Different cardiovascular protective effects of quercetin administered orally or intraperitoneally in spontaneously hypertensive rats


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We tested whether the administration procedure of quercetin affects its metabolite profile and antihypertensive activity. Spontaneously hypertensive rats (SHR) were randomly assigned to four experimental treatments: (1) 1 mL of 1% methylcellulose by oral gavage and 2% DMSO i.p. (control group); (2) 10 mg kg⁻¹ quercetin by oral gavage once daily and 2% DMSO i.p.; (3) 10 mg kg⁻¹ quercetin by oral gavage divided in two daily doses (5 + 5 at 12 h intervals) and 2% DMSO i.p.; (4) 1 mL of 1% methylcellulose by oral gavage and 10 mg kg⁻¹ quercetin i.p. injection. Rats were treated daily for 5 weeks. Single dose and two daily doses, in a long-term oral treatment were equally efficient, both restoring the impaired aortic endothelium-dependent vasodilatation and reducing mesenteric contractile response to phenylephrine, systolic blood pressure, heart rate, and heart and kidney hypertrophy. Attenuation of vascular NADPH oxidase-driven $O_2^-$ production was also found in orally treated rats. Intraperitoneal administration reduced, to lesser extent than oral administration, the increased systolic blood pressure, being without effect to the endothelial dysfunction and vascular oxidative stress. In contrast, greater levels of metabolites were quantified following intraperitoneal compared to oral administration at any time point, except for higher plasma methylated quercetin aglycone in oral as compared to intraperitoneal administration at 2 but not at 8 h. In conclusion, oral quercetin was superior to intraperitoneal administration for the protection from cardiovascular complications in SHR. No differences were found between the oral administration as a single daily dose or divided into two daily doses.

Introduction

The health benefits of consuming fruits and vegetables are often attributed, in part, to their high content of polyphenolic compounds. The consumption of polyphenols in a plant-derived diet can be several times higher than the consumption of other phytochemicals and vitamins. Among the polyphenols, flavonoids have attracted considerable attention. Epidemiological studies have found that an increased intake of dietary flavonoids is associated with a decreased mortality related to ischaemic heart disease. Quercetin (3,3',4',5,7-pentahydroxyflavone), a member of the flavonoid family, is one of the most widely found dietary polyphenolic compounds in foods including vegetables, fruits, tea, and wine. Several experimental studies have shown that quercetin exerts several biological effects including improved vascular reactivity and antihypertensive effects. These antihypertensive effects are accompanied by a reduction of associated end-organ damage (cardiac hypertrophy, kidney histological alterations, proteinuria, vascular remodelling and endothelial dysfunction), as well as a decrease in the markers of oxidative stress in plasma, liver and urine.

A quercetin-supplemented diet increased nitric oxide production in rat aorta and reduced blood pressure and cardiac hypertrophy in rats with aortic constriction. However, Carlstrom et al. reported no effects of this diet in spontaneously hypertensive rats (SHR). This result contrasts with other reports on cardiovascular protection when quercetin was delivered via oral gavage. Furthermore, it has been shown that the metabolite profiles of quercetin in the plasma of rats are significantly affected by the administration procedure. Thus, the bioavailability of quercetin aglycone could be a determining factor of its beneficial effects in vivo. Therefore, in this study we sought to determine whether different dosage regimens of quercetin (1 or two daily oral doses or 1 intraperitoneal dose) lead to different metabolite profiles and different efficiency to attenuate...
the severity of hypertension, vascular dysfunction and oxidant stress that develops in SHR.

