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The Effect of Reducing Endoplasmic Reticulum Stress by Tauroursodeoxycholic Acid on *Caenorhabditis elegans* Lifespan

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Abstract

Endoplasmic reticulum (ER) stress is involved in many diseases including diabetes, cancer and aging. Tauroursodeoxycholic acid (TUDCA) is a chemical chaperone that reduces ER stress. *Caenorhabditis elegans* (*C. elegans*), a widely used model organism in aging studies, also suffers from ER stress. In this study, we tested the effect of TUDCA on *C. elegans* life span with the aim to decipher the contribution of ER stress on *C. elegans* aging. We tested TUDCA at the concentrations of 0.625, 1.25, 2.5, 3.75, 5, 7.5, 10, 15 and 20 mM. It showed a negative effect on the life span at all of the tested concentrations except 3.75 mM. At this concentration it had no toxicity nor an effect on longevity. One of the previously reported critical micellar concentrations (CMC) of TUDCA is closer to this concentration. Therefore TUDCA's mechanism of action could be explained, at least in part, by its micelle formation. It could have exerted negative effects at both lower and higher concentrations than CMC. If it had a positive effect on life span through ameliorating ER stress at the CMC, this could have been neutralized by its partial toxicity. Our results can be helpful in future studies on TUDCA which is a very important and promising therapeutical agent.

INTRODUCTION

The endoplasmic reticulum (ER) is a multifunctional intracellular organelle involved in the synthesis and folding of secretory and membrane proteins, lipid biosynthesis, calcium storage/release. ER has a very important role in protein homoeostasis. Since ER's protein processing capacity is limited, the unfolded and misfolded proteins can accumulate and cause ER stress. The impairment of ER function by ER stress upregulates a signaling pathway called the unfolded protein response (UPR). The activation of UPR attenuates translation in order to decrease the load on the folding machinery, and induces transcription of chaperones and folding enzymes to increase folding. ER stress has been suggested in the mechanisms of a wide range of diseases including aging, cancer and diabetes [1-5].

The UPR functions well in young but appears to be perturbed in aged animals and age related diseases. The expression and activity of many components of the UPR decrease with age with the contribution of oxidative damage [5]. Fibroblasts from long-lived Snell dwarf mutant mice and naked

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mole-rats (NMR) are more sensitive to ER stress than normal short-lived mouse cells. This suggests that differences in the UPR might be involved in the modulation of cell survival and aging rate [6].

Caenorhabditis elegans (*C. elegans*) is also subject to ER stress. ER stress is involved in the mechanisms of aging and UPR contributes to lifespan extension in *C. elegans* [7-9]. Silencing of APY-1, a *C. elegans* apyrase involved in the protection against ER stress through UPR signalling, causes premature aging [8].

Tauroursodeoxycholic acid (TUDCA) has been used for thousands of years in traditional Chinese medicine as one of the main constituents of black bear bile in the treatment of several diseases [10]. It is a cytoprotective bile acid having chaperone like properties. Another chemical chaperone is sodium 4-phenylbutyrate (4PBA). TUDCA and 4PBA were both shown to protect against ER stress and alleviate leptin resistance in brain [11]. TUDCA was shown to have a protective effect at 10 mM concentration against rotenone induced toxicity in *C. elegans* models of Parkinson disease [12].

In this study we aimed to test the effect of TUDCA on *C. elegans* life span in order to assess the role of ER stress in organismal aging.

MATERIALS AND METHODS

Bristol N2 (wild-type) *C. elegans* strain and *E. coli* OP50 were obtained from the Caenorhabditis Genetic Center at the University of Minnesota. The life span analysis experiments were performed according to the standart protocol described by Sutphin and Kaeberlein [13] except the concentrated OP50 bacteria were killed by incubating at 65°C for 30 minutes. TUDCA (Calbiochem) was added to both NGM and lawn of bacteria to allow complete exposure of animals. Tested final concentrations of TUDCA were 0.625, 1.25, 2.5, 3.75, 5, 7.5, 10, 15 and 20 mM. The worms were grown at 20°C. The escaping animals from the petri dishes were excluded from the study.

RESULTS AND DISCUSSION

Since the reported most protective concentration of TUDCA against rotenone toxicity was 10 mM in *C. elegans* [12], we first tested 5, 10 and 20mM concentrations. At all of these concentrations, the life span of worms were shorter than controls (data not shown).

