Journal of Thermal Biology 86 (2019) 102446

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: http://www.elsevier.com/locate/jtherbio

Protective effect of exogenous 8-oxo-2′-deoxyguanosine on *Drosophila melanogaster* larval stages under heat shock

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ARTICLE INFO

Keywords:
Heat shock
Exogenous 8-oxo-2′-deoxyguanosine
Bioregulation
Protective effect

ABSTRACT

The influence of exogenous 8-oxo-2′-deoxyguanosine on the development of *Drosophila melanogaster* under normal conditions, and under the influence of short-term heat shock was studied. It was shown that 8-oxo-2′-deoxyguanosine was not toxic at concentrations of up to 1 μM. A tendency to accelerate larval development and fly emergence was observed under the influence of this compound in our experiments. Short-term heat shock causes a 50–80% decrease in the number of larvae that complete development. The addition of exogenous 8-oxo-2′-deoxyguanosine to the food before thermal influence negates this effect and brings the levels of the imago emergence indicators back to the basal level. The obtained results are further evidence of the possible bioregulatory and adaptogen functions of 8-oxo-2′-deoxyguanosine.

1. Introduction

8-oxo-2′-deoxyguanosine (8-oxo-dG) is a product formed upon the oxidation of 2′-deoxyguanosine–one of the four main nucleotides of DNA–by reactive oxygen species like the hydroxyl radicals. It is formed spontaneously in the cells of all aerobic organisms both as a part of DNA and in the cytoplasmic triphosphate pool. According to numerous studies, the 8-oxo-dG content in the DNA increases in response to stressful and traumatic conditions [Ke et al., 2016], infections [Yeniova et al., 2015], inflammation [Kobayashi et al., 2015], oncological diseases [Valavanidis et al., 2009], aging [Nie et al., 2013], and other conditions that case intense oxidative cellular metabolism [Hara et al., 2016] or cause active oxygen species to enter from outside the cell [Jornot et al., 1998]. 8-oxo-dG has been used for over 20 years as a biomarker of oxidative DNA damage. This biomarker—apart from other uses—is of diagnostic and predictive value when choosing the dosage of radio- and chemotherapy [Haghdoost, 2005] and for treating autoimmune [Bashir et al., 1993] and inflammatory diseases [Barollo et al., 2011].

However, extensive data [Marmiy and Esipov, 2015; Chernikov et al., 2017] indicating the significant biological role of 8-oxo-2′-deoxyguanosine were obtained from 2006 to 2017. Its role cannot be reduced to the function of an oxidative metabolism by-product and damage marker. In experiments carried out by a group of Korean researchers in mice and cellular cultures, high concentrations of 8-oxo-dG exerted pronounced anti-inflammatory [Kim et al., 2006a] and antiallergic [Kim et al, 2011] effects, reduced the severity of autoimmune diseases symptoms [Hong et al., 2013], and increased the resistance towards adverse environmental factors like UV-radiation and a hypoxia [Lee et al., 2013]. When studying the mechanisms of these phenomenon, the same group of researchers successfully identified an 8-oxo-dGTP complex with RAC-1 protein. This protein initiates the inflammatory activation pathway of macrophages and neutrophils [Huh et al., 2012]. The formation of a stable complex with 8-oxo-dG inhibits RAC-1 activity. This means that it also inhibits the full complex of inflammatory reactions: the production of active oxygen forms and inflammatory cytokines and the proliferation and adhesion of macrophages. This fact, along with the identification of STAT-1 and STAT3 [Kim et al., 2006b] phosphorylation inhibition due to 8-oxo-2′-deoxyguanosine action, explains its anti-inflammatory and immunomodulatory effects, but not its protective and adaptogenic effects. The presence of the latter is shown by publications of independent groups reporting an increase in cellular culture survival rates under the influence of 8-oxo-dG in starvation, radiation, and a hypoxia conditions [Kostyuk et al., 2013].

We assumed that exogenous 8-oxo-dG could lead to DNA repair enzyme activation, active oxygen form neutralization, and other cellular protective mechanisms. This compound can act as a specific signal for the cell to inform it of oxidative damage severity reaching a level requiring protective system mobilization. In this case the spectrum of

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https://doi.org/10.1016/j.jtherbio.2019.102446
Received 11 April 2019; Received in revised form 21 August 2019; Accepted 28 October 2019
Available online 31 October 2019

possible 8-oxo-dG protective activity should be much broader and cover a great variety of both damaging factors and organisms that produce the protective effect. Previously, we succeeded in obtaining data indicating a decrease in 8-oxo-dG content as a portion of cell culture DNA under the influence of exogenous 8-oxo-dG [Marmiy et al., 2016]. This result supports the above hypothesis.

The objective of this study was to identify the protective effect of 8-oxo-2′-deoxyguanosine. Heat-shocked *Drosophila melanogaster* Mg (Diptera, Drosophilidae) was used as the model organism. Larvae at the I–III stages of development were subjected to thermal stress. The effect of shock temperature and 8-oxo-2′-deoxyguanosine was estimated based on the number of adult flies produced and the number of the preimaginal development stages. Adult *D. melanogaster* were subjected to a similar procedure. In this case, 8-oxo-dG content in the DNA was measured in addition to survival.

