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Pomegranate juice sugar fraction reduces macrophage oxidative state, whereas white grape juice sugar fraction increases it

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Abstract

The antiatherogenic properties of pomegranate juice (PJ) were attributed to its antioxidant potency and to its capacity to decrease macrophage oxidative stress, the hallmark of early atherogeneis. PJ polyphenols and sugar-containing polyphenolic anthocyanins were shown to confer PJ its antioxidant capacity. In the present study, we questioned whether PJ simple or complex sugars contribute to the antioxidative properties of PJ in comparison to white grape juice (WGJ) sugars.

Whole PJ decreased cellular peroxide levels in J774A.1 macrophage cell-line by 23% more than PJ polyphenol fraction alone. Thus, we next determined the contribution of the PJ sugar fraction to the decrease in macrophage oxidative state. Increasing concentrations of the PJ sugar fraction resulted in a dose-dependent decrement in macrophage peroxide levels, up to 72%, compared to control cells. On the contrary, incubation of the cells with WGJ sugar fraction at the same concentrations resulted in a dose-dependent increment in peroxide levels by up to 37%. The two sugar fractions from PJ and from WGJ showed opposite effects (antioxidant for PJ and pro-oxidant for WGJ) also in mouse peritoneal macrophages (MPM) from control as well as from streptozotocin-induced diabetic Balb/C mice.

PJ sugar consumption by diabetic mice for 10 days resulted in a small but significant decrement in their peritoneal macrophage total peroxide levels and an increment in cellular glutathione content, compared to MPM harvested from control diabetic mice administrated with water. In contrast, WGJ sugar consumption by diabetic mice resulted in a 22% increment in macrophage total peroxide levels and a 45% decrement in cellular glutathione content.

Paraoxonase 2 activity in macrophages increases under oxidative stress conditions. Indeed, macrophage paraoxonase 2 activity was decreased after PJ sugars supplementation, but increased after WGJ sugars supplementation.

We conclude that PJ sugar fraction, unlike WGJ sugar fraction, decreases macrophage oxidative state under normal and under diabetic conditions. These antioxidant/antiatherogenic effects could be due to the presence of unique complex sugars and/or phenolic sugars in PJ.

Keywords: Pomegranate; White grape juice; Atherosclerosis; Diabetes; Paraoxonase; Macrophage; Oxidative stress; Glucose; Fructose; Polyphenols; Sugar

1. Introduction

The pomegranate tree, which is said to have flourished in the Garden of Eden, has been extensively used as a folk medicine in many cultures. However, this folk medicine found its reinforcement in today's scientific medicine. Pomegranate juice (PJ) was found to inhibit low-density lipoprotein (LDL) oxidation, macrophage foam cell formation and atherosclerosis development in atherosclerotic mice [1,2]. Moreover, PJ consumption for 3 years by patients with carotid artery stenosis reduced common carotid intima-media thickness, blood pressure and LDL oxidation [3]. Similarly, PJ consumption by diabetic patients resulted in antioxidative effects in their serum and their monocytes-macrophages [4]. These beneficial effects of the PJ were attributed to the antioxidative properties of pomegranate polyphenols [5] and sugar-containing polyphenolic tannins and anthocyanins [6].

Oxidative stress is thought to play a key role in early atherogenesis and in macrophage foam cell formation

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which is the hallmark of the early atherosclerotic lesion [7,8].

Oxidative stress is associated with lipid peroxidation in lipoproteins and in arterial cells, including macrophages [9,10]. These "oxidized macrophages" are characterized by increased peroxide levels, decreased glutathione content, and increased capability to oxidize LDL [11,12]. These properties of the cells increase their capability to accumulate cholesterol and to form macrophage foam cells [13–15].

Type 2 diabetes is a major risk factor for the development of coronary artery disease (CAD) and premature atherosclerosis [16]. In diabetes, the postprandial phase is characterized by a rapid and large increase in blood glucose levels. Postprandial hyperglycemic episodes in diabetic patients are closely associated with increased oxidative and nitrosative stress, and are a most important factor in the onset and progress of vascular complications, both in Type 1 and 2 diabetes mellitus [17–19]. Fructose, however, was found to increase plasma antioxidant capacity after apple consumption, due to increment in plasma urate levels [20].

