Minireview

Extension of life span by down-regulation of enzymes catalyzing tryptophan conversion into kynurenine: Possible implications for mechanisms of aging

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Impact Statement

Present review suggests the causative link between down-regulation of kynurenine (Kyn) formation from tryptophan (Trp) and life span prolongation. Down-regulation of the enzymes that regulate kynurenine formation from tryptophan (tryptophan- and indoleamine 2, 3-dioxygenases and ATP-binding cassette transporter) might slow down aging and prevent/treat aging-associated disorders and metabolic syndrome. Already available for human use inhibitors of rate-limiting enzymes of Kyn formation from Trp might be tried for antiaging interventions.

Abstract

The end products of catabolism of tryptophan (Trp), an essential amino acid, are known to affect mechanism(s) of aging, a neurodegenerative condition. This review focuses on the possible role of the initial step of Trp catabolism, kynurenine (Kyn) formation from Trp, in aging mechanism(s). Rate-limiting enzymes of Trp conversion into Kyn are tryptophan 2,3-dioxygenase 2 (TDO) or indoleamine 2,3-dioxygenase (IDO). Aging is associated with up-regulated production of cortisol, an activator of TDO, and pro-inflammatory cytokines, inducers of IDO. The other rate-limiting enzyme of Kyn formation from Trp is ATP-binding cassette (ABC) transporter that regulates Trp availability as a substrate for TDO. Inhibitors of TDO (alpha-methyl tryptophan) and ABC transporter (5-methyltryptophan) extended life span of wild-type *Drosophila*. Life span prolongation was observed in TDO knockdown of *Caenorhabditis elegans* and in TDO or ABC transporter–deficient *Drosophila* mutants. Down-regulation of enzymes catalyzing Kyn conversion into kynurenic acid (KYNA) and 3-hydroxykynurenine decreases life span. Considering that down-regulation of Methuselah (MTH) gene prolonged life span, aging-accelerating effect of KYNA, a GPR35/MTH agonist, might depend on MTH gene activation. Mice treated with TDO inhibitor, benserazide, an ingredient of anti-Parkinson medication carbidopa, and TDO-deficient *Drosophila* mutants were resistant to inducement of aging-associated Metabolic Syndrome by high-sugar or high-fat diets. Up-regulation of Kyn formation was associated with accelerated aging and increased mortality in human subjects. Trp–Kyn pathway is evolutionary conserved (from yeasts, through insects, worms, vertebrates to humans). Further studies might explore possible antiaging effect of down-regulation of Kyn formation from Trp by dietary, pharmacological, and genetic interventions.

Keywords: Aging, benserazide, Drosophila, kynurenine, metabolic syndrome, tryptophan

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Cover letter

This mini-review was presented at the ICNA meeting, September 2022. It summarizes studies initiated by our discovery of life extension effect of down-regulation of kynurenine formation from tryptophan (according to Clarivate publication, it is the sixth most cited paper in *Journal of Neural Transmission* in 2010). There are increasing numbers of studies of kynurenine's role in aging. However, this pathway still not generally recognized as one of the mechanism(s) of aging. This review will stimulate further studies in this direction. Already available for human use inhibitors of rate-limiting enzymes of kynurenine formation from tryptophan might be tried for antiaging interventions. Non-signing author, Dr Navrotska, has read and approved the manuscript.

Introduction: tryptophan–kynurenine–niacin pathway

One of the end products of catabolism of tryptophan (Trp) is niacin, a precursor of nicotinamide adenine dinucleotide (NAD+) (Figure 1). Trp–Kynurenine (Kyn) catabolism is the only *de novo* NAD+ biosynthetic pathway in humans. Niacin deficiency in human subjects results in the disease