Methods

Animals and experimental groups

The present investigation conforms to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1996), and the procedures were approved by our institutional review board (Comité de Experimentación Animal, Universidad de Granada, ref. 2066/10). Twenty-week old, male SHR rats were obtained from Harlan Laboratories (Barcelona, Spain). All rats were maintained, five per cage, at a constant temperature (24 ± 1 °C), with a 12-hour dark/light cycle and on standard rat chow. An adaptation period of two weeks for vehicle administration and blood pressure measurements was allowed before the initiation of the experimental protocols. Forty SHR were randomly assigned to four experimental groups (n = 10 each): (1) 1 mL of 1% methylcellulose administered by an intragastric probe (oral gavage) and 2% DMSO i.p. (control group), (2) 10 mg kg⁻¹ quercetin by oral gavage once daily and 2% DMSO i.p. (Q1Od), (3) 10 mg kg⁻¹ quercetin by oral gavage divided in two daily doses (5 + 5 at 12 h intervals) and 2% DMSO i.p. (Q2Od), and (4) 1 mL of 1% methylcellulose by oral gavage and 10 mg kg⁻¹ quercetin i.p. (Q11Pd). Rats were treated daily for five weeks. The quercetin treatment was stopped two days before the end of the experiment in order to study the long-term effects of quercetin without the involvement of the effects of acute administration. 

In order to study the metabolite profile and antihypertensive activity of quercetin by the administration procedure another set of experiments were performed. Male SHR were given quercetin (10 mg kg⁻¹) either orally as a suspension in 1% methylcellulose or intraperitoneally as a solution in 2% DMSO for two weeks. The last day of treatment the animals were sacrificed either at 2 h or 8 h after the administration of the flavonoid. Plasma samples were collected and frozen at −80 °C until analysis.

Blood pressure measurements

Systolic blood pressure (SBP) was measured weekly 18–20 h after administration of the drugs in conscious, pre-warmed, restrained rats by tail-cuff plethysmography.²⁸

Cardiac and renal weight indices

At the end of the experimental period, animals were anaesthetized with 2.5 mL kg⁻¹ equitensin (i.p.) and blood was collected from the abdominal aorta. The heart and kidneys were excised, cleaned and weighed. The atria and the right ventricle were then removed and the remaining left ventricle weighed. The cardiac, left ventricular and renal weight indices were calculated by dividing the heart, left ventricle and kidney weight by the body weight.

Vascular reactivity studies

The fourth branch of the mesenteric artery (1.7–2 mm in length) and descending thoracic aortic rings (2–3 mm) were dissected and mounted in a Mulvany wire myograph (model 610M, Danish Myo Technology, Aarhus, Denmark) and organ chambers, respectively, filled with Krebs solution (composition in mM: NaCl 118, KCl 4.75, NaHCO₃ 25, MgSO₄ 1.2, CaCl₂ 2, KH₂PO₄ 1.2 and glucose 11) at 37 °C and gassed with 95% O₂ and 5% CO₂. Each mesenteric artery was stretched to its individual optimal lumen diameter, i.e., the diameter at which it developed the strongest contractile response to 62.5 mM K⁺, using a diameter-tension protocol.²⁸ Changes in arterial tone were recorded via a PowerLab/8A30 recording unit (ADInstruments Pty Ltd., Australia), and analyzed using Chart 5.0 acquisition system (ADInstruments). Concentration response curves to phenylephrine (10⁻⁷ M–10⁻⁴ M) and a vasoconstrictr response to KCl 125 mM were performed in intact vessels.

Aortic rings were stretched to 2 g of resting tension by means of two L-shaped stainless-steel wires inserted into the lumen and attached to the chamber and to an isometric force-displacement transducer (Letigraph 2000), respectively, as previously described.²⁸ The concentration-relaxation response curves to acetylcholine (10⁻⁹ M–10⁻⁵ M) were performed in aortic rings pre-contracted by 10⁻⁶ M phenylephrine. In endothelium-denuded aorta, the concentration-relaxation response curves to nitroprusside (SNP, 10⁻⁹ M–10⁻³ M) were performed in the dark in rings without endothelium pre-contracted by 10⁻⁶ M phenylephrine.