Serum paraoxonase 1 (PON1), an HDL-associated lactonase was found to possess antioxidative properties, probably due to its ability to decrease macrophage oxidative stress [21,22]. PON1 was shown to be decreased under oxidative state, as shown in atherosclerotic as well as diabetic patients [23–25]. In contrast, another member of the Paraoxonase family, paraoxonase 2, is increased in macrophages under oxidative stress [26].

As complex sugars (tannins and anthocyanins), as well as simple sugars (fructose) were shown to possess antioxidative properties, we hypothesized that the PJ sugar fraction could contribute to the juice's antioxidativity. The aim of the present study was to characterize the effect of the sugar fraction that was purified from PJ on macrophage oxidative status, in comparison to white grape juice sugar fraction. We questioned whether this fraction would exhibit beneficial antioxidative properties, like the PJ, or will exhibit negative hyperglycemic effects under diabetic conditions.

2. Methods

2.1. Extraction of sugar and polyphenol fractions from pomegranate juice (PJ) and from white grape juice (WGJ)

For the extraction, C18 sorbent column was used (Varian HF Bondesil C18 resin sorbent). Sugar fraction was eluted with distilled water and total polyphenols were eluted from the column with 1% acidified (food-grade acetic acid) ethanol.

2.2. Mice and sugar supplementation

Twelve male Balb/C mice at the age of 3 months were injected intraperitoneally with streptozotocin (STZ) (200 mg/kg in 0.05 M sodium citrate, pH 4.5) within 5 min of preparation as previously described [27]. Serum glucose levels were determined within 1 week by using a glucometer (Accu-Check Sensor, Roche Manheim, Germany) and mice with serum glucose levels under 190 mg/dl were administrated with a second injection.

Diabetic mice were randomly divided into 3 groups of 4 mice each. One group designated "Control" drank tap water, the two other groups were supplemented via their drinking water with PJ sugar fraction or WGJ sugar fraction (containing 30 mg of glucose per day per mouse) for a period of 10 days. At the end of this period, the mice were sacrificed and blood sample were collected from the retroorbital plexus (under isoflurane anesthesia) and also peritoneal macrophages were harvested after thioglycollate injection as described below.

2.3. Cells

2.3.1. J-774A.1 murine macrophage-like cell line

This cell line was purchased from the American Type Culture Collection (ATCC, Rockville, MD). J-774A.1 cells were plated at 5×10^5 cells/well in 12-well dish in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% fetal calf serum (FCS), 100 U penicillin/ml, 100 μg streptomycin/ml, and 2 mM glutamine. At the next day, the cells were washed and the media was replaced to low glucose (1.0 g/l = 5 mM) serum-free (SF) DMEM (control) or low glucose SF DMEM with PJ sugar fraction or WGJ sugar fraction containing 2.8 mM glucose for 18 h at 37 °C.

2.3.2. Mouse peritoneal macrophages (MPM) isolation

MPM were harvested from mice peritoneum 4 days after intraperitoneal injection of thioglycollate (3 ml, 40 g/l), as previously described [22]. Cells were plated at 1×10^6 cells/well in 12-well dish and treated under the same conditions as described for the J774A.1 macrophages. For the in vitro experiments, the cells were washed at the next day and the media was replaced to high glucose (4.5 g/l) SF DMEM (control) or high glucose SF DMEM with PJ sugar fraction or WGJ sugar fraction containing 2.8 mM glucose for 18 h at 37 °C.

MPM from the mice that were administrated with sugars were plated and washed similarly, with no further treatments.

2.4. Paraoxonase (PON) activities

2.4.1. Serum PON1 activity towards phenylacetate (arylesterase)

This activity was measured spectrophotometrically at 270 nm [28]. The E_{270} for the reaction was 1310 M^{-1} cm^{-1}. One unit of paraoxonase activity is equal to 1 μmol of phenylacetate hydrolyzed/ml/min.

Serum PON1 activity towards paraoxon (*paraoxonase*) is specific for PON1 and was assessed by measuring *p*-nitrophenol liberation at 412 nm [28]. One unit of paraoxonase activity produces 1 nmol of *p*-nitrophenol per minute.