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Pomegranate juice decreases amyloid load and improves behavior in a mouse model of Alzheimer's disease

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Although there are no proven ways to delay onset or slow progression of Alzheimer's disease (AD), studies suggest that diet can affect risk. Pomegranates contain very high levels of antioxidant polyphenolic substances as compared to other fruits and vegetables. Polyphenols have been shown to be neuroprotective in different model systems. We asked whether dietary supplementation with pomegranate juice (PJ) would influence behavior and AD-like pathology in a transgenic mouse model. Transgenic mice (APP_{sw}/Tg2576) received either PJ or sugar water control from 6 to 12.5 months of age. PJ-treated mice learned water maze tasks more quickly and swam faster than controls. Mice treated with PJ had significantly less (~50%) accumulation of soluble $A\beta_{42}$ and amyloid deposition in the hippocampus as compared to control mice. These results suggest that further studies to validate and determine the mechanism of these effects, as well as whether substances in PJ may be useful in AD, should be considered.

Keywords: Alzheimer; Transgenic; Mouse; Diet; Pomegranate; Amyloid; Plaque; Behavior; Spatial learning; Polyphenol; Antioxidant

Introduction

Alzheimer's disease (AD) is the most common cause of dementia and affects more than 10% of individuals over the age of 65. Although there are currently no proven ways to delay the onset or slow the progression of AD, epidemiological and experimental evidence suggests that diet can affect the risk for AD and alter amyloid-β (Aβ) levels. For example, a high cholesterol diet has been shown to increase levels of Aβ and apoE (key constituents of the plaques deposited in the brains of AD patients) in the brains of rabbits (Wu et al., 2003) and APP transgenic mice (Refolo et al., 2000). It has been hypothesized that diets high in carbohydrates may alter metabolism of cellular membrane proteins (e.g., APP) and trigger excessive cell signaling cascades, leading to neuronal damage (Henderson, 2004). Other research suggests that dietary intake of aluminum may increase the risk of developing AD (Newman, 1992; Roberts et al., 1998; Rogers and Simon, 1999) and that diets deficient in magnesium can produce cognitive deficits in mice (Bardgett et al., 2005). Importantly, mounting evidence suggests that diet can also decrease the risk for developing AD (Mattson, 2000; Pope et al., 2003). Caloric restriction appears to be neuroprotective in mouse models of AD (Love, 2005; Wang et al., 2005), perhaps by decreasing the accumulation of Aβ deposits (Patel et al., 2005). Another recent study suggests that increased dietary intake of niacin may slow the progression of cognitive decline in AD (Morris et al., 2004).

Foods containing high levels of antioxidants may also slow the progression of AD, possibly by preventing or neutralizing the damaging effects of free radicals (Kostrzewa and Segura-Aguilar, 2003; Polidori, 2003). The essential fatty acids contained in fish oil (e.g., docosahexaenoic acid/DHA) may be neuroprotective in humans (Grant, 2000, 2003; Horrocks and Yeo, 1999; Peers, 1990). Recent studies have also shown beneficial effects of DHA on learning in a rat model of AD (Hashimoto et al., 2002, 2005) and on both plaque deposition and dendritic pathology in aged APP_{sw} transgenic mice (Calon et al., 2004; Lim et al., 2005). Chronic dietary administration of the antioxidant vitamin E has been shown to reduce Aβ deposits in APP_{sw} mice (Sung et al.,

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2004), and epidemiological evidence suggests that high intake of food-based vitamin E is associated with a lower incidence of AD in humans (Morris et al., 2005).

Phytochemicals are nonnutritive bioactive chemicals found in plants (especially pigments) that can have beneficial effects on health. Phytochemicals like polyphenols (including the phenolic acids and flavonoids) have been shown to have antioxidant properties and to suppress inflammatory and other pathways (Aggarwal and Shishodia, 2004; Joseph et al., 2005). Quercetin, a flavonoid polyphenol found in several fruits and vegetables, was recently shown to protect against oxidative stress *in vitro* (Heo and Lee, 2004), and curcumin, a polyphenol found in the curry spice turmeric, was shown to lower levels of oxidized proteins and plaque burden in APP_{sw} mice (Lim et al., 2001). Green tea, another food high in polyphenols, may also be neuroprotective (Weinreb et al., 2004), and one of its flavonoid components, epigallocatechin-3-gallate, decreased Aβ levels in APP_{sw} mice (Rezai-Zadeh et al., 2005). Dietary supplementation with blueberries, also rich in polyphenols, has been shown to improve Y-maze performance, but not plaque deposition, in APP+PS1 transgenic mice (Joseph et al., 2003).