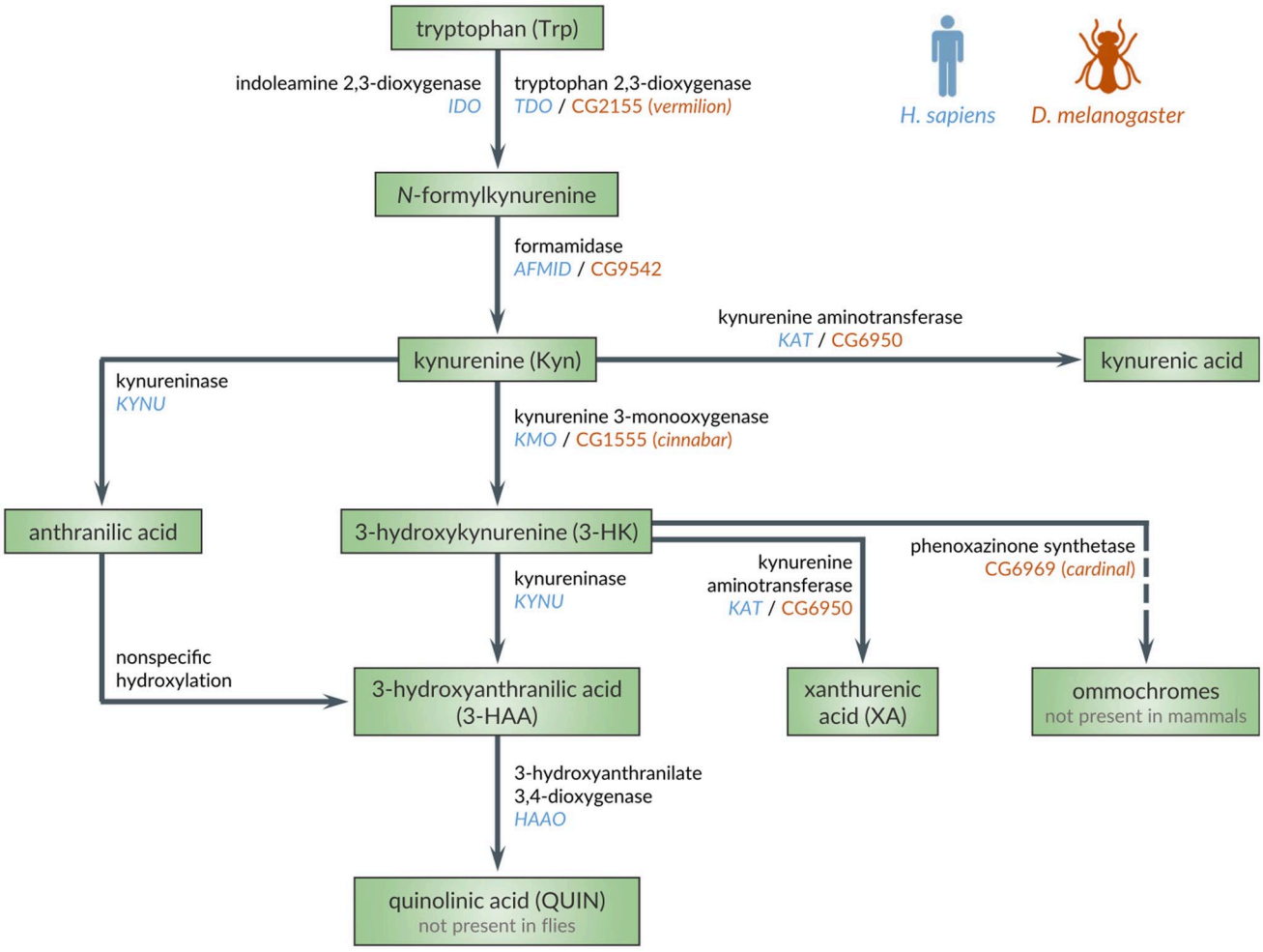

Figure 1. Kynurenine catabolism in *Homo sapiens* and *Drosophila melanogaster*.[22]

of four "D": dermatitis, diarrhea and dementia, and, if not treated, death. Niacin/NAD+ synthesis was suggested to be involved in the regulation of longevity and health span.[1] This review focuses on the possible role of the initial steps of Trp–Kyn–Niacin pathway in aging mechanism(s).

Inhibitors of enzymes of tryptophan conversion into kynurenine prolong life span

The initial step of Trp–Kyn pathway is Trp conversion into Kyn (Figure 1). The rate-limiting enzymes of Kyn formation from Trp are tryptophan 2,3-dioxygenase 2 (TDO) or indoleamine 2,3-dioxygenase (IDO). Trp availability as a substrate for Kyn formation is another rate-limiting step in Kyn formation because Trp has to enter the cell to interact with intracellularly located TDO. Trp transport into cells is regulated by ATP-binding cassette (ABC), a member of a superfamily of enzyme pumps that hydrolyze ATP in exchange for translocation of substrates across cellular membranes.[2]

Aging is associated with up-regulated production of cortisol[3,4] and pro-inflammatory cytokines, for example, interferon-gamma (IFNG).[5] Cortisol activates TDO while IFNG transcriptionally induces IDO.[6] We reported that alpha-methyl tryptophan, the TDO inhibitor,[7] and 5-methyltryptophan, the ABC transporter inhibitor, prolonged life span of wild-type *Drosophila melanogaster* (Figure 2).[8] Our finding was corroborated by observations of life span extension effect of berberine, a direct TDO inhibitor,[9,10] and minocycline, indirect TDO inhibitor,[11,12] in wild-type *Drosophila*. Furthermore, life span prolongation effect of ibuprofen was suggested to depend on inhibition of Trp import in yeast[13] and on down-regulation of neuronal TDO in mice.[14] Inhibition of Trp conversion into Kyn in *Drosophila* might contribute to the life span extending effect of mifepristone, a synthetic steroid with antiobesity and antidiabetic effects in mammals.[15]

