Redox Biology 14 (2018) 305–315

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Research Paper

Epigallocatechin-3-gallate promotes healthy lifespan through mitohormesis during early-to-mid adulthood in *Caenorhabditis elegans*

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ARTICLE INFO

Keywords:
Epigallocatechin-3-gallate
Healthy lifespan
Mitohormesis
Early-to-mid adulthood
AMPK
C. elegans

ABSTRACT

The green tea polyphenol epigallocatechin-3-gallate (EGCG) is widely consumed as a dietary supplement. Its potential properties include slowing aging and extending lifespan, although how exactly this is achieved remains unclear. Here, we report that EGCG promoted healthy lifespan in *Caenorhabditis elegans* when administered throughout or only at early-to-mid adulthood. Specifically, EGCG extended lifespan in an inverted U-shaped dose-response manner. The life-extending mechanism was stimulated by EGCG-induced production of reactive oxygen species (ROS). Additionally, EGCG triggered mitochondrial biogenesis to restore mitochondrial function. The EGCG-induced increase in lifespan depends on known energy sensors such as AMPK/AAK-2, as well as SIRT1/SIR-2.1 and FOXO/DAF-16. Interestingly, aging decreased the response to EGCG and progressively neutralized its beneficial effects on longevity. Collectively, our findings link EGCG to the process of mitohormesis and suggest an inducible, AMPK/SIRT1/FOXO-dependent redox signaling module that could be invoked in different contexts to extend healthy lifespan. Its effectiveness is higher in younger adults and declines with age.

1. Introduction

Epigallocatechin-3-gallate (EGCG) is the major bioactive polyphenol in green tea (*Camellia sinensis* L.). EGCG has been shown to participate in the regulation of various metabolic processes [1] and has lifespan-extending properties in non-disease animal models [2–5]. It has also been shown to improve disease phenotypes in animal models of cancer (reviewed in [6]), neurodegeneration (reviewed in [7]), and metabolic syndrome (reviewed in [8]). This suggests that EGCG acts on common pathways involved in a variety of aging-associated processes. Because of its demonstrated health benefits on lifespan in invertebrates, such as *Drosophila melanogaster* [2] and *Caenorhabditis elegans* [4,9], as well as in the mammal *Rattus norvegicus* [3], EGCG may be a bona fide anti-aging phytochemical.

It should be noted that previous studies on the impact of EGCG on *C. elegans* lifespan have been inconclusive, as they claimed either a positive or no effect on survival under normal conditions [4,9–11]. These discrepancies may be attributed to experimental design and EGCG dosage. Longevity has been extensively correlated with resistance to stress, and studies in *C. elegans* revealed that EGCG could protect against several stressors [5,11]. In particular, the beneficial effect of EGCG is thought to be directly related to its intrinsic antioxidant properties, however other redox effects have also been documented (reviewed in [12]). Whereas numerous specific targets of EGCG have been identified and characterized [1,6,8,12], the exact mechanism by which EGCG affects longevity remains poorly understood.

The energy-sensing AMP-activated protein kinase (AMPK) is a well-established longevity factor [13] and an attractive candidate to mediate EGCG's effect on lifespan in *C. elegans*. The induction of AMPK by EGCG has been demonstrated both *in vivo* and *in vitro* (reviewed in [8]). Recently, EGCG has been proposed to inhibit gluconeogenesis by activating AMPK and in this way mediate lifespan extension in *D. melanogaster* [2]. Additionally, EGCG may promote longevity also through other energy sensors, such as sirtuin 1 (SIRT1) and the forkhead box O proteins (FOXOs) [3,4]. However, whether AMPK, SIRT1, or FOXO mediate directly lifespan extension by EGCG is still unknown and the link between EGCG and these three important regulators is not well established.

In this work, we report that EGCG affected longevity in an inverted U-shaped dose-response manner. A moderate level of EGCG extended

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http://dx.doi.org/10.1016/j.redox.2017.09.019
Received 12 September 2017; Received in revised form 26 September 2017; Accepted 27 September 2017
Available online 29 September 2017

healthy lifespan mainly during early-to-mid adulthood. This occurred somewhat counterintuitively through the transient induction of reactive oxygen species (ROS), and subsequent activation of an antioxidant response. We also show that AAK-2, the homolog of AMPK in *C. elegans*, acted as a feedback regulator that modulated longevity in response to transiently increased ROS levels. This triggered SIRT1/SIR-2.1- NAD^+- and FOXO/DAF-16-dependent activation. Moreover, EGCG improved mitochondrial biogenesis and restored mitochondrial function in a way that was dependent on AMPK/AAK-2 and SIRT1/SIR-2.1. Finally, EGCG-induced benefits declined with age. Overall, these findings provide new insights on the role of EGCG or other plant-derived polyphenols in healthy aging.

2. Materials and methods

2.1. Chemicals

EGCG (98% pure) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored in water solution at −20 °C. All other chemicals and reagents were also purchased from Sigma-Aldrich unless otherwise stated. For all experiments, stock solutions were freshly prepared in distilled water, unless specified, and sterilized by filtration through 0.2-μm pore size membranes prior to administration.

