

The Study on Antioxidation and Mechanism of 2 - Bromo - 4'- Nitroacetophenone

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Abstract

The 2-Bromo-4'-nitroacetophenone is an important compound which is widely used in the modification of methionine residues in alpha-chymotrypsin, organic synthesis materials and pharmaceutical intermediates. Our laboratory recently found that the 2-bromo-4'-nitroacetophenone can extend the lifespan of the *C. elegans*. Therefore, we want to know whether this compound has the anti-oxidative ability.

In this paper, we used three different concentrations of 2-bromo-4'-nitroacetophenone to feed normal *C. elegans* and the mutants (*daf-16*). we found that 2-bromo-4'-nitroacetophenone has antioxidant effect and this effect is dependent on Insulin pathway.

Keywords: *C. Elegans*, 2-bromo-4'-nitroacetophenone, anti-oxidation

1. Introduction

Upon stepping into the twenty-first century, how to solve the problem of aging is attracting more and more people's attention. The current majority of the country's population reached or exceeded the total population for more than 10 percent. With the lifting living standard, how to solve the problem of aging will be the arduous task facing mankind (Zhuang et al., 2016; Weiming Zhang et al., 2013). From the biological point of view, aging is the decline of body structure and ability with the time passing by. From the pathological point of view, aging is due to the body's cell state emergency caused damage, resulting in the body's immune response to decline and metabolism (Jiong Wu et al., 2017; Guo SH et al., 2007;).

The essence of aging is the process of making the various tissues and organs of the organism unable to meet the needs of the organism as the age increases. Although aging is the inevitable result of nature (Nie GJet et al., 2002, Gami MS &Wolkow CA, 2006), it is found

that the process of aging can be effectively carried out by some biological means (Wilkinson DS et al., 2012; Sangha JS et al., 2012) . In this experiment, we used the *C. elegans* as a model organism to feed normal *C. elegans* and mutants with 2-bromo-4'-nitroacetophenone and found that 2-bromo-4'- Nitroacetophenone has antioxidant properties. The mechanism is mainly through the control of the insulin pathway to achieve.

2. Materials and Methods

2.1 Reagents and C. elegans Strain Preparation

The 2-bromo-4'-nitroacetophenone is obtained from Sigma-Aldrich (St. Louis, MO, USA), and its purities is 95%. It was dissolved in M9 solution and then diluted by M9.

Nematodes used were wild-type *C. elegans* N2, mutants of *daf-16* originally obtained from Caenorhabditis Genetics Center. They were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 at 20°C as described.

Gravid nematodes were washed off the plates into centrifuge tubes, and were lysed with a bleaching mixture (0.45 M NaOH, 2% HOCl). Age synchronous populations of L1-larvae nematodes were obtained by the collection. Nematodes were washed with a modified S medium (Ren HR et al,2011; Brenner S, 1974) . Exposures were performed from L1-larvae to adult (prolonged exposure) in K medium of 96-well sterile tissue culture plates at 20°C incubator in the presence of food. Paraquat was added forty eighteen hours later (Donkin S & Williams PL, 2012).

2.2 Locomotion behavior

To determine the Locomotion behavior of lifespan. Head thrash and body bend were used as endpoints (Akesson Chet al., 2003). The number of head wobbles and body bending times of *Caenorhabditis elegans* in the specified time were observed.

During the locomotion behavior assay, the examined nematodes were transferred into the assay plate containing K medium on top of the agar. After a recovery time of 1 min, head thrashes, and body bends were counted for 1 min and 20s respectively. (Kim J et al., 2008; Louppis A P et al., 2010).

2.3 Stress assay

To assay thermal stress resistance, worms pre-treated with the 2-bromo-4'-nitroacetophenone for 48-hr were transferred to the 35°C condition, and then the lifespan was measured and analyzed as described above. To determine antioxidant activity, paraquat was added to *Caenorhabditis elegans* pre-treated with 2-bromo-4'-nitroacetophenone for 48 hours, and then the longevity was measured and analyzed as described above (Honda Y et al., 1999; Sutphin GL et al., 2012) .

2.4 Statistical analysis

All data in this article are expressed as mean \pm standard error (S.E.M.). Generate charts using Microsoft Excel (Microsoft Corporation, Redmond, WA). Statistical analysis was performed

using SPSS 12.0 (SPSS Inc., Chicago, USA). Differences between groups were determined by analysis of variance (ANOVA). Probability levels 0.05 and 0.01 are considered statistically significant.

3. Results

3.1 2-Bromo-4'-nitroacetophenone has antioxidant effect on *C. Elegans*.

