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Black tea theaflavins extend the lifespan of fruit flies

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ABSTRACT

Black tea extract (BTE) is a mixture of epicatechins and theaflavins. The present study investigated the effect of BTE on the lifespan of *Drosophila melanogaster*. Results showed the mean lifespan was significantly extended from 51 to 56 days upon BTE treatment. Gene expression of superoxide dismutase (SOD1 and SOD2), catalase (CAT), and methuselah (MTH) was characterized by an increase in young and then a decrease in aged fruit flies. Higher gene expression of SOD1 and CAT was observed in the BTE-treated group than the control flies. However, BTE exerted a minimal effect on the expression of SOD2 and MTH genes. Dietary fat could induce oxidative stress and shorten the maximum lifespan to 15 days, while addition of 10 mg/ml BTE into diet extended it to 28 days. Paraquat and H_2O_2 challenge tests demonstrated that BTE prolonged the survival time only for Oregon-R wild type flies but not for SOD^{n108} or Cat^{n1} mutants. This suggests that the lifespan-prolonging activity of BTE is mediated at least in part through SOD and CAT.

1. Introduction

Interest in the relationship between diet and ageing is growing. Research has shown that a moderate reduction of nutrient intake and/or dietary calorie restriction extends the lifespans of rodents (McCay et al., 1935), fruit flies (Partridge et al., 2005), nematode worms (Lee et al., 2006), and yeast (Lin et al., 2002). The proposed underlying mechanisms responsible for lifespan extension are that dietary restriction may retard growth, reduce body fat, decrease the metabolic rate, and attenuate oxidative stress (Masoro, 2009). Some earlier studies have also suggested that besides total energy intake, the composition of nutrients in the diet also affects the lifespan and ageing of an organism (Piper and Bartke, 2008).

Dietary antioxidants have become popular supplements in prevention of ageing (Willis et al., 2009). Oxygen is essential to aerobic organisms because it functions as a final electron acceptor. However, oxygen can continuously generate reactive oxygen species (ROS), which are believed to be one of the causes of an organism's ageing (Gutteridge and Halliwell, 2000). Aerobic organisms possess an antioxidant enzyme system, which includes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase, to remove the ROS in cells (Cutler, 1991). In addition, dietary antioxidants, including ascorbic acid, vitamin A, vitamin C, α-tocopherol and plant flavonoids, are also responsible for scavenging the ROS in cells (Ames et al., 1993). Both endogenous antioxidant enzymes and exogenous dietary antioxidants build a defense base to terminate the propagation of free radical reactions, limit the formation of new free radicals and slow down the ageing process.

Fruit fly, *Drosophila melanogaster*, is one of the most commonly used models to investigate the genetic determinants of ageing (Minois, 2006). The *Methuselah* (*MTH*) gene has been shown to be involved in longevity in fruit flies, although its underlying mechanism remains poorly understood (Lin et al., 1998). Green tea is an excellent source of dietary antioxidants as it contains four epicatechin derivatives: (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC). We have previously demonstrated that green tea extracts are able to prolong the lifespan of fruit flies (Li et al., 2007). Black tea is also rich in antioxidants. In addition to EGCG, EGC, ECG, and EC, black tea also contains four theaflavin derivatives: theaflavin-1 (TF_1), theaflavin-3-gallate (TF_2A), theaflavin-3′-gallate (TF_2B), and theaflavin-3,3′-digallate (TF_3). In the present study we investigated whether black tea extract (BTE) has the capacity to scavenge free radicals and prolong the lifespan of *D. melanogaster*. In particular, we focused on the interaction

Abbreviations: CAT, catalase; CuZnSOD or SOD1, copper–zinc containing superoxide dismutase; BTE, black tea extract; LPO, lipid hydroperoxide; MTH, methuselah; MnSOD or SOD2, manganese containing superoxide dismutase; SOD, superoxide dismutase; ROS, reactive oxygen species.

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between BTE and gene expression of the endogenous antioxidant enzymes SOD and CAT in *D. melanogaster*.

2. Materials and methods

2.1. BTE and lard fatty acids

BTE was purchased from Siming Natural Plant Co., Zhejiang, China. According to the supplier, BTE (theaflavins-enriched) was prepared by enzymatic oxidation of green tea polyphenols (Tu and Xia, 2004). Tea epicatechin and theaflavin were quantified using a Shimadzu LC-10AD HPLC (Tokyo, Japan) as we described previously (Su et al., 2003). In brief, BTE mixture (10 μl, 0.5 mg/ml) was injected onto column (Hypersil ODS, 250 × 4.6 mm, 5 m, Alltech, Deerfield, IL, USA) via a Rheodyne valve (Shimadzu, Tokyo, Japan). The mobile phase consisted of 2% acetic acid in water (vol/vol) (Solvent A) and acetonitrile (Solvent B). After the injection of the sample, Solvent B was increased from 8% to 15% over 28 min, to 31% over additional 52 min and then back to the starting ratio over an additional 5 min. The flow rate was maintained at 1.0 ml/min. The individual catechin and TF were monitored at 280 nm and quantified using (+)-catechin as an internal standard.