2. Materials and methods

Reagents: 2′-deoxyguanosine, bromine, dimethylformamide, sodium acetate, acetic anhydride, N-methyl imidazole, ammonium acetate, Tris-HCl, EDTA (Sigma-Aldrich, USA), RNAase A (Qiagen, Netherlands), Nuclease P1 (USBiological, USA), Proteinase K (Thermo Fisher Scientific, USA), alkaline phosphatase (Biosolve, Netherlands), chloroform, ethanol, and pure isoamyl alcohol (Chimmed, Russia).

To prepare food for the flies, baker's yeast (60 g/l), crushed raisin (45 g/l), food sugar (50 g/l), semolina (35 g/l), agar (8 g/l), and propionic acid (2 ml/l) were used. The yeast was inactivated by boiling for 2.5 h. The raisin was cooked for 1 h. The semolina and agar were cooked for 15 min. Propionic acid was added to the food after cooking was finished.

Equipment: Thermostats NESLAB FTC350 was used to perform the heat shock. Analytical HPLC of DNA hydrolyzate samples was done using a Beckman chromatograph (USA) equipped with two pumps of Beckman 114 M. A variable wavelength UV detector "KNAUER" (Germany) (detection was used at a wavelength of 254 nm and a cell thickness of 2 mm). An Altex 210 A Valve "Beckman" injector; a Beckman 421 A controller; an electrochemical detector made by Khimavtomatika (Russia) research and manufacturing association were also used. Acetonitrile procured from Biosolve was used for HPLC. An ULTRASPHERE ODS column (5 μm, 4.6 × 250 mm; Beckman) was used. UV spectra were registered using a Helios α "Unicam" spectrometer (Great Britain) at a range of wavelengths from 220 to 300 nm. Samples were precipitated using a Sigma 202 MK "Sigma" centrifuge (USA) and in a Speed Vac Concentrator "Savant" device (USA). 8-oxo-dG samples were prepared using an Ohaus EX-124 analytical balance with subsequent spectrophotometric control.

8-oxo-2′-deoxyguanosine was synthesized using the method developed by our group [Marmiy and Esipov, 2017]. This method includes 8-bromo-2′-deoxyguanosine acylation using a mixture of acetic anhydride with sodium acetate in anhydrous dimethylformamide and pyridine, followed by alkaline hydrolysis. 8-bromo-2′-deoxyguanosine was obtained using a published technique [Bodepudi et al., 1992] consisting of 2′-deoxyguanosine interaction with bromine water. Obtained 8-oxo-dG was purified using preparative reverse-phase HPLC using ethanol-water mixture as an eluent. The product purity was confirmed using NMR and LC-MS.

The experimental flies were stored in test tubes with a diameter of 20 mm containing 4 ml of the food. The food was moistened with a suspension of living baker's yeast before placing the flies on the surface.

2.1. Biological experiment

A heterogeneous box population of *D. melanogaster* at the Biological evolution department of the Biology faculty of Lomonosov Moscow State University served as the starting material. Flies were taken from this population as parents, placed as couples into test tubes with food, left for five days to reproduce and lay eggs, and removed from the test tubes.

Four experimental groups of flies were used in the experiment:

1) Control group: food without additives at 22 °C. Designation: t-/oxo-
2) 8-oxo-dG: food containing 1 μM of 8-oxo-dG at 22 °C. Designation: t-/oxo+.
3) Heat shock: food without additives, heat shock for 1 h 38 °C on the 5th day from beginning experiment, and 22 °C for the rest of time. Designation: t +/oxo-.
4) 8-oxo-dG + heat shock: food containing 1 μM 8-oxo-dG, heat shocked for 1 h 38 °C on the 5th day, and 22 °C for the rest of time. Designation: t+/oxo +.

The number of test tubes (family couples) in experimental variants is indicated in Table 1.

When studying the influence of 8-oxo-dG, the nucleoside in the mother solution (14 mg/ml) was added to liquid food to a final concentration of 1 μM. The mixture was stirred to homogenize and distributed into test tubes. Subsequently, the parental couples of the test groups were placed into the tubes.

In groups "t+/oxo-" and "t+/oxo+," the test tubes were heated to 38 °C for 1 h on the 5th day of the experiment after removing the parents. Next, the test tubes were returned to the room with normal temperature. By this time, some of the larvae reached the final 3rd age. Others were of an earlier age or still in the egg stage.

The emergent imagoes were calculated on the 9th, 11th, 13th, 15th and 18th days of the experiment. The flies were etherized and frozen for subsequent DNA isolation from tissues. The time for collecting the flies was restricted to 18 days to prevent the emergence of the next generation individuals developing from eggs deposited by the first offspring. The cycle of *D. melanogaster* development from egg to imago at 22 °C is 12 days. Thus, the 18-day time restriction for collecting flies guarantees that there are no second-generation flies when calculating the breed. Only a small number of the flies are not calculated because in normal conditions most of them emerge on the third to fifth day after the start of breeding.