The treatment is carried out with 2-bromo-4'-nitroacetophenone throughout the life of the larval stage. We can see the 2-Bromo-4'-nitroacetophenone has an antioxidant effect on *C. Elegans*(Figure 1).

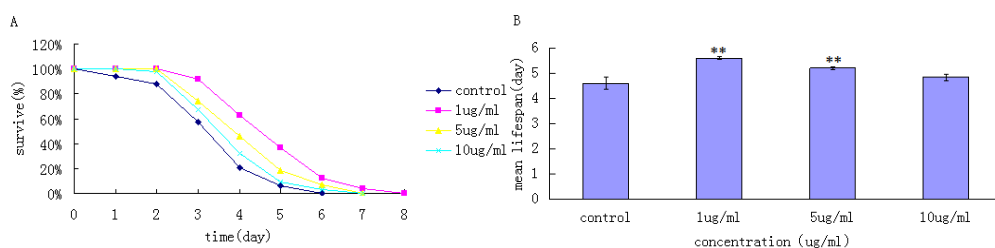


Figure 1. The 2-Bromo-4'-nitroacetophenone has antioxidant properties. (A) Survival curve of nematodes after pretreatment at different concentrations of 2-Bromo-4'-nitroacetophenone in paraquat-containing medium. (B) The average lifespan after treatment of the *Caenorhabditis elegans* with 2-Bromo-4'-nitroacetophenone in paraquat-containing medium. (*P < 0.05, **P < 0.01)

3.2 The 2-Bromo-4'-nitroacetophenone has improves the locomotion behavior of *Caenorhabditis elegans*.

We studied the effects of 2-bromo-4'-nitroacetophenone on the locomotion behavior of *C. elegans* and found that the nematode treated with 2-bromo-4'-nitroacetophenone significantly improved the motor behavior of *C. elegans*(Figure 2).

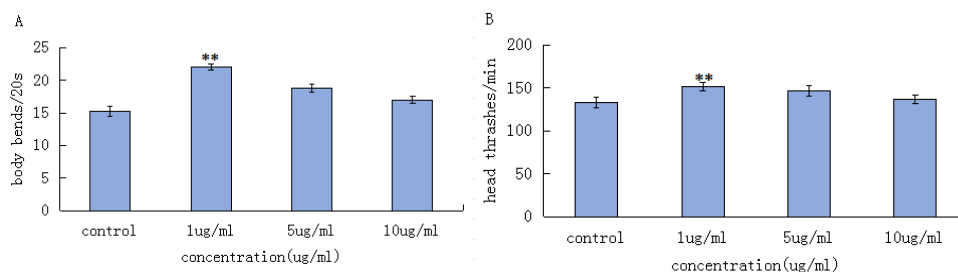


Figure 2. The 2-bromo-4'-nitroacetophenone improves the locomotion behavior and reproductive capacity of *Caenorhabditis elegans*. (A) Progeny of animals exposed to 2-bromo-4'-nitroacetophenone on locomotion behavior as indicated by head thrashes. (B) Progeny of animals exposed to 2-bromo-4'-nitroacetophenone on locomotion behavior as indicated by body bends. (*P < 0.05, **P < 0.01)

3.3 2-bromo-4'-nitroacetophenone improves the heat stress of *Caenorhabditis elegans*

The *C. elegans* treated with 2-bromo-4-nitroacetophenone were transferred to a temperature of 35°C to determine 2-bromo-4'-nitroacetophenone have resistance of *C. Elegans*. The mean lifespan the effect of *C.elegans* treated with 2-bromo-4'-nitroacetophenone Increased. Indicating that the 2-bromo-4-nitroacetophenone improved the heat stress of the *C. Elegans* (Figure 3).

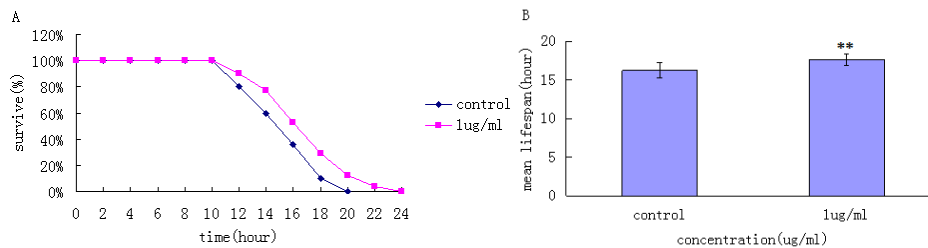


Figure 3. The 2-bromo-4-nitroacetophenone improves the heat stress of *caenorhabditis elegans*.. (A) Survival curves of *C. elegans* pretreated with 1 ug/ml or without of 2-bromo-4'-nitroacetophenone in 35 °C. (B) Mean lifespans of *C. elegans* pretreated with or without 2-bromo-4'-nitroacetophenone in 35 °C. (*P < 0.05, **P < 0.01)

3.4 2-bromo-4'-nitroacetophenone has antioxidant effect and this effect is dependent on Insulin pathway

Compared with the control group, the mutants of *C. elegans* had significantly lower antioxidant activity, indicating that 2-bromo-4'-nitroacetophenone on *Caenorhabditis elegans* oxidation resistance through control of genes *daf-16* to achieve.(Figure 4)(Xu X et al., 2007; I.N. et al 2003).

