High-molecular Weight Polyphenols from Black Tea Increase Lifespan in *Caenorhabditis elegans* via the Forkhead Transcription Factor DAF-16

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Summary

Mitochondrial activation factors (MAFs) are the high-molecular weight polyphenols extracted from black tea, which cause the increase of mitochondrial membrane potential and amounts of intracellular ATP. Our previous studies using mice have shown that the application of MAFs causes a number of beneficial effects, such as decreases of the blood sugar level and the amounts of hepatic fatty acid.
In this report we demonstrate that the treatment of MAFs extended the mean lifespan of the nematode *Caenorhabditis elegans* by up to 18%. The MAFs-induced longevity was cancelled in the loss-of-function mutant of the forkhead transcription factor DAF-16. DAF-16 is known as the key regulator for the insulin-dependent longevity in *C. elegans*. These results suggest that MAFs have clear and reproducible effects during ageing via a part of the conventional longevity pathway.

Introduction

Tea is the most popular beverage all over the world. It has been reported that it has beneficial physiological effects in several aspects, including antioxidation, antiobesity, acceleration of lipid metabolism, anti-carcinoma activity and antimutagencity. However, it is still unclear which chemical components are critical for these beneficial effects.
Our group had recently reported that high-molecular weight polyphenols from black tea, designated mitochondrial activation factors (MAFs), increase mitochondrial membrane potential and level of cellular ATP (Fujihara et al., 2007).
To investigate the physiological effects of the application of MAFs to organisms *in vivo*, we have chosen the nematode *Caenorhabditis elegans* as a model organism. *C. elegans* is a well-established model for studies of ageing (Kenyon, 2010). Several studies provide evidence that *C. elegans* responds upon exposure to various natural compounds, including low-molecular weight polyphenols such as resveratrol, which increases stress resistance and/or extends lifespan (e.g., Saul et al., 2009). In this report, we demonstrate that the exposure of MAFs leads to increased mean lifespan of *C. elegans*. Furthermore, we also show that the MAFs-induced longevity depends on the evolutionarily conserved forkhead transcription factor DAF-16, which plays a crucial role in regulating lifespan.

Materials and methods

Caenorhabditis elegans were maintained on NGM agar and fed with *Escherichia coli* strain OP50 (Sulston and Hodgikin, 1988). N2 (wild type) and *daf-16(mu86)* strains were used (Lin et al., 2001). All aging assays were performed at 25ºC. Preparation of MAFs form black tea extract was performed as previously described (Fujihara et al., 2007). Various concentrations of MAFs dissolved in 0.5% DMSO, and 0.5% DMSO alone (for the control) were added to molten agar NGM during plate preparation. Plates also contained 40 micromolar 5-Fluoro-2'-deoxyuridine (FUdR) to prevent progeny-production. Animals were exposed to MAFs and FUdR from newly-hatched adults, and were transferred to fresh plates at every 2nd day for the first 9 days and then at every 6th day after the 10th

day.

Results and discussion

We investigated the effect of various concentrations of MAFs (0.005, 0.01, and 0.05 mg/ml) on ageing in wild type N2 *C. elegans* strain. We found that 0.05 mg/ml MAFs exposure leads to maximum increased mean lifespan of wild type worms up to 18% (Fig. 1a). The MAFs-induced longevity requires the forkhead transcription factor DAF-16 (Fig. 1b), which is the key regulator for the insulin-dependent longevity in *C. elegans* (Lin et al., 2001). We postulate that the effect of MAFs upon lifespan in *C. elegans* would be caused by a modulation of a signaling component of the insulin/IGF pathway, whose roles is well conserved beyond animal species. We are now studying which signaling molecule in the insulin/IGF pathway is the actual target of MAFs.

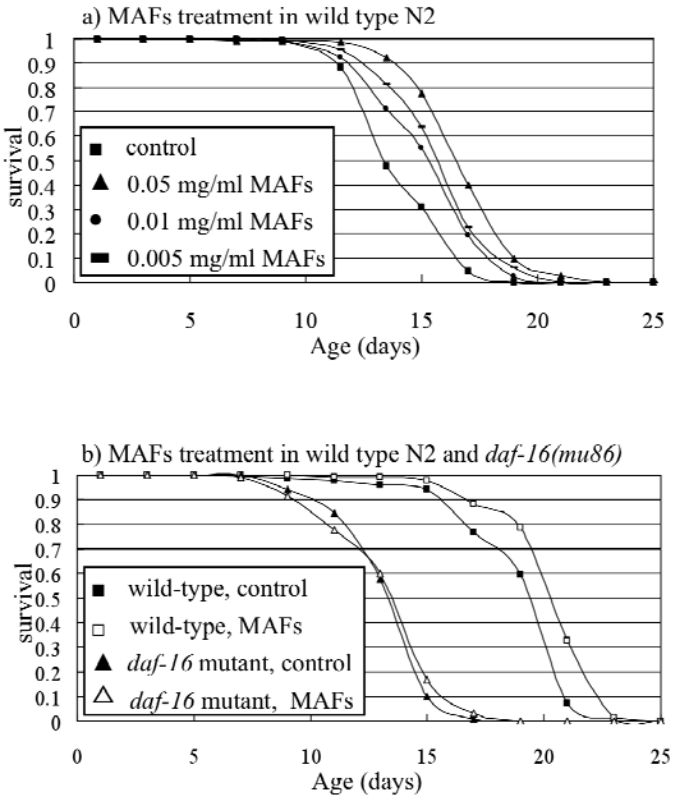

Figure 1. (a) Lifespan of wild type N2 in control DMSO and 0.005, 0.01, or 0.05 mg/ml MAF. (b) Lifespan of wild type N2 and *daf-16(mu86)* in DMSO or 0.05 mg/ml MAF.

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