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A catechin-enriched green tea extract prevents glucose-induced survival reduction in *Caenorhabditis elegans* through *sir-2.1* and *uba-1* dependent hormesis

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ABSTRACT

Hyperglycemia is a hallmark of diabetes mellitus which leads to the onset of complications in the long term. Green tea through its high content of polyphenolic catechins, on the other hand, is suggested to prevent or at least delay such detrimental complications. In the present study we fed the nematode *Caenorhabditis elegans* on a liquid medium supplemented with 10 mM glucose in the absence or presence of a catechin-enriched green tea extract (CEGTE). After exposure of young adults for 48 h survival was subsequently measured under heat stress at 37 °C. Whereas CEGTE at 0.01% did not affect the survival of wild type nematodes, it completely reversed the glucose-induced survival reduction. Those effects were not achieved through the monomeric catechins included in CEGTE. RNA interference (RNAi) for *sir-2.1* not only prevented the survival extension by CEGTE under simultaneous glucose exposure but also caused a further reduction of survival. Likewise, the knockdown of *uba-1*, encoding the only E1-ubiquitin-activating enzyme in *C. elegans*, proved that UBA-1 is essential for the survival extension by CEGTE and that its loss of function changes CEGTE from a survival extending into a survival reducing extract. Stimulation of the proteasome by CEGTE was finally proven through measurements of the proteolytic cleavage of a fluorogenic peptide substrate.
To conclude, our studies provide evidence that CEGTE reverses glucose-induced damage in *C. elegans* through activation of adaptive responses mediated by SIR-2.1 and proteasomal degradation. The hormetic mode of action is revealed by a reduction of survival once the adaptive processes were blocked.

1. Introduction

Enhanced blood glucose levels, known as hyperglycemia, are recognized as the major cause for the development of late onset diabetic complications [1]. Chronic hyperglycemia imposes damage in various cell types and is strongly correlated with microvascular complications, including retinopathy, nephropathy, and neuropathy [2]. Altogether, prolonged elevated plasma glucose levels are associated with premature death in animals and humans [3]. Polyphenolic compounds as present in larger amounts in plant foods, have been suggested to prevent the development of long-term diabetes complications [4]. Besides displaying antioxidative and anti-inflammatory activities, through which polyphenols could interfere with increased ROS and inflammation as a consequence of enhanced glucose levels [5], specific influences on glucose transport and

Abbreviations: CEGTE, catechin-enriched green tea extract; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; MW, molecular weight; NGM, nematode growth medium; RNAi, RNA interference; SGLT, sodium-dependent glucose transporter; Suc-LLVY-AMC, succinyl-leucyl-leucyl-valine-aminomethyl-coumarine.

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metabolism has also been described [4]. Those very specific effects include inhibition of α-glucosidase and α-amylase [6,7], the key enzymes for the digestion of dietary carbohydrates, or the inhibition of SGLT-1 and SGLT-2, which are mainly responsible for intestinal glucose absorption and renal glucose reabsorption [7,8]. Moreover, selected polyphenols have been demonstrated to regulate the key pathways of carbohydrate metabolism and hepatic glucose homeostasis including glycolysis, glycogenesis and gluconeogenesis, which are usually impaired in diabetes [4,9,10]. The major green tea polyphenol constituents, the catechins, were described to affect many of those metabolic dysregulations of diabetes [4,11]. Although intervention studies provide evidence that green tea consumption could be beneficial in preventing late onset diabetic complications in animal models [4,12], more simple models are required in order to explore quickly the underlying molecular mechanisms.

Using the nematode *Caenorhabditis elegans* as a model organism we have previously shown that it enables the fast screening of the effects of various plant extracts on glucose toxicity [13]. The most potent extracts in reverting the survival reduction caused by exposure versus enhanced glucose levels were also proven to be effective with regard to revert the glucose-induced impairment of the proteasome [13]. In a more mechanistic study we have shown that the polyphenol quercetin, also prevents the glucose-induced survival reduction in *C. elegans*, most likely through *sir-2.1* triggered activation of the proteasome [14].

