Endothelium-Independent Vasodilator Effects of the Flavonoid Quercetin and Its Methylated Metabolites in Rat Conductance and Resistance Arteries

FRANCISCO PÉREZ-VIZCAÍNO, MANUEL IBARRA, ANGEL L. COGOLLUDO, JUAN DUARTE, FRANCISCO ZARAGOZÁ-ARNÁEZ, LAURA MORENO, GUSTAVO LÓPEZ-LÓPEZ, and JUAN TAMARGO

Department of Pharmacology, School of Medicine, University Complutense of Madrid, Madrid (F.P-V., A.L.C, F.Z-A., L.M., J.G.L-L.); Department of Pharmacology, School of Pharmacy, University of Alcalá, Madrid (M.I.); and Department of Pharmacology, School of Pharmacy, University of Granada, Granada (J.D.), Spain

Received January 18, 2002; accepted February 28, 2002 This article is available online at http://jpet.aspetjournals.org

ABSTRACT

The flavonoid quercetin is metabolized into isorhamnetin, tamarixetin, and kaempferol, the vascular effects of which are unknown. In the present study, the effects of quercetin and its metabolites were analyzed on isometric tension in isolated rat thoracic and abdominal aorta, in isolated intact and β-escin-permeabilized iliac arteries, and on perfusion pressure in the isolated mesenteric resistance vascular bed. In noradrenaline-pretreated vessels, the four flavonoids produced a vasodilator effect, which was inversely correlated with the diameter of the vessel studied; i.e., quercetin, isorhamnetin, tamarixetin, and kaempferol were 5-, 25-, 4-, and 6-fold, respectively, more potent in the resistance mesenteric bed (−log IC50 = 5.35 ± 0.15, 5.89 ± 0.11, 5.34 ± 0.10, and 5.66 ± 0.06, respectively) than in the thoracic aorta (−log IC50 = 4.68 ± 0.08, 4.61 ± 0.08, 4.73 ± 0.11, and 4.81 ± 0.13, respectively; n = 4–6). The vasodilator responses of quercetin and isorhamnetin were not significantly modified after removal of the endothelium in the thoracic aorta or in the mesenteric bed. Furthermore, the guanylate cyclase inhibitor ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; 10−6 M), the adenylate cyclase inhibitor SQ22536 [9-(tetrahydro-2-furanyl)-9H-purin-6-amine; 10−6 M], KCl (40 mM), or ouabain (10−3 M) had no effect on isorhamnetin-induced vasodilation in the mesenteric bed. In permeabilized iliac arteries stimulated with Ca22+ (pCa of 5.9), isorhamnetin was also significantly more potent (−log IC50 = 5.27 ± 0.15) than quercetin (−log IC50 = 4.56 ± 0.15). In conclusion, quercetin and its metabolites showed vasodilator effects with selectivity toward the resistance vessels. These effects are not due to or modulated by endothelial factors and are unrelated to changes in cytosolic Ca2+.

Flavonoids comprise a large group of secondary metabolites widely distributed throughout the plant kingdom, including food plants. The daily flavonoid intake in the human diet (mainly from onions, apples, grapes, wine, tea, berries, herbs, and spices) is highly variable, with estimations ranging from 23 mg (flavonols plus flavones; Hertog et al., 1993b) to more than 500 mg (total flavonoids; Kuhnau, 1976; Manach et al., 1996). Epidemiological studies including over 120,000 patients (Hertog et al., 1993a, 1995, 1997; Hertog, 1996; Knekt et al., 1996; Rimm et al., 1996; Yochum et al., 1999; Hirvonen et al., 2001) have shown an inverse association between dietary flavonoid intake and mortality from coronary heart disease.

Among dietary flavonoids, quercetin is by far the most abundant representing approximately 60% of the total intake (Hertog et al., 1993b). A very wide range of biological actions of quercetin have been reported (Middleton et al., 2000). In fact, these drugs exert antioxidant (Rice-Evans and Packer, 1998), antiaggregant (Gryglewski et al., 1987), and vasodilator effects (Ko et al., 1991; Duarte et al., 1993a,b, 1994), which may help explain their cardiovascular protective effects (Duarte et al., 2001c). In addition, it has been recently reported that quercetin exerts anti-inflammatory effects and reduces left ventricular hypertrophy, endothelial dysfunction, and the plasma and hepatic oxidative status in spontaneously hypertensive rats (Duarte et al., 2001a,b).