2.2. C. elegans strains

The following strains were obtained from the Caenorhabditis Genetics Center (University of Minnesota, MN, USA): N2 (Bristol, wild type), BA17 (*fem-1(hc17) IV*), DA465 (*eat-2(ad465) II*), RB754 (*aak-2(ok524) X*), MR507 (*aak-2(rr48) X*), VC199 (*sir-2.1(ok434) IV*), IU7 (*rrf-3(pk1426) II*; *sir-2.1(ok434) IV*), MIR13 (*sir-2.1(ok434) IV*; *aak-2(ok524) X*), CF1038 (*daf-16(mu86) I*), CB1370 (*daf-2(e1370) III*), CF1588 (*daf-16(mu86) I; daf-2(e1370) III*), TJ1052 (*age-1(hx546) II*), MQ130 (*clk-1(qm130) III*), CW152 (*gas-1(fc21) X*), MQ887 (*isp-1(qm150) IV*), MQ1333 (*nuo-6(qm200) I*), TK22 (*mev-1(kn1) III*), SJ4100 (*hsp-6*::GFP), SJ4058 (*hsp-60*::GFP + *lin-15*(+)) SJ4103 (*myo-3*::GFP(mit)), and TJ356 (*daf-16*p::*daf-16*a/b::GFP + *rol-6*(su1006)). Strains were cultivated on standard nematode growth medium (NGM) seeded with *Escherichia coli* OP50. All experiments were performed at 20 °C unless stated otherwise.

2.3. Lifespan experiments and oxidative stress resistance assays

Lifespan assays were performed according to standard protocols unless otherwise indicated. In brief, at the prefertile young adult stage (Day 0), age-synchronized nematodes were transferred to NGM plates with 50 μM 5-fluoro-2′-deoxyuridine (FUdR) and 100 μg/mL ampicillin.

For oxidative stress experiments, prefertile (Day 0) age-synchronized worms were transferred to NGM plates (with or without EGCG) containing 50 μM FUdR for 2 days (or 6 days) and subsequently to plates containing 5 mM paraquat (PQ).

Surviving and dead animals were counted daily until all individuals had died. Worms that failed to respond to a gentle touch were scored as dead. All lifespan experiments were conducted in a double-blind manner. The SPSS 18.0 (Demo version, Armonk, NY, USA) statistical analysis package was used for all lifespan and stress resistance statistics. Lifespan assays and stress resistance experiments were analyzed using the Kaplan-Meir test, and *P* values were calculated with the log-rank test.

2.4. Quantitative RT-PCR for mRNA and DNA quantification

Total RNA was isolated using Trizol (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized using a cDNA synthesis kit (TaKaRa Bio, Dalian, China). Quantitative RT-PCR was carried out using SYBR Premix Ex Taq (TaKaRa Bio, Kyoto, Japan) and the Rotor Gene Q real-time PCR cycler (Qiagen, Hilden, Germany). Expression of the *ama-1* and *act-1* genes was used as endogenous control to normalize the amount of mRNA obtained from a target gene. Samples were run in triplicate, and primers are listed in Table S1.

Quantification of the mtDNA copy number in worms was performed by real-time PCR as previously described. Briefly, relative values for *nd-1* and *act-3* (or *18S*) were compared within each sample to generate a ratio representing the relative level of mtDNA per nuclear genome. The results obtained were confirmed with a second mitochondrial gene *MTCE.26*. The average of at least three technical repeats was used for each biological data point. Primer sequences are listed in Table S1.

2.5. Quantification of ROS production

ROS formation was quantified with dichlorofluorescein diacetate (DCF-DA) (Beyotime Institute of Biotechnology, Haimen, China) (Ex 488 nm/Em 525 nm). Briefly, worms were maintained and treated as described above. After exposure to EGCG, worms were washed off the plates with cold M9 buffer. OP50 were removed by three to five repeated washes. Hydrogen peroxide production was quantified using the Amplex Red hydrogen peroxide kit (Invitrogen) (Ex 550 nm/Em 590 nm).

2.6. Superoxide dismutase and catalase activity assay

Superoxide dismutase (SOD) and catalase (CAT) activity was measured spectrophotometrically using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. Protein content was determined using a commercially available kit (BCA; Auragene, Changsha, China).

2.7. Determination of ATP

Worms were harvested and immediately shock-frozen in liquid nitrogen. The frozen pellet was ground in a nitrogen-chilled mortar. The ATP level was analyzed using a commercially available kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. For normalization of the luminescence signal, protein content was determined as described above.

2.8. Oxygen consumption assays

Oxygen consumption was measured using the Seahorse XF96 apparatus (Seahorse Bioscience, North Billerica, MA, USA) as described previously [14]. Respiration rates were normalized to the number of worms in each individual well. Each experiment was repeated at least twice.

2.9. NAD^+ measurement

For NAD^+ quantification, worms were collected in M9 buffer, washed five times to remove residual bacteria, flash-frozen in liquid nitrogen, and stored at −80 °C until analysis. NAD^+ levels were determined using a commercial kit (Enzychrom, BioAssays Systems, Hayward, CA, USA) following the manufacturer's instructions.

2.10. Confocal microscopy and image processing

Worms were immobilized with 6 mM solution of tetramisole hydrochloride in M9 and mounted on 6% agarose pads on glass slides. Images were acquired using a Zeiss LSM 710 upright confocal microscope (Carl Zeiss AG, Jena, Germany) under non-saturating exposure conditions. For each condition, multiple worms were observed and imaged. Image processing was performed using ImageJ software (NIH, Bethesda, MD, USA).