NADPH oxidase activity

The lucigenin-enhanced chemiluminescence assay was used to determine NADPH oxidase activity in intact aortic rings, as previously described.²⁷ Aortic rings from all experimental groups were incubated for 30 min at 37 °C in HEPES-containing physiological salt solution (pH 7.4) of the following composition (in mM): NaCl 119, HEPES 20, KCl 4.6, MgSO₄ 1, Na₂HPO₄ 0.15, KH₂PO₄ 0.4, NaHCO₃ 1, CaCl₂ 1.2 and glucose 5.5. Aortic production of O₂⁻ was stimulated by addition of NADPH (100 μM). Rings were then placed in tubes containing physiological salt solution, with or without NADPH, and lucigenin was injected automatically at a final concentration of 5 μM to avoid known artifacts when used a higher concentrations. NADPH oxidase activity were determined by measuring luminescence over 200 s in a scintillation counter (Lumat LB 9507, Berthold, Germany) in 5-s intervals and was calculated by subtracting the basal values from those in the presence of NADPH. Vessels were then dried, and dry weight was determined. NADPH oxidase activity is expressed as relative luminescence units (RLU)/min mg⁻¹ dry aortic tissue.

Western blots analysis

Aortic homogenates were run on a sodium dodecyl sulphate (SDS)-polyacrilamide electrophoresis. Proteins were transferred to polyvinilidene difluoride membranes (PVDF). Membranes were incubated overnight at 4 °C with polyclonal rabbit anti-p47phox (1 : 1000, Millipore), monoclonal mouse anti-p22phox (1 : 1000, Santa Cruz Biotechnology), polyclonal rabbit NOX4 (1 : 1000, Novus Biologicals), or polyclonal rabbit NOX1 (1 : 2000, Novus Biologicals). Antibody binding was detected by an ECL system (Amersham Pharmacia Biotech, Amersham, United Kingdom).
Extraction and analysis of quercetin metabolites from rat plasma

Plasma samples were extracted with the same volume of methanol/0.5 M acetic acid (80 : 20, v/v) for 30 min at 25 °C in an ultrasonic bath, and then centrifuged for 3 min at 3500g. The supernatant was collected and the pellet submitted to the same process further two times, assisted by sonication (1 min) using a Microson™ XL-2000 ultrasonic cell disruptor (Misonix Inc., New York, USA). The methanolic extracts obtained from 6 rats were pooled and dried in a centrifugal concentrator micVac (GeneVac, Ipswich, United Kingdom). The residue was dissolved in 120 μL acetonitrile/water (30 : 70 v/v) and centrifuged (5 min, 3500g) previous to its injection (100 μL) in HPLC-DAD-ESI/MS.

HPLC-DAD-ESI/MS analyses were carried out 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. An Ascentis™ RP-Amide 3 μm (2.1 × 150 mm) column thermostatted at 30 °C 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⁻¹. 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 analyses were performed in a Finningan™ LCQ (Thermoquest, San Jose, CA, USA) equipped with an ESI source and an ion trap mass analyser, which were controlled by the LCQ Xcalibur software. Both the auxiliary and the sheath gases were nitrogen at flow rates of 20 and 80 L min⁻¹, respectively. The source voltage was 4.5 kV, the capillary voltage was 39 V, and the capillary temperature was 195 °C. Spectra were recorded in positive ion mode between m/z 150 and 1500. The mass spectrometer was programmed to record a full mass and MS² scans of the most abundant ion in the full mass, using a normalised energy of collision of 45%. Quantitative analysis of quercetin and conjugated metabolites was performed from their chromatographic peaks recorded at 370 nm by comparison with calibration curves obtained by injection of increasing concentrations of quercetin and quercetin 3-O-glucuronide.

Statistical analysis.