A limitation for the understanding and relevance of these findings was the scarce and conflicting data on the pharmacokinetics of flavonoids. However, recently, several studies in rats and humans have revealed that oral quercetin is relatively well absorbed, and it is metabolized mainly by methylation in the 3′ or 4′ hydroxyl groups rendering isorhamnetin and tamarixetin (Fig. 1),

ABBREVIATIONS: PMA, phorbol 12-myristate 13-acetate; DMSO, dimethyl sulfoxide; pCa, plasma calcium; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; SQ22536, 9-(tetrahydro-2-furanyl)-9H-purin-6-amine; NO, nitric oxide.
respectively, and by dehydroxylation yielding kaempferol (Manach et al., 1998; Boyle et al., 2000). Although not as abundant as quercetin, these flavonoids can also be found in plants; e.g., isorhamnetin can be found in Ginkgo biloba leaves, garlic, and wine, whereas kaempferol is also widely distributed (Scalbert and Williamson, 2000). These metabolites, which can be further conjugated with glucuronide and sulfate, present a long-lasting elimination half-life (about 25 h) and accumulate after repeated daily dosages (Hollman et al., 1996). After chronic administration of quercetin to rats, the ratio of quercetin to isorhamnetin to tamarixetin in plasma is 1:5:1 (Morand et al., 1998). Thus, given this pharmacokinetic profile, the in vivo biological effects of quercetin might be partly due to its metabolites.

Unfortunately, the pharmacology of quercetin metabolites has been scarcely studied. In addition, to our knowledge, there is no information about the effects of dietary flavonoids in resistance arteries. Therefore, in the present study we have analyzed the vasodilator effects of quercetin and its metabolites in rat conductance and resistance vessels and further characterized the mechanism of their vascular smooth muscle relaxant effect.

Materials and Methods

All the experiments have been carried in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

Vascular Contractility. Male Wistar rats (250–300 g) were killed by a blow to the head and then exsanguinated. The descending thoracic aorta, abdominal aorta, and iliac arteries were rapidly dissected and placed in Krebs’ solution of the following composition: 118 mM NaCl, 5 mM KCl, 25 mM NaHCO3, 1.2 mM MgSO4, 2 mM CaCl2, 1.2 mM KH2PO4, and 11 mM glucose at pH 7.4. After excess fat and connective tissue had been removed, the arteries were cut into rings (2–3 mm long). In some arteries the endothelium was mechanically removed by gently rubbing the intimal surface of the rings with a metal rod. The rings were suspended horizontally by means of two parallel L-shaped stainless steel holders inserted into the lumen in 5-ml organ baths filled with Krebs’ solution, bubbled with a 95% O2-5% CO2 gas mixture, and maintained at 37°C. One holder served as anchor and the other was attached to an isometric force-displace-
1.3 \times 10^{-6} \text{ M (pCa = 5.9)}\) to induce a submaximal (60–70% of maximal) contraction, which averaged 44 ± 6% of the initial effect of KCl (n = 15). Then the relaxant effects of quercetin and isorhamnetin (3 \times 10^{-6}, 3 \times 10^{-5}, and 10^{-4} \text{ M}) were tested by cumulative addition.

**Drugs.** The following drugs were used: isorhamnetin and tamarixetin ( Extrasynthese, Genay, France); quercetin, kaempferol, acetylsalicylic acid, PMA, ouabain, sodium deoxycholate, and noradrenaline bitartrate (Sigma Chemical, Alcobendas, Madrid, Spain); ODQ (Tocris Cookson, Bristol, UK); and SQ22536 (Sigma/RBI, Alcobendas, Madrid, Spain). Stock solutions of quercetin, isorhamnetin, tamarixetin, kaempferol, ODQ, and PMA were prepared in DMSO, and all other drugs were prepared in distilled deionized water; further dilutions were made in Krebs’ solution.

