GENETICS

Effect of Phenol Inducing the Antioxidant Responsive Element on *Drosophila Melanogaster* Lifespan

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The effect of hydrophilic synthetic antioxidant TC-13 (3-(3'-tert-butyl-4'-hydroxyphenyl) propylthiosulfonate sodium) inducing the antioxidant-responsive element on the lifespan of *Drosophila melanogaster* was studied. Addition of 1% TC-13 to diets prolonged the lifespan of long-lived *D. melanogaster* Canton S strain females and males, but not of short-lived Oregon R insects and reduced the mean lifespan of *D. melanogaster* males of the $lgl^{558}OR/Cy$ strain containing a recessive lethal mutation of tumor suppressor in the heterozygotic state. The geroprotective effects of TC-13 synthetic phenol antioxidant depended on *D. melanogaster* genotype and gender.

Key Words: *lifespan; Drosophila melanogaster; phenol antioxidant*

Manipulations with the genome aimed at modification of antioxidant defense enzyme activities change animal lifespan (LS) in many cases [7,12]. This is in good agreement with the free radical theory of aging suggested in 1956 by D. Harman [9]. Attempts at LS modulation with exogenous antioxidants lead to far less unambiguous and often contradictory results [3, 11]. A concept of active defense from oxidant exposure with participation of the so-called antioxidant responsive element (ARE) explaining this phenomenon was recently proposed [2]. LS did decrease in animals knocked out by some elements of the ARE system signal pathway (Nrf2 transcription factor and its cytoplasmatic inhibitor Keap1) [13]. However, no attempts at modifying the LS by ARE stimulation by exogenous antioxidants were undertaken.

By adding sulfur-containing ionogenic fragments into the structure of bromine-substituted alkylphenols we synthesized structurally related series of compounds with different degree of OH group shielding, containing alkyl substitutes with sulfonate or thiosulfonate groups in the *para* position. These compounds are highly hydrophilic and are characterized by bifunctional antioxidant effects, because in addition to the phenol OH group they have sulfur-containing fragments with antiperoxide activity. Antiradical and antioxidative effects of the new compounds were studied in different experimental systems [5]. Studies on the model of carrageenin-induced edema in rats showed high anti-inflammatory activity of partially hindered phenol with thiosulfonate group in the *para*-propyl substitute 3-(3'-tert-butyl-4'-hydroxyphenyl)propylthiosulfonate sodium (TC-13; Fig. 1) [1]. Relationship between TC-13 anti-inflammatory effect and capacity to induce ARE-regulated expression of glutathione-S

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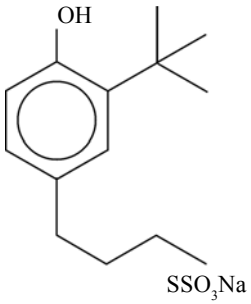

Fig. 1. Chemical structure of TC-13.

transferase P1 gene was shown in cell cultures and animal experiments [1].

We studied the possibility of using TC-13 (bifunctional phenol antioxidant), an effective inductor of ARE, as an instrument for studies of ARE involvement in LS regulation in *D. melanogaster*.

MATERIALS AND METHODS

TC-13 was synthesized on the basis of 3-(3',5'-di-tert-butyl-4'-hydroxyphenyl)propanol-1 [1].

The study was carried out at 25°C on inbred *D. melanogaster* strains from the collection of Laboratory of Population Genetics, Institute of Cytology and Genetics. We used two normal strains, Canton S (CS) and Oregon R (OR), and $lgl^{558}OR/Cy$ strain (558OR) containing a recessive lethal mutation in tumor suppressor *lethal*(2)-*giant larvae* gene – allele lgl^{558} in a heterozygotic status (*Cy* is *Curly* dominant marker in lethal-free chromosome). The insects were narcotized with ether and females and males of each strain were selected under a binocular magnifying glass within 24 h after imago release. The selected *D. melanogaster* were placed in tubes with nutrient medium, up to 50 flies per tube. The females were kept separately from males.

