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Structure–activity relationships of flavonoids for vascular relaxation in porcine coronary artery

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Abstract

Flavonoids are polyphenolic compounds that are widespread in the plant kingdom, and structure–activity relationships (SAR) for vascular relaxation effects were examined for 17 of them using porcine coronary arteries. Density functional theory was employed to calculate the chemical parameters of these compounds. The order of potency for vascular relaxation was as follows: flavones (apigenin and luteolin) ⩾ flavonols (kaempferol and quercetin) > isoflavones (genistein and daidzein) > flavanon(ol)es (naringenin) > chalcones (phloretin) > anthocyanidins (pelargonidin) > flavan(ol)es ((+)-catechin and (−)-epicatechin). SAR analysis revealed that for good relaxation activity, the 5-OH, 7-OH, 4′-OH, C2=C3 and C4=O functionalities were essential. Comparison of rutin with quercetin, genistin with genistein, and puerarin with daidzein demonstrated that the presence of a glycosylation group greatly reduced relaxation effect. Total energy and molecular volume were also predictive of their relaxation activities. Our findings indicated that the most effective relaxing agents are apigenin, luteolin, kaempferol and genistein. These flavonoids possess the key chemical structures demonstrated in our SAR analysis.

Keywords: Flavonoid; Structure–activity relationship; Chemical parameter; Relaxation activity; Porcine coronary artery

1. Introduction

Flavonoids are polyphenolic compounds that are widespread in the plant kingdom, and they are important components of many traditional Chinese medicine and phyto-medicine. They have diverse pharmacological effects, such as anticancer, antioxidant, anti-aging, and antibacterial properties (Terao et al., 1994; Bos et al., 1996; Guo et al., 1999; Meng and Wang, 2001). Recently many studies have focused on their cardiovascular effects (Vitor et al., 2004; Stocker and O'Halloran, 2004). Epidemiological reports have demonstrated that people can have lower incidence of heart diseases if they have a high dietary intake of flavonoids (Knekt et al., 2002; Jenkins et al., 2003) and this may help explain the lower mortality of coronary heart diseases in some Asian countries (Lock et al., 1988). Furthermore, other studies have demonstrated that some flavonoids produced concentration-dependent relaxation responses in contracted arterial rings (Guerrero et al., 2002; Fusi et al., 2003). These relaxations are in part mediated by stimulation of nitric oxide release from the endothelium (Kim et al., 2000; Mishra et al., 2000; Zhang et al., 2002). However, the majority of the relaxation is attributed to direct action of the flavonoids on the vascular smooth

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muscle (Mishra et al., 2000; Zhang et al., 2002; Ajar et al., 2003).

Since different flavonoids have different relaxation effects, the structure–activity relationships (SAR) of flavonoids have become the subject of many investigations (Hiipakka et al., 2002; Taubert et al., 2002; Chen et al., 2003; van Zanden et al., 2004). For an understanding of the three-dimensional microscopic interaction and binding between a ligand and a receptor, detailed analysis in SAR is important in drug design and synthesis (Costa et al., 1995; Vaya and Tamir, 2004). Certain chemical parameters may be responsible for their molecular interactions (Camargo et al., 2002). Therefore, SAR studies using quantum chemical parameters have become important in qualitative and quantitative analyses of three-dimensional molecular interactions (Dewar et al., 1985; Souza et al., 2003). However, few investigations have examined the relationships between chemical parameters and vascular relaxation of flavonoids. The present study focuses on SAR analysis of 17 natural compounds (**1**–**17**) using structural parameters