Lard fatty acids were prepared according to the method previously described (Li et al., 2008). In brief, 35 g potassium hydroxide was ground into power and dissolved into 2 L of methanol solution containing 130 g of lard. The mixture was heated for 2 h in a water bath under a gentle stream of nitrogen gas to prevent oxidation. The methanol was removed in a rotary evaporator. The resultant saponified mixture was then acidified using 10% sulfuric acid to precipitate the free fatty acids followed by washing five times with distilled water. The free fatty acids were then stored at −20 °C.

2.2. Fly strains

The fly strains used in this study included Oregon-R-C (OR), SOD^{n108}/TM3 (SOD^{n108}), and OE^{-}/SM5 x Cat^{n1}/TM3 (Cat^{n1}) (Bloomington *Drosophila* Stock Center, Department of Biology, Indiana University, Bloomington, IN, USA). OR is a wild type fly which was used in all experiments unless specified otherwise. SOD^{n108} is a mutant with one pair of single SOD gene on 3L chromosome knocked out, while Cat^{n1} is a mutant with the CAT gene on chromosome 3L knocked out by a point mutation.

2.3. Diet

A standard diet was prepared according to a previously described formulation (Li et al., 2007; Roberts and Standen, 1998). In brief, 1000 ml diet contained 105 g cornmeal, 21 g yeast, 105 g glucose, and 13 g agar. Ethyl-4-hydroxybenzoate (0.4%) was added into the diet to prevent mold growth. BTE was added into the basal diet at 5 and 10 mg/ml, respectively. For the fat-induced mortality experiments, the fatty acids derived from lard were added into the basal diet at 10% on a weight basis. The mixture was cooked and poured into each vial (5 ml each). For rearing the stocks, 15 ml of the basal diet was poured and set into a vial. For the experimental flies, 5 ml of the basal or experimental diets was prepared per vial. BTE was obtained from Professor You-Ying Tu, Tea Research Institute of Zhejiang University, Hangzhou, China, while the lard fatty acids were prepared according to a previously described method (Li et al., 2008).

2.4. Effect of BTE on longevity of OR flies fed the basal diet

Male flies (2-day-old) developed from eggs were divided into three groups, with 200 flies in each group, and were reared in 10 vials (20 flies per vial). The first group was maintained on the basal diet, while the other two groups were fed one of the two diets containing 5 or 10 mg BTE/ml. The dead flies were counted every 2–3 days and the remaining flies were transferred to a new vial containing the same diet. The feeding lasted 76 days. The two sets of experiments described above were similarly repeated and the fruit flies were killed at various time points to quantify the expression of SOD, CAT, and MTH.

2.5. Effect of BTE on food intake and body weight of OR flies fed on standard diet

Quantification of food intake by a fruit fly is technically difficult because they are small insects and evaporation of moisture from foods cannot be avoided during incubation. Therefore, the change in average body weight per fly was used as an indication of whether BTE could affect the food intake of fruit flies. In brief, flies in each vial were anesthetized by carbon dioxide and then weighed in a balance (Mettler Toledo AG285, Switzerland) at day 0, 15, 25, 35, 45, and 55. The average body weight per fly in each group was recorded.

An alternative method to measure food intake was to use the gustatory assay as previously described (Bahadorani and Hilliker, 2008; Bahadorani et al., 2008). In brief, 60 newly eclosed male flies were collected (20 per vial) and reared on a standard diet for 5 days and then starved for 24 h on Kimwipes paper soaked with distilled water. Afterward, flies were maintained on the standard or BTE-supplemented diet containing 0.2% sulforhodamine B sodium salt (Acid-Red) for 2 h. The flies were anesthetized by carbon dioxide, and the degree of abdomen redness was blind-scored using a grading scale ranging from grade 0 (colorless abdomen) to grade 5 (fully red abdomen). Food intake was compared on the basis of the difference in the degree of abdomen redness between the control and BTE-fed group.

2.6. Paraquat treatment

Paraquat (1,1′-dimethyl-4,4′-bi-pyridinium dichloride; Pq^{2+}) (Sigma, St. Louis, MO, USA) is able to generate superoxide anion radicals (Michaelis and Hill, 1933). To examine the resistance of flies to superoxide-induced stress, both OR flies (n = 400 in 20 vials) and SOD^{n108} mutant flies (n = 400 in 20 vials) were maintained on either the standard control diet or an experimental diet containing 10 mg BTE/ml. All the flies were raised at 25 °C. At day 25, the fruit flies in the two groups were first starved for 2 h, and then transferred to new vials containing a filter paper saturated with 1 ml of 20 mM paraquat diluted in a 6% glucose solution. The number of dead flies was counted every 4–6 h until all the flies were dead.

2.7. Hydrogen peroxide (H_2O_2) treatment

H_2O_2 is able to generate a hydroxyl radical in the presence of some metal ions, and was therefore also used to examine the resistance of flies against OH-induced oxidative stress. OR flies (n = 400) and Cat^{n1} mutant flies (n = 400) were maintained on either the standard control diet or an experimental diet containing 10 mg BTE/ml and incubated at 25 °C. Similarly, the fruit flies in the two groups were first starved for 2 h, and then were transferred to new vials containing a filter paper saturated with 1 ml of 30% H_2O_2 diluted in a 6% glucose solution at day 25. The number of dead flies was counted every 4–6 h until all the flies were dead.

2.8. Effect of BTE on longevity of wild type flies fed on a high-fat diet

Two-day-old male flies were divided into three groups with 200 flies in each group, and reared in 10 vials (20 flies per vial). The first