led to the development of an industry hoping to find and develop longevity mimetics as potential therapeutic agents against age-related disease (Mercken et al., 2012). Work from model organisms like *C. elegans* has identified numerous compounds that extend lifespan by influencing conserved longevity mechanisms and we wondered if these compounds would be effective against age-dependent proteotoxicity. To evaluate these compounds we turned to a *C. elegans* model of age-dependent motor neuron

toxicity (Vaccaro et al., 2012a) and tested 11 compounds reported to extend lifespan. We identified 6 compounds that reduced mutant transactive response (TAR) DNA-binding protein-43 (TDP-43) neuronal toxicity and might be useful as candidates for testing and drug development in mammalian models of neurodegeneration.

2. Methods

2.1. *C. elegans* strains and genetics

Standard methods of culturing and handling worms were used. Worms were maintained on standard nematode growth media plates streaked with OP50 *E. coli*. All strains were scored at 20 °C. Mutations and transgenes used in this study were: *daf-16(mu86)*, *hsf-1(sy441)*, *rff-3(pk1426)*, *sir-2.1(ok434)*, and *xqls133[unc-47::TDP-43[A315T];unc-119(+)]*. Most of the strains were obtained from the *C. elegans* Genetics Center (University of Minnesota, Minneapolis, MN, USA). Mutants or transgenic worms were verified by visible phenotypes, polymerase chain reaction analysis for deletion mutants, sequencing for point mutations, or a combination thereof. Deletion mutants were outcrossed a minimum of 3 times to wild type N2 worms before use.

2.2. Paralysis assays

Worms were counted as paralyzed if they failed to move when prodded with a worm pick. Worms were scored as dead if they

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failed to move their head after being prodded in the nose and showed no pharyngeal pumping. For the paralysis tests worms grown on the specific compound from hatching were transferred to the appropriate experimental plate for scoring.

2.3. Neurodegeneration assays

For scoring of neuronal processes, TDP-43 transgenic animals were selected at day 9 of adulthood for visualization of motor neurons processes in vivo. Animals were immobilized in M9 with 5 mM levamisole and mounted on slides with 2% agarose pads. Neurons were visualized using a Leica DM6000 microscope and a Leica DFC 480 camera. A minimum of 100 animals were scored per treatment over 4–6 trials. The mean and standard error of the mean were calculated for each trial and 2-tailed *t* tests were used for statistical analysis.

2.4. RNAi experiments

RNA interference (RNAi)-treated strains were fed *E. coli* (HT115) containing an empty vector or *skn-1* (T19E7.2) RNAi clones from the ORFeome RNAi library (Open Biosystems). RNAi experiments were performed at 20 °C. Worms were grown on Nematode Growth Media enriched with 1 mM isopropyl- β -D-thiogalactopyranoside. All RNAi paralysis tests were performed using a TDP-43

[A315T];*rrf-3(pk1426)* strain. To minimize developmental effects, L4 worms were grown on plates with either *skn-1*(RNAi) or empty vector and assayed for paralysis as adults. *skn-1*(RNAi) activity was confirmed by the observation of lethal and sterile phenotypes in the progeny of treated animals.

2.5. Protein extraction

Worms were lysed in radioimmunoprecipitation assay buffer (150 mM NaCl, 50 mM Tris pH 7.4, 1% Triton X-100, 0.1% sodium dodecyl sulfate, 1% sodium deoxycholate) plus 0.1% protease inhibitors (10 mg/mL leupeptin, 10 mg/mL pepstatin A, 10 mg/mL chymostatin). Nematodes were lysed with a 27 $\frac{1}{2}$ syringe 10–15 times, incubated on ice for 10 minutes then moved at room temperature for 10 minutes and finally centrifuged at 16,000g for 10 minutes. Protein quantification was performed using a BCA protein assay kit (Thermo Scientific). For TDP-43 transgenic worms, soluble and insoluble fractions were obtained using methods previously described (Liachko et al., 2010; Neumann et al., 2006), with modifications. Briefly, worms pellets were homogenized with a pellet mixer (Disposable Pellet Mixer and Cordless Motor, VWR) in 1 volume (wt/vol) of low-salt buffer (Benedetti et al., 2008) (10 mM Tris, 5 mM Ethylene Diamine Triacetic Acid (EDTA), 10% sucrose, pH 7.5) and centrifuged at 25,000g for 30 minutes at 4 °C. The supernatant represents the low salt (LS) fraction, containing the soluble proteins.