To study the protective effect of 8-oxo-dG under heat shock in *D. melanogaster* imagoes, we randomly selected flies from the general population and placed 10 of them into a test tube with food. The food used was either the regular food or contained 8-oxo-dG at a final concentration of 1 μM depending on the experimental group. The experimental groups were similar to those in the study of larvae.

After keeping for seven days, the flies in the "t+/oxo-"and "t+/oxo+" groups were heated to 38 °C for 1 h.

Calculation of the surviving flies was done on the first, second, and third days after heating began to estimate instant and delayed death.

Table 1
Results of ANOVA due to heat shock, 8-oxo-2′-deoxyguanosine, and their combined action on the overall emergence of *Drosophila melanogaster*.

Factors	The quantity of degrees of freedom	Mean square	F value	Level of significance
Experiment on larvae				
Heat shock	1	30668.9	176.63	$p < 0.001$
8-oxo-2′-deoxyguanosine	1	15624.4	89.99	$p < 0.001$
Interaction of factors	1	2136.9	12.31	$p < 0.001$
Random error	125	173.6		
Experiment on imagoes				
Heat shock	1	888,306	441,641	$p < 0,001$
8-oxo-2′-deoxyguanosine	1	209,306	104,061	$p < 0,001$
Interaction of factors	1	209,306	104,061	$p < 0,001$
Random error	156	2011		

Some of the test tubes were used to measure the ratio of 8-oxo-dG/dG in fly DNA. For this purpose, we used flies that survived until the end of heating.

2.2. Analysis of 8-oxo-dG content in Drosophila melanogaster DNA

Isolation of the fly DNA was done using a technique [Esipov et al., 2010] developed previously in our laboratory. This technique used proteolysis, step phenol-less extraction, sedimentation of nucleic acids, RNase treatment, and enzyme hydrolysis. The 8-oxo-dG content in DNA was determined using HPLC with electrochemical detection previously developed and validated in our laboratory.

2.3. Statistical analysis

Data processing was done using Excel 2007 and Statistica v.10 software.

Mean values and standard deviations for all studied parameters were calculated in Excel (2007).

Deviations from the normal distribution of emergent *D. melanogaster* flies in individual test tubes were evaluated using the Kolmogorov-Smirnov (K–S) criterion.

The impact of the studied factors on the survival of *D. melanogaster* was analyzed using two-factor ANOVA and accounting for factor interactions using Statisticav.10 software. The first factor was the presence or absence of heat shock, and the second was the presence or absence of 8-oxo-dG in the feed.

When processing the results of the study on the effect of heat shock and 8-oxo-dG on the larval stages of Drosophila, multiple pairwise comparisons were made between several experimental variants using Dunn's criterion.

This criterion includes an adjustment for multiple comparisons. In addition, it was chosen because it is non-parametric, meaning that it does not require sample distributions to be normal. In the "Statistica 10" package that was used for statistical data processing, the Dunn's criterion is located in the "Comparing multiple indep samples (groups)" section of the "Nonparametric Statistics" module.

Before combining the results of experiments performed at different times, we performed a test on the homogeneity of these samples. This was done using the nonparametric Mann-Whitney test, an analogue of the parametric *t*-test, because the distribution in the two samples differs from normal. The homogeneity test showed that, according to the number of surviving flies, there was no considerable difference between experiments performed at different times ($U = 3140.5$, $p = 0.84$).

3. Results and discussion

3.1. Influence of heat shock on the emergence of Drosophila melanogaster flies

The influence of elevated temperatures considerably reduces the number of emergent imagoes. Incubation for 1 h at 37–38 °C decreases the total quantity of emergent imagoes by 50–80% compared to the control quantity (see Fig. 1). The dispersion values within one experiment option are probably determined both by random factors and by the genetic heterogeneity of the material. Thus, the dispersion reflects the variability of the studied population with respect to the temperature shock response.

Massive larvae death in a short time (right after the end of heating) at this temperature was scarcely observed. On the other hand, the temperature shock caused subsequent death (darkening) of pupae, indicating the effect of this factor on fly metamorphosis. Its impact on the other preimaginal stages like the egg must not be ruled out because the complete, normal *D. melanogaster* development cycle from egg to imago at 22 °C can be maintained within an interval from the time of temperature shock influence to the end of calculation of adult fly emergence (13 days).

The mortality of larvae is up to 90–100% when using higher temperatures (39–40 °C) during the same time (imago emergences are single). Both quick death (dead motionless larvae right after removal of test tubes from the thermostat) and delayed death at the pupation stage occur. The effect of the 45–50 °C temperature during the same time leads to complete and quick death of all larvae and eggs.

Based on the imago emergence dynamics curve (see Fig. 2), larvae at late development stages close to pupation are most vulnerable to the temperature effect. They almost completely die by the imago stage. Larvae subjected to temperature shock at early stages are much more likely to finish their life cycle. The visually observed "dead" pupae and the "senior" larvae do not alternatively explain a similar form of the curve of imago emergence with a delay of larvae development and later transition to pupation.