Results are expressed as means ± SEM and n reflects the number of animals. Statistically significant differences between groups were calculated by Students’ t test for unpaired observations or for multiple comparisons by an ANOVA followed by a Newman Keuls test. P < 0.05 was considered statistically significant. Concentration-response curves were fitted to a logistic equation and from these plots the maximal effect (Eₘₐₓ) and the negative logarithm of the concentration producing half maximal response (pD₂) were calculated.

Results

Blood pressure, cardiac and renal hypertrophy

Chronic administration (five weeks) of oral quercetin (10 mg kg⁻¹) resulted in a progressive decrease in SBP from the first week of treatment (Fig. 1A). The effect tended to be more efficacious when given as a single daily dose (Q1Od) than when the dose was divided into two administrations at 12 h intervals (Q2Od), but this difference was not significant. Chronic oral quercetin treatments also significantly reduced heart rate (HR) after the second (Q1Od group) or third (Q2Od group) week compared to control rats (final HR, 392 ± 9 and 400 ± 8 bpm, respectively, vs. 425 ± 10 bpm in control rats, Fig. 1B). Likewise, in the group treated with quercetin by intraperitoneal injection (Q1IPd) a decrease in SBP and HR was observed, which was statistically significant only in the last two weeks of treatment. No statistical differences between Q1IPd and Q2Od in both parameters were found. However, we found a different time course and less intensive anti hypertensive effect in the Q1IPd group as compared to the Q1Od group (final decrease in SBP: 13.5 ± 1.6 vs. 18.1 ± 2.4%, respectively, P < 0.05).

At the end of the experimental period, animals of the oral groups showed significantly decreased left ventricular and renal weight indices, as compared with the animals of the control group. No significant modifications of these parameters were observed in the rats receiving quercetin intraperitoneally (Fig. 2).

Effects on responses to vasoconstrictors in mesenteric resistance arteries

Both oral treatments of quercetin caused a downward shift in the phenylephrine concentration response-curve in mesenteric arteries, without affecting pD₂ (Fig. 3A, Table1) and the response to 125 mM KCl was also reduced as compared to the control group (Fig. 3B). In contrast, the chronic intraperitoneal administration of quercetin did not affect either the phenylephrine or the KCl response (Fig. 3, Table 1).
Effects on endothelial function and NOX subunits expression in thoracic aorta

NADPH increases lucigenin-enhanced luminescence in normal aortic rings, which was almost abolished by previous incubation for 30 min with the flavoprotein inhibitor DPI (10 μM) (not shown), showing that external NADPH increased NADPH oxidase activity in vascular tissue. NADPH oxidase activity was decreased significantly in aortic rings from groups treated with oral quercetin as compared to control rats, being without effect in rats treated intraperitoneally (Fig. 5A). Chronic oral but not chronic i.p. treatments with quercetin reduced protein expression of the NADPH subunits p47^{phox} (Fig. 5B), NOX1 (Fig. 5D) and NOX4 (Fig. 5E). Any changes in p22^{phox} protein expression were detected after treatments with quercetin (Fig. 5C).

Quercetin metabolites in rat plasma from two administration routes

The plasma samples obtained after 2 and 8 h of oral (intragastrical) or intraperitoneal administration of quercetin (10 mg kg^{-1}) were analyzed by HPLC-DAD-MS (Fig. 6). Peaks identified as quercetin metabolites in the chromatograms yielded a common product ion at m/z 303 and showed absorption spectra with maximum wavelength of absorption around 360–370 nm. Identities of the compounds were tentatively assigned according to their pseudomolecular ions, i.e., methylquercetin ([M + H]^+ at m/z 317), methylquercetin glucuronide ([M + H]^+ at m/z 493), methylquercetin sulfate ([M + H]^+ at m/z 397) and methylquercetin diglucuronide ([M + H]^+ at m/z 655). Curiously, none of the two detected methylquercetins corresponded toisorhamnetin or tamarixetin, as checked by comparison with authentic standards. The concentrations of the quantifiable metabolites are shown in the Table 2.

