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Extension of life span of *Drosophila melanogaster* by the inhibitors of tryptophan-kynurenine metabolism

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Upregulation of kynurenine (KYN) formation from tryptophan (TRY) was associated with aging in animal and human studies. TRY-KYN metabolism is affected by the activities of TRY 2,3-dioxygenase 2 (TDO) and ATP-binding cassette (ABC) transporter regulating TRY access to intracellular TDO. We studied the effects of TDO inhibitor, alpha-methyl tryptophan (aMT) and ABC transported inhibitor, 5-methyl tryptophan (5MT), on the life span of wild strain female Drosophila flies (Oregon-R). aMT and 5MT prolonged mean and maximum life span (by 27% and 43%, and 21% and 23%, resp.). The present results are the first observation of the extension of life span of *Drosophila melanogaster* by inhibitors of TRY-KYN metabolism, and in line with literature and previous studies on prolonged life span of TDO- and ABC-deficient female Drosophila mutants. Inhibition of TDO and ABC transporter activity might offer the new target for anti-aging interventions.

Introduction

Tryptophan (TRY) is an amino acid participating in biosynthesis of proteins and methoxyindoles (serotonin and melatonin) (reviewed in ref. 1). TRY 2,3-dioxygenase 2 (TDO) is a rate-limiting enzyme of the major non-protein route of TRY metabolism: the cleavage of indole ring of TRY with the formation of formyl-kynurenine, and subsequently, kynurenine (KYN).[2] Since TDO is intracellular enzyme,[3] TRY must enter cell to be available as a substrate for KYN formation. Cellular uptake of TRY is facilitated by ATP-binding cassette (ABC) transporter.[4] Thus, besides TDO, ABC transporter is a rate-limiting factor of TRY conversion into KYN.[5]

Animal and human studies suggested that aging is associated with upregulation of TRY-KYN metabolism. Thus, plasma KYN/TRY ratio (marker of activity of KYN formation from TRY) is increased with aging.[6,7] Increased formation of KYN derivative, kynurenic acid, was observed in aged rat brain[8,9] and in human serum.[10] One of the mechanisms of aging-associated upregulation of TRY-KYN metabolism might be the increased production of cortisol,[11] the inducer of TDO. Association between TRY-KYN metabolism and aging might be further supported by the observations of the increased rate of TRY conversion into KYN in obesity, diabetes, atherosclerosis, menopause, major depression and other aging-associated medical and psychiatric disorders (including metabolic syndrome).[12,13]

TRY-KYN pathway and related genes were described in *Drosophila melanogaster*.[14] The end product of TRY-KYN pathway in Drosophila is brown eye pigment.[15] TDO is the rate-limiting enzyme of KYN formation from TRY in Drosophila, as in the other species. Life spans of TDO-deficient (vermilion) [16,17] and of ABC transport impaired (white) eye mutants of *Drosophila melanogaster* were longer than of wild type flies.[17] This data are in line with the observation of high mortality in humans with increased plasma KYN/TRY ratio at the entry of the 10-year prospective study of nonagenarians.[18] Therefore, we suggested that prolongation of life span might be associated with the slow rate of KYN formation from TRY.[13,17]

To further evaluate this hypothesis we were interested to study the effect of TDO inhibitor, alpha-methyl tryptophan (aMT) [19] and of ABC transporter inhibitor, 5-methyltryptophan (5MT),[5] on the life span of wild type Oregon flies.

Results

Effect of alpha-methyl tryptophan. Treatment with aMT (0.46 mM) did not affect the life span of Drosophila (data not shown).

Treatment with higher concentration of aMT (18.3 mM) increased mean survival time (by 27%, $p < 0.0001$) and maximum life span (by 23%, $p < 0.0001$, two way ANOVA) (**Table 1**).

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Table 1. Inhibitors of tryptophan—kynurenine metabolism and life span of female *Drosophila melanogaster* (Oregon)

	Control (days) (n = 50)	aMT (days) (n = 51)	5MT (days) (n = 58)
Mean	40.1	51.7*	48.5*
Std. Err.	1.4	0.9	1.0
Maximum	53	65*	46*

Concentrations of aMT (18.3 mM) and 5MT (34.5 mM); *increase in days in comparison with control group; $p < 0.0001$, two-way ANOVA.

