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The flavonoid quercetin induces acute vasodilator effects in healthy volunteers: Correlation with beta-glucuronidase activity

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ABSTRACT

Quercetin exerts vasodilator, antiplatelet and antiproliferative effects and reduces blood pressure, oxidative status and end-organ damage in hypertensive humans and animal models. We hypothesized that oral quercetin might induce vasodilator effects in humans and that they might be related to the deconjugation of quercetin-3-O-glucuronide (Q3GA). *Design*: double blind, randomized, placebo-controlled trial. Fifteen healthy volunteers (26 ± 5 years, 6 female) were given a capsule containing placebo, 200 or 400 mg of quercetin in random order in three consecutive weeks. At 2 h a dose-dependent increase in Q3GA was observed in plasma (~0.4 and 1 μM for 200 and 400 mg, respectively) with minor levels of quercetin and isorhamnetin. No changes were observed in blood pressure. At 5 h quercetin induced and increase in brachial arterial diameter that correlated with the product of the levels of Q3GA by the plasma glucuronidase activity. There was an increase in urinary levels of glutathione but there was no increase in nitrites plus nitrates. Quercetin and isorhamnetin also relaxed human umbilical arteries *in vitro* while Q3GA was without effect. In conclusions, quercetin exerts acute vasodilator effects *in vivo* in normotensive, normocholesterolemic human subjects. These results are consistent with the effects being due to the deconjugation of the metabolite Q3GA.

Introduction

Flavonoids constitute a large class of polyphenols found in plant-derived foodstuffs which includes several subclasses; such as flavonols; flavones; flavanones; flavanols; anthocyanidins; isoflavones; dihydroflavonols and chalcones [1]. Among them; the flavonol quercetin is one of the most abundant and widely distributed. Epidemiological studies have found an inverse association between dietary flavonol and flavone intake and the risk of several diseases; including cancer; cardiovascular disease; and neurodegenerative disorders [2–4] which may account; at least partly; for the health effects of fruits and vegetables. The beneficial effect of flavonols on cardiovascular disease is supported by continuously growing evidence based on animal studies and short term clinical trials [5,6]. One of the main mechanisms by which flavonols are thought to lower cardiovascular risk is *via* its vasodilator and antihypertensive effect [7–11]. This might have a large impact on global mortality and morbidity since in 2010 elevated blood pressure was estimated to be the most important risk factor while a diet low in fruits is in the top four for disease burden globally [12]. In rats and humans quercetin lowers blood pressure in hypertensive but not in normotensive subjects; an effect which seems to be independent of endothelial function or angiotensin-converting enzyme

Abbreviations: CE, collision energy; DAD, diode array detector; DP, declustering potential; EP, entrance potential; FMD, flow-mediated dilatation; iso-PGF2α, 8-iso-prostaglandin F2α; MS, mass spectrometer; NO, nitric oxide; NO_x, NO_2^- and NO_3^-; Q3GA, quercetin-3-O-glucuronide.

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Table 1
Baseline characteristics.

N	16
Age, years	25.8 ± 5.2
Males (%)	10 (62.5)
Body mass index, kg/m^2	24.0 ± 4.7
Systolic blood pressure, mmHg	117.1 ± 3.6
Diastolic blood pressure, mm	69.3 ± 2.9
Total cholesterol, mg/dL	169.1 ± 30.6
LDL cholesterol, mg/dL	92.8 ± 7.0
HDL cholesterol, mg/dL	57.1 ± 2.3
Triglycerides, mg/dL	93.1 ± 44.8
Fasting glucose, mg/dL	84.2 ± 5.8
Creatinemia, mg/dL	0.87 ± 0.20

Values are mean SD unless indicated otherwise.

activity [13,11]. Quercetin induces arterial vasodilation in isolated arteries from several animal species [7,14] but to our knowledge its effects on human arterial tone have not been tested so far *in vitro* or *in vivo*.

Quercetin may be administered orally in its glycosylated forms as they are regularly present in foods or as an aglycone in food supplements [15]. The major metabolites of quercetin in human plasma are quercetin-3-O-glucuronide (Q3GA), quercetin-3′-O-sulfate and isorhamnetin-3-O-glucuronide (3′-methyl-quercetin-3-O-glucuronide) while minimal concentrations of free aglycone are found in plasma or urine [16]. However, the time-course of the biological effects of quercetin does not correlate with the presence in plasma of these metabolites. In fact, these metabolites are usually less active than the parent aglycones and often totally inactive *in vitro* when tested for short periods of time [17]. However, the glucurono-conjugated metabolites of quercetin can be hydrolyzed by β-glucuronidase in several tissues, releasing the parent aglycone which accumulates intracellularly [18–22]. Therefore, at time periods longer than 1–2 h, Q3GA induces vasorelaxation *in vitro* [21] and reduces blood pressure *in vivo* [23] and both effects can be prevented by inhibition of β-glucuronidase. Thus conjugation is a reversible process and, at least regarding the vasodilator and antihypertensive effects in laboratory animals, the conjugation–deconjugation cycle appears to be an absolute requirement [24]. We hypothesized that oral quercetin might induce vasodilator effects in humans and that they might be related to the deconjugation of Q3GA.