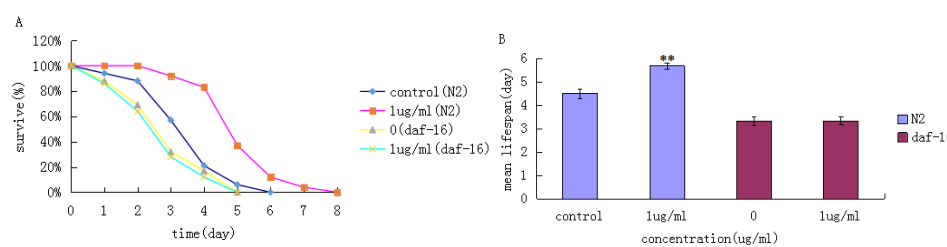


Figure 4. Antioxidant properties in wild-type and mutants exposed to 2-Bromo-4'-nitroacetophenone . (A) Survival curves of N2 and *daf-16* pretreated with different densities of 2-Bromo-4'-nitroacetophenone in paraquat-containing medium. (B) Mean lifespans of N2 and *daf-16* pretreated with different densities of 2-Bromo-4'-nitroacetophenone in paraquat-containing medium. (*P < 0.05, **P < 0.01)

Compared with the normal wild-type *C. elegans*, the transformed body of *C. elegans* had a much weaker body curvature and head swing. This further confirms our conclusion. (Figure

5).

In summary, we can demonstrate that daf-16 participates in the antioxidant regulation of *C. elegans* by mutant daf-16, that is, the antioxidant activity of *C. elegans* is mainly controlled by genes. (Figure 4 and Figure 5).

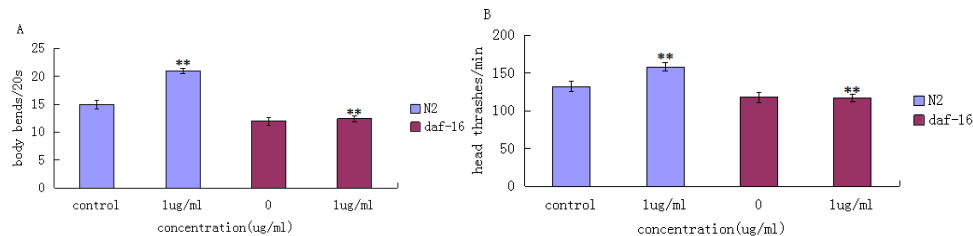


Figure 5. Locomotion behaviors in wild-type and mutants exposed to EP. (A) Effects of 2-Bromo-4'-nitroacetophenone on locomotion behavior in daf-16 and N2 as indicated by body bends. (B) Effects of 2-Bromo-4'-nitroacetophenone on locomotion behavior in daf-16 and N2 as indicated by head thrashes. (*P < 0.05, **P < 0.01)

4. Discussion

In this experiment, we found that 2-bromo-4'-nitroacetophenone had a significant antioxidant effect, and reached its best at 1ug/ml concentration [Figure 1]. In addition, compared with the control group, the nematode treated with 2-bromo-4'-nitroacetophenone significantly improved the motility of the *Caenorhabditis elegans* during the aging process [Figure 2]. These results indicate that 2-bromo-4'-nitroacetophenone has a potential anti-oxidative effect.

Under the thermal stress conditions, the *C. elegans* treated with 2-bromo-4'-nitroacetophenone were able to survive for longer periods under the same conditions compared to the control group [Figure 3), the condition of 2-bromo-4'-nitroacetophenone has the effect of improving heat stress.

As can be seen from Figure 4, under the same conditions, the survival rate of daf-16 nematode is reduced. From the above data, the compound 2-bromo-4'-nitroacetophenone Control of nematode life is achieved mainly through the control of the corresponding genes, in which daf-16 nematode life is of the greatest impact. That is to say 2-bromo-4'-nitroacetophenone has antioxidant effect and this effect is dependent on Insulin pathway.

Correspondingly, after the mutation of the *C. elegans*, its movement behavior will be correspondingly reduced, and these data further confirmed our conclusion. However, the mechanism of controlling the life of nematodes is very complex and requires more experiments to verify.

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