**Statistical Analysis.** Results are expressed as means ± S.E.M. of measurements in n preparations from different animals. The \(-\log IC_{50}\), the drug concentration that inhibited 50% of the contractile response, was calculated in each concentration-response curve by linear regression analysis with the concentrations producing 20 to 80% inhibition of the contractile response. Statistically significant differences were calculated by an analysis of variance, followed by a Newman–Keuls test. \( P < 0.05 \) was considered statistically significant. The internal diameter of the vessels in Fig. 3 is the mean value measured in a microscope in cross-sections of four vessels fixed in formalin (10%). For this purpose, the value for the mesenteric bed was taken from the thinnest arterial branch dissected from the preparation.

**Results**

**Flavonoid-Induced Vasodilation: Selectivity toward Resistance Arteries.** Noradrenaline induced a sustained vasoconstriction in the isolated vessels and in the perfused mesenteric bed (Table 1). Quercetin, isorhamnetin, tamarixetin, and kaempferol induced a concentration-dependent relaxation in all vessels precontracted by noradrenaline (the \(-\log IC_{50}\) values are shown in Table 1). The four flavonoids produced a more marked relaxant response in the mesenteric bed than in the thoracic aorta (Fig. 2), and the selectivity was significantly more marked for isorhamnetin compared with kaempferol, quercetin, or tamarixetin (25-, 5-, 4-, and 6-fold more potent, respectively, in the mesenteric bed versus the thoracic aorta). Thus, the potency of isorhamnetin was higher than that of quercetin in the abdominal aorta, the iliac artery, and the mesentery (\( P < 0.05 \)). The plot of the potency (\(-\log IC_{50}\)) of the flavonoids versus the vessel diameter shows a good inverse correlation between both parameters (Fig. 3). The slopes of these plots were significantly different from zero. Therefore, these drugs showed selectivity for the resistance compared with conductance arteries, isorhamnetin showing the highest degree of selectivity. Addition of flavonoids (up to 3 \times 10^{-5} \text{ M}) had no effect on baseline tension in isolated rings or baseline perfusion pressure in the mesenteric bed (n = 3–4). In the mesenteric bed, after the concentration-response curves to the flavonoids were finished, washing in normal Krebs’ solution for at least 1 h restored the contractile effect of noradrenaline (not shown).

**Endothelial Dependence.** The vasodilator effects of flavonoids were analyzed in mechanically denuded thoracic aorta and deoxycholate-denuded mesenteric beds compared with intact vessels. Endothelial removal did not significantly affect the contractile response to noradrenaline (1323 ± 127 mg and 57 ± 11 mm Hg, respectively), but the relaxant response to acetylcholine (10^{-6} \text{ M}) was almost abolished in the rat aorta (60 ± 6% and 4 ± 2%, in intact and denuded preparations, respectively; \( n = 15, P < 0.01 \)) and in the mesenteric bed (41 ± 4% versus 3 ± 1% before and after deoxycholate treatment, respectively, at 1 nmol of acetylcholine; \( n = 12, P < 0.01 \)). However, the relaxant response to quercetin and isorhamnetin was similar in endothelium-denuded (\(-\log IC_{50} = 4.87 ± 0.07\), \( n = 8 \)) and 4.54 ± 0.11, \( n = 7 \), respectively, in the aorta and 5.09 ± 0.14, \( n = 5 \), and 5.81 ± 0.09, \( n = 7 \), respectively, in the mesenteric bed) compared with endothelium-intact preparations (Fig. 4).

**Effects of ODQ, SQ22536, KCl, Ouabain, and PMA on Flavonoid-Induced Vasodilation in the Mesenteric Bed.** The increase in perfusion pressure induced by noradrenaline in the presence of the guanylate cyclase inhibitor ODQ (10^{-6} \text{ M}), the adenylate cyclase inhibitor SQ22536

![Fig. 2. Relaxant effects of quercetin (A), isorhamnetin (B), tamarixetin (C), and kaempferol (D) in isolated endothelium-intact rat thoracic aortic rings and perfused mesenteric bed. Values are means ± S.E.M. (n = 4–8), * \( P < 0.05 \) versus aorta.](image)

**TABLE 1**

Potency (\(-\log IC_{50}\)) of quercetin and its metabolites in isolated endothelium-intact vascular preparations

