Atherosclerosis 216 (2011) 327–333

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Açaí juice attenuates atherosclerosis in ApoE deficient mice through antioxidant and anti-inflammatory activities☆

Chenghui Xie[a,b,1], Jie Kang[a,b,1], Ramona Burris[a], Matthew E. Ferguson[a], Alexander G. Schauss[c], Shanmugam Nagarajan[a,d], Xianli Wu[a,b,*]

[a] USDA Arkansas Children's Nutrition Center, University of Arkansas for Medical Sciences, 15 Children's Way, Little Rock, AR 72202, USA
[b] Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, 15 Children's Way, Little Rock, AR 72202, USA
[c] AIBMR Life Science Inc., 4117 S Meridian, Puyallup, WA 98373, USA
[d] Department of Immunology, University of Arkansas for Medical Sciences, 15 Children's Way, Little Rock, AR 72202, USA

ARTICLE INFO

Article history:
Received 8 December 2010
Received in revised form 18 February 2011
Accepted 20 February 2011
Available online 24 February 2011

Keywords:
Açaí
Euterpe oleracea Mart.
Inflammation
Antioxidant enzyme
apoE-deficient mice
Atherosclerosis
Cytokine

ABSTRACT

Objective: Açaí fruit pulp has received much attention because of its high antioxidant capacity and potential anti-inflammatory effects. In this study, athero-protective effects of açaí juice were investigated in apolipoprotein E deficient (apoE$^{-/-}$) mice.

Methods and results: ApoE$^{-/-}$ mice were fed AIN-93G diet (CD) or CD formulated to contain 5% freeze-dried açaí juice powder (AJ) for 20 weeks. The mean lesion areas in the aorta for apoE$^{-/-}$ mice fed AJ were 58% less ($P < 0.001$) compared to that for CD fed mice. HDL-cholesterol was higher in AJ fed mice. Biomarkers of lipid peroxidation, including F_2-isoprostanes and isomers of hydroxyoctadecadienoic acids and hydroxyeicosatetraenoic acids were significantly lower in serum and in liver of AJ fed mice. Expression of the two antioxidant enzyme genes, Gpx3 and Gsr, were significantly up-regulated in the aorta from AJ fed mice. The activity of GPX, GSR and PON1 increased in serum and/or liver of mice fed AJ. In the second experiment, ApoE$^{-/-}$ mice were fed CD or AJ for 5 weeks. Serum levels, gene expression and protein levels of the two proinflammatory cytokines TNF-α and IL-6 in the resident macrophages with or without LPS stimulation were lower in mice fed AJ. SEAP reporter assay determined that AJ reduced NF-κB activation.

Conclusion: Reducing lipid peroxidation through boosting antioxidant enzymes and inhibiting pro-inflammatory cytokine production are proposed as major underlying mechanisms for the athero-protective effects of the açaí juice tested in these experimental *in vivo* models.

1. Introduction

Cardiovascular diseases remain the leading cause of death not only in the United States but also in most of the industrialized world [1,2]. It has long been recognized that a diet rich in fruits and vegetables may have beneficial effects on cardiovascular diseases, largely attributed to their antioxidant and anti-inflammatory properties [3–5]. However, *in vivo* experimental evidence that consumption of specific fruits and vegetables reduces the risk of cardiovascular disease based on plausible underlying mechanisms remain scarce.

Açaí (*Euterpe oleracea* Mart.) belongs to the family Arecaceae (palm tree). It is indigenous to South America especially in the Amazon flood plains. Açaí pulp has received much attention in recent years as one of the new "superfruits". It exhibits high antioxidant capacity and has been used as a food ingredient with functional contributions to the diet beyond its basic nutritional composition [6–8]. In a recent paper, diet supplementation with açaí pulp was found to improve biomarkers of oxidative stress resulting in a hypocholesterolemic effect in rats [9]. This suggested that consumption of açaí could improve antioxidant status and provide athero-protective effects in an animal model of hypercholesterolemia. Açaí pulp or açaí juice has also been shown to possess anti-inflammatory activity [10]. Freeze-dried açaí pulp inhibited the activity of cyclooxygenase (COX)-1 and -2 *in vitro*, with greater

Abbreviations: AJaçaí, juice; ApoE$^{-/-}$, apolipoprotein E deficient; GPX, glutathione reductase; GSR, glutathione reductase; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; 12/15-LOX, 12/15-lipoxygenase; IL-6, interleukin-6; ORAC, oxygen radical absorbance capacity; PON1, paraoxonase 1; TNF-α, tumor necrosis factor-alpha.