The insects were treated with the antioxidant in two concentrations, 1 and 0.2%. The solution was added to drosophila diets every 3 days by applying 10 μl with a micropipette onto the surface of fresh nutrient medium in the tube. Every 3 days, the flies were transferred to fresh medium with the antioxidant and mortality was recorded until the end of the vital cycle in all flies of the strain. Controls were kept on nutrient medium without antioxidant.

The significance of differences between the LS of experimental and control flies was evaluated using Student's *t* test [6].

TABLE 1. Lifespan of Different Drosophila Strains in the Control and under Conditions of TC-13 Antioxidant Treatment

Strain (gender)	TC-13 concentration	*n*	*M*±*m*	ΔM, %	Day of death	
					50%	90%
CS (females)	0 (control)	306	58.2±1.0	0	63	75
	1%	322	62.4±1.0*	+6.7	69	78
	0.2%	312	56.1±1.1	-3.7	63	75
CS (males)	0 (control)	272	46.8±1.1	0	51	66
	1%	330	50.8±1.0*	+7.9	57	69
	0.2%	287	42.8±1.2*	-9.3	51	63
OR (females)	0 (control)	289	43.7±0.7	0	51	51
	1%	385	42.7±0.7	-2.3	48	54
	0.2%	450	43.6±0.6	-0.2	48	51
OR (males)	0 (control)	294	39.8±0.7	0	45	48
	1%	328	40.5±0.6	+1.7	45	45
	0.2%	488	38.0±0.7	-4.7	42	45
558OR (females)	0 (control)	190	41.3±0.9	0	48	51
	1%	417	40.7±0.4	-0.6	45	48
558OR (males)	0 (control)	188	431±0.5	0	45	48
	1%	407	39.6±0.5*	-8.8	42	45

Note. *$p<0.05$ compared to the control.

RESULTS

The effect of TC-13 on drosophila LS depended on compound concentration, strain genotype, and insect gender. Insects of each of the three studied strains differently reacted to addition of the same doses of TC-13 to the diet. Positive effect on LS was found in only long-lived CS insects receiving 1% TC-13 solution. A paradoxical fact was revealed: a 5-fold lower concentration of TC-13 (0.2%) led to a significant reduction of the mean LS in males in comparison with the control. This effect could be due to oxidation of thiosulfonate group under aerobic conditions with the formation of hydroperoxide: long-term exposure to TC-13 in a low concentration could produce a pro-oxidant effect.

The reaction to TC-13 in short-lived OR insects was more or less neutral in both genders. However, zero oncosuppressor heterozygosity (*lgl*^{558}*OR/Cy* genotype) reduced LS in response to addition of TC-13 in an experimental concentration of 1%. Shortening of LS in response to treatment with 1% TC-13 in the *lgl*^{558}*OR* strain was presumably caused by combined effect of TC-13 and heterozygosity of males and females by *lgl* gene mutation. It is known that apoptosis is suppressed in various tissues of drosophila *lgl* mutants [8]. On the other hand, active oxygen and nitrogen forms initiate apoptosis, reducing tumor transformation of cells. Addition of antioxidant to the diets of this drosophila genotype reduces the content of endogenous pro-oxidants and the efficiency of apoptosis further decreases, which leads to reduction of the mean LS and modification of survival profile.

We previously showed that TC-13 is an effective inductor of the Nrf2/Keap1 signal pathway [1]. Studies on the Nrf2 knockout mice (*nrf2*$^{-/-}$ genotype) showed shortening of the mean LS in animals: high mortality from various (mainly inflammatory) diseases was observed in these animals after the age of 20 weeks [10]. The mean LS of *Keap1*-heterozygotic *D. melanogaster* males was prolonged by 8-10%, while the same genetic modification was inessential for the female LS [13]. By the present time numerous studies on animals have persuasively demonstrated that in addition to the positive effect (including that on LS), antioxidants induce the development of tumor processes [4]. Our comparative study of TC-13 effects on wild phenotype drosophila and the *lgl*^{558}*OR* strain liable to tumor formation also showed that the antioxidant prolonged the LS of wild phenotype flies and reduced it in *lgl*^{558}*OR* insects. Hence, the study provided an evidence calling for special attention to therapeutic use of antioxidants.

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