Table 1 pD_{2} and E_{max} values of the contractile responses to phenylephrine in mesenteric arteries from all experimental groups calculated from data in Fig. 3A

<table>
<thead>
<tr>
<th>Groups</th>
<th>pD_{2} (−log EC_{50})</th>
<th>E_{max} (mN)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>5.60 ± 0.09</td>
<td>25.1 ± 2.0</td>
</tr>
<tr>
<td>Q1Od</td>
<td>5.56 ± 0.05</td>
<td>19.6 ± 2.1</td>
</tr>
<tr>
<td>Q2Od</td>
<td>5.54 ± 0.04</td>
<td>19.7 ± 2.1</td>
</tr>
<tr>
<td>Q1IPd</td>
<td>5.67 ± 0.10</td>
<td>22.7 ± 2.8</td>
</tr>
</tbody>
</table>

* SHR vehicle (Cont, n = 9), quercetin 1 oral dose/day (Q1Od, n = 10), quercetin 2 oral doses/day (Q2Od, n = 9) and quercetin 1 i.p. dose/day (Q1IPd, n = 9) groups. Data are expressed as mean ± SEM. * P < 0.05 vs. SHR Cont group.

Effects on nitric oxide mediated-vasodilator responses to the NO donor SNP in vessels pre-contracted with phenylephrine

Aortas from SHR showed significantly reduced endothelium-independent vasodilator responses to the NO donor SNP in vessels pre-contracted with phenylephrine (Fig. 4B).

![Fig. 4](image-url) Effects of quercetin on endothelial function. Vascular relaxant responses induced by acetylcholine (Ach, A) in endothelium-intact and sodium nitroprusside (SNP, B) in endothelium-denuded aortae pre-contracted by 1μM phenylephrine (Phe) from SHR vehicle (Cont, n = 9), quercetin 1 oral dose/day (Q1Od, n = 10), quercetin 2 oral doses/day (Q2Od, n = 9) and quercetin 1 i.p. dose/day (Q1IPd, n = 9) groups. Data are expressed as mean ± SEM. * P < 0.05 vs. SHR Cont group. # P < 0.05 vs. Q1IPd group.
administration at 2 h, and continued being prominent after 8 h, at which other methylquercetin glucuronides were also observed. A sulfated methylquercetin was also detected as a minor metabolite in both oral and intraperitoneal assays at 8 h. Greater levels of total metabolites were quantified following intraperitoneal than oral administration at the two time points.

**Discussion**

Herein, we describe for the first time that the route (oral vs. intraperitoneal) of chronic administration of quercetin alters the time course and the efficacy of its antihypertensive effects in SHR and its protective effect in target organs, such as heart, kidneys and endothelium, being oral administration more effective than intraperitoneal injection. In contrast, greater levels of metabolites were quantified following intraperitoneal compared to oral administration at any time point, except for higher plasma methylated quercetin aglycone in oral as compared to intraperitoneal administration at 2 but not at 8 h. These data confirm that the method of quercetin delivery is a critical determinant of the biological effect.

We found that under the same dose level of quercetin, the efficacy of a single administration (10 mg kg\(^{-1}\)) and two daily doses (5 + 5 mg kg\(^{-1}\) at 12 h intervals) was comparable; both dosage regimens reduced similarly blood pressure, and heart and kidney hypertrophy, and restored the impaired aortic endothelium-dependent vasodilation. If any, differences favoured the single vs. the two daily doses. Clinical trials reporting the antihypertensive effects of quercetin have used a single dose (150 mg,\(^{28}\)) or two daily doses (2 × 365 mg,\(^{18}\)) but they have not been directly compared. If our data could be translated to humans, a single daily dose should be equivalent (or possibly better) than repeated doses. We speculate that this might also have nutritional relevance because it suggests that a single flavonoid-rich meal per day might be sufficient to afford cardiovascular protection.