Note that in the statistical analysis of the group subjected to heat

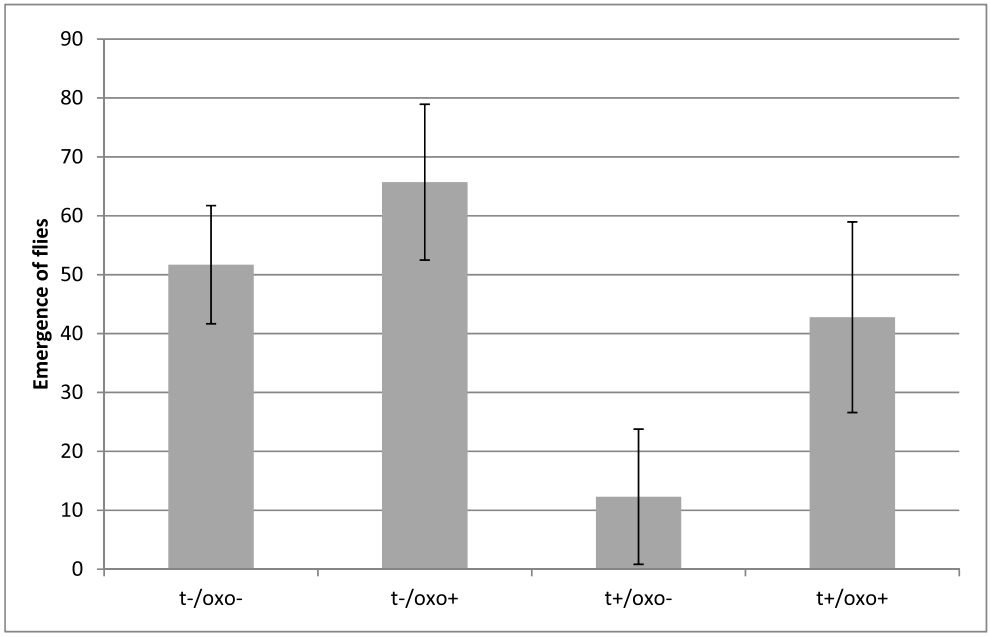

Fig. 1. Average emergence of flies in experimental variants testing the effect of heat shock and protective effect of exogenic 8-oxo-2′-deoxyguanosine. Group: t-/oxo-: flies that received food without additives at 22 °C; t-/oxo +: flies that received food containing 1 μM of 8-oxo-dG at 22 °C; t +/oxo-: flies that received food without additives, heat shocked for 1 h (38 °C) on the 5th day, and at 22 °C for the rest of time; t +/oxo +: flies that received food containing 1 μM of 8-oxo-dG, heat shocked (38 °C) for 1 h on the 5th day, and at 22 °C for the rest of time. The differences between the t-/oxo+ and t+/oxo + groups are not significant. The differences between all the other groups are significant: $p < 0.05$, analysis with the use of Dunn's criterion (Table 2).

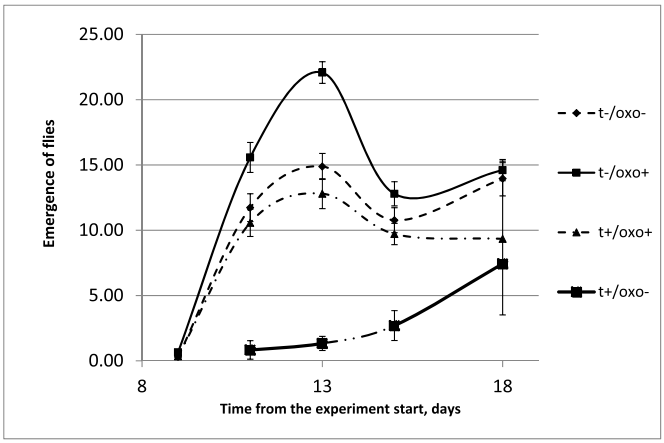

Fig. 2. Dynamics of imago emergence in experimental groups. Group: t-/oxo-: flies that received food without additives at 22 °C; t-/oxo+: flies that received food containing 1 μM of 8-oxo-dG at 22 °C; t+/oxo-: flies that received food without additives, heat shocked (38 °C) for 1 h on the 5th day, and at 22 °C for the rest of time; t+/oxo+: flies that received food containing 1 μM of 8-oxo-dG, heat shocked (38 °C) for 1 h on the 5th day, and at 22 °C for the rest of time.

shock had pronounced deviations from the normal distribution that were not present in three other experimental groups (see Fig. 3). This indicates that there can be interfamily differences in shock resistance in our population. However, there are very few of these families, and the effect manifests itself in distribution asymmetry or deviation from normality. Test tubes with low imago emergence prevail in the heat shock group, and there are almost no points with moderate emergence typical of the other groups.

Table 2
Multiple comparison by Dunn's criterion (Q) *.

