Archives of Biochemistry and Biophysics xxx (2016) 1–11

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Identification and quantification of novel cranberry-derived plasma and urinary (poly)phenols

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ARTICLE INFO

Article history:
Received 1 January 2016
Received in revised form
22 January 2016
Accepted 26 January 2016
Available online xxx

Keywords:
Cranberry
(Poly)phenols
Bioavailability
Absorption
Metabolism
Excretion

ABSTRACT

Cranberries are a rich source of (poly)phenols, in particular proanthocyanidins, anthocyanins, flavonols, and phenolic acids. However, little is known about their bioavailability in humans. We investigated the absorption, metabolism, and excretion of cranberry (poly)phenols in plasma and urine of healthy young men after consumption of a cranberry juice (787 mg (poly)phenols). A total of 60 cranberry-derived phenolic metabolites were identified using UPLC-Q-TOF-MS analysis with authentic standards. These included sulfates of pyrogallol, valerolactone, benzoic acids, phenylacetic acids, glucuronides of flavonols, as well as sulfates and glucuronides of cinnamic acids. The most abundant plasma metabolites were small phenolic compounds, in particular hippuric acid, catechol-*O*-sulfate, 2,3-dihydroxybenzoic acid, phenylacetic acid, isoferulic acid, 4-methylcatechol-*O*-sulfate, α-hydroxyhippuric acid, ferulic acid 4-*O*-sulfate, benzoic acid, 4-hydroxyphenyl acetic acid, dihydrocaffeic acid 3-*O*-sulfate, and vanillic acid-4-*O*-sulfate. Some benzoic acids, cinnamic acids, and flavonol metabolites appeared in plasma early, at 1–2 h post-consumption. Others such as phenylacetic acids, benzaldehydes, pyrogallols, catechols, hippuric and dihydrocinnamic acid derivatives appear in plasma later (T_{max} 4–22 h). The 24 h urinary recovery with respect to the amount of (poly)phenols consumed was 6.2%. Our extensive description of the bioavailability of cranberry (poly)phenols lays important groundwork necessary to start understanding the fate of these compounds in humans.

1. Introduction

Cranberries (for example *Vaccinium macrocarpon* Ait.) are a rich source of (poly)phenol compounds, in particular proanthocyanidins (PAC), anthocyanins, flavonols, and phenolic acids [1–4]. The most abundant compounds in cranberries are PAC, which in contrast to other foods such as blueberries, grapes, or apples, contain A-type PAC interflavan bonds [5], which are thought to have different biological activities than B-type PAC [6–8], although the clinical significance of these findings remains unknown. Anthocyanins, such as cyanidin and peonidin, are also abundant in cranberry fruit, although commonly used processing techniques can dramatically lower their presence in products such as cranberry juice [9,10] and lead to the formation of anthocyanin-PAC which have limited bioavailability [11–13]. The most abundant phenolic acid in cranberry is benzoic acid, with other hydroxybenzoic and hydroxycinnamic acids such as *p*-coumaric and ferulic acids also present in lower amounts [14].

In recent years, the potential health benefits of cranberry consumption have gained public attention. In particular, cranberries have been suggested to possess beneficial effects, most notably the prevention of urinary tract infections [15] and promotion of cardiovascular health [16,17]. However, strong evidence from human intervention trials is still lacking, due to the small number and heterogeneity of the studies that have been conducted so far [18,19]. In addition, before investigating potential mechanisms of

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http://dx.doi.org/10.1016/j.abb.2016.01.014
0003-9861/

action, the main cranberry-derived (poly)phenol metabolites need to be identified and their absorption, distribution, metabolism, and excretion (ADME) established.

A limited number of studies have investigated the ADME of cranberry (poly)phenols in humans [20–25]. Some of these studies have focused only on the analysis of structurally-related anthocyanin metabolites [20,21], which are present in very low amounts in plasma in comparison to their phenolic acid metabolites [26,27]. Due to the difficulty and cost of obtaining authentic standards from commercial sources, the existing studies which investigated cranberry-derived phenolic acid metabolites have used either enzymatic treatment with glucuronidase and sulfatase to cleave the glucuronide and sulfate moieties from phase II metabolites [24], or have used the aglycone counterpart for quantification, which leads to inaccurate results [28].

In the present work, we aimed to comprehensively investigate the bioavailability of cranberry (poly)phenols by using authentic standards of phenolic acid metabolites to accurately identify and quantify individual cranberry-derived metabolites in plasma and urine.