In contrast to our method of administering quercetin via oral gavage, other ways of incorporating quercetin directly into the diet and allowing *ad libitum* consumption have been employed. A quercetin-supplemented diet increased nitric oxide production in rat aorta\(^{20}\) and reduced blood pressure and cardiac hypertrophy in rats with aortic constriction.\(^{21}\) However, Carlstrom *et al.*\(^{22}\) did not find any effect of a quercetin-supplemented diet while the oral administration of quercetin *via* gavage was effective in preventing cardiovascular complications in SHR. Those authors explained the different behaviour by the higher peak plasma quercetin levels achieved after gavage as compared to those achieved in diet-supplemented quercetin. Similarly, Kawai *et al.*\(^{24}\) found that the profile of metabolites and the antioxidative capacity in the plasma of rats was significantly affected by the administration procedure. Thus, plasma extracts rich in conjugated metabolites from free-access supplemented-quercetin diet were less effective regarding their *in vitro* antioxidant activity towards LDL oxidation than those from the oral group.

Compounds administered i.p. are absorbed primarily through the portal circulation and, therefore, must pass through the liver where they may be metabolized before reaching other organs.\(^{29}\) In contrast, compounds administered orally are subject to potential degradation and metabolism by enzymes from the host.
and the microbiota in the gastrointestinal lumen. During intestinal absorption, compounds are subject to potential metabolism in the epithelial cells before they reach the portal circulation and finally the liver. Therefore, hepatic and peripheral metabolism is common to oral and i.p. administration while intraintestinal and epithelial metabolism are exclusive to the oral treatment. As a rule, intraperitoneal administration is expected to lead to higher drug bioavailability. Quercetin glycosides are known to be hydrolyzed by the microbiota, releasing the free aglycone which can be further degraded into simpler polyphenols or phenolic acids.30,31 During absorption quercetin can be also conjugated in the intestinal wall.12,32 Finally, quercetin or its metabolites are subject to hepatic metabolism (methylation and conjugation with glucuronic acid and sulphate,34) and further metabolism in peripheral tissues (deconjugation and oxidation into phenolic acids,35,50).

In the present study we found that the route of administration is a critical determinant for the time course and effectiveness of the cardiovascular protection in SHR. In fact, chronic quercetin administration intraperitoneally also reduced SBP, but only after three weeks of treatment, and in this case it was not accompanied by a protection in target organs and endothelial function. This lack of protective effects in the morphology of the heart and kidneys despite low SBP at the end of the experiment (five weeks) compared to control might be explained by an insufficient time to regress organ hypertrophy. The ex vivo response to vasoconstrictors (KCI and phenylephrine) was also reduced in mesenteric vessels only after chronic oral quercetin, as compared to control and intraperitoneal administration. In vessels from SHR and essential hypertensive patients, an enhanced production of endothelial superoxide anion has been described,13,23,38,39 and further metabolism in peripheral tissues (deconjugation and oxidation into phenolic acids,35,50).

The present study also revealed that both the absolute amount and the profile of quercetin metabolites in plasma after oral administration was different to those found following intraperitoneal administration. As could be expected from a more direct administration route, concentrations of metabolites as a whole were higher after 2 or 8 h of intraperitoneal administration compared to the oral one, indicating a higher bioavailability. However, this absolute higher bioavailability did not predict a higher efficacy, indicating that the profile of the metabolites is essential for the activity. A number of different compounds were identified consistent with the previously reported complex metabolism of quercetin.40,46,47 In SHR, quercetin aglycone was undetectable and there was a strong methylation of the flavonoid at 2 and 8 h regardless of the administration route, supporting previous observations in rats and humans that indicate that quercetin is mainly metabolized by methylation. However, 3′ and 4′ methyl derivatives, i.e.isorhamnetin and tamarixin, have been the metabolites usually reported46,47 while, surprisingly, in the present study none of the two detected unconjugated methylvquercetins were found as the main metabolites in plasma 2 h after oral administration accompanied by a glucuronidated.