Compared variants of various experiments		Q	Significance level
t-/oxo-	t-/oxo+	2.91	$p < 0.05$
t-/oxo-	t+/oxo-	5.39	$p < 0.001$
t-/oxo-	t+/oxo+	1.33	n.s.
t-/oxo+	t+/oxo-	8.94	$p < 0.001$
t-/oxo+	t+/oxo+	4.64	$p < 0.001$
t+/oxo-	t+/oxo+	4.41	$p < 0.001$

3.2. Influence of 8-oxo-dG on the development of Drosophila melanogaster

When adding 8-oxo-dG in the nutrient medium, no toxic effects were observed in parent flies or developing larvae. Moreover, a tendency for the number of drosophilae that successfully completed development cycle increased compared to the reference group. This is especially noticeable at the middle and early stages on the 11th and 13th days of the experiment (see Fig. 2). Unfortunately, the experiment design does not allow us to determine whether this is caused by an increase in the parent couple fertility, improvement of larvae survival rate, or by development acceleration.

Theoretically, the effect of toxic stress release in larvae that fall behind in their development under the effect of the metabolites of quickly growing senior flies is probable. However, this hypothesis requires additional test.

3.3. Protective effect of 8-oxo-dG on the larval stages of Drosophila melanogaster according to the heat shock model

Drosophila larvae that received 8-oxo-2′-deoxyguanosine in food

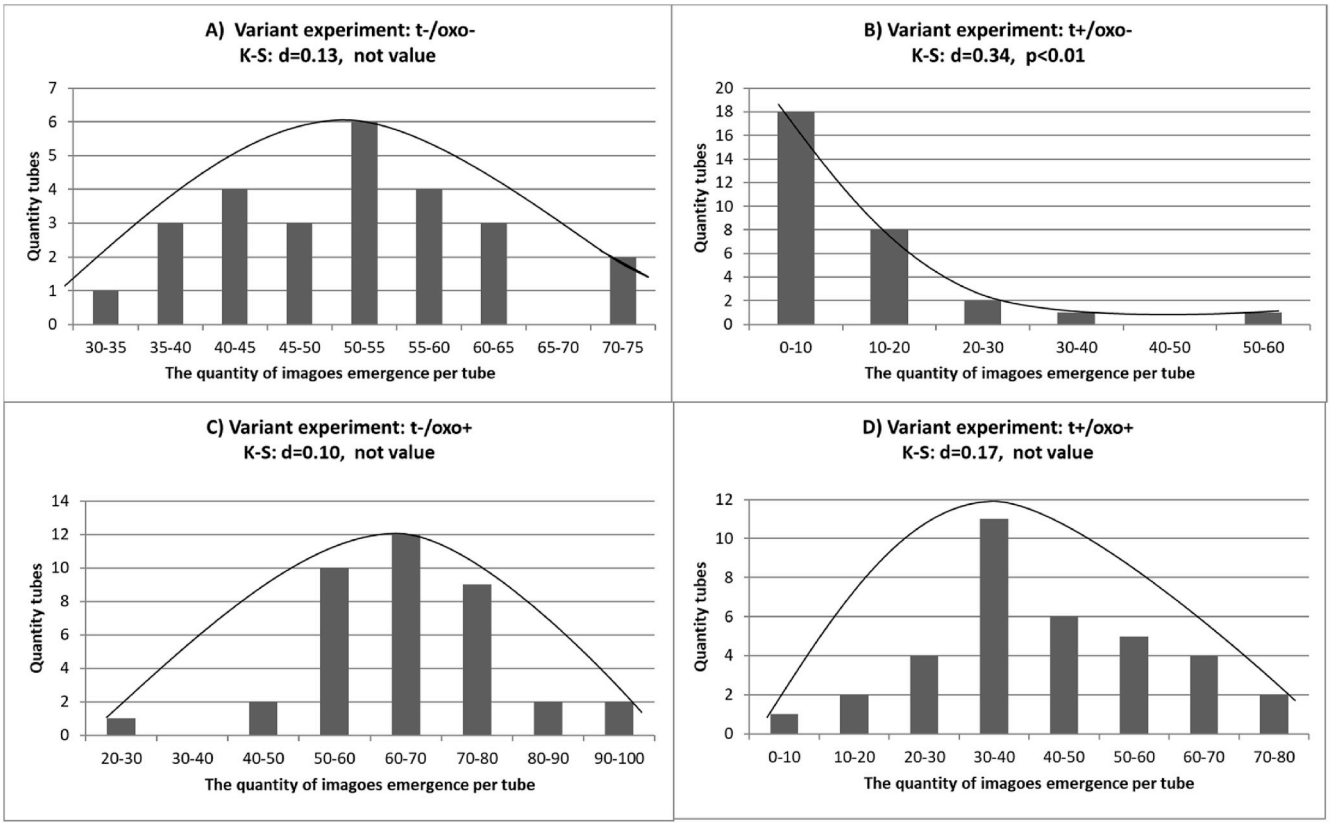

Fig. 3. Distribution of tubes by the number of *Drosophila melanogaster* imagoes emerging in different experimental variants. The graphs show the results of the test for deviation from the normal distribution of the K–S criterion. The deviation is fixed only for the variant of the experiment t/oxo- (B) Analysis of distributions of overall emergence by test-tubes in experimental groups.

