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Responses to a single dose of different polyphenols on the microcirculation and systemic circulation in rats

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ABSTRACT

Numerous studies suggest that the ingestion of polyphenols can potentially confer haemodynamic benefits. The association between different polyphenolic chemical structures and their physiologic effects on systemic haemodynamic variables and microcirculation are less well known. This study investigated the effects of a single 10 mg/kg body weight dose of different polyphenolic compounds on haemodynamic and microcirculatory parameters. Key results from laser Doppler flowmetry showed that flavan-3-ols, theaflavins, and quercetin significantly increased cremaster artery blood flow. Flavan 3-ols, theaflavins, (–)-epicatchin, daidzein, and cyanidin significantly elevated mean blood pressure, and flavan-3-ols significantly increased the heart rate. In contrast, hesperidin, epigallocatechin gallate, trans-resveratrol, gnetin C or curcumin did not result in any detectable changes. Our results suggest that polyphenols can contribute variably to alterations in the haemodynamic and microcirculatory status in rats depending on their characteristic chemical structures.

1. Introduction

Polyphenols are widely distributed in fruits, vegetables, nuts, seeds, and beverages, which can impart colour and taste to edible plants or processed foods derived from plant materials (Cheynier, 2005). Several thousand polyphenolic compounds have been identified in plants. These compounds are classified into different groups on the basis of the number of phenol rings they contain and the structural elements that bind these rings together (Bravo, 1998).

Numerous studies have established that some polyphenols can contribute to the reduction of cardiovascular disease risk (Arts & Hollman, 2005; Scalbert, Johnson, & Saltmarsh, 2005). The results of numerous epidemiological studies have suggested that the frequency of polyphenol intake is negatively correlated with the risk of coronary heart disease, myocardial infarction, and stroke (Lecour & Lamont, 2011; Visioli & Davalos, 2011). In addition, intervention trials have shown that repeated ingestion of polyphenol-rich foods resulted in a significant hypotensive effect in healthy and mildly-hypertensive subjects (Galleano, Pechanova, & Fraga, 2010;

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Medina-Remón, Estruch, Tresserra-Rimbau, Vallverdú-Queralt, & Lamuela-Raventos, 2013; Michalska et al., 2010; Rodrigo, Gil, Miranda-Merchak, & Kalantzidis, 2012). These previous data have shown that the ingestion of polyphenol-containing foods supported an improvement in endothelial function, as detected by flow-mediated dilation (FMD) and/or an elevation of nitric oxide (NO) metabolites levels in the blood. Alteration of endothelial function was observed not only after repeated ingestion of polyphenols, but also by a single dose of polyphenol-rich food. Several of these studies indicated that endothelial function was altered a few hours after the ingestion of polyphenol-containing foods (Li, Tian, Zhao, Chen, & Cui, 2013; Matsusima et al., 2013; Wong et al., 2011). In addition, Hooper et al. (2012) performed a meta-analysis that showed a single dose of chocolate was significantly more effective at increasing FMD than repeated ingestion. In our previous study in rats, we found that a single dose of polyphenol, containing the polyphenolic fraction derived from cocoa (the flavan-3-ol fraction), resulted in a rapid increase in skeletal muscle blood flow, a transient rise in blood pressure and heart rate, induction of phosphorylation of aortic endothelial nitric oxide synthase (eNOS), and an elevation of nitrate and nitrite levels in the blood (Inagawa, Aruga, Matsumura, Shibata, & Osakabe, 2014). According to these perception, initial haemodynamic changes were important to elucidation of the effect of polyphenols on circulating system.

To date, polyphenols have been studied in relation to their effects on circulation, but not enough is known about the correlations between polyphenols and their potential to alter haemodynamic as a consequence of their structure–activity relationship. In this rodent study, we compared a single dose of several different polyphenols and the response of systemic circulation and microcirculation by measuring blood pressure, heart rate, and blood flow in the cremaster artery using laser Doppler flowmetry.