The life span of worms treated with 5 mM TUDCA was most closer to controls. Therefore, we then tested TUDCA at the concentrations of 0.625, 1.25, 2.5, 5, and 7.5 mM. The survival of worms was again lower than controls in all groups. Interestingly we observed the best survival rates closer to controls at 2.5 and 5mM (Figure 1).

Since TUDCA had a worsening effect on life span of animals at the concentrations ≤ 2.5 and ≥ 5 mM, we tested 3.75 mM concentration. There was no difference between the controls and TUDCA treated group at this concentration (Figure 2).

In this study, we detected the optimum non-toxic concentration of TUDCA in *C. elegans* as 3.75 mM. The fact that TUDCA had a negative effect on *C. elegans* life span at the concentrations both lower and higher than 3.75 mM suggests that its beneficial effects, if there are any, could have been less stronger than its toxicity at these levels. TUDCA could have been neutral or, more likely, its toxicity could have neutralized its benefits at 3.75 mM concentration.

In one of the early studies concerning the critical

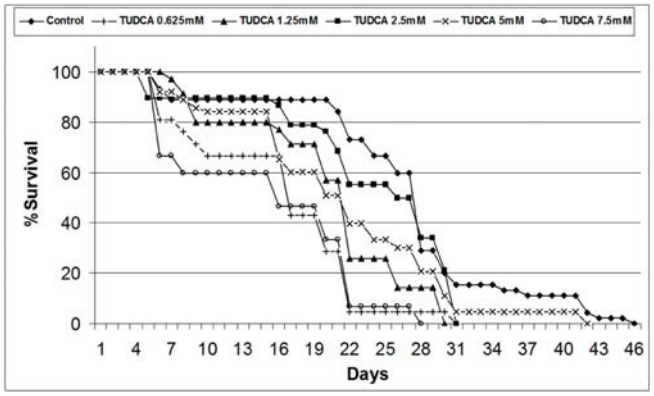

Figure 1. The life span of *C. elegans* at different concentrations of TUDCA (n=45 in control, TUDCA groups: n=21 in 0.625 mM, n=35 in 1.25 mM, n=38 in 2.5mM, n=63 in 5 mM, n=15 in 7.5 mM).

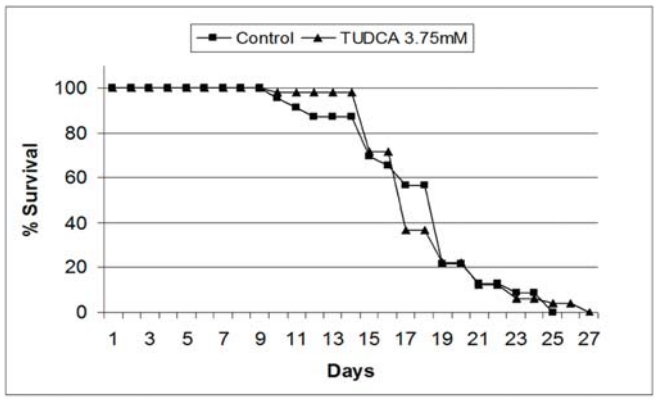

Figure 2. The effect of TUDCA on *C. elegans* life span at 3.75 mM concentration (n=23 in control, n=49 in TUDCA groups).

micellar concentrations (CMC) of TUDCA a concentration of 3 mM which is closer to 3.75 mM was reported as its CMC. But it should be kept in mind that CMC is not an absolute value and can change depending on the composition of solutions or mediums [14,15]. Therefore, we think that the beneficial effects of TUDCA might be mediated, at least partly, by its micelle formation that likely helps to keep in solution the unfolded or misfolded proteins. If it has a positive effect on life span of *C. elegans* by reducing ER stress at the CMC, this could have been neutralized by the partial toxicity of its molecules that are not part of the micelles.

CONCLUSION

Based on our results, it can not be argued that ER stress has no effect on *C. elegans* aging. Previous studies suggest a very important role of ER stress in aging [5]. Therefore, the effect of other chemical chaperons like 4PBA or other interventions that reduce ER stress [11] have to be tested in model organisms' life span before drawing a conclusion.

Our findings can be helpful in future studies addressing other effects of TUDCA in *C. elegans*. Since TUDCA is a very important chemical agent concerning human health [11,16-21]. There will surely be many studies to test TUDCA in model organisms.

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