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Dietary broccoli mildly improves neuroinflammation in aged mice but does not reduce lipopolysaccharide-induced sickness behavior

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ABSTRACT

Aging is associated with oxidative stress and heightened inflammatory response to infection. Dietary interventions to reduce these changes are therefore desirable. Broccoli contains glucoraphanin, which is converted to sulforaphane (SFN) by plant myrosinase during cooking preparation or digestion. Sulforaphane increases antioxidant enzymes including NAD(P)H quinone oxidoreductase and heme oxygenase I and inhibits inflammatory cytokines. We hypothesized that dietary broccoli would support an antioxidant response in brain and periphery of aged mice and inhibit lipopolysaccharide (LPS)–induced inflammation and sickness. Young adult and aged mice were fed control or 10% broccoli diet for 28 days before an intraperitoneal LPS injection. Social interactions were assessed 2, 4, 8, and 24 hours after LPS, and mRNA was quantified in liver and brain at 24 hours. Dietary broccoli did not ameliorate LPS-induced decrease in social interactions in young or aged mice. Interleukin-1β (IL-1β) expression was unaffected by broccoli consumption but was induced by LPS in brain and liver of adult and aged mice. In addition, IL-1β was elevated in brain of aged mice without LPS. Broccoli consumption decreased age-elevated cytochrome b-245 β, an oxidative stress marker, and reduced glial activation markers in aged mice. Collectively, these data suggest that 10% broccoli diet provides a modest reduction in age-related oxidative stress and glial reactivity, but is insufficient to inhibit LPS-induced inflammation. Thus, it is likely that SFN would need to be provided in supplement form to control the inflammatory response to LPS.

1. Introduction

Aging is accompanied by chronic low-grade inflammation and increased oxidative stress, both of which are common factors in the pathology of chronic diseases [1,2]. Chronic inflammation leads to cognitive deficits and increases likelihood of developing neurodegenerative disease [3]. The aging brain is highly sensitive to inflammatory mediators generated in the

Abbreviations: ARE, antioxidant response element; CYBB, cytochrome b-245 β; GFAP, glial fibrillary acidic protein; HMOX1, heme oxygenase I; IL, interleukin; LPS, lipopolysaccharide; MHC-II, major histocompatibility complex II; NFκB, nuclear factor κ light chain enhancer of activated B cells; NQO1, NAD(P)H quinone oxidoreductase; Nrf2, nuclear factor erythroid 2–related factor; SFN, sulforaphane.

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periphery, evidenced by the molecular and behavioral changes that follow a peripheral immune stimulus such as infection, lipopolysaccharide (LPS) endotoxin, or stress [4–6]. In fact, LPS-challenged aged mice exhibit exacerbated inflammation in the brain compared with adult mice [6,7]. Exaggerated expression of inflammatory mediators associated with immune activation in the aged signifies a need to identify interventions that attenuate age-related inflammation and oxidative stress both centrally and peripherally.

The nuclear factor erythroid 2–related factor 2 (Nrf2) pathway is the primary transcriptional regulator of the cellular antioxidant response and is increasingly implicated in longevity and protection from inflammation. Declining Nrf2 activity may also be involved in the deleterious neurocognitive decline associated with aging [8–10]. The broccoli-derived bioactive sulforaphane (SFN) elicits activation of the Nrf2 antioxidant pathway, which protects tissues from toxic and carcinogenic insult by promoting transcription of genes containing the antioxidant response element (ARE) [11–13]. Because of the cytoprotective nature of Nrf2, activation of the Nrf2 pathway may be a good therapeutic target for reducing oxidative and immune stress associated with chronic low-grade inflammation. In addition to evoking a Nrf2-dependent antioxidant response, SFN also displays anti-inflammatory effects in vitro, which generates further interest in SFN and foods rich in SFN as potential therapeutic candidates for chronic inflammatory diseases [14,15]. As highlighted in a recent review article, the beneficial effects of SFN have also been demonstrated in a number of experimental animal models, with evidence strongly suggesting that SFN is a versatile treatment for inflammation and oxidative stress [16].