<table>
<thead>
<tr>
<th>Diameter(^a)</th>
<th>Noradrenaline-Induced Contraction</th>
<th>Quercetin</th>
<th>Isorhamnetin</th>
<th>Tamarixetin</th>
<th>Kaempferol</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm</td>
<td>(-\log IC_{50})</td>
<td>(-\log IC_{50})</td>
<td>(-\log IC_{50})</td>
<td>(-\log IC_{50})</td>
<td>(-\log IC_{50})</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>1.60 ± 0.05</td>
<td>4.68 ± 0.08</td>
<td>4.61 ± 0.08</td>
<td>4.73 ± 0.11</td>
<td>4.81 ± 0.13</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>0.95 ± 0.03</td>
<td>4.70 ± 0.07</td>
<td>5.02 ± 0.10*</td>
<td>4.82 ± 0.05</td>
<td>5.10 ± 0.15*</td>
</tr>
<tr>
<td>Iliac artery</td>
<td>0.60 ± 0.01</td>
<td>4.98 ± 0.06</td>
<td>5.27 ± 0.05*</td>
<td>5.16 ± 0.07</td>
<td>5.39 ± 0.17*</td>
</tr>
<tr>
<td>Mesenteric bed</td>
<td>0.125 ± 0.007</td>
<td>5.35 ± 0.15</td>
<td>5.89 ± 0.11*</td>
<td>5.34 ± 0.10</td>
<td>5.66 ± 0.06</td>
</tr>
</tbody>
</table>

\(^a\) Diameter represents the luminal diameter measured in arteries from four age-matched rats.

\(^*\) \( P < 0.05 \) versus quercetin.
KCl (40 mM), or the Na+/K+ ATPase inhibitor ouabain (10⁻³ M) was not significantly different from that induced by noradrenaline alone (Table 2). None of these treatments had any significant effect on the relaxant effects of quercetin or isorhamnetin (Fig. 5, Table 2). Addition of PMA (3 × 10⁻⁷ M) to resting preparations induced a slowly developing increase in perfusion pressure, which reached a plateau within 1 h and was not different from that induced by noradrenaline (P > 0.05). The relaxant effects of quercetin were similar in PMA-stimulated compared with noradrenaline-stimulated mesenteric beds, whereas isorhamnetin-induced vasodilation was less potent (P < 0.05) in PMA-stimulated mesenteric beds (Table 2).

Effects of K⁺ Channel Blockers on Quercetin-Induced Vasodilation in the Aorta and Iliac Artery. The possible role of K⁺ channels in flavonoid-induced vasodilation was analyzed using K⁺ channel blockers. Addition of tetraethylammonium (10 mM), 4-aminopyridine (1 mM), or glibenclamide (10⁻⁶ M) to aortae or iliac arteries prestimulated with noradrenaline had no significant effect on tone (except 4-aminopyridine in iliac arteries, which evoked a contraction of 19 ± 2% versus 5 ± 3% in time-matched controls). The relaxant effects of quercetin were similar (P > 0.05) in control (−log IC₅₀ 4.56 ± 0.10, n = 10, and 4.97 ± 0.13, n = 7, in the aortae and iliac arteries, respectively) and in the presence of the K⁺ channel blockers tetraethylammonium (10 mM; −log IC₅₀ 4.49 ± 0.11, n = 5, and 5.01 ± 0.13, n = 7, respectively), 4-aminopyridine (1 mM; 4.87 ± 0.25, n = 4, and 5.06 ± 0.07, n = 5, respectively), and glibenclamide (10⁻⁶ M; 4.45 ± 0.09, n = 5, and 4.89 ± 0.13, n = 7, respectively).

Permeabilized Iliac Arteries. In permeabilized iliac arteries increasing pCa from 8 (relaxing solution) to 5.9 induced a submaximal contractile response averaging 225 ± 23 mg (n = 15). Under these conditions, addition of quercetin or isorhamnetin (3 × 10⁻⁶ M, 3 × 10⁻⁵ M and 10⁻⁴ M) induced a concentration-dependent relaxant response (Fig. 6). As occurred in intact arteries, isorhamnetin was significantly more potent than quercetin. The calculated −log IC₅₀ value for isorhamnetin (5.27 ± 0.15, n = 6) was similar to that obtained in intact arteries stimulated by noradrenaline (Table 1). In contrast, quercetin was slightly but significantly (P < 0.05) less potent in permeabilized (−log IC₅₀ = 4.56 ± 0.15, n = 9) than in intact arteries (Table 1).