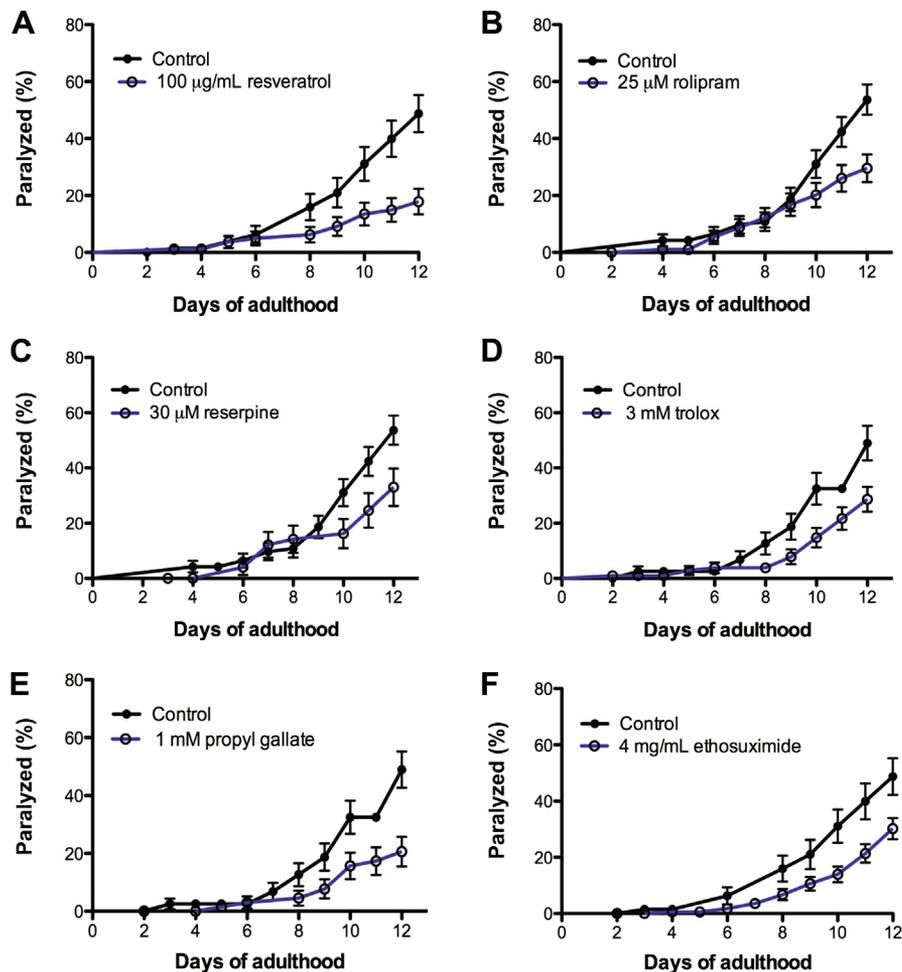


Fig. 1. Lifespan-extending compounds reduce mutant TAR DNA-binding protein-43-induced paralysis. Compounds that reduced mutant TAR DNA-binding protein-43-induced motility defects and paralysis compared with untreated control animals included: (A) 100 µg/mL resveratrol ($p < 0.001$); (B) 25 µM rolipram ($p < 0.001$); (C) 30 µM reserpine ($p < 0.05$); (D) 3 mM trolox ($p < 0.01$); (E) 1 mM propyl gallate ($p < 0.01$); (F) 4 mg/mL ethosuximide ($p < 0.01$). See also [Supplementary Table 1](#).

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