Genetically induced down-regulation of enzymes of Kyn formation from Trp prolong life span

Our finding of the life span extending effect of TDO and ABC-transporter inhibitors were corroborated by observation that TDO knockdown prolonged lifespan of *Caenorhabditis elegans*.[16,17] TDO is encoded by the *vermilion* (*v*) gene, and ABC transporter is encoded by the *white* (*w*) gene in flies. Life spans of TDO deficient *v* and ABC transporter deficient *w Drosophila* mutants were longer than that of wild-type flies (Figure 3).[18]

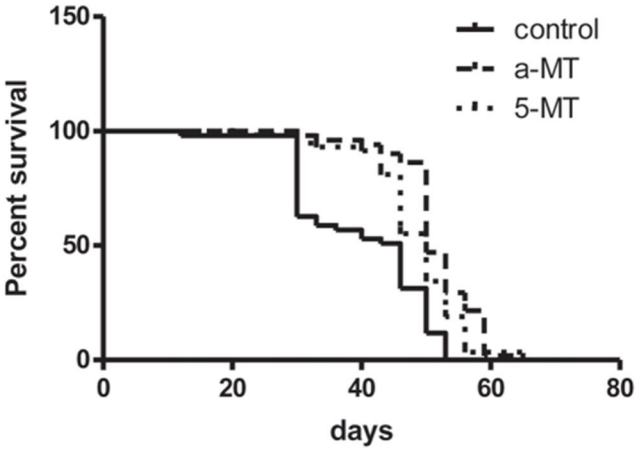

Figure 2. Survival time of *Drosophila melanogaster* (Oregon) treated with alpha-methyl (aMT) and 5-methyl (5MT) tryptophan.[8]

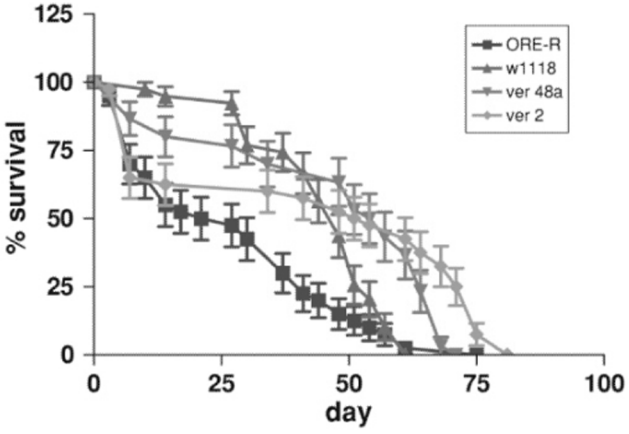

Figure 3. Survival time of *Drosophila melanogaster* mutants with impaired formation of kynurenine.[18]
Ore-R: Oregon-R; w1118: white; ver 48a: vermillion; ver2: vermillion hypomorph.

Extension of life span depends on decreased formation of kynurenine

Inhibition and knockdown of TDO and ABC transporter not only decreases formation of Kyn but attenuates Trp catabolism as well. Therefore, life span extension, induced by inhibition of TDO/ABC transporter, may depend on increase in Trp and/or on decrease in Kyn formation. Indeed, Trp attenuates age-dependent decline of muscle function in flies.[16] However, further analysis revealed that the effect of Trp on age-dependent decline of muscle function was independent from Trp-related regulation of lifespan in flies.[16] The notion of different regulation of aging and age-dependent decline of motor function is further supported by the disappearance of the significant advantage of long-lived *Drosophila* mutants in maintaining of sustained flight in old age (over 30 days) flies.[19] Furthermore, analysis of Trp's effect on TDO has to consider the possibility of TDO activation by increasing doses of Trp, a substrate for TDO.[6] Indeed, while low doses of Trp (1 nM) increase life span and attenuate age-dependent decline of muscle function, the increased doses of Trp (5 nM) were more efficient against age-dependent decline of muscle function, than on life span extending effect of Trp. Increase in Trp dose (to 10 nM) decreases life span, most likely, because of TDO activation.[20]