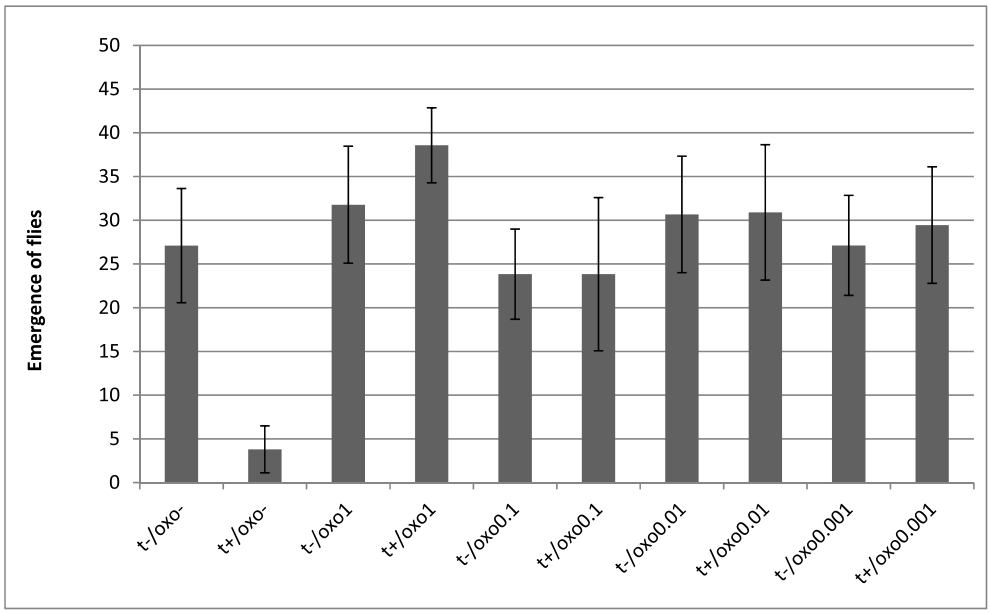

Fig. 4. Investigation of the concentration-dependence of the protective effect of 8-oxo-dG. Groups: t-/oxo-control, cultivation on standard feed at normal temperature; t + /oxo-heat shock for 1 h (38 °C), standard food; t + /oxo1 - heat shock for 1 h (38 °C) and cultivation with 1 μM 8-oxo-dG; t-/oxo1 - cultivation with 1 μM 8-oxo-dG; t + /oxo0.1 - heat shock for 1 h (38 °C) and cultivation with 0.1 μM 8-oxo-dG; t-/oxo0.1-cultivation with 0.1 μM 8-oxo-dG; t + /oxo0.01 - heat shock for 1 h (38 °C) and cultivation with 0.01 μM 8-oxo-dG; t-/oxo0.01 - cultivation with 0.01 μM8-oxo-dG; t + /oxo0.001 - heat shock for 1 h (38 °C) and cultivation with 0.001 μM 8-oxo-dG; t-/oxo0.001 - cultivation with 0.001 μM 8-oxo-dG. The errors of the mean are indicated.

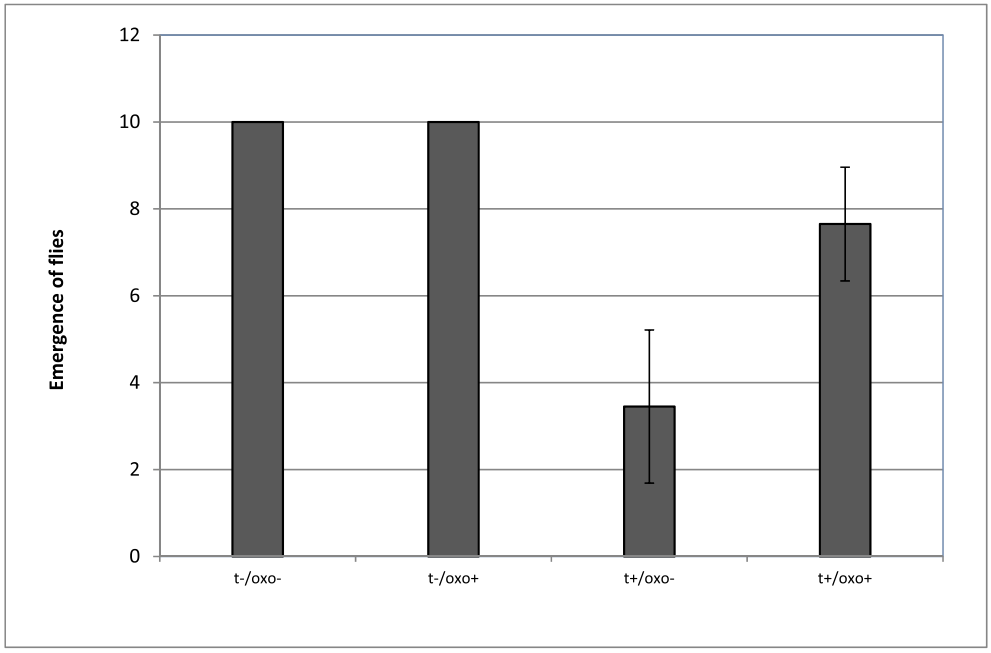

Fig. 5. The average number of surviving flies per test tube in experimental groups. Groups: t-/oxo- - intact flies; t-/oxo + - flies receiving 8-oxo-dG with food for 7 days; t+/oxo-flies heat shocked at 38 °C for 1 h; t +/oxo + - flies that received 8-oxo-dG with food for 7 days and heat shocked at 38 °C for 1 h. Mean errors are indicated. There are significant differences (the Dunn test, $P < 0.001$) between the groups t-/oxo- and t+/oxo-, t+/oxo- and t+/oxo+.

were 3–4 times as resistant to heat shock than control group larvae. Temperature increase to 38 °C within 1 h practically did not affect the total number of the imagoes that emerged from the test tubes with 8-oxo-dG-enriched food (see Fig. 1). Thus, the expected protective effect of 8-oxo-dG in heat shock was confirmed experimentally.

