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Article

Albuterol extends lifespan of *Caenorhabditis elegans*

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SUMMARY

The objective of this experiment is to determine if the medication albuterol has a positive impact on the lifespan of *C. elegans*. We hypothesize that if albuterol is added to the diet of *C. elegans*, then the lifespan of *C. elegans* will increase. *C. elegans* were separately incubated in the medication albuterol at 20 µg/ml at the start of the L1 phase of their life cycle. Then they were closely monitored and counted daily throughout the duration of their lifespan. Albuterol increased the mean lifespan of *C. elegans* by 4.31 ± 0.13 days, compared to the control group and increased the outer range of the *C. elegans* lifespan. The medication albuterol increased the lifespan of *C. elegans*. The method of which this occurred is still unknown. Further testing is required to deduce whether mitochondrial biogenesis was the contributing factor or esophageal function.

INTRODUCTION

A prominent theory used to explain the aging process is the "free radical theory of aging" (1). This theory is based on findings that the cumulative oxidative damage caused by oxygen free radicals, a byproduct of cellular respiration occurring in mitochondria, speeds up the aging process (2-3). Specifically, during cellular respiration, oxygen free radicals are released as a side product of oxidative phosphorylation, the process used to generate ATP. These free radicals subsequently destroy a variety of cellular components, leading to accumulation of damage and the "aging process" in which bodily function gradually declines over time leading to death (1). Oxidative stress is also known to play a key role in the onset and/or promotion of cancer, cardiovascular disease, rheumatoid arthritis, nephropathy and other diseases (4).

There is evidence that various antioxidants can counteract oxygen free radicals and reduce the rate of aging (2, 5-6). Antioxidants can therefore extend the healthspan, the length of time a cell properly functions, and increase lifespan of organisms. Interestingly, decreased food intake, or "caloric restriction," has been shown to extend lifespan in a range of animals. In the nematode *Caenorhabditis elegans*, for example, a 40% reduction in caloric intake results in a substantial increase in lifespan (7-8). One hypothesis for this effect is that caloric restriction leads to a decreased production of oxygen-free radicals (7). Other manipulations shown to increase longevity in *C. elegans* include polyphenols (9) and heat shock (10).

Several compounds in a family of beta-adrenergic agonists have been known to induce a process called mitochondrial biogenesis which induces replication of the mitochondria and the regulation of oxygen free radical production. This process is known to reduce pathological oxidative stress (11). One example of such an agonist is the medication albuterol, a bronchodilator commonly used in the treatment of asthma (12). The focus of our investigation was to ask whether albuterol treatment alters the average lifespan of *C. elegans*, with the specific hypothesis that addition of albuterol to the diet of C. *elegans* increases lifespan of the animal. Albuterol was shown in this study to increase the lifespan of *C. elegans*. This would mean that albuterol's effect on free radical production could possibly correlate to human cells and therefore increase human lifespan.

RESULTS

We compared the lifespan of *C. elegans* under normal culture conditions and in culture medium containing 1µg/ml albuterol. Each culture contained approximately 80-120° *C. elegans* (strain SS104) with a mutation in *glp-4* which causes sterility when the worms are grown at 25°C but are fertile at 15°C (13). To avoid the complication of scoring the progeny of these self-fertilizing nematodes and the need to transfer the adults away from the progeny, worms were grown at 25°C for experimentation, and at 15°C to maintain the strain. In Trial 1, our experimental data indicated that albuterol increased the mean lifespan of *C. elegans* compared to the control group and extended the upper range (22 days compared to 16 days, respectively) of the lifespan (**Figure 1**). The albuterol group survival mean (10.83 ± 0.61 days) was 3.43 days longer than that of the control (7.41 ± 0.32 days), resulting in an increase in lifespan of 46.25% (**Table 1 and 3**; *p*-values: <0.0001 (log-rank), <0.0001 (Wilcoxon), and <0.0001 (Tarone-Ware)). The experimental data in Trial 1 recorded a Cox Proportional