2. Materials and methods

2.1. *Materials*

The flavan-3-ol fraction was provided by Meiji Co., Ltd (Tokyo, Japan) and was prepared from cocoa using a method described in a previous report (Osakabe et al., 1998). In brief, the cocoa powder was defatted with n-hexane and the residue was extracted with acetone. The n-butanol dissolved fraction of the extract was subsequently applied to a Diaion HP2MG column (Mitsubishi Kasei Co. Ltd., Tokyo, Japan). The fraction eluted with 80% ethanol was collected, freeze-dried, and used in the experiments. The concentration of catechins and procyanidins in this fraction was determined by high-performance liquid chromatography (HPLC) (Natsume et al., 2000). Flavan-3-ol fraction contained 4.56% (+)-catechin, 6.43% (–)-epicatechin, 3.93% procyanidin B2, 2.36% procyanidin C1, and 1.45% cinnamtannin A2. The theaflavin (TF) fraction was prepared from green tea leaves. The crude TF mixture was purchased from Yaizu Suisankagaku- Industry Co. Ltd., Shizuoka, Japan., which was prepared by incubating catechins derived from green tea leaves in the presence of polyphenol oxidase, The purified TF mixture was prepared, as follows: the crude TFs was solubilized in water and the catechins and caffeine in the solution were excluded with medium pressure column chromatography with a reverse-phase preparative column and with the gradient mobile phase, 10–80% acetonitrile. These TF were further purified by preparative HPLC with the Capcell Pak C18 (20 mm × 250 mm, Shiseido, Japan) and a mobile phase of 22% acetonitrile in the presence of 0.05% phosphoric acid. The aqueous solutions containing the respective TFs were treated with a Sep-Pak C18 cartridge, eluted with ethanol, and lyophilized. The concentration of each TF in the mixture was analyzed by HPLC. The resulting mixture contained 4.6% TF (TF1), 16.36% theaflavin-3-*O*-gallate (TF2A), 11.2% theaflavin 3'-*O*-gallate (TF2B) and 26.2% of theaflavin-3,3'-di-*O*-gallate (TF3). (–)-Epicatechin (purity assessment by HPLC, >90%), hesperidin (purity assessment by HPLC, >90%), quercetin (purity assessment by HPLC, >95%), daidzein (purity assessment by HPLC, >95%) and curcumin (purity assessment by HPLC, >97%) were purchased from Tokyo Chemical Industry (Tokyo, Japan), cyanidin (purity assessment by HPLC, >98%) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), epigallocatechin gallate (EGCG, purity assessment by HPLC, >98%) was purchased from the Cayman Chemical Company (Ann Arbor, MI, USA). *Trans*-resveratrol (purity assessment by HPLC, >97%) and gnetin C (purity assessment by HPLC, >97%) were provided by Yamada Bee Company, Inc. (Okayama, Japan). The chemical structures used in this study are shown in Fig. 1. Urethane and Krebs-Ringer bicarbonate buffer were purchased from Sigma Chemicals (St. Louis, MO, USA).

2.2. *Animals and diets*

This study was approved by the Animal Care and Use Committee of Shibaura Institute of Technology (Saitama, Japan). All animals received humane care under the guidelines of this institution. Male Wistar rats weighing 200–250 g (8–10 weeks old) were obtained from Saitama Experimental Animal Supply (Tokyo, Japan). The rats were housed in a temperature-regulated room (23–25 °C) with controlled lighting (12-h light and dark cycles). A basal Oriental MF diet was obtained from the Oriental Yeast, Co. Ltd., Tokyo, Japan.

2.3. *Experimental procedures*

The animals were fed the basal diet for 4 days. Eighteen to 24 animals were randomly allocated into each treatment group. The change in blood flow in the cremaster artery was measured in 10–12 animals and the changes in blood pressure and heart rate were determined in the other animals in each group. Under anesthesia using urethane (1 g/kg body weight, s.c.), the cremaster muscle was exteriorized and carefully spread out over an optically clear viewing pedestal, and the tissue surface was superfused with Krebs-Ringer bicarbonate buffer (pH 7.3–7.4) and 95% N_2–5% CO_2 at 37 °C, a tail cuff was attached, and an indwelling gastric feeding tube was inserted into the stomach of each animal. After a post-surgical 15 min of equilibration period, blood flow in the cremaster muscle was measured by a laser Doppler blood perfusion imager (Periscan PIM-2, Perimed Co. Ltd.) for 15 min. Distilled water (vehicle), or polyphenols suspended in distilled water, were administered to the animals via the feeding tube and blood flow in the cremaster artery was