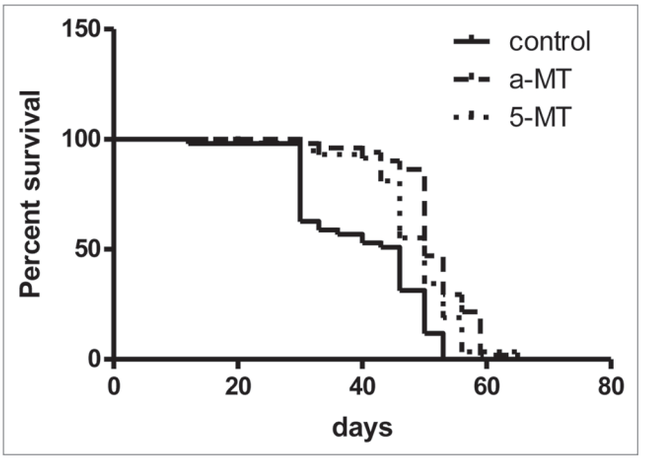

Figure 1. Survival time of *Drosophila melanogaster* (Oregon) treated with alpha-methyl (aMT) and 5-methyl (5MT) tryptophan ($p < 0.0001$, Logrank test).

Maximum life span of female control group was 53 days. 25% (13 out of 51) flies treated with high concentrations of aMT survived longer than 53 days (up to 65 days) (**Fig. 1**).

Effect of 5-methyl tryptophan. Treatment with 5MT (2.4 mM) did not affect the life span of Drosophila (data not shown).

Treatment with higher concentration of 5MT (34.5 mM) increased mean survival time (by 21%) and maximum life span (by 23%) of flies ($p < 0.0001$, two way ANOVA) (**Table 1**).

Maximum life span of female control group was 53 days. 19% (11 out of 58) female flies treated with high concentrations of 5MT survived longer than 53 days (up to 65 days) (**Fig. 1**).

Discussion

This is the first (as far as we know) observation of the prolongation of life span in Drosophila by TDO inhibitor, aMT, and by ABC transporter inhibitor, 5MT. The effect appeared to be a dose-dependent: prolongation of life spam was observed only with high but not low concentrations of aMT and 5MT. Intrathorasic administration of aMT inhibited TDO in domestic flies,[19] and TRY-KYN metabolism is similar in domestic flies and *Drosophila melanogaster*: in both species the end product of TRY-KYN pathway is brown eye pigment, ommochrome. Therefore, aMT effect on life span is, most likely, related to inhibition of TDO. However, the direct assessment of TDO activity is needed to proof this suggestion. The present results are in line with the previous observations of prolonged life span of TDO-deficient eye color mutants (vermilion) of Drosophila.[16,17]

Aging might be affected by the manipulations of the upstream biochemical pathways of TRY metabolism. Thus, reducing the TRY content of the diet extended maximum life span in mammals.[20] Since TRY is required for the synthesis of proteins and methoxyindoles (serotonin, N-acetylserotonin and melatonin),[1] dietary reductions of TRY might not be advisable. It is noteworthy that TRY participation in protein biosynthesis is not affected by inhibition of ABC transporter.[5]

TRY has to enter the cell to interact with intracellular TDO.[3] TRY shares the same ABC transporter with guanine, the initial substrate for formation of the red eye pigment of Drosophila.[21] Since the eye color of wild stock Drosophila depends on a combination of red and brown pigments, mutants with impaired ABC transporter of both guanine and TRY have no eye pigment, and hence, have white colored eyes.[22] Prolonged life span was observed in white mutants of Drosophila.[17] ABC transporter is encoded by white gene, and inhibition of ABC transporter by 5MT was observed in in vitro experiments.[5] Since TRY is transported by ABC transporter, administration of 5MT might prolong life span by inhibiting TRY-KYN metabolism. However, ABC transporter might be involved in transport of KYN,[5] 3-hydroxy KYN,[4] and cGMP.[23] Further studies with the assessment of the effect of 5MT on the content of TRY, KYN, and its metabolites are needed to explain the effect of 5MT on life span.

In conclusion, prolongation of life span was observed in wild stock flies treated by the inhibitors of TDO and of ABC transporter. Both treatments supposed to limit TRY conversion into KYN. This data are in agreement with previously observed extension of life span in Drosophila mutants with deficient TDO[16,17] or impaired ABC transporter,[17] and with neuroprotective effect of genetic inhibition of TDO.[24] Importantly, in difference with genetic mutations, pharmacological interventions increased not only mean survival time but maximum life span as well. Inhibition of TRY conversion into KYN might be a target for anti-aging intervention.

Future studies might explore the effect of potential inhibitors of TDO and ABC transporter among tryptophan derivatives[25,26] on the life span of Drosophila and other species.

Methods

Female wild-type stock Oregon of *Drosophila melanogaster* from the collection of V.N. Karazin Kharkiv National University was used in the experiments and maintained at 23°C on a standard Drosophila medium consisting of sugar, yeast, agar and semolina. Two concentrations of aMT (alpha-DL-methyl tryptophan) (0.46 mM or 18.3 mM) or 5MT (5-methyl-DL-tryptophan) (2.4 mM and 34.5 mM) (Sigma Aldrich Chemical Co., USA) were added to nutrition medium of experimental groups. To examine life span, 1-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. The study was carried out between April and June.

Statistics. The data were analyzed using two ways ANOVA and Logrank test.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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