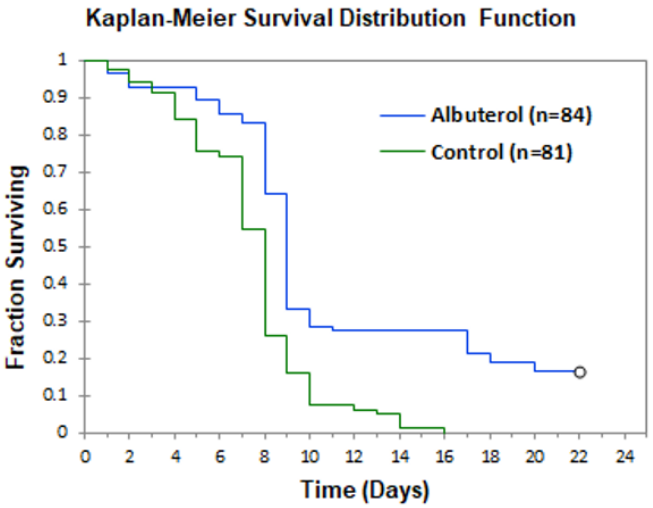

Figure 1. Survival distribution function representing the fraction of *C. elegans* remaining alive after each consecutive day for Trial 1. The first group of *C. elegans* was treated with 1 µg/ml of albuterol and the total worms surviving was measured 5 days of the week. The second group was not treated and the total worms remaining was measured 5 days of the week.

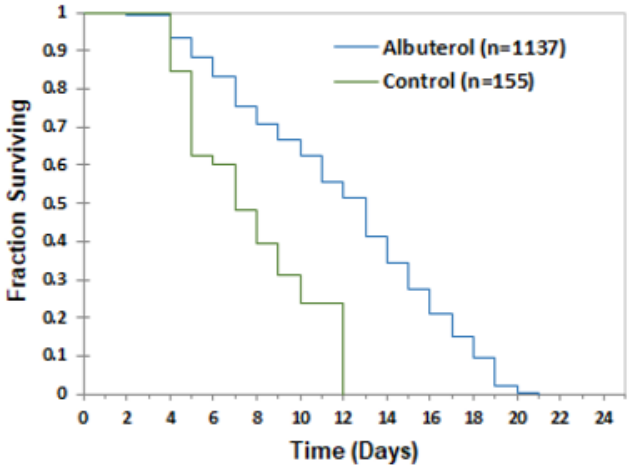

Figure 2. Survival distribution function representing the fraction of *C. elegans* remaining alive after each consecutive day for Trial 2. The first group of *C. elegans* was treated with 1 µg/ml of albuterol and the total worms surviving was measured 5 days of the week. The second group was not treated and the total worms remaining was measured 5 days of the week.

Hazard ratio of 2.99, meaning at any point in time, a control worm is 2.99 times as likely to die in comparison to a worm treated with albuterol. (Upper 95% Bound: 3.23, Lower 95% Bound: 1.64) An associated *p*-value < 0.0001 was also recorded.

In Trial 2, our experimental data indicated that albuterol increased the mean lifespan of *C. elegans* compared to the control group and extended the upper range (22 days compared to 14 days respectively) of the lifespan (**Figure 2**). The albuterol group survival mean (13.30 ± 0.13 days) was 4.31 days longer than that of the control (9.00 ± 0.25 days), resulting in an increase in lifespan of 47.9% (**Tables 2-3**; *p*-values: <0.0001 (log-rank), <0.0001 (Wilcoxon), and <0.0001 (Tarone-Ware)). The experimental data in Trial 2 also recorded a Cox Proportional Hazard ratio of 3.140, meaning at any point in time, a control worm is 3.14 times as likely to die in comparison to a worm treated with albuterol. (Upper 95% Bound: 3.75, Lower 95% Bound: 2.63) An associated *p*-value < 0.0001 was also recorded. The control lifespan observed was typical of *C. elegans*. Thus, albuterol appears to substantially slow the aging rate in this experimental treatment. The second trial is a repetition of the first pilot trial using a larger sample size. The control population in trial 2 was decreased due to time constraints.

Table 1. Test for the equality of the survival distributions in trial 1 (df = 1).

Statistic	Observed value	Critical value	*p*-value	alpha
Log-rank	31.948	3.841	< 0.0001	0.050
Wilcoxon	23.463	3.841	< 0.0001	0.050
Tarone-Ware	26.787	3.841	< 0.0001	0.050

Table 2. Test for the equality of the survival distributions in trial 2 (df = 1).

Statistic	Observed value	Critical value	*p*-value	alpha
Log-rank	188.276	3.841	< 0.0001	0.050
Wilcoxon	155.691	3.841	< 0.0001	0.050
Tarone-Ware	171.294	3.841	< 0.0001	0.050

DISCUSSION

The results of our experiment support the hypothesis that albuterol, which is known to induce mitochondrial biogenesis and relax smooth muscles, increases the lifespan of *C. elegans*. Further research is required to determine the biochemical pathway of albuterol in its extension of the *C. elegans* lifespan.

