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Dimethyl sulfoxide and dimethyl formamide increase lifespan of *C. elegans* in liquid

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ABSTRACT

Lifespan extension through pharmacological intervention may provide valuable tools to understanding the mechanisms of aging and could uncover new therapeutic approaches for the treatment of age-related disease. Although the nematode *Caenorhabditis elegans* is well known as a particularly suitable model for genetic manipulations, it has been recently used in a number of pharmacological studies searching for compounds with anti-aging activity. These compound screens are regularly performed in amphipathic solvents like dimethyl sulfoxide (DMSO), the solvent of choice for high-throughput drug screening experiments performed throughout the world. In this work, we report that exposing *C. elegans* to DMSO in liquid extends lifespan up to 20%. Interestingly, another popular amphipathic solvent, dimethyl formamide (DMF), produces a robust 50% increase in lifespan. These compounds work through a mechanism independent of insulin-like signaling and dietary restriction (DR). Additionally, the mechanism does not involve an increased resistance to free radicals or heat shock suggesting that stress resistance does not play a major role in the lifespan extension elicited by these compounds. Interestingly, we found that DMSO and DMF are able to decrease the paralysis associated with amyloid-β_{3-42} aggregation, suggesting a role of protein homeostasis for the mechanism elicited by these molecules to increase lifespan.

1. Introduction

A number of genes that influence lifespan have been identified in invertebrates (Murphy et al., 2003). Some of these genes also appear to modulate mammalian lifespan (Bluher et al., 2003; Holzenberger et al., 2003). A well-characterized pathway influencing aging is the insulin/insulin-like growth factor signaling pathway (ILS). The major constituents of this pathway: *daf-2*, *age-1*, *akt1* and *daf-16* and their mammalian homologs IGF-R1, PI3K, PKB and forkhead/winged-helix transcription factors have been identified (Kenyon, 2001).

This pathway relies on the activation of AGE-1 kinase by DAF-2, leading to phosphorylation of DAF-16 by AKT protein kinase (Kenyon, 2001). In the absence or during reduced activity of DAF-2, DAF-16 is no longer phosphorylated and undergoes nuclear translocation. Once in the nucleus, DAF-16 activates the transcription of a series of pro-longevity genes, including those that confer increased resistance to oxidative stress and thermotolerance (Murphy et al., 2003).

Alternatively, the over-expression in *Caenorhabditis elegans* of molecular chaperones, like HSP-16.2, also leads to an increase in thermotolerance and survival (Walker and Lithgow, 2003).

Interestingly, the first increase in survival observed in mammals was attained through dietary restriction (DR) more than eight decades ago (McCay, 1933). Mimicking DR in *C. elegans*, by reducing the amount of bacteria they are fed, increases lifespan but also produces reductions in body and brood size, and delays reproduction (Chen et al., 2009; Steinkraus et al., 2008). Although, the insulin-like growth factor (IGF) levels appear to be reduced in DR mammals (Dunn et al., 1997), there is no direct evidence that lifespan extension triggered by DR is mediated through ILS pathway. However, it has been shown that the target of rapamycin (TOR) pathway plays a critical role in DR mediated effects in invertebrates (Katewa and Kapahi, 2010). TOR is a serine/threonine kinase involved in the regulation of a number of key physiological processes that can be inhibited by rapamycin. A recent report showing lifespan extension by rapamycin in adult treated mice

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(Harrison et al., 2009) suggested that the TOR pathway could also mediate the beneficial effects of DR in mammals. Additionally, ablation of SIRT1, a member of the sirtuins family of NAD^+-dependent protein deacetylases, blocks the DR-produced lifespan extension in invertebrates, perhaps through a modulation of the somatotropic axis (Katewa and Kapahi, 2010). Despite the advances in this field and probably due to the fact that diverse forms of DR exist, the mechanisms activated by DR to increase lifespan are still not fully understood.

Protein aggregation is a common feature of aging and several age-related pathologies, collectively known as conformational diseases (CD). Although it is not clear how protein aggregation occurs, dramatic alterations in the balance of protein synthesis, protein folding and protein degradation (together representing "protein homeostasis") are likely to play important roles in this process. As a consequence, modified proteins tend to accumulate into soluble oligomers and insoluble aggregates that may actively influence cell function. This suggests a critical role for protein homeostasis in controlling aging and disease (Morimoto and Cuervo, 2009).

The fact that genetic or dietary manipulations have dramatic effects on aging rates leads to the question of whether or not pharmacologic interventions mimicking such mutations might also extend lifespan. Indeed, although *C. elegans* is known as a model particularly suitable for genetic manipulations, a number of pharmacological interventions on the aging process have been demonstrated in this system (Alavez et al., 2011; Benedetti et al., 2008; Evason et al., 2005; Honda et al., 2010; Melov et al., 2000; Onken and Driscoll, 2010; Powolny et al., 2011). Some of them have focused on the effects of natural products since some of these compounds are likely to be more translatable. Some examples include extracts from *Ginkgo biloba* and blueberry phenols, which have been shown to increase stress resistance and lifespan (Wilson et al., 2006; Wu et al., 2002). The potential for natural products is also highlighted by resveratrol, a compound found in many dietary plants and red wine that appears to extend lifespan in multiple species. Resveratrol is an activator of SIR2 in yeast and increases lifespan of budding yeast, Drosophila, and the worm (Kang et al., 2002; Wood et al., 2004). Despite that these results are no free of controversy (Bass et al., 2007), several benefits have been reported by the use of resveratrol in laboratory mice but no increase in lifespan has been observed for mice on a normal laboratory diet (Miller et al., 2011).