Considering the imago emergence dynamics curve (Fig. 2), we noted that 8-oxo-dG eliminates temperature's harmful effect on larvae that developed at late stages. The fly emergence curve for flies grown on food enriched with 8-oxo-dG and heat shocked has a shape similar to that for the groups grown at normal temperature. Maxima of imago emergences in all these three groups fall on the 13th day of the experiment, but the flies grown on food without additives and subjected to heat shock emerge at later stages.

The dispersion analysis of the combined influence of heat shock and 8-oxo-2′-deoxyguanosine (see Table 2) showed considerable interaction between the factors. 8-oxo-dG increases fly emergence after temperature shock more markedly than with no shock.

This can be due to preventive intensification of the heat shock protein expression or other factors providing heat shock resistance under the influence of 8-oxo-dG. It is commonly known that "hardening" of organisms and cells by preliminary temperature increase that does not cause critical effects causes an increase in resistance to sublethal shock in the next time period. Heat shock proteins are produced as this takes place. These proteins enable the organism to endure heat shock. They play a significant role in cell metabolism at normal temperatures too, but they are vital during heat shock. However, this hypothesis also requires

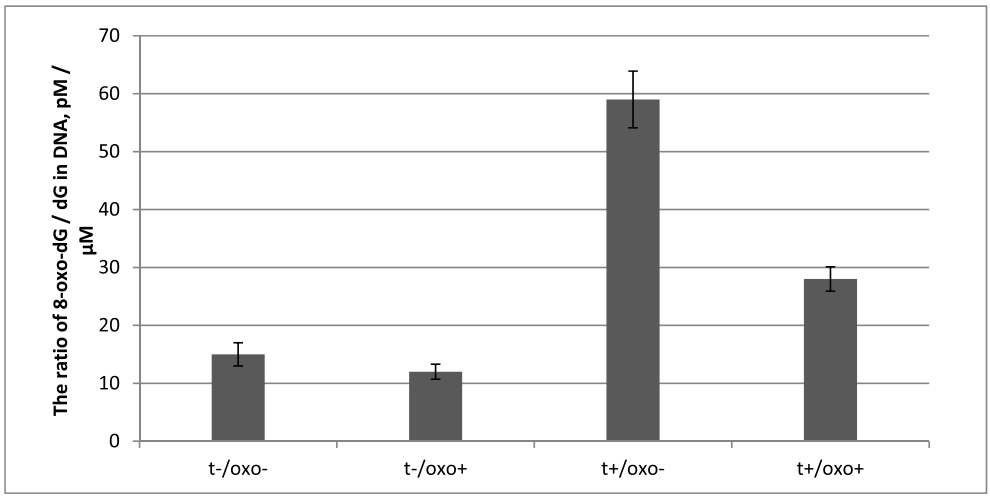

Fig. 6. The ratio of 8-oxo-dG/dG in the DNA of flies of experimental groups.
Groups: t-/oxo- - intact flies; t-/oxo + - flies receiving 8-oxo-dG with food for 7 days; t+/oxo-flies subjected to heat shock at 38 °C for 1 h; t+/oxo + - flies that received 8-oxo-dG with food for 7 days and heat shocked at 38 °C for 1 h. Mean errors are indicated. The Kruskal-Wallis test showed a statistically significant effect of temperature ($H = 8.308$, $p < 0.01$). The effect of 8-oxo-dG is not significant. ($H = 0.923$, $p = 0.337$). Multiple pairwise comparisons of the experimental variants according to the Dunn criterion at different combinations of temperature and oxo revealed a statistically significant difference between the variants "t+/oxo-" and "t-/oxo+" ($Z' = 2.717$, $p < 0.05$). The rest of the compared variants are not statistically different.

additional tests.

It should be also noted that the distribution of imago emergence by test tubes in the group with the combined heat shock and 8-oxo-dG is normal (see Fig. 3). This means that the addition of 8-oxo-dG negates the selective effect of heat shock on the heterogeneous *Drosophila* population.

3.4. Dependence of the protective effect of 8-oxo-dG on the larval stage of *Drosophila melanogaster* on its concentration

In the range of concentrations from 1 nM to 1 µM, no difference in the intensity of 8-oxo-dG-mediated protective effects on *D. melanogaster* larval stages under the conditions of heat shock was observed (see Fig. 4). 8-Oxo-dG in all the studied concentrations in this range increased the survival rate of flies subjected to heat shock up to the indicators of the control group.

