Determine the optimum quantity of antioxidant substance for expanding the lifespan of *Drosophila*

KONISHI Kyoka HIGASHI Fumika HONDA Kazuki MIYASHITA Daisuke YAMAGISHI Fumiya

Abstract

Our goal is to determine the optimum quantity of antioxidant substance to extend *Drosophila*'s lifespan with the aim of discovering a key to improve the length of a human lifespan. We put the focus on the potential of antioxidant action to remove ROS (Reactive Oxygen Species: a high reactivity by-product of the oxygen consuming process in aerobic creatures). We think that an excessive intake of antioxidant substance shortens *Drosophila*'s lifespan.

１. Background and purpose

In recent years, many people have been interested in "anti-aging". We thought that reducing oxidative stress in cells of living things might lead to anti-aging, so we focused on this stress.

Oxidative stress is the harmful function caused by oxidative reactions and it results from the balance between active oxygen and antioxidant substance. Also active oxygen has a disadvantage which promotes aging and an advantage which protects us from bacteria. Therefore we thought that we should not simply reduce the amount of active oxygen.

The purpose of our study is to discover an antioxidant substance which can efficiently reduce the stress of living things, and the optimum quantity of this substance.

We used *Drosophila* because its life cycle is short and breeding them is easy. Also according to previous study, *Drosophila* and human have a similar digestive organ.

２. Methods

<Experiment 1: Change of the survival rate by intake of antioxidant substances>

We gave olive oil which contains much vitamin E as an antioxidant substance or iron which causes oxidative stress to *Drosophila* and then we examined the survival rate of *Drosophila*, which were given stress.

1. We bred *Drosophila* in normal culture and we used newly hatched male of *White* for our experiments.

2. A: normal culture, B: antioxidant substance culture (olive oil culture), C: iron culture. We bred only 16 males in each of these cultures for 3 days.
3. After 2, we bred *Drosophila*, which had been bred in A, B and C at 32 degreess for 5 days and then recorded the survival rate.

The size of a male *Drosophila* is smaller than that of a female and the entire and the section of the abdomen on the backside of male is black. Generally the lifespan of *Drosophila* is as long as 80 days, but according to the previous research, the lifespan is only about 2 days when we breed them at a high degrees.

<Experiment 2: conducting quantitative analysis of iron which *Drosophila* took into the body>

According to the previous research, we are sure that *Drosophila* take iron into their body but to confirm that the cause of the rapid decrease in the number of Drosophila is iron, we examine the amount of iron with chelate reagent which Drosophila take into body.

A. in normal culture for one day
B. in culture with 5%Fe for one day
C. in culture with 10%Fe for one day
D. in culture with 10%Fe for two days

under each of these conditions, we bred 16 *Drosophila* for two days at 25 degrees, and put some samples from the *Drosophila*'s abdomen with them with them. We examined the amount of iron by the absorbance method with 110-phenanthroline. We use the reaction between Fe2+ and 1,10-phenanthroline. If Fe2+ was detected, it turned red. We used (NH4)2Fe(SO4)2・6H2O to make a calibration curve.

① We dissolved 7g of (NH4)2Fe(SO4)2・6H2O in water, added 2ml of HCl 6mol/L and diluted to 1L (Iron ion standard solution)

②We dissolved 0.24g of 1,10-phenanthrolinehydrochloride in 200ml of water.

② We mixed 0.84g of CH_3COONa dissolved in 100g of water into 0.1mol/L of CH_3COOH. We mixed an equal volume of them. (Acetate buffer solution)

③ We dissolved 10g of hydroxyl ammonium chloride in 100ml of water.

④ We diluted the iron ion standard solution 100 times, and put 5ml, 15ml, and 25ml into measuring flask(50ml).

⑥We added $HONH_3Cl$ solution to each solution.

⑦We added 1,10-phenanthroline solution to each solution.

⑧We added 10ml of acetate buffer to each solution.

⑨We diluted it with refined water and measured the absorbance of a sample solution by using the absorptiometer (510nm).

⑩We put 16 Drosophila which were raised under A~D conditions into 2ml of acetic buffer