### Table 2 Quercetin metabolites detected in plasma of rats 2 and 8 h after the administration of quercetin (10 mg kg⁻¹)²

<table>
<thead>
<tr>
<th>Assay</th>
<th>Metabolites (tentative identity)</th>
<th>Concentration at 2 h (μM)</th>
<th>Concentration at 8 h (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>N.D.⁶</td>
<td>0.08</td>
<td>0.6415</td>
</tr>
<tr>
<td>Oral</td>
<td>Quercetin diglucuronide</td>
<td>0.40</td>
<td>Tr</td>
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<tr>
<td></td>
<td>Methylquercetin glucuronide 1</td>
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<tr>
<td></td>
<td>Methyl quercetin 1</td>
<td></td>
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<tr>
<td></td>
<td>Methylquercetin 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methylquercetin sulfate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>Methylquercetin glucuronide 1</td>
<td>2.26</td>
<td>Tr</td>
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<tr>
<td></td>
<td>Methylquercetin glucuronide 2</td>
<td></td>
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<tr>
<td></td>
<td>Methylquercetin glucuronide 3</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Methylquercetin sulfate</td>
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<tr>
<td></td>
<td>Methylquercetin 2</td>
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<td></td>
</tr>
</tbody>
</table>

² Tr. compound detected but not in sufficient amounts for routine quantification. ⁶ Not detected.
methylquercetin at 8 h. In contrast, rat plasma after intraperitoneal administration contained essentially high levels of glucuronidated methylquercetins and relatively minor amounts of sulphated and unconjugated metabolites.

Our previous results also showed that quercetin conjugated metabolites (quercetin-3-glucuronide, quercetin-3′,O-sulfate orisorhamnetin-3-O-glucuronide) have no direct acute vasorelaxant effect in isolated rat aorta or in mesenteric arteries (unpublished). But recently, we have shown that, in vitro conditions, long term (3 h) incubation with quercetin-3-glucuronide induced a progressive inhibitory effect on vascular contraction in a concentration- and time-dependent manner. Moreover, we found that quercetin-3-glucuronide lowered SBP in vivo in SHR. These effects were prevented by inhibition of β-glucuronidase demonstrating that this was due to local deconjugation of the glucuronide and subsequent quercetin accumulation. Nevertheless, concentrations of quercetin-3-glucuronide higher than quercetin aglycone were required to produce vasorelaxant effects.

Taken together, the differences in efficacy of the two routes of administration might be related to the different profiles in plasma metabolites, with higher levels of methylquercetin aglycones and lower of conjugated (glucuronidated) metabolites after oral administration at least at 2 h. However, we cannot discard that other undetected metabolites can be responsible for the higher efficacy of the oral administration. Recently, an interesting hypothesis has been raised proposing that polyphenols are metabolized by the resident microbiota in the colon and the metabolites entering the systemic circulation may be responsible for the in vivo effects. Our results showing that intraperitoneal administration of quercetin is effective in lowering SBP indicate that the intestinal metabolism by enzymes from the host or the microbiota is not essential for the activity of quercetin if we exclude a possible role of metabolism of excreted compounds in the bile followed by re-absorption. However, the higher activity of orally administered quercetin is consistent with the view that intestinal metabolism enhances the efficacy of quercetin as antihypertensive.

**Conclusion**

In conclusion, we found that chronic oral quercetin induced a faster and stronger decrease in SBP than intraperitoneal quercetin, and that there were no significant differences in the efficacy of oral quercetin antihypertensive activity when given as a single daily dose or when it was divided into two daily doses. Moreover, target organ protection was not afforded at five weeks by intraperitoneal quercetin, possibly related to the slower effect on SBP. The overall bioavailability, considered as the sum of detected metabolites, was higher after intraperitoneal administration. However, we found higher plasma levels of methylquercetins, as aglycones, during the early hours after the oral administration which might account for the higher efficacy of the oral route.

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