Berberine Attenuated Aging-Accelerating Effect of High Temperature in Drosophila Model

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Abstract

We have observed that berberine prolonged life span and improved viability of pupae and climbing activity of imagos of wild-type Drosophila melanogaster maintained at 23°C. As a continuation of our studies of berberine effect on life span, we were interested to evaluate the effect of berberine of life span in flies maintained at a higher temperature (28°C) known to accelerate aging in wild type flies. Considering that genetically or pharmacologically induced deficiency of TRP conversion into KYN prolonged life span in a Drosophila model, we compared the effects of berberine, a powerful inhibitor of kynurenine (KYN) formation from tryptophan (TRP), on life span in wild type and in Drosophila melanogaster mutants (vermilion) with deficient TRP-KYN metabolism maintained at 23°C and 28°C. High (28°C) ambient temperature decreased life span in both wild type and vermilion flies. Aging accelerating effect of high temperature was more pronounced in Oregon than in vermilion flies (−60% vs. −40% decrease of mean life span, resp). Berberine attenuated the aging-accelerating effect of high temperature. Effect of berberine was more pronounced in Oregon (+46%) than in vermilion (+22%) flies. The obtained data suggested the possible involvement of TRP-KYN metabolism in the aging-acceleration effect of the high temperature and in protective effect of berberine.

Keywords
Berberine; Life Span; Drosophila Melanogaster; High Temperature; Kynurenine

1. Introduction

Up-regulation of kynurenine (KYN) pathway of tryptophan (TRP) metabolism was suggested as one of the mechanisms of neurodegenerative disorders of aging [1-3]. We found that Drosophila melanogaster mutants with deficient formation of KYN, vermilion and white, had longer life span than wild type flies (Oregon) [4]; and that inhibitors of TRP-
KYN metabolism, alpha-methyl-TRP and 5-methyl-TRP, prolonged life span of wild-type flies [5]. Inhibition of TRP-KYN metabolism exerted neuroprotective effect in flies [6]. Furthermore, inhibitors of TRP-KYN metabolism available for human use, berberine, isoquinoline alkaloid isolated from Berberis aristata, a major herb widely used in Indian and Chinese systems of medicine, is a strong inhibitor of the rate-limiting enzyme of TRP-KYN metabolism the active ingredient of an herbal medicine [7, 13] and minocycline, an antibiotic with anti-inflammatory effects, prolonged life span and stimulated locomotor activity (negative geotaxis) of wild type flies [8-10].

As a continuation of our studies of berberine effect on life span we were interested to evaluate the effect of berberine of life span in flies maintained at a higher temperature (28°C). It is known that life span is temperature dependent, and flies are living faster at the higher temperature [11], i.e. there is an inverse relationship between life span and temperature [12]. Our working hypothesis was that berberine protects against the aging-accelerating effect of high ambient temperature. To check this hypothesis we compared the effect of berberine on life span of Oregon flies kept at 23°C and 28°C.

Considering that berberine is a strong inhibitor of kynurenine formation from tryptophan [13], and that genetically or pharmacologically induced deficiency of TRP conversion into KYN prolongs life span [4, 5, 14], we compared the effects of berberine on life span in wild type and KYN formation deficient mutants (vermilion) of Drosophila melanogaster maintained under 23°C and 28°C.

2. Methods

Wild-type stock Oregon of Drosophila melanogaster and mutant stock vermilion from the collection of V. N. Karazin Kharkiv National University were used in the experiments. The study was carried out between June and August.

Flies were maintained at 23°C in a 12:12 light: dark period on a standard Drosophila medium consisting of sugar, yeast, agar and semolina. Berberine (Sigma Aldrich Chemical Co, USA) was added to nutrition medium in the dose of 1 mM (0.4 mg/ml of nutrition medium) at a larvae stage. Effective doses of berberine were selected by us in a previous investigation [7].

Flies collected in control and berberine variants were divided into two groups, one of which maintained at 23°C and the other at 28°C.

Life span evaluation: one day old adult flies (males) 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 obtained data were statistically analyzed using Wilcoxon rank-sum test and two ways ANOVA test.
3. Results

Effect of impaired formation of KYN on life span. Life span of vermillion male flies maintained under 23°C was longer (by 43%) than life span of Oregon flies in accord with our previously published data [4] (Table 1).