Recently we reported that curcumin, the main component of the Indian spice turmeric, among other compounds, is also able to increase lifespan through a mechanism that involves the regulation of protein homeostasis (Alavez et al., 2011). This result has been recently confirmed by an independent group that proposes that the antioxidant properties of curcumin are also important for its effect on lifespan (Liao et al., 2011).

The increase in lifespan of *C. elegans* resulting from the treatment with free radical scavengers such as the *Ginkgo biloba* extract EGb 761, the flavonoid component tamarixetin (Wu et al., 2002), or a synthetic superoxide dismutase/catalase mimetic (Melov et al., 2000) stresses the importance of the role of free radicals during the aging process. The fact that another group has failed to observe a lifespan increase with dismutase/catalase mimetic (EUK-8) under apparently similar conditions (Keaney and Gems, 2003) suggest the importance of unknown environmental factors in the mechanisms of action elicited by a drug, particularly during chronic treatment.

An interesting study showing that the nervous system could have a preponderant role in controlling aging has been reported (Evason et al., 2005). From a screen of a total of 19 FDA-approved drugs, the anticonvulsant ethosuximide, and several derivatives, were able to increase lifespan in worms.

Taken together, these studies demonstrate that *C. elegans* is an excellent model for drug screening assays. Indeed, several high-throughput assays have been performed in this nematode to uncover a higher amount of compounds with potential anti-aging properties in mammals (Gill et al., 2003), and to shed light on important biological pathways (Burns et al., 2006; Kwok et al., 2006). Interestingly, all the high-throughput assays have been performed in liquid using DMSO as solvent. Additionally, most of the commercially available compound libraries are created with DMSO as the compound solvent.

In this work we report that the treatment of *C. elegans* hermaphrodites in liquid cultures with 0.9% dimethyl sulfoxide (DMSO) or 0.75% dimethyl formamide (DMF), triggers lifespan extension up to 20 and 50% respectively. A similar increase in longevity was observed when DAF-2, AGE-1 and DAF-16 mutant worms were treated with either compound. This increase in lifespan was associated with reduction in brood size and a small decrease in body size. Our data suggest that DMSO and DMF mediated longevity does not act through the ILS pathway, a DR mechanism, a stress response, or by acting as free radical scavengers. However, both compounds decrease the paralysis phenotype associated to amyloid-β_{3-42} aggregation suggesting that the modulation of protein homeostasis could play a role in the mechanism activated by DMSO and DMF to extend lifespan in *C. elegans*.

2. Materials and methods

2.1. C. elegans *strains and culture conditions*

All the strains used in the present study; N2, DR1572 *[daf-2 (e1368) III]*, TJ1052 *[age-1(hx546)II]*, DR26 *[daf-16 (m26) I]*, CL2070 *dvIs70 Is[hsp-16.2::gfp; rol-6(su1006)]*, CF1139 *[daf-16(mu86)I;(daf-16::GFP)]* were provided by the Caenorhabditis Genetic Center. The stocks were maintained on standard nematode growth media (NGM) agar plates at 20 °C. All the assays were performed in liquid culture unless otherwise stated. Worms were grown in 15 mm flat-bottomed wells in 24-well Costar plates (Corning Inc., Corning, NY, USA) in a final volume of 0.5 ml of S-media. Alternatively, a large scale culture was performed in 35 mm flat-bottomed wells in 6-well Costar plates (Corning Inc., Corning, NY, USA). All cultures were incubated at 20 °C and agitated at 20 rpm/min to allow correct oxygenation. The food source used was the *Escherichia coli* strain OP50 (obtained from the Caenorhabditis Genetics Center), at a final concentration of 2×10^9 cells/ml of S-media. Alternatively, the concentration of 6×10^8 cells/ml was used for the induction of the DR. Bacterial concentration was estimated by colony titration.

2.2. Survival assays

Unless otherwise indicated, all experiments were initiated from the egg-stage of N2 hermaphrodites. The same culture conditions were used for mutant worms. Briefly, synchronous cultures of hermaphrodites were set up by transferring 5 young egg-layers per well into 24 well Costar plates, from NGM agar plates into the liquid media using a sterile platinum wire. Following a 4 h laying period at 20 °C, egg-layers were discarded and DMSO and DMF were added at concentrations of 0.9% and 0.75%. Alternatively, when testing the effect of the compounds during the post-laying period, worms were grown in liquid, starting from egg-stage, and the culture media was supplemented with either DMSO or DMF starting from the end of egg laying period. In the case of DR worms, the egg-layers were allowed to lay eggs for 4 h in the presence of 2×10^9 OP50 cfu/ml of S-media. Following that period, the eggs were transferred into a new well supplemented with 6×10^8 OP50 cfu/ml of S-media and the compounds were added.

On the second day of culture, between 20 and 25 larvae were randomly selected and transferred to fresh wells with either DMSO or DMF. Control animals were transferred to wells that did not contain either compound. Worms were transferred 6 days a week and dead individuals were scored, excluding bagged worms (animals with larvae hatched inside) or those injured during the transfer. All worms were assessed individually for their survival by successive flushing using a 10 μl pipette. Worms unresponsive were scored as dead. Two different batches of DMSO and DMF were tested with identical results. All experiments were run in duplicate and data was analyzed with Kaplan–Meier log rank test for survival analysis (Prism 4, Graphpad Software) with $p < 0.05$ considered as significant.

2.3. Fertility measurements

During the survival assay the egg-layers were transferred daily to the fresh wells containing 0.5 ml of culture media. After 24 h, the remaining progeny were scored individually for each well 24 h later in order to avoid counting un-hatched embryos. First, larvae were resuspended by rocking the Plate 3 times, a 50 μl sample