In the present study, we tested a catechin-enriched green tea extract (CEGTE) for its potential to prevent glucose toxicity in *C. elegans* by assessing the survival of nematodes at 37 °C. RNA interference (RNAi) was used investigate the role of *sir-2.1* and of key components of the proteasome for adaptations to the glucose imposed stress. Finally, we assessed the effect of CEGTE on the chymotrypsin-like activity of the proteasome, according to the release of a fluorophore in the presence and absence of a specific proteasome inhibitor.

2. Methods and materials

2.1. Reagents

SYTOX green nucleic acid stain was obtained from Life Technologies (Karlsruhe, Germany). Suc-LLVY-AMC and all other materials used were from Sigma-Aldrich (Steinheim, Germany).

2.2. Plant material

CEGTE is a spray dried extract from leafs of *Camellia sinensis* L. (Kuntze), family Theacea, enriched with catechins to a total amount of at least 60% that was provided by Plantextrakt GmbH & Co. KG (Vestenbergsgreuth, Germany), batch no. 19202077. CEGTE contained 76.9% total catechins and 37.9% EGCG, respectively, as determined using HPLC (Table 1). Therefore, the extract was dissolved and analyzed against external standards.

For extraction, 0.5 g extract are dissolved in 25 ml hot water (70 °C). After the solution has cooled down, 5 ml acetonitrile and 20 ml water are added. The sample solution is diluted 1:20 with stabilizing solution and subsequently centrifuged at 13,400 rpm for 3 min. The supernatant solution is used for HPLC measurement.

Table 1
Lead compounds of CEGTE were analyzed by HPLC and are given in % of the dry extract. RT: retention time.

Lead compound	RT (min)	Content (%)
Epicgallocatechin	8.250	5.88
Catechin	10.832	1.53
Epicatechin	16.821	3.64
Epigallocatechin gallate	18.988	37.85
Epicatechin gallate	23.979	14.58
Gallocatechin	5.844	3.24
Gallocatechin gallate	19.976	10.21
Total catechins		76.92
Caffeine	15.994	10.23
Theobromine	6.419	0.16
Theogallin	3.931	0.05
Gallic acid	4.338	0.18

Catechins are separated by an RP-phenyl-hexyl column using an acetonitrile–water gradient and detected by UV-detector. The flow rate was 1 ml/min. For calibration a caffeine working standard with specific response factors for the various catechin derivatives is used (Fig. 1).

2.3. C. elegans and bacterial strains

C. elegans wild type strain N2, variation Bristol, and *Escherichia coli* OP50 were obtained from *C. elegans* Genetics Center (CGC, University of Minnesota, USA). Nematodes were maintained on nematode growth medium (NGM) agar plates seeded with *E. coli* OP50 at 20 °C according to standard protocols [15]. For all experiments synchronous populations were used that were obtained by bleaching [16]. *E. coli* HT115 RNAi clones were purchased from Source Bioscience (Cambridge, UK) and included a negative control (L4440), *sir-2.1* (R11A8.4), and *uba-1* (C47E12.5).

2.4. RNAi experiments

RNAi experiments were performed in liquid cultures as described [17,18]. In brief, expression of gene-specific dsRNA in the corresponding RNAi strain was induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 4 h at 37 °C. Subsequently bacteria cells were washed and resuspended in NGM liquid added with 40 μg/ml kanamycin to inactivate *E. coli* HT115. A volume of 10 μl M9-buffer containing 10 synchronized L1 larvae was dispensed into each well of a 96-well-plate (Greiner Bio-One, Frickenhausen, Germany) to which 46 μl of *E. coli* HT115-NGM suspension were added. In general, L1 larvae reached the adult stage within 3 days of incubation with agitation at 20 °C. At this stage nematodes were treated with effectors for 48 h.

2.5. Quantitative real-time PCR

Total RNA was extracted from 10,000 worms using Trizol. One-step-real-time PCR reactions were performed in triplicate using 1 μl of RNA template (10 ng/μl), Brilliant II SYBR Green QRT-PCR Mastermix and appropriate primers in a CFX™ Real-