Journal of Nutrition & Intermediary Metabolism 11 (2018) 1–8

Contents lists available at ScienceDirect

Journal of Nutrition & Intermediary Metabolism

journal homepage: http://www.jnimonline.com/

Effects of consumption of whole grape powder on basal NF-κB signaling and inflammatory cytokine secretion in a mouse model of inflammation

Sonni-Ali Miller [a], Jason A. White [a], Rupak Chowdhury [a], Dominique N. Gales [a], Berhanu Tameru [a], Amit K. Tiwari [b], Temesgen Samuel [a, *]

[a] Department of Pathobiology, Tuskegee University, College of Veterinary Medicine, 1200 W. Montgomery Road, Tuskegee AL 36088, United States
[b] Department of Pharmacology & Experimental Therapeutics, College of Pharmacy & Pharmaceutical Sciences, Frederic and Mary Wolfe Center, The University of Toledo – Health Science Campus, 3000 Arlington Ave., MS 1015, Toledo, OH 43614, United States

ARTICLE INFO

Article history:
Received 4 August 2017
Received in revised form
13 November 2017
Accepted 13 November 2017
Available online 22 November 2017

Keywords:
Whole grape powder
Inflammation
NF-kB
Diet
In vivo
Mouse

ABSTRACT

Dietary consumption of polyphenol-rich fruits, such as grapes, may reduce inflammation and potentially prevent diseases linked to inflammation. Here, we used a genetically engineered murine model to measure Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) activity and pro-inflammatory cytokine secretion to test the hypothesis that oral consumption of whole grape formulation reduces inflammatory signaling in the body. NF-κB luciferase reporter mice were divided into two groups, one which was fed an experimental diet formulated with 4% (w/w) whole grape powder (WGP) or another which was fed a control diet formulated with 3.6% glucose/fructose (w/w) combination. Simulated inflammation was induced in the mice by intraperitoneal injection of lipopolysaccharide (LPS). *In vivo* imaging was used to determine the effect of each diet on NF-κB activity. We found that there were no significant differences in weight gain between the WGP and control diet groups. However, there was a statistically significant ($p < 0.0001$) difference in the progression of basal levels of NF-κB signaling between mice fed on control or WGP diet. There were no significant differences in NF-κB reporter indices between WGP- and control-diet groups after either acute or repeated inflammatory challenge. However, terminal blood collection revealed significantly ($p < 0.01$) lower serum concentrations of the inflammatory cytokines Interleukin-6 (IL-6) and Tumor Necrosis Factor alpha (TNFα) only among WGP diet mice subjected to acute inflammatory challenge. Overall, these data suggest that while diets supplemented with WGP may suppress steady-state low levels of inflammatory signaling, such a supplementation may not alleviate exogenously induced massive NF-κB activation.

1. Introduction

Chronic, persistent inflammation is known to be associated with most chronic diseases such as diabetes, kidney disease, asthma, arthritis, cardiovascular diseases, Alzheimer's disease and cancer [1–4]. These chronic conditions are the leading causes of human and companion animal morbidity and mortality both within the United States (U.S.) and worldwide [2]. The human health and economic consequences of these illnesses are immense. According to the U.S. Centers for Disease Control and Prevention (CDC), 50% of all adults in the U.S. suffer from at least one type of chronic illness, with approximately 75% of all deaths within the U.S. attributed to these chronic diseases [2,5]. Though dietary and medical interventions may be able to reduce these numbers, with the continued rise in the obesity epidemic in the U.S., there is no indication that these diseases will be eradicated in the near future [6,7]. Although many efforts have been placed on costly treatments of these chronic conditions, the investment in prevention of these conditions is still below the threshold for health outcome effectiveness. Recently, in recognition of the seriousness of the problem,

* Corresponding author. Tuskegee University, College of Veterinary Medicine, Patterson Hall A408, Tuskegee, AL 36088, United States.
E-mail addresses: smiller@mytu.tuskegee.edu (S.-A. Miller), jwhite@tuskegee.edu (J.A. White), rchowdhury3191@mytu.tuskegee.edu (R. Chowdhury), dgales8709@mytu.tuskegee.edu (D.N. Gales), tameru@mytu.tuskegee.edu (B. Tameru), amit.tiwari@utoledo.edu (A.K. Tiwari), tsamuel@tuskegee.edu (T. Samuel).

https://doi.org/10.1016/j.jnim.2017.11.002

research on the molecular process of inflammation and the development of preventive and therapeutic strategies has come to the forefront in biomedical research (*NCI provocative questions,* http://provocativequestions.nci.nih.gov).

One of the primary molecular drivers of inflammatory signaling in cells is the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB), a dimeric protein which, in response to stimuli, translocates from the cytoplasm to the nucleus to initiate the expression of several genes which collectively promote multiple inflammation-associated pathologic processes [8–12]. Studies from our group and others have also shown the pathways and molecular regulation of NF-κB activation through various regulatory interactions [13–15]. In particular, canonical NF-κB signaling pathways induced by tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) play an important role in the pathogenesis of multiple chronic inflammatory diseases including inflammatory bowel disease (IBD), asthma, and chronic obstructive pulmonary disease (COPD) [12,16]. Therefore, inhibition of NF-κB activity and/or the stimuli that induce NF-κB activity have become central themes for anti-inflammatory research [17–22]. Supporting evidence for the role of NF-κB driven inflammation in malignancies also comes from genetic models of diminished NF-κB signaling [8,23]. As a result of its role in driving inflammation, the NF-κB pathway has also been targeted in therapeutic development and in various clinical trials [24–31].

