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The extended life span of *Drosophila melanogaster* eye-color (white and vermilion) mutants with impaired formation of kynurenine

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Abstract

Animal and human studies suggest that aging is associated with increased formation of kynurenine (KYN) from tryptophan (TRY). The rate-limiting factors of TRY–KYN metabolism are transmembrane transport of TRY, and activity of enzyme, TRY 2,3-dioxygenase (TDO2). Eye-color mutants, white (w1118) (impaired TRY transport) and vermilion (v48a and v2) (deficient TDO activity), were compared with wild-type Oregon-R (Ore-R) strain of *Drosophila melanogaster*. Female 1-day-old adult flies maintained on a standard medium, and acclimatized to 12-h light:12-h dark cycle were collected, and then regularly transferred to fresh medium every 3–4 days. The number of dead flies was recorded at the time of transfer. Forty flies were studied in each experimental group. The life span of w1118 (mean = 45.5 days), and v48a (mean = 47.6 days) and v2 (mean = 43.8 days), were significantly longer than of wild-type Ore-R flies (27.1 days) ($p < 0.001$, Logrank test). There were no differences in life span between w1118 and v48a and v2 mutants. Present results suggest that prolongation of life span may be associated with slow rate of KYN formation from TRY.

Keywords

Drosophila; Aging; Kynurenines

Introduction

Tryptophan (TRY) is an amino acid participating in biosynthesis of proteins and methoxyindoles (serotonin and melatonin). The major non-protein route of TRY metabolism is the cleavage of its indole ring with the formation of kynurenine (KYN) (Oxenkrug 2007).

Animal and human studies suggest that aging is associated with upregulation of TRY–KYN metabolism. KYN/TRY ratio was found to be increased with aging in humans when comparing three age groups (34–60, 61–71, and 72–93 years) (Frick et al. 2004) and nonagenarians with 45-year-old subjects (Pertovaara et al. 2006). Increased formation of KYN derivative, kynurenic acid, was observed in aged rat brain (Moroni et al. 1988; Gramsbergen et al. 1992) and in human serum (Urbanska et al. 2006). The higher rate of TRY conversion into KYN at the entry into the study was predictive of higher mortality in 10-year prospective study of nonagenarians (Pertovaara et al. 2006). Association between TRY–KYN metabolism and aging might be further supported by observation of the

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increased rate of TRY conversion into KYN in obesity, diabetes, atherosclerosis, menopause and other components of aging-associated metabolic syndrome (Oxenkrug 2009).

Drosophila is well suited for studies of the effect of TRY–KYN metabolism on aging since TRY–KYN pathway and related genes were described in *Drosophila melanogaster* (Savvateeva-Popova et al. 2003). However, genes impacting TRY–KYN pathways have not been considered among genetic pathways influencing longevity in *Drosophila* (Seroude 2002). There is only one study evaluating the life span of *Drosophila* mutants with impaired kynurenine pathway of TRY metabolism (Kamyshev 1980). TRY conversion into KYN occurs in pigmented eyes of *Drosophila* (Tearle 1991). The rate-limiting enzyme of this reaction in *Drosophila*, as in the other species, is tryptophan 2,3-dioxygenase (TDO2). The X-linked vermilion (v) mutants have deficient TDO activity (Beadle and Ephrussi 1936). Life spans of *v* flies were 33% longer than of wild-type Canton-S flies (Kamyshev 1980). This data in line with the above mentioned observation of high mortality in humans with increased KYN/TRY ratio (Pertovaara et al. 2006). Therefore, prolongation of life span might be associated with the slow rate of KYN formation from TRY. Since TDO is an intracellular enzyme (Kudo et al. 2001), TRY must enter the pigment cell to be available as a substrate for KYN formation. Thus, besides TDO2, transmembrane transport of TRY is another rate-limiting factor of TRY conversion into KYN. The deficient transmembrane transport of TRY underlines impaired formation of KYN from TRY in white (*w*) mutant of *D. melanogaster* (Sullivan and Sullivan 1975).

To further assess the effect of impaired conversion of TRY into KYN on life span, the present study compared life spans of the *w* mutant of *D. melanogaster* with the TDO2-deficient *v* mutants and wild-type Oregon-R flies.

Materials and methods

Wild-type Oregon-R (Ore-R), and eye-color mutants with impaired transmembrane transport of TRY (w^{1118}) and deficient TDO2 activity [v^{48a} and hypomorph v^{2} (Searles et al. 1990)] of *D. melanogaster* were obtained from the Bloomington Drosophila Stock Center (http://flystocks.bio.indiana.edu). Flies were raised at 25°C, 30% humidity at 12-h light:12-h dark (LD 12:12) cycle on a standard Drosophila medium consisting of cornmeal, agar, brewers yeast, dextrose, sucrose and wheat germ. One-day-old adult flies were collected, and then regularly transferred to fresh medium every 3–4 days. The number of dead flies was recorded at the time of transfer. Forty flies were studied in each experimental group. The study was carried out between January and March.

Results

Ore-R had statistically significant shorter survival time (mean = 27.1 days) than v^{2} (mean = 43.8 days), v^{48a} (mean = 47.6 days) and w^{1118} (mean = 45.5 days) (Logrank test) (Fig. 1).