Thus, it can be assumed that exogenous 8-oxo-dG affects *Drosophila melanogaster* under heat shock according to the alarm mechanism. First, it starts certain biochemical ways providing thermotolerance. Even nanomolar concentrations of the exogenous nucleoside is sufficient for signal transmission. Small GTPase proteins are probable mediators in the alarm chain. There are data in the literature indicating the ability of 8-oxo-dG to interact with these proteins and change their activity [Choi et al., 2007]. Small GTPases participate in cell viability regulation, particularly under stress conditions, and in morphological rearrangement.

3.5. Protective effect of exogenous 8-oxo-dG on Drosophila melanogaster imagoes under heat shock

Heating to 38 °C for 1 h resulted in the death of about 60% of the flies (see Fig. 5). On average, 85% of them died even during the heating: by the time the test tubes were extracted from the thermostat they were already dead. The remaining flies died on the first day after the heat shock. No deaths were observed on the third and subsequent days.

The flies that received food with 8-oxo-dG within a week before the heat shock showed a much higher survival rate. The share of dead flies in this group was about 30%, which is half the flies who had received food without additives during this week. The death of the flies in the t+/oxo + group was instant, and delayed effects were not observed.

Thus, the experiment showed pronounced protective effects 8-oxo-dG under heat shock after preventive application. In addition, no toxic effects of 8-oxo-dG were noted in any of the groups during the experiment.

3.6. Influence of exogenous 8-oxo-dG on 8-oxo-dG/dG ratio in Drosophila melanogaster DNA under normal or heat shock conditions

The 8-oxo-dG/dG ratio in DNA is a recognized and accepted biomarker of oxidative damage. It increases under stress, diseases, and pathologies [Valavanidis et al., 2009]. We expected a significant increase in the 8-oxo-dG/dG ratio in the DNA of heat shocked flies. Indeed, in the DNA of *D. melanogaster* imagoes in the first three days after the thermal shock (the t+/oxo-group), it was almost 4-fold higher than the control group (see Fig. 6).

Currently, the effect of exogenous 8-oxo-dG on the 8-oxo-dG/dG ratio in DNA remains a matter of dispute. In spite of the fact that experiments with marked 8-oxo-dG suggested the impossibility of embedding it in the DNA [Kim and Chung, 2006], a number of researchers noted an increase in the DNA oxidative damage level under the effect of exogenous 8-oxo-dG [Hyun et al., 2000]. But they used cell lines defective for DNA repair proteins (in particular, a line of myeloid leukemia cells KG-1). However, we do not know from the literature of similar experiments on entire organisms. Our experiment did not show obvious differences between the 8-oxo-dG levels in the DNA of flies of groups "t-/oxo-" (control) and "t-/oxo+" (receiving food with 8-oxo-dG). In other words, exogenous 8-oxo-dG did not result in 8-oxo-dG/dG increase in DNA.

In case of the t+/oxo + group, the 8-oxo-dG/dG ratio in DNA was approximately 2.5-fold lower than the t+/oxo-group. That is, the protective effect of 8-oxo-dG is evident also with respect to oxidative DNA damage under heat shock.

Thus, not only did we find an increase in the content of 8-oxo-dG in *Drosophila melanogaster* DNA under the effect of the exogenous oxidized nucleoside, but we also showed a considerable reduction in this indicator in flies subjected to heat shock in the case of 8-oxo-dG introduction.

In addition, analysis of imago DNA grown from the larvae that survived the experiment of article 3.3 did not reveal differences in 8-oxo-dG levels between the experimental groups.

This experiment has confirmed – at the organismal level – the impossibility of embedding exogenous 8-oxo-dG in cellular DNA even against the background of actively occurring replication. The flies grown up from the very beginning of their development on the food enriched with 8-oxo-dG did not show a enrichment of this modified nucleotide in DNA. No developmental irregularities or other visually noticeable deviations were found among them. Similar results have been previously obtained when cultivating cells in a medium enriched both with 8-oxo-dG and 8-oxo-dGTP. Moreover, an enzyme hydrolyzing the phosphate group of 8-oxo-dGTP and preventing its use by polymerases has been found [Henderson et al., 2010]. Our data additionally confirm that

exogenous 8-oxo-dG has no mutagenic activity.

In addition, heat shock at early stages of development did not lead to oxidative DNA damage accumulation in *Drosophila* imagoes. It is likely that tissue rearrangements in the course of metamorphosis level the "load" of damage and mutations accumulated by the larval stages.

4. Conclusion

The hypothesis of possible protective activity of 8-oxo-dG in the conditions of heat shock was confirmed experimentally. The mechanism of this effect remains open and requires further study.

The additional confirmation of 8-oxo-dG biological activity can serve as a powerful argument in favor of the considerable regulatory role of this biogenic compound. Apparently, the heterogeneous population of *Drosophila* has genotypes with higher and lower intensity of response to exogenous 8-oxo-dG. It is still impossible to tell which genes are responsible for the response to exogenous 8-oxo-dG. However, such individual differences in the response indicate that this nucleoside does not work as a "direct" antioxidant or protector nor due to its own physical and chemical properties. Rather, it acts as a signal starting certain physiological and biochemical response from a cell or even from an organism in general.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.102446.

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