Whole grape powder (WGP) contains several bioactive ingredients, including resveratrol, flavonoids, anthocyanins, cathechins, and other compounds. Several studies have documented that these ingredients contained in WGP possess anti-inflammatory and health promoting properties [32–41]. However, less is known about the activity of WGP as a composite entity that possesses a combinatorial activity of its ingredients. Therefore, studies using whole grape powder may help to elucidate and fully exploit the benefits from synergy among the individual bioactive components. This study was designed to determine the bioactivity of WGP *in vivo* by using a reporter mouse model for NF-κB-driven inflammatory signaling.

2. Materials and methods

2.1. Experimental animals and groups

Five-week old female BALB/c-Tg(Rela-luc)31Xen (cat. no. 10499-F, n = 40) reporter mice were purchased from Taconic Biosciences® (Hudson, NY) and housed in groups of 5 per cage in a 12 h light/12 h dark cycle, temperature-controlled room. After arrival, animals were allowed to acclimatize for 10 days before the start of experiments. At the end of the quarantine period the animals were randomly grouped into WGP (n = 20) and control (n = 20) diet groups. After 3 weeks on their assigned diets, 2 cages (10 animals) from each group were randomly assigned to either acute or repeat challenge group. Bodyweights of animals were recorded once a week and daily during the repeat challenge period. Animals were housed at the Tuskegee University Comparative Medicine Resource Center animal facility (Tuskegee Institute, AL) and all experiments described herein were reviewed and approved by the Tuskegee University Animal Care and Use Committee.

2.2. Whole grape powder (WGP)

Whole Grape Powder formulated from lyophilized red, green, blue-purple grapes was provided in aluminum bags by the California Table Grape Commission (Fresno, CA, USA). The bags were kept frozen at −40 °C until use in feed formulation and during the entire study duration. The phytochemical and nutritional compositions of WGP used in this study are shown in Tables 1 and 2. The grape powder contains about 90% sugar in equal proportion of glucose and fructose.

2.3. Feed formulation

Powdered rodent diet was purchased from (Teklad® T.2018M.15, Harlan Laboratories, Indianapolis, IN) and formulated in-house into mashed feed supplemented by addition of 4% WGP (experimental, w/w) or 3.6% sugar (control, 1:1 w/w mixture of glucose and fructose). The powdered feed was combined with fresh WGP or control supplement every day in the morning, made into paste using deionized water and then molded into 14 g balls which were placed daily on mice cage feeders. Five balls were placed in each cage daily for the entire duration of the study. Any remaining feed material was discarded before the fresh balls were placed.

2.4. Induction of inflammatory stimuli

To induce inflammation in the mice, we injected lipopolysaccharide (LPS, L3024, Sigma-Aldrich, Co., St. Louis, MO) intraperitoneally (i.p.). Acute inflammatory challenge was induced by one time administration of 0.5 mg/kg LPS. Repeat challenge inflammation was induced by administration of 0.25 mg/kg LPS every other day during the final week of the study. Luminescence images for both acute and repeat challenges were taken 4 h after the LPS injections.

2.5. In vivo imaging and sera collection

BALB/c-Tg(Rela-luc)31Xen mice are genetically engineered model mice carrying a reporter construct which expresses luciferase enzyme when NF-kB signaling is activated in the body. Subsequent injection of luciferin, a substrate for luciferase, results in a luminescent signal measured by *in vivo* imaging. Animals were administered luciferin substrate solution (XenoLight D-Luciferin, PerkinElmer, Santa Clara, CA) equivalent to 15 μg/kg of animal weight via i.p. injection. After 15 min, animals were placed in the imager in supine position and imaged two at a time using IVIS Lumina XR *In Vivo* Imaging System (PerkinElmer, Santa Clara, CA). *In vivo* luminescence images were taken before the diet provision, and then once a week for the remainder of the study period except for those animals on repeat challenge schedule, which were imaged on each of the challenge days. Luminescence measurements were collected and analyzed as average radiance (photon/sec/cm2/sr) for

Table 1
Phytochemical analysis of the WGP powder used in the study.[a]

Compounds	Total	Individual
Catechins	mg/kg	
Catechin		19.59 mg/kg ± 1.06
Epicatechin		8.77 mg/kg ± .71
Anthocyanins	mg/kg	
Peonidin		148.75 mg/kg ± 8.19
Cyanidin		20.1 mg/kg + /- 1.33
Malvidin		127.77 mg/kg ± 8.33
Flavonols		
Kaempferol		1.03 mg/kg ± .16
Isorhamnetin		1.06 mg/kg ± .11
Quercetin		14.44 ± 1.20
Taxifolin		1.87 mg/kg ± .10
Stilbenes		
Resveratrol		0.85 mg/kg ± .16
Total Polyphenols in gallic acid equivalents		326 mg/100 g

[a] Data provided by the supplier. *Note*: This analysis does not represent the complete phytochemical profile of grapes. Abbreviations: kg = kilogram, g = gram, mg = milligrams, mcg = micrograms, IU = international unit.