☆ Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

* Corresponding author at: Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, 15 Children's Way, Little Rock, AR 72202, USA. Tel.: +1 501 364 2813; fax: +1 501 364 3161.
E-mail address: wuxianli@uams.edu (X. Wu).

[1] These two authors contributed equally to this work.

0021-9150/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2011.02.035

Table 1
Body weight, body composition and serum lipid profiles in mice fed CD or AJ.[a]

Parameters	CD	AJ
Body weight (g)[b]	25.0 ± 1.73	27.4 ± 1.61***
Energy Intake (kcal/kg body weight 0.75)	183.8 ± 14.1	194.2 ± 19.8
Body composition[c]		
Body fat (%)	22.4 ± 4.2	24.9 ± 3.7
Lean body mass (%)	62.7 ± 7.6	61.5 ± 4.8
Lipid profile[d]		
Total cholesterol (mmol/L)	13.5 ± 1.0	14.4 ± 1.7
HDL-cholesterol (mmol/L)	0.88 ± 0.06	1.04 ± 0.10**
LDL-cholesterol (mmol/L)	12.4 ± 1.1	13.3 ± 1.7
Triglyceride (mmol/L)	1.30 ± 0.14	1.48 ± 0.24

[a] Data expressed as mean ± SD.
[b] $n = 15$.
[c] $n = 8$.
[d] $n = 6$.
** $P < 0.01$.
*** $P < 0.001$.

efficacy against COX-1 [8]. Açaí extracts inhibited lipopolysaccharide- and interferon-g-induced nitric oxide production by reducing the expression of inducible nitric oxide synthase (iNOS) expression [11]. Consumption of a juice blend containing açai as the main component has demonstrated *in vivo* anti-inflammatory properties in human subjects based on a randomized, double-blind, placebo controlled cross-over study [7]. These findings suggest possible cardio-protective properties of açaí.

In this study, the athero-protective effects of a freeze-dried and frozen açaí pulp juice mixture were studied in the apolipoprotein deficient (apoE$^{-/-}$) mouse model. The apoE$^{-/-}$ mouse model has been a useful model to study the mechanisms of action related to atherosclerosis in cardiovascular research [12]. The underlying mechanisms were also explored with primary emphases on the fruit's antioxidant and anti-inflammatory activities.

2. Materials and methods

2.1. Experimental materials and animal

The açaí juice blend was provided by MonaVie, LLC (South Jordan, UT). This blend contains açaí (*Euterpe oleracea* Mart.) freeze-dried and frozen pulp (Earthfruits, South Jordan, UT) as the predominant ingredient. This juice blend has been characterized as containing polyphenols as major phytochemicals previously [7]. The chromatograms of polyphenol profiles of this açaí juice and freeze-dried açaí pulp were presented as supplemental data (Fig. S1). To precisely control the intake, the juice blend was lyophilized and the dried powder was used to make the pellet diet.

AIN-93G (CD) or AIN-93G incorporated with 5% freeze-dried açaí juice powder (AJ) was made by Harlan Teklad (Madison, WI). To eliminate caloric density as a confounding variable, all diets were formulated to be isocaloric and isonitrogenous. Both diets consisted of 20% casein as the protein source and the diets in different groups had the same levels of protein, essential amino acids, calories, vitamins and minerals. The diet formulation is shown in supplemental data (Table 1).