2. Materials and methods

2.1. Materials

Homovanillic acid sulfate sodium salt, caffeic acid 3-*O*-β-D-glucuronide, caffeic acid 4-*O*-β-D-glucuronide, dihydro caffeic acid 3-*O*-sulfate sodium salt, dihydro caffeic acid 3-*O*-β-D glucuronide diammonium salt, ferulic acid 4-*O*-β-D-glucuronide disodium salt, ferulic acid 4-*O*-sulfate disodium salt, dihydro ferulic acid 4-*O*-sulfate sodium salt, dihydro ferulic acid 4-*O*-β-D-glucuronide, isoferulic acid 3-*O*-sulfate disodium salt, isoferulic acid 3-*O*-β-D-glucuronide, dihydro isoferulic acid 3-*O*-sulfate disodium salt, dihydro isoferulic acid 3-*O*-ß-D-glucuronide and (5R)-5-(3′,4′-dihydroxyphenyl)-γ-valerolactone-4′-O-sulfate sodium salt were obtained from Toronto Research Chemicals (Toronto, Canada). The valerolactone was provided without information regarding the exact position of the sulfate and will be designated in this work as (5R)-5-(3′-hydroxyphenyl)-γ-valerolactone-4′-O-sulfate, according to the NMR spectrum provided by the supplier. Kaempferol-3-*O*-ß-D-glucuronide was obtained from Extrasynthese (Genay, France). 1-Methylpyrogallol-*O*-sulfate, 2-methylpyrogallol-*O*-sulfate, 4-methylcatechol-*O*-sulfate, 4-methylgallic-3-*O*-sulfate, catechol-*O*-sulfate, pyrogallol-*O*-1-sulfate, pyrogallol-*O*-2-sulfate and vanillic acid-4-*O*-sulfate were kindly provided by Dr Cláudia Nunes dos Santos and Dr Rita Ventura, and their synthesis has been described elsewhere [29]. All the polyphenol and phenolic acid aglycones were obtained from Sigma–Aldrich Co. (Steinheim, Germany) and 2-, 3- and 4-hydroxyhippuric acids were purchased from Enamine (Kiev, Ukraine). Acetic acid was from Carl Roth (Karlsruhe, Germany) and Oasis HLB μElution plates (2 mg sorbent per well, 30 μm) were from Waters (Eschborn, Germany). Milli-Q system (Merck KGaA, Darmstadt, Germany) ultra pure water was used. Unless otherwise stated, all chemicals and reagents were obtained from Sigma–Aldrich Co. (Steinheim, Germany).

2.2. Human study design

Ten healthy young men between 18 and 35 years old were recruited and the study was conducted between January and September 2015. Blood samples were collected using an intravenous cannula before and after 1, 2, 4, 6, 8 consumption of 450 ml of cranberry juice containing 787 mg of total (poly)phenols (Table 1). An additional blood sample was collected 24 h post-consumption. Urine was collected over 24 h (0–8 and 8–24 h) after consumption of the cranberry juice. Volunteers were asked to follow an anthocyanin-free diet for 1 week before the study started and a low (poly)phenol diet for 3 days before and during the study. Volunteers were asked to exclude all fruits and vegetables and beverages such as cocoa, tea, coffee, wine and beer from their diet. Volunteers fasted for 12 h before the study day. A white bread cheese sandwich was given to the volunteers together with the cranberry juice and after 8 hours post-consumption. Volunteers were also given a low polyphenol meal to take home for dinner (consisting of fish and white rice) and were asked not to eat anything else until the 24 h blood sample and the urine collection was completed. Compliance with the diet was determined via a 24 h-dietary recall and via interview. Water was the only drink allowed *ad libitum*. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Duesseldorf Research Ethics Committee (ref: 14-012). This study was part of a study registered with the National Institutes of Health (NIH)-randomized trial records held on the NIH ClinicalTrials.gov website (NCT02517775).

Table 1
(Poly)phenol composition of cranberry juice. BL-DMAC corresponds to the 4-(dimethylamino)cinnamaldehyde (DMAC) assay used for quantification of proanthocyanidins (PAC) using procyanidin A2 as a standard and in OS-DMAC a proprietary standard was used. The total sum of (poly)phenols * used here included OS-DMAC which is more accurate then BL-DMAC [11].

	mg/450 ml
Phenolic acids	**24.5**
Benzoic acid	7.8
2-Hydroxybenzoic acid	0.1
3,4-Dihydroxybenzoic acid (Protocatechuic acid)	1.1
Gallic acid	0.1
Vanillic acid	1.0
Cinnamic acid	1.0
p-Coumaric acid	6.9
Caffeic acid	1.2
Ferulic acid	0.0
Chlorogenic acid	5.2
Flavan-3-ols	**5.0**
Catechin	0.5
Epicatechin	4.5
Flavonols	**31.3**
Quercetin	8.5
Quercetin-3-*O*-rhamnoside	4.6
Quercetin-3-*O*-galactoside	3.9
Myricetin	8.2
Myricetin-3-*O*-rhamnoside	3.2
Myricetin-3-*O*-galactoside	2.9
Anthocyanins	**16.2**
Cyanidin-3-arabinoside	6.8
Cyanidin-3-galactoside	1.7
Cyanidin-3-glucoside	0.0
Peonidin-3-arabinoside	2.1
Peonidin-3-galactoside	2.0
Peonidin-3-glucoside	0.0
Proanthocyanidins	
BL-DMAC	242.5
OSC-DMAC	710.5
Total phenolics (Folin method)	517.5
Total sum (poly)phenols*	**787.5**

2.3. Plasma and urine collection

Plasma was obtained by whole-blood centrifugation (EDTA-containing vacutainers) at 1800 *g* for 15 min at 4 °C, and spiked with 2% formic acid before storage at −80 °C. Ascorbic acid was added to the urine containers (3.75 g/2 l container) and acidification with formic acid until pH 2.5 was achieved. Urine containers were kept in an opaque cool bag with ice blocks at all times.

Please cite this article in press as: R.P. Feliciano, et al., Identification and quantification of novel cranberry-derived plasma and urinary (poly) phenols, Archives of Biochemistry and Biophysics (2016), http://dx.doi.org/10.1016/j.abb.2016.01.014