There were no statistically significant differences in survival time of w^{1118}, v^{2} and v^{48a} flies.

Discussion

The results of the present study confirm the earlier observation of prolonged life span of TDO2-deficient *v* mutants in comparison with wild-type Canton-S *D. melanogaster* (Kamyshev 1980). In addition, the present study evaluated the impact of impaired transmembrane transport of TRY on life span of *Drosophila*. Conversion of TRY into KYN in *D. melanogaster* is an initial step of biosynthesis of brown eye pigment, ommochrome (Tearle 1991). The red pigments, drosopterins, (synthesized from guanine) contribute to pigmentation of the eye of *D. melanogaster* as well (Mackenzie et al. 1999). The w^{1118}

mutant of *D. melanogaster* is deficient in transport of both TRY, the initial substrate for biosynthesis of brown pigments, ommochromes, and guanine, the initial substrate for biosynthesis of red pigments, drosopterins, in pigment cells (Sullivan et al. 1980). The present study revealed that deficient transmembrane transport of TRY is associated with prolonged survival time of w^{1118} mutant in comparison with wild-type Ore-R strain of *D. melanogaster*. Since white mutants have deficient transport of guanine, the initial substrate for red eye pigment, their prolonged survival time might depend not only on TRY but guanine transport deficiency as well. While such a possibility could not be ruled out based on available data, some other factors might affect life span of white mutant, the parent strain of Methuselah Drosophila (Borycz et al. 2008; Petrosyan et al. 2007).

The possible mechanisms of the effect of impaired TRY conversion into KYN on life span are not clear. Since TRY is an initial substrate of melatonin biosynthesis, one might suggest that decreased utilization of TRY for KYN formation might increase the formation of melatonin that is known to prolong life span of flies (Bonilla et al. 2002). The effect of down-regulation of TRY–KYN pathway on life span might be linked with the effects of nicotinamide adenine dinucleotide (NAD+), the final product of TRY–KYN pathway, on sirtuins genes that require NAD+ for their deacetylase or ADP-ribosyl transferase activity (Guarente 2007). The other possibility is the impairment of post-KYN metabolism. Post-KYN metabolism in *D. melanogaster* consists of two competitive routes: formation of 3-hydroxyKYN (catalyzed by KYN-3-hydroxylase), and, consequently, ommochromes (catalyzed by phenoxazinone synthase); or formation of KYNA (catalyzed by aminotransferases) (Howells et al. 1977; Savvateeva-Popova et al. 2003) (Fig. 2).

Considering the neurotoxic, anti-cholinergic and free radical-generating properties of KYN and its derivatives (summarily called “kynurenines”), their increased production might contribute to initiation and/or maintaining of aging processes (Oxenkrug, 2007). However, there was no difference between life spans of wild-type Canton-S flies and mutants with deficient post-KYN metabolism: scarlet (deficient transmembrane transport of KYN); cinnabar (deficient KYN 3-hydroxylase activity resulted in accumulation of KYN and KYNA), and cardinal (deficient phenoxazinone synthase resulted in excess of 3-hydroxy-kynurenine) in the only one study addressing this issue (Kamyshev 1980).

Therefore, literature data and the results of the present study suggest that prolongation of life span in *D. melanogaster* is associated with impaired transmembrane transport of TRY resulting in diminished availability of TRY as a substrate for KYN formation (w^{1118} mutant) or impaired TDO2 activity (*v* mutants). The present results suggest that *D. melanogaster* mutants with deficient TRY–KYN metabolism might be considered as an experimental model for studies of certain aspects of aging mechanisms. Drosophila model may have some relevance for humans considering that major components of Drosophila’s TRY– KYN metabolism are conserved in humans (Savvateeva-Popova et al. 2003); that upregulation of TRY–KYN metabolism was associated with aging in rodents and humans (see “Introduction”); and that anti-aging effect of competitive TDO2 inhibitor melatonin (Walsh and Daya 1997) was reported in *Drosophila* (Bonilla et al. 2002; Anisimov et al. 1997), and rodents and humans (Karasek 2004).

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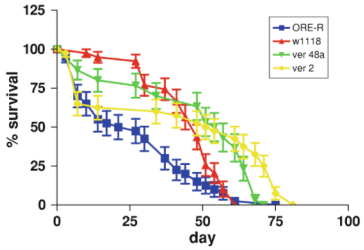

Fig. 1.
Survival time of *Drosophila melanogaster* mutants with impaired formation of kynurenine. *ORE-R* Oregon-R, *w1118* white, *ver 48a* vermilion, *ver2* vermilion hypomorph

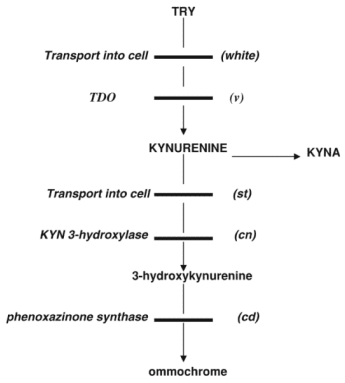

Fig. 2.

Kynurenine pathway of tryptophan metabolism in *Drosophila melanogaster*. *TRY* tryptophan, *TDO TRY 2,3-dioxygenase*, *KYNA* kynurenic acid, *v* vermilion, *st* scarlet, *cn* cinnabar, *cd* cardinal