Materials and methods

Subjects and experimental design

Seventeen healthy volunteers participated in the study. All subjects were healthy, had no evidence of chronic disease, had not taken any medication or vitamin supplement and were advised not to take flavonoid rich foods from a list provided 48 h before each intervention. The baseline characteristics are shown in Table 1. All subjects had normal values of blood pressure, electrolytes, hepatic enzymes, urea, uric acid, homocysteine and blood cells.

The acute effects of two doses of quercetin aglycone (200 and 400 mg) were assessed in a randomized double-blind placebo-controlled crossover study. The doses were chosen as they represent a maximal amount of flavonoids which could be achieved by eating flavonoid-rich foods but lower than those present in most food supplements (1 g/day is generally used). During the initial visit subjects were interviewed, gave informed written consent, received the instructions and blood samples were taken for routine biochemical and hematological analysis. The treatments consisted in a single administration of a capsule containing 200 and 400 mg of quercetin or placebo (capsule with rice starch indistinguishable from active treatments). The subjects received each of the three treatments in random order 1 week apart on the same day of the week and at the same time of the day. On the treatment day, a blood sample was collected at time 0 (baseline) and 2 h and a urinary sample at time 0 and 5 h after oral administration of the capsules. We choose 2 h to collect plasma samples because after oral ingestion of quercetin aglycone, the median plasma concentrations of total quercetin peaked at 60–360 min in healthy volunteers [25–27,11]. One subject was excluded because only received one treatment, other subject only received placebo and 200 mg quercetin. No adverse effects after quercetin or placebo intake were found.

Brachial artery diameter measurements

Participants rested for 15 min in the supine position. A blood pressure cuff was placed on the upper right arm proximal to the elbow but distal to the placement of an ultrasound Doppler probe on the brachial artery. All measurements were performed by the same trained ultrasound operator. The brachial artery was assessed 2–3 cm above the elbow using external B-mode ultrasound imaging and arterial diameter was analyzed off-line using automatic edge-detection (Sonosite, Titan, 7.0 MHz linear transducer). Angle-corrected and intensity-weighted mean velocities (V_{mean}) were determined using Logic 7 software. Brachial artery blood flow was calculated using the formula blood flow = $V_{mean}\pi$(arterial diameter/2)$^2 \times 60$. Three blood pressure measurements were performed in the left arm using an automatic sphygmomanometer (Omron, Spain).

Analysis of quercetin and metabolites in plasma

Plasma samples (300 μL) supplemented with ascorbic acid (30 μL of 10 mM aqueous solution) and apigenin 7-O-glucoside as internal standard (40 μL of 10 μM solution in 50% acetonitrile in water), were extracted and analyzed as described [23]. Briefly, samples were treated with acetonitrile/0.5 M acetic acid (80:20, v/v) and centrifuged. The pellet was submitted to a similar process twice with acetonitrile and the combined supernatants were dried. Extracts were dissolved in acetonitrile/water (30:70, v/v) and analyzed in a Hewlett-Packard 1100 chromatograph (Agilent Technologies, Waldbronn, Germany) with a quaternary pump and a diode array detector (DAD) coupled to an HP Chem Station (rev. A.05.04) data-processing station as described. An Ascentis TM RP-Amide 3 μm (2.1 × 150 mm) column was used. The solvents used were: (A) 0.1% formic acid, and (B) acetonitrile. An elution gradient was established from 15% to 50% B over 15 min, isocratic 50% B for 10 min, from 50% to 75% B over 3 min, isocratic 75% B for 10 min, and re-equilibration of the column, at a flow rate of 0.2 mL/min. Double online detection was carried out in the DAD using 370 nm as a preferred wavelength and in a mass spectrometer (MS) connected to HPLC system *via* the DAD cell outlet. MS detection was performed in a API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an ESI source and a triple quadrupole-ion trap mass analyzer that was controlled by the Analyst 5.1 software. Zero grade air served as the nebulizer gas (30 psi) and turbo gas for solvent drying (400 C, 40 psi). Nitrogen served as the curtain (20 psi) and collision gas (medium). Both quadrupoles were set at unit resolution. The ion spray voltage was set at −4500 V in the negative mode. Method settings used were: declustering potential (DP) −40 V, entrance potential (EP) −10 V, collision energy (CE) −50 V, and cell exit potential −3 V. In order to obtain the fragmentation pattern of the parent ion(s), enhanced product ion mode was also performed using the following parameters: DP −50 V, EP −6 V, CE −25 V, and collision energy spread 0 V. Quantitative determination of the assayed quercetin and conjugated metabolites was performed from their chromatographic peaks at 370 nm by comparison with calibration curves of standard compounds either commercial