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Original Research

Grape powder treatment prevents anxiety-like behavior in a rat model of aging

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ABSTRACT

Earlier, we have reported that grape powder (GP) treatment prevented pharmacologic and psychological stress-induced anxiety-like behavior and memory impairment in rats. Protective effects of GP were attributed to its antioxidant effects. In this study, we tested the hypothesis that age-associated behavioral and cognitive deficits such as anxiety and memory impairment will be ameliorated with GP treatment. Using a National Institute of Aging recommended rodent model of aging, we examined a potentially protective role of antioxidant-rich GP in age-associated anxiety-like behavior and memory impairment. Male Fischer 344 rats were randomly assigned into 4 groups: young rats (3 months old) provided with tap water or with 15 g/L GP dissolved in tap water for 3 weeks, aged rats (21 months old) provided with tap water or with GP-treated tap water for 3 weeks (AG-GP). Anxiety-like behavior was significantly greater in aged rats compared with young rats, GP-treated young rats, or aged control rats ($P < .05$). Also, GP treatment prevented age-induced anxiety-like behavior in AG-GP rats ($P < .05$). Neither short-term nor long-term age-associated memory deficits improved with GP treatment in AG-GP rats. Furthermore, aged rats showed increased level of physiological stress (corticosterone) and increased oxidative stress in the plasma (8-isoprostane) as well as in selected brain areas (protein carbonylation). Grape powder treatment prevented age-induced increase in corticosterone levels and plasma 8-isoprostane levels in aged rats ($P < .05$), whereas protein carbonylation was recovered in the amygdala region only ($P < .05$). Grape powder by regulating oxidative stress ameliorates age-induced anxiety-like behavior in rats, whereas age-associated memory deficits seem unaffected with GP treatment.

1. Introduction

It is well documented that aging is accompanied with decline in cognitive and emotional functions [1]. Several interventions have been proposed over the years to promote healthy aging including moderate physical exercise and a balanced diet [2]. Many studies have suggested that polyphenolic compounds present in fruits and vegetables rich in color such as grapes help

Abbreviations: EPM, elevated plus maze; CTGC, California Table Grape Commission; GP, grape powder; OFT, open-field test; RAWM, radial arm water maze test.

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in coping with anxiety and improving memory and cognition during aging [3,4].

These health benefits are proposed to occur via the antioxidant and anti-inflammatory activities of fruits and vegetables [5]. In particular, a lot of attention has been focused on potential health benefits of grapes [6]. Several studies have reported beneficial effects of grapes on cardiovascular activities [7], as well as on mental well-being [3,4,8]. Antioxidant properties of grapes attributed predominantly to its numerous polyphenolic constituents including resveratrol are well characterized [9–13] and largely believed to be responsible for its beneficial effects. Multiple signaling pathways involving antioxidant [14,15], anti-inflammatory [16], and/or antiapoptotic [17] mechanisms are purported to enable the protective effect of grapes. Recently, using a pharmacologic model of oxidative stress [14], we established that California Table Grape Commission (CTGC) provided grape powder (GP) treatment ameliorated oxidative stress–induced anxiety-like behavior, memory impairment, and hypertension in rats [18]. This study has prompted us to further investigate the protective effects of this GP in a nonpharmacologic model and examine whether beneficial effects of GP are limited to pharmacologically induced models of oxidative stress or extend to other genetic models that are well known to be associated with oxidative stress [19].

It is well recognized that oxidative stress, which results when the production of reactive oxygen species overwhelms antioxidant defense system [1], is critical for aging. There is extensive evidence suggesting the involvement of oxidative stress in aging processes of the brain [1]. Relevant to this, oxidative stress has been implicated in Alzheimer disease, Parkinson disease, and several other age-related neurodegenerative illnesses [20]. Numerous epidemiologic studies have suggested that dietary supplementation with antioxidant-rich fruit or vegetable extracts might decrease the enhanced vulnerability to oxidative stress that occurs during aging leading to improvements in motor and cognitive behavior [21]. Therefore, in order to fully investigate the protective effects of grapes on age-related anxiety and memory deficits, it must be tested in an aging model. A National Institute of Aging recommended that rodent F344 model of aging seems a good fit for this study because this is known to have elevated oxidative stress and has an aged phenotype [22–24].

Effects of nutritional intervention including tea, fruit, and vegetable extracts on cognitive and motor function have been tested in the F344 model by others [25,26]. However, 2 of the most pronounced age-associated behaviors, that is, anxiety and cognition, have not been examined in this model. Protective effects of grapes on simultaneous occurrence of these behaviors also are not known. In the present study using the F344 rodent model of aging, we examined the role of antioxidant-rich GP, provided by the CTGC in age-associated anxiety-like behavior and memory impairment, and also examined the level of physiological stress and levels of oxidative stress systemically as well as in specific areas of the brain including the prefrontal cortex, hippocampus, and amygdala. These regions are implicated in regulation of anxiety [27] and cognition [28], amenable to nutritional intervention [18], and also regarded as oxidative stress–susceptible regions [29].

Finally, this study using the National Institute of Aging-recommended rodent F344 model of aging will test the hypothesis that age-associated behavioral and cognitive deficits such as anxiety and memory impairment are ameliorated with GP treatment.

2. Methods and materials

2.1. *Freeze-dried GP*

Freeze-dried GP was provided by the CTGC. The powder was received in small sealed plastic bags and stored at –80°C. Grape powder solution was prepared fresh daily as published previously [18] by dissolving the powder in tap water at a concentration of 15 g/L. This GP dose produced most pronounced effects on rat behavior as reported previously [27]. Detailed composition and purity of this powder have been described in Allam et al [18].

2.2. *Animals*

All experiments were conducted in accordance with the National Institutes of Health guidelines using approved protocols from the University of Houston Animal Care Committee. Three-month-old young male Fischer 344 rats (250-275 g) and 21-month-old male Fischer 344 rats (400-450 g) were purchased from Charles River, Wilmington, MA, USA. These rats were housed with a 12-hour light, 12-hour dark cycle (lights on at 0600 hours) in a climate-controlled room with food and water provided ad libitum. After arrival at the animal research facility, all rats were allowed 1 week for acclimatization.

2.3. *Experimental design*

Male Fischer 344 rats were assigned into 4 groups (8-10 rats/group). (1) Young rats (Y-CON), (2) GP-treated young rats (Y-GP; provided with 15 g/L GP dissolved in tap water for 3 weeks), (3) aged control rats (AG-CON), and (4) GP-treated aged rats (AG-GP; provided with 15 g/L GP dissolved in tap water for 3 weeks). The Y-GP and AG-GP were pretreated with GP for 3 weeks prior to behavior testing and continued to receive GP-treated water until euthanized with decapitation. All rats were subjected to anxiety-like behavior tests followed by memory test. Upon conclusion of behavior and cognition tests, rats were killed by decapitation. Blood and brain tissues were collected, and corticosterones and indices of oxidative stress were measured as previously [14,15,30] (Fig. 1).

2.4. *Anxiety-like behavior tests*

First, elevated plus maze (EPM) was conducted followed by open-field tests (OFTs), as previously published [14,15].

2.4.1. *Elevated plus maze*

The less amount of time spent in open arms is considered as a measure of anxiety-like behavior. A standard rat EPM with 2 walled arms and 2 open arms extending 43 cm from a 10-cm central area (Med Associates Inc, St Albans, VT, USA) was used. The arms of the maze were approximately 90 cm above the floor. The rat's movements were tracked visually. Each session was