Kynurenine down-stream catabolism and life span

Kynurenine amino transferase (KAT) catalyzes further Kyn conversion into kynurenic acid (KYNA), and kynurenine 3 monooxygenase (KMO) catalyzes Kyn conversion into 3-hydroxykynurenine (3-HK), the intermediate substrate for NAD+ biosynthesis (Figure 1). Life span of KAT and KMO-deficient natural *Drosophila* mutants was shorter than that of wild-type flies.[21] Therefore, extension of life span depends on down-regulation of the enzymes catalyzing Kyn formation from Trp but not on deficiency of the enzymes catalyzing down-stream catabolism of Kyn. Notably, we observed that administration of KYNA increased the lethality of pupae of wild-type flies but not of KMO-deficient mutants. Our data suggested that the toxic effect of exogenous KYNA might depend on the presence of 3-HK.[22]

Kynurenic acid and Methuselah mutation

It was reported that down-regulation of Methuselah (MTH) gene or reduced signaling via MTH receptor prolonged life span and enhanced stress resistance of MTH flies.[23] MTH gene encodes a class of proteins called G protein-coupled receptors (GPR35).[24] Considering that KYNA is an agonist to GPR35, down-regulation of KYNA formation (as a consequence of decreased availability of Kyn) may contribute to life span extension of MTH flies.[25] In addition, down-regulation of ABC transporter may contribute to prolongation of life span of MTH mutants considering that *w* is a parental strain for MTH.[19] Notably, elevated formation of KYNA was suggested to be causatively linked to major psychopathology of schizophrenia.[26]

Down-regulation of kynurenine formation from tryptophan attenuates insulin resistance in a mouse and *Drosophila* models

Aging-associated Metabolic Syndrome (MetS) (insulin resistance [IR], excessive body weight gain, dyslipidemia) has common mechanisms with aging.[27] Benserazide, a TDO inhibitor, and an ingredient of Carbidopa, an anti-Parkinson drug, attenuates development of IR, dyslipidemia, and bodyweight gain in a mouse model of MetS.[28]

The life cycle of *Drosophila* has four distinct stages: eggs, larva, pupa, and imago (adult). *Drosophila* MetS model was built on the observation that flies reared on a high-sugar diet (HSD) developed IR in larvae stage and diabetes in imago.[29] Notably, development of IR extends larva stage, and, consequently, delays the emergence of pupae from larvae. We observed shorter duration of larva stage in HSD-treated *v* and *w* mutants in comparison with wild-type flies.[30,31] Our finding suggests that down-regulation of Kyn formation from

Trp, in addition to life span extension, delays HSD-induced development of aging-associated MetS. Further studies have to explore whether down-regulation of Kyn formation from Trp might be utilized for the prevention and treatment of aging-associated IR, obesity, and dyslipidemia.[32]

Activation of kynurenine formation from tryptophan is associated with accelerated aging

As reviewed above, down-regulation of Kyn formation from Trp extends life span and attenuates development of aging-associated disorders in flies, *C. elegans*, yeasts, and mice. The effect of up-regulated Kyn formation on aging was analyzed, mainly, in human studies. High blood Kyn/Trp ratio, an index of increased TDO/IDO activity, was associated with aging[33,34] and predicted higher mortality rate within 10 years of observation in a prospective study of nonagenarians.[35] High Kyn/Trp might be a consequence of aging-associated elevation of production of TDO activator, cortisol.[4] Notably, in humans, Kyn formation from Trp is catalyzed by pro-inflammatory cytokines, for example, IFNG-induced IDO.[5] Therefore, increased formation of Kyn from Trp might be a consequence of IDO induction by aging-associated increase of IFNG production.[32] IFNG, concurrently with IDO, induces rate-limiting enzyme of pteridines biosynthesis from guanine, one of the four main nucleobases found in the nucleic acids DNA and RNA. Blood level of stable and water-soluble pteridine derivate, neopterin, strongly correlates with Kyn level, and used as a marker of IFNG-induced activation of IDO (rather than stress-induced activation of TDO).[33] We observed strong correlation between elevated serum neopterin levels and mortality risk among the elderly Boston community.[36] Notably, the prevalence of low producer (A) alleles of IFNG (+874) T/A gene in nonagenarian women in comparison with men might contribute to higher longevity in women.[37] On the contrary, the presence of high producer (T) alleles increases the risk of development of depression and IR, induced by interferon treatment of hepatitis C virus patients.[38,39] In conclusion, reviewed data suggest the causal link between down-regulation of Kyn formation from Trp and prolongation of life span in vertebrates, while up-regulation of Kyn formation is associated with aging acceleration and increased mortality in human subjects. Trp–Kyn pathway is evolutionary conserved (from yeasts, through insects, worms, vertebrates to humans). Further studies are required to assess the possible antiaging effect of dietary, pharmacological, and genetic interventions-induced down-regulation of Kyn formation from Trp.

AUTHORS' CONTRIBUTIONS

All authors participated in the design, interpretation of the studies, and analysis of the data and review of the manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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