Pomegranates contain very high levels of polyphenols as compared to other fruits and vegetables (Kelawala and Anantha-narayan, 2004; Wang et al., 2004; Xu et al., 2005). Dietary supplementation of pregnant mice with pomegranate juice was recently shown by our laboratory to protect against neurodegeneration in neonatal mice subjected to hypoxic–ischemic brain injury (Loren et al., 2005). Therefore, we asked whether dietary supplementation with pomegranate juice would influence AD-like pathology and behavior in a mouse model of AD.

Materials and methods

Animals—Transgenic experiments

Beginning at 6 months of age, transgenic mice expressing a form of the amyloid precursor protein (APP) that causes early-onset familial AD (APP_{sw}/Tg2576) (Hsiao et al., 1996) received in their drinking bottles pomegranate juice (PJ) from a single lot of PJ concentrate (PomWonderful; Los Angeles, CA) diluted 1:160 or 1:80 in filtered water. Since the PJ concentrate is 4 times more concentrated than regular strength PJ sold commercially, the dilutions of concentrate are approximately equivalent to dilutions of 1:40 or 1:20 of non-concentrated PJ. The mice drank an average of 5 ml of fluid per day. This amount of PJ at these dilutions is roughly equivalent on a mg/kg basis to a human drinking one versus two 8-ounce glasses of PJ per day. The amount of polyphenols consumed per day was estimated to be ~0.3–0.6 mg. Control APP_{sw} mice received sugar water that mimicked the sugar content of the 1:40 PJ (85% sucrose, 7.5% D-(+)-glucose, 7.5% D-fructose). Previous data from our laboratory have shown that polyphenolic substances in PJ are detectable in the plasma of mice pups whose mothers drank PJ but not in pups whose mothers drank sugar water (Loren et al., 2005). Because there were no statistically significant differences between the 1:40 and 1:20 PJ groups for any of the behavioral or neuropathological assessments, the two PJ groups were grouped together for purposes of data analysis. Additionally, there were no weight differences between any of the groups. Behavioral testing began at 11.5 months of age, and the mice continued their treatment regimen throughout testing.

Animals—Wildtype experiments

Wildtype (non-transgenic) control littermates of the APP_{sw} mice were fed PJ (1:40 dilution) or vehicle (sugar water control) beginning at 3–5 months of age. There were no weight differences between the groups. Behavioral testing began after 3 weeks of treatment, and the mice continued their treatment regimen throughout testing.

Pomegranate juice preparation

Pomegranates (Wonderful variety) were picked by hand, washed, and stored in tanks. The fruit was crushed and squeezed. The juice was filtered, pasteurized, concentrated and stored at −18°C. The composition of PJ that was used in these experiments was assessed as follows. PJ was fractionated by low-pressure chromatography on Sephadex LH-20. Five different fractions were obtained by sequential elution with aqueous/organic solvents and then analyzed by high-performance liquid chromatography (HPLC), diode array ultraviolet detection (DAD), electrospray ionization-mass spectrometry (ESI-MS), and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (J. Reed, University of Wisconsin, Madison, personal communication). PJ consisted of 84% water, 14% carbohydrates, 0.48% ash, 0.4% citric acid, 0.1% protein, 0.02% fat and 1% other, including polyphenols (phenolic acids and flavonoids). Phenolic acids included 115 ppm ellagic acid and 5 ppm gallic acid. Flavonoids included 1880 ppm hydrolysable tannins (e.g., gallotannins, ellagitannins, punicalagin) and 369 ppm anthocyanins and their glycosides (e.g., cyanidin, delphinidin, pelargonidin).

Water maze

The learning and memory abilities of the mice were tested in the Morris water maze, as described (Hartman et al., 2005). Briefly, this test of spatial navigation learning requires the mouse to find a hidden (submerged) platform in a pool of water using visual cues from around the room. As performance improves, escape latency and swim path length generally decrease. The water maze consisted of a metal pool (118 cm diameter) in a well-lit room filled to within 10 cm of the upper edge with water made opaque by the addition of white non-toxic tempera paint. The pool contained a round platform (22 cm diameter) that the mice could step on to escape the water. For each trial, a mouse was released nose against the wall into the pool at one of four release points and allowed to find the platform. All trials lasted a maximum of 60 s, at which point the mouse was manually guided to the platform. An overhead camera recorded the animals' swim paths, allowing for quantification of distance, latency, proximity to target and swimming speed by a computer (Polytrack; San Diego Instruments, San Diego, CA).

CUED water maze

The CUED (visible platform) task was used to assess sensorimotor and/or motivational deficits that could affect performance during the SPATIAL water maze task. For this task, the surface of the escape platform was visible (5 mm above the surface of the water), and a 10 cm tall pole capped by a red tennis ball was placed on top of the platform to make its location even more obvious. The walls of the room were kept bare, although the room geometry, experimenter and computer system were obvious