Albuterol functions by relaxing airway smooth muscles allowing for easier breathing in humans with obstructive lung diseases such as asthma and chronic obstructive pulmonary disease (14). Research suggests mitochondrial biogenesis, an effect of albuterol, improves cell survival (15).

Side effects of albuterol include nervousness or shakiness, headache, throat or nasal irritation, and muscle aches. Other more serious effects include tachycardia and heart palpitations (16). These effects must be taken into consideration when examining the results. An unanswered question is whether albuterol could have a similar effect on human longevity as in

Table 3. Comparison of average lifespan (days) for each trial.

Condition	Trial 1	Trial 2
Control	7.41	9.00
Albuterol	10.83	13.30

C. elegans, and if so, whether the possible beneficial effects of a long-term course of albuterol outweigh the adverse effects. No prior studies have been conducted that suggest albuterol extends longevity in any organism.

If the action of albuterol on *C. elegans* was through reduced feeding due to relaxed muscle function, the observed effect might reflect the impact of caloric restriction in the animal's lifespan, rather than the direct effect of albuterol on mitochondrial biogenesis. To determine the mechanism of action in the extension of *C. elegans* lifespan, further experiments would need to be conducted. As stated previously, the lifespan extension may be due to mitochondrial alterations or caloric restriction. To test this, we can assay the lifespan of *glp-4; eat-2* mutants, which have altered feeding behavior casing less intake due to the inhibition of pharyngeal pumping in the presence of food (17). When exposed to albuterol, if the longevity of the *glp-4; eat-2* double mutant displays a similar trend as the singular *glp-4* mutant, the finding would point towards a mechanism of action of caloric restriction. However, if the longevity of the double mutant in the presence of albuterol is further increased then it would indicate that albuterol may act to extend lifespan through mitochondrial alterations. This study indicated albuterol extended the lifespan of C. elegans which could suggest albuterol having similar effects on other organisms.

MATERIALS AND METHODS

C. elegans strains and maintenance

Caenorhabditis elegans strain SS104 was cultivated using standard lab practices (18). This strain possesses a mutation in *glp-4* which causes sterility when the worms are grown at 25°C but are fertile at 15°C (*13*). To avoid the complication of scoring the progeny of these self-fertilizing nematodes and the need to transfer the adults away from the progeny, worms were grown at 25°C for experimentation, and at 15°C to maintain the strain. Worms were grown in petri dishes containing 10 mL Nematode Growth Medium (NGM) (19) made according to standard protocol, with a total of 12 petri dishes containing approximately 100 worms each. Petri dishes were seeded with *E. coli* to serve as food for the worms.

C. elegans synchronization

A synchronized population of worms is obtained to assure all nematodes are at the same developmental stage. (20). Briefly, mixed stage worms and embryos were harvested from a plate of SS104 worms grown at 15°C in 1.5 mL of osmotically balanced M9 Buffer made according to standard lab practices (19). The worms and embryos were pelleted and the supernatant containing the bacteria was discarded and the worms were suspended in 1 mL of M9 buffer. Fresh alkaline bleach solution (0.5 mL; 1 mL Clorox bleach mixed with 0.5 mL 5M NaOH) was added to the worm and embryo suspension and mixed constantly by inversion until the suspension turned clear. The embryos were pelleted in a microcentrifuge, and the supernatant was discarded. This process was repeated three more times to remove all traces of bleach. The embryo suspension was then placed overnight on an agar plate devoid of bacterial food to generate a synchronous population of L1 larvae.

Albuterol treatment

To test the effect of albuterol on *C. elegans* lifespan, generic 2 mg oral albuterol tablets (Sun Pharmaceutical Industries, Inc.) were crushed and combined with sterile distilled water to obtain a concentration of 20 μg/mL. The albuterol solution (0.5 mL) was then spread on 10 of the 12 seeded plates to give a final plate concentration of 1 μg/mL albuterol. The plates were allowed to dry overnight.

The synchronized L1 larvae were suspended in M9 buffer, and 1 mL of the worm suspension was pipetted onto each of 12 labeled NGM plates with *E. coli*. After the plates dried, they were incubated at 25°C. A microscope was used to count the total number of living worms in each dish, and these counts were recorded in a daily table. Moving worms were counted as "Living," whereas still worms were counted as "Dead." Each plate was covered immediately after counting and returned to 15°C. The count continued daily until all worms were dead. All of the petri dishes were placed in the same incubator in order to account for external variables, such as temperature and light exposure, and the worms were counted at approximately the same time each day. All lifespans are given as the mean ± SD in days.

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