Discussion

Previous reports have shown that quercetin exhibits vasodilator effects in isolated rat aorta (Duarte et al., 1993a,b). In the present study we report for the first time that 1) the metabolites of quercetin, isorhamnetin, tamarixetin, and kaempferol, also induced vascular smooth muscle relaxation with potency similar to or higher than that of the parent compound; 2) the relaxant effects of quercetin and its metabolites were markedly augmented in resistance compared with conductance arteries; 3) the effects of isorhamnetin, the most potent and long-lasting metabolite in plasma, were endothelium-independent and were not modified by inhibition of
guanylate cyclase, adenylate cyclase, or Na\(^+\)/K\(^+\) ATPase, or by increasing the extracellular concentration of KCl; and 4) isorhamnetin showed a similar vasodilator potency in permeabilized iliac arteries at constant [Ca\(^{2+}\)] and in intact artery preparations.

Resistance arteries are responsible for regulation of arterial pressure and local blood flow. Quercetin and its metabolites were more potent in the mesenteric resistance vascular bed than in conductance arteries, and, in fact, an inverse correlation was found between the potency of the flavonoid and the internal diameter of the vessel studied. Selectivity for resistance arteries is also a typical feature of Ca\(^{2+}\) channel blockers and K\(^+\) channel openers (Cauvin et al., 1988), whereas nitrates are more potent in large than in small arteries. Furthermore, significant vasodilator responses were observed in the mesenteric bed at concentrations of flavonoids of \(3 \times 10^{-7} - 10^{-6}\) M, which corresponded with a range of concentrations of quercetin that are normally reached in plasma after a single flavonoid-containing meal (0.3 – 2.2 \(\times 10^{-6}\) M; reviewed by Scalbert and Williamson, 2000). On the basis of relative potency and the relative plasma concentrations of quercetin and its metabolites, we decided to further analyze the effects of isorhamnetin and compare them with those of quercetin.

Many endogenous mediators exert vasodilator effects through the release of endothelium-derived factors such as NO. Flavonoids scavenge superoxide anions and thus might protect NO from superoxide-induced inactivation (Rice-Evans and Packer, 1998). However, quercetin also scavenges NO (Van Acker et al., 1995) and inhibits the expression and/or the activity of the inducible NO synthase (Kim et al., 1999; Middleton et al., 2000). Since our initial report (Duarte...
et al., 1993b), the endothelial dependence of the vasodilator effects of quercetin-related flavonoids in rat conductance arteries has been studied by several groups. The relaxation induced by quercetin and other related flavonoids was endothelium-independent (Duarte et al., 1993a; Fitzpatrick et al., 1993) or very weakly (less than 2-fold shift) inhibited by endothelial removal (Chen and Pace-Asciak, 1996). In the present paper, we found that the vasodilator effects of quercetin and its main metabolite isorhamnetin are endothelium-independent, not only in the rat aorta, but also in the mesenteric resistance vascular bed. However, these results do not exclude an effect of quercetin on endothelial function because after administration for 5 weeks to spontaneously hypertensive rats, quercetin restored endothelium-dependent relaxation (Duarte et al., 2001b).

A possible role of cyclic nucleotides in flavonoid-induced vasodilation was tested using ODQ and SQ22536, selective inhibitors of soluble guanylate cyclase and adenylate cyclase, respectively. High KCl was used as a classic pharmacological maneuver to inhibit K⁺ channel-dependent hyperpolarization, and ouabain was used as an inhibitor of the Na⁺/K⁺ ATPase. None of these treatments had any effect on isorhamnetin-induced vasodilatation in the mesenteric bed excluding these signaling pathways as potential mechanisms of action. In addition, the absence of effect of the nonselective K⁺ channel blockers, tetraethylammonium and 4-aminopyridine, and the K⁺ channel inhibitor glibenclamide on quercetin-induced relaxation in isolated intact aortae and iliac arteries confirmed that K⁺ channels were not involved.