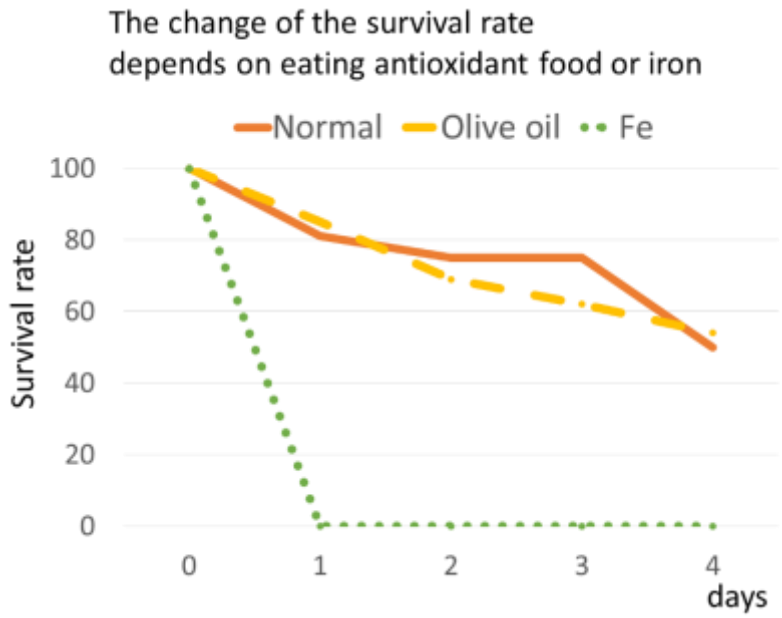
solution and mixed it. Next we took 2ml of each solution and conducted ⑥~⑨ operations.

⑪Similarly we made a sample as a control experiment.

⑫We measured the absorbance of the sample solution and standard sample with the absorptiometer on 510nm wavelengths.

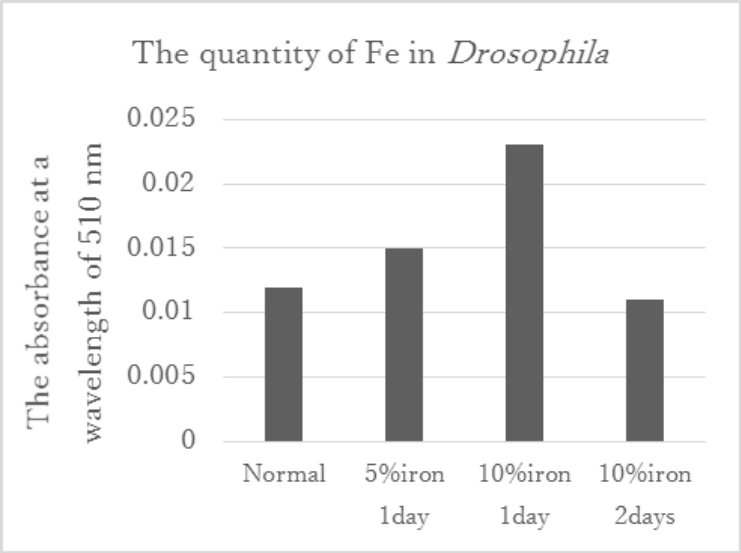
3. Result

<Experiment 1: Change of the survival rate by uptake of antioxidant substances>



Drosophila growing in iron culture died out in the first day. There was no difference between the survival rate of *Drosophila* growing in normal culture and the survival rate of *Drosophila* growing in olive oil culture.

<Experiment 2: conducting quantitative analysis of iron which *Drosophila* took into the body>



We found that there is a direct relationship between the absorbance at a wavelength of 510 nm and iron concentration from the experiment using the standard iron sample. So we measured the quantity of iron in *Drosophila* with an absorbance meter. The quantity of iron in *Drosophila* growing in B and C cultures was more than that in *Drosophila* growing in A culture. It means that *Drosophila* ate the iron from the culture. We found from the result of B and C that the higher the concentration of iron culture we gave the more iron *Drosophila* ate. And we found that the concentration of iron in *Drosophila* which were bread in iron culture for two days was lower than that in *Drosophila* which were bread in iron culture for a day.

４. Consideration

According to experiment 1, the lifespan of the *Drosophila* which were bread in iron cultures were shortened. This is because the amount of active oxygen in *Drosophila* was increased and they destroyed *Drosophila*'s cells. According to the experiment2, we revealed that the *Drosophila* absorbed iron by eating food with which contains it. And we also revealed that the *Drosophila* not only eat but also drain iron. However there were no differences of lifespan between normal cultures and antioxidant cultures. We think there are three reasons why this occurred

Ⅰ, the experimental period was too short.

Ⅱ, the antioxidant substances in the *Drosophila* did not work.

Ⅲ, the *Drosophila* did not absorb antioxidant substances.

In our experiment, we could not investigate the amount of antioxidant substances they actually absorbed. So we can say that the normal culture had antioxidant substances originally and they extended the lifespan of *Drosophila*. What we want to do in the

future is to make it clear the amount of antioxidant substances and active oxygens they really take in.

５．The future prospect

We are going to conduct more experiments in order to investigate what kind of antioxidant substances is most appropriate to expand *Drosophila*'s lifespan. We will use some antioxidant substances we can get easily: green tea, soy sauce, and soy milk. We need to use various substances in the experiments and compare these effect on prolongation of *Drosophila*'s life.

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７．Key words

Drosophila, culture