2.2. Animal protocol

The animal protocol was approved by the Animal Care and Use Committee of the Arkansas Children's Hospital Research Institute. ApoE$^{-/-}$ mice (female, 4 weeks old) of the C57BL/6 genetic background were obtained from Jackson Laboratory (Bar Harbor, ME). Animals were maintained in sterile micro-isolator cages and fed autoclaved-pelleted diet ad libitum and water.

In the first experiment, apoE$^{-/-}$ mice were fed either CD or AJ (15/group) for 20 weeks. Food intake and body weight were recorded weekly. NMR analysis was performed at day 171 to measure body fat and lean body mass content following procedures described before [13]. At the end of 24 weeks, animals were euthanized using CO_2 and serum, plasma and tissue samples (aorta, macrophage, heart and liver) were collected and stored at −70 °C until analysis.

In the second experiment, apoE$^{-/-}$ mice were fed either CD or AJ (15/group) for 5 weeks. Food intake and body weight were recorded weekly. At the end of experiment, animals were euthanized using CO_2 and resident macrophage collected.

2.3. Isolation and treatment of resident macrophages

Resident macrophages were collected using harvest medium (Dulbecco's phosphate-buffered saline) without any eliciting agents. Cells were plated in RPMI-1640 supplemented with 10% (v/v) FBS, 2 mM L-glutamine, penicillin, streptomycin, and sodium pyruvate. Non-adherent cells were removed after 2 h and macrophages used after 48 h. Cells were then treated with or without LPS (Invitrogen, San Diego) for 18 h. Supernate of the cultured macrophages was used to measure protein levels of TNF-a and IL-6, and the cell lysate was used for TNF-α and IL-6 gene expression analyses.

2.4. Serum lipid analysis

Serum total, LDL-, HDL-cholesterol, and triglyceride were determined by colorimetric assay using diagnostic reagents from Synermed (Montreal, Quebec, Canada) [13].

2.5. Aortic lesion analysis

Lesion analysis of the descending aorta (enface analysis) was carried out as described [13] and performed independently by two individuals blinded to the study design. The results are reported as percentage of the total descending aorta area containing lesions.

2.6. LC–MS/MS analysis of lipid peroxidation biomarkers

Analyses of lipid peroxidation markers, including 8-isoprostaglandin $F_{2\alpha}$, hydroxyeicosatetraenoic acids (HETEs) and hydroxyoctadecadienoic acids (HODEs), were carried out in an HPLC–MS/MS system based on the method modified from published procedures [14,15]. Sample preparation of serum and liver was based on the method described by our lab [16]. Prior to extraction, three internal standards, 8-isoprostaglandin $F_{2\alpha}$-d_4, 12(S)-HETE-d_8 and 13(S)-HODE-d_6 (Cayman Chemical, Ann Arbor, MI) were added to 30 μL of serum or 100 μL of liver homogenate.

Analysis was carried out using the Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) coupled with a 4000 Q TRAP™ mass spectrometer (Applied Biosystems, Forest City, CA). Separation was performed on a Phenomenex Synergi Max-RP column (150 mm × 3 mm, 4 μm) using a flow rate of 0.4 mL/min. The solvent consisted of (A) 0.2% (v/v) of formic acid in water and (B) methanol. The 19 min gradient was as follows: 22–20% A (1–2 min), 20–20% A (2–18 min), 20–22% A (18–19 min). Multi-reaction monitoring (MRM) mode scan was used for quantitation. The transitions monitored were mass to charge ratio (m/z): m/z 295 → 171 for 9-HODE; m/z 295 → 195 for 13-HODE; m/z 319 → 163 for 8-HETE; m/z 319 → 167 for 11-HETE; m/z 319 → 179 for 12-HETE; m/z 353 → 193 for 8-isoprostaglandin $F_{2\alpha}$; m/z 327 → 184 for 12(S)-HETE-d_8; m/z 299 → 198 for 13(S)-HODE-d_4; m/z 353 → 197 for 8-isoprostaglandin $F_{2\alpha}$-d_4. The mass spectrometer equipped with