An increase in intracellular Ca²⁺ concentrations ([Ca²⁺]i) and the subsequent Ca²⁺-calmodulin-dependent activation of myosin light chain kinase is the main determinant of smooth muscle contraction (Somlyo and Somlyo, 2000). However, multiple studies have shown that agonists can also modulate contractile force by increasing the myofilament sensitivity to Ca²⁺ or through Ca²⁺-independent pathways (Somlyo and Somlyo, 2000). The involvement of protein kinases such as protein kinase C or Rho kinase in the signaling cascades of Ca²⁺ sensitization in intact and permeabilized arteries has been reported (Martínez et al., 2000; Somlyo and Somlyo, 2000). Quercetin did not modify the ⁴⁵Ca²⁺ efflux induced by noradrenaline (Duarte et al., 1994), and at 3 × 10⁻⁵ M, it only weakly inhibited ⁴⁵Ca²⁺ influx induced by KCl in rat aorta (J. Duarte and F. Pérez-Vizcaíno, unpublished observations). The potent vasodilator effects of quercetin and isorhamnetin in permeabilized iliac arteries at constant [Ca²⁺]i, confirmed that changes in [Ca²⁺]i, are not required for its vasodilator effect. Furthermore, the vasodilator effects without changes in [Ca²⁺]i, in the absence of vasoconstrictor agonists or other Ca²⁺-sensitizing agents, strongly suggest that they are related to direct interactions with the contractile proteins. In fact, flavonoids, including quercetin and kaempferol, are potent inhibitors of myosin light chain kinase in vascular smooth muscle, showing IC₅₀ values of 1 and 0.45 µM, respectively (Hagiwara et al., 1988; Rogers and Williams, 1989), which corresponded to the range of concentrations producing their vasodilator effects in the present study. Unfortunately, the effects of isorhamnetin and tamarixetin on myosin light chain kinase are unknown. However, quercetin, kaempferol, and isorhamnetin have also been reported to inhibit Ca²⁺-sensitizing mechanisms for smooth muscle contraction such as protein kinase C (Middletom et al., 2000). Accordingly, the vasoconstriction induced by protein kinase C activator PMA was inhibited by quercetin and related flavonoids in the rat aorta (Duarte et al., 1993a,b) and in the mesenteric bed (present results). From these results, it is tempting to speculate that the primary mechanism of flavonoid-induced vasodilation results from inhibition of protein kinases such as myosin light chain kinase and, possibly, other kinases involved in Ca²⁺-sensitizing mechanisms including protein kinase C. These flavonoid-sensitive mechanisms could play a more important role in vasoconstriction in small arteries, which might explain the selectivity of flavonoids for the resistance vessels.

In conclusion, quercetin and its metabolites show potent vasodilator effects in the isolated resistance mesenteric vascular bed, showing selectivity for the resistance vessels. These vasodilator effects are not caused or modulated by endothelial factors or cyclic nucleotides and are not related to changes in [Ca²⁺]i. The present results showing that quercetin metabolites are at least as potent as the parent compound as vasodilators, together with their reported long plasma half-lives, suggest that the effects of quercetin in vivo result mostly from its metabolites. Flavonoid-induced vasodilation in resistance vessels may explain its antihypertensive effects.

Fig. 6. Relaxant effects of quercetin and isorhamnetin on isolated iliac arteries permeabilized with β-escin and contracted with a free Ca²⁺ concentration of 1.3 µM (i.e., pCa 5.9). Panel A shows a typical recording obtained with quercetin, and panel B shows the averaged values for the two drugs (means ± S.E.M., n = 6–9). * P < 0.05, isorhamnetin versus quercetin.
and might contribute to the reduction of mortality due to ischemic heart disease observed in epidemiological studies.

References


Address correspondence to: Francisco Pérez-Vizcaíno, Department of Pharmacology, Inst. Farmacología y Toxicología (CSIC), Facultad de Medicina, Universidad Complutense de Madrid, 28040 Madrid, Spain. E-mail: fperez@eucmail.ucm.es