Effect on high ambient temperature on life span. High ambient temperature (28°C) decreased life span of Oregon flies in accordance with literature data (see above) (Figure 1). High ambient temperature decreased life span of vermillion flies as well (Figure 2). The effect of high temperature on life span was less pronounced in vermillion (~40%) than in Oregon (~60%) flies (Table 1).

Berberine and life span of flies maintained at high temperature. Berberine attenuated the effect of exposure to 28°C on life span in Oregon (Figure 1) and vermillion flies (Figure 2). Berberine prolonged the life span of Oregon flies by 46% in comparison with control flies kept at 28°C and by 22% in vermilion flies kept at 28°C (Table 1).

4. Discussion

The main finding of our study is berberine-induced attenuation of aging-accelerating effect of high temperature in Drosophila melanogaster model. We previously reported that berberine prolonged the life span of Drosophila flies kept at 23°C [7]. The present data indicate that berberine may enhance the ability of fruit flies to resist stress caused by high temperature, since its addition to larvae attenuated the aging-accelerating effect of high temperature. The free radical theory of aging hypothesizes that oxygen-derived free radicals are responsible for the age-related damage at the cellular and tissue levels [15], and at temperature-accelerated aging these processes might be especially intensive [11, 12]. So the protective effect of berberine may be dependent on its anti-oxidant activity [16].

The alternative mechanism of observed protective effect of berberine against aging-accelerating action of high temperature may be related to berberine-induced inhibition of TRP-KYN metabolism. Berberine is a stronger inhibitor of TRP conversion into KYN than a “standard” inhibitor, 1-methyl-TRP [13]. Genetic and pharmacological inhibition of TRP-KYN pathway extends life span of Drosophila. Flies with mutations white (impaired transmembrane transport of TRP into cells where its metabolism occurs) and vermilion (deficiency of rate-limiting enzyme of TRP-KYN pathway, TRP 2,3-dioxygenase) had longer life spans than wild-type flies [4, 14]. Among pharmacological inhibitors of KYN formation from TRP used in our experiments were alpha-methyl-TRP and 5-methyl-TRP, berberine and minocycline, all of them prolonged the life span of wild-type Drosophila melanogaster [5, 7, 8].

Berberine-induced attenuation of aging-accelerating effect of high temperature (28°C) was less pronounced in vermillion (22%) than in Oregon flies (46%) (Table 1) might suggest the involvement of TRP-KYN pathway in mechanisms of high temperature effect on the life span. This suggestion might be further confirmed by less pronounced protective effect of berberine against high temperature-induced acceleration of aging in vermillion than in wild-type flies (Table 1). Present data warrant further studies of the involvement of TRP-KYN
metabolism in mechanisms of high temperature-induced acceleration of aging and protective effect of berberine and other inhibitors of TRP-KYN metabolism.

The results of present and our previous studies of berberine action on life span, viability and stress-resistance of drosophila indicate that berberine is a potentially good candidate drug for anti-aging intervention and attenuation of stressful conditions impact.

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Figure 1.
Berberine effect in Oregon flies. OREC23°C: Oregon male controls flies kept at 23°C; OREC28°C: Oregon male controls flies kept at 28°C; OREBe28°C: Oregon male flies kept at 28°C with addition of berberine.
Figure 2.
Berberine effect in vermilion flies. vmc23°: vermilion male controls flies kept at 23°C; vmc28°: vermilion male controls flies kept at 28°C; vmBe28°: vermilion male flies kept at 28°C with addition of berberine.
# Table 1

Life span at different temperatures and effect of berberine, in drosophila stocks

| Stock     | Experimental groups |          |          |          |
|-----------|---------------------|----------|----------|----------|
|           |                     | 23°C     | 28°C     | 28°C + berberine |
| Oregon    | 19.94 ± 1.24* n = 168 | 7.85 ± 0.64 n = 150 | 11.51 ± 1.35** n = 98 |
| Vermillion| 28.52 ± 1.65* n = 100 | 17.36 ± 0.57 n = 94  | 21.19 ± 0.43# n = 92 |

* Mean ± standard error; n = number of flies; P = 0.001 vs. 28°C and 28°C + berberine;
** P = 0.001 vs. 28°C;
# P = 0.001 vs. 28°C.