Significant advances have been made in understanding the biochemical mechanisms underlying SFN-mediated activation of Nrf2 and its physiological effects, but minimal research has examined whether whole broccoli consumption influences age-associated inflammation. Broccoli provides a rich dietary source of vitamins, minerals, and flavonoids, but the unique nature of its health-promoting benefits, including cancer prevention and increased endogenous antioxidant production, has been associated with its naturally high levels of glucoraphanin [17–19]. Glucoraphanin is enzymatically hydrolyzed to the bioactive isothiocyanate SFN during crushing, chewing, or digestion of broccoli. Frequent intake of broccoli is associated with lowered risk of cancer and elevation of antioxidant enzymes [20,21]. Therefore, clinical research involving dietary supplementation with broccoli has focused primarily on chemoprevention and detoxification through activation of phase II enzymes. Despite the accumulating evidence that SFN reduces inflammatory markers in cell culture and protects against oxidative stress during brain injury in vivo, the effects of dietary broccoli on peripheral and central inflammation in adult and aged animals have not been thoroughly investigated. Our objective was to examine whether dietary broccoli reduces LPS-induced inflammatory markers in brain or liver of aged mice, and whether dietary broccoli could alter the sickness behavior response to LPS. We hypothesized that dietary broccoli would support an antioxidant response in brain and periphery of aged mice and inhibit LPS-induced inflammation and sickness behavior. To test this hypothesis, we used a preclinical murine model to investigate whether 4 weeks of dietary supplementation was sufficient to decrease markers of inflammation and reduce sickness behavior in adult and aged mice challenged with LPS. Sickness behavior and molecular inflammatory response have been well characterized in our model of LPS-challenged aged mice, and these measurements will provide useful information for determining whether broccoli supplementation attenuates behavioral complications of inflammation. A reduction in LPS-induced proinflammatory markers in the broccoli-supplemented mice would indicate that broccoli is a suitable dietary addition to temper inflammation.

2. Methods and materials

2.1. *Animals and experimental diets*

Adult (4-month-old) and aged (18-month-old) BALB/c mice reared in-house were individually housed in a temperature-controlled environment with a reversed-phase light/dark cycle (lights on 8:00 PM). During the 28-day experimental period, mice were given ad libitum access to water and diet consisting of AIN-93M or AIN-93M + 10% freeze-dried broccoli (Table). Soy oil was replaced with corn oil to mitigate any potential anti-inflammatory effects derived from increased omega-3 fatty acid content of soy oil. The broccoli used in the diet provided 5.22 μmol SFN/g as determined by laboratory hydrolysis using the methods described by Dosz and Jeffery [22]. Therefore, it is estimated that mice fed the 10% broccoli diet were exposed to 0.5 μmol glucoraphanin per gram of diet consumed, providing up to 0.5 μmol SFN/g, depending on the extent of glucoraphanin hydrolysis. To diminish the potential for degradation of glucosinolates from the broccoli-containing diet, we replaced both diets every other day. Body weight was

Table – Ingredient composition of the diets fed to mice

	AIN-93M	AIN-93M + 10% broccoli
Protein	14.7%	14.7%
Carbohydrate	75.9%	75.9%
Fat	9.4%	9.4%
Freeze-dried broccoli (*Brassica oleracea L. cv* "Green Magic")	–	100 g
Casein	140 g	113.6 g
Corn starch	495.7 g	473.8 g
Maltodextrin 10	125 g	110.1 g
Sucrose	100 g	99.1 g
Cellulose	50 g	25.7 g
L-Cystine	1.8 g	1.8 g
Mineral mix	35 g	35 g
Vitamin mix	10 g	10 g
Choline bitartrate	2.5 g	2.5 g
Corn oil	40 g	36.5 g

Broccoli was grown at the University of Illinois, Urbana. All other diet ingredients were purchased from Harlan Laboratories (Indianapolis, Indiana). Nutrient content of broccoli was obtained from the US Department of Agriculture Nutrient Database for Standard Reference.