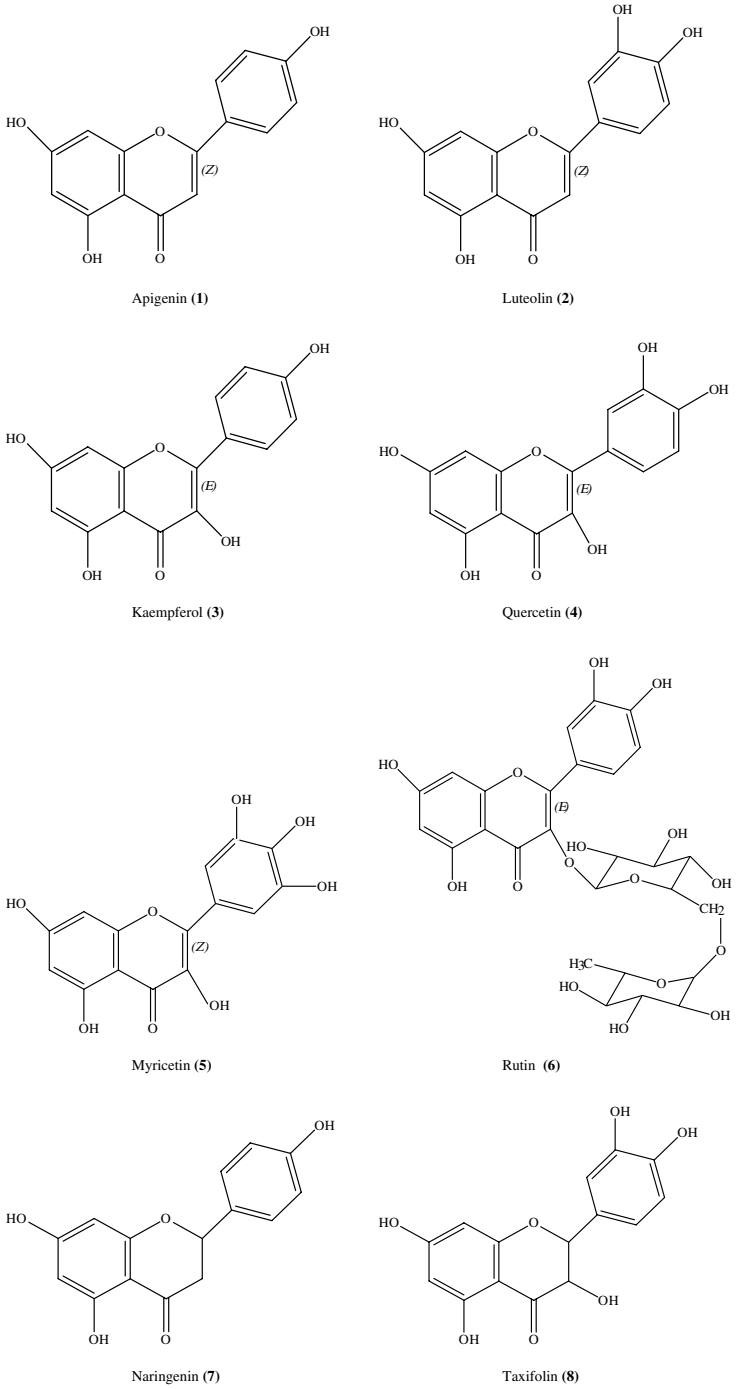
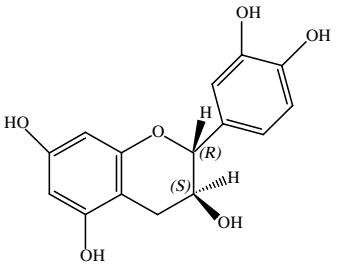
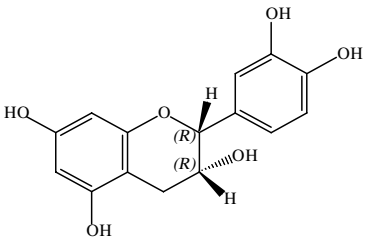


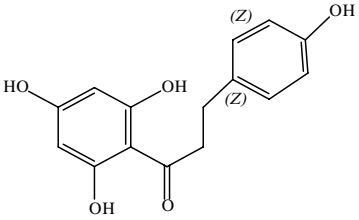
Fig. 1. Molecular structures of the 17 flavonoids* studied. *The numbering system for these flavonoids is as follows:



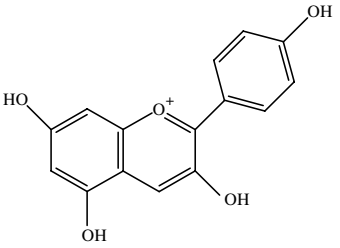
(+)-Catechin **(9)**



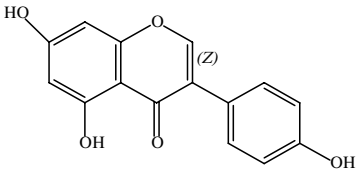
(-)-Epicatechin **(10)**



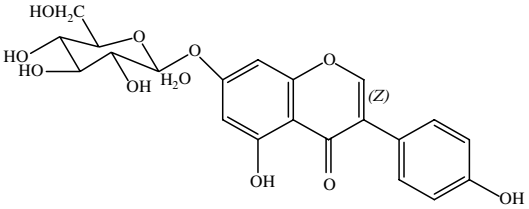
Phloretin **(11)**



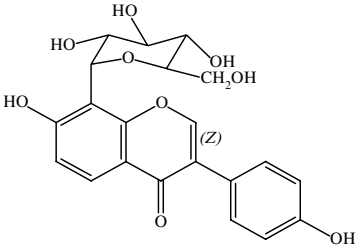
Pelargonidin **(12)**



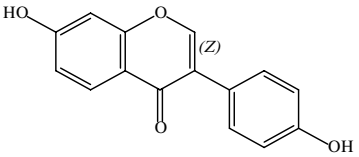
Genistein **(13)**



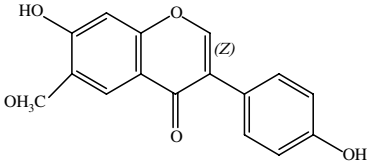
Genistin **(14)**



Puerarin **(15)**



Daidzein **(16)**



Glycetein **(17)**

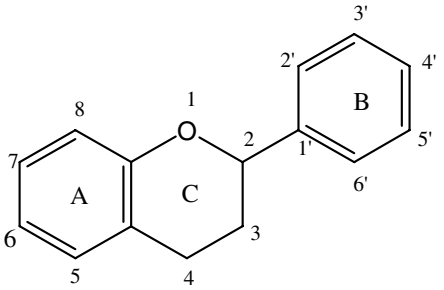


Fig. 1 (*continued*)

Table 1
Names and classes of natural flavonoids in this study, and relaxation properties

Number	Class	Chemical name	Compound name	% Relation		Relaxation effect
				30 μM	100 μM	
1	Flavones	4′,5,7-trihydroxy flavone	Apigenin	114.5 ± 3.5*	118.5 ± 3.9*	Good
2		3′,4′,5,7-tetrahydroxy flavone	Luteolin	107.6 ± 2.7*	114.2 ± 2.6*	Good
3	Flavonols	3,4′,5,7- tetrahydroxy flavone	Kaempferol	108.2 ± 2.7*	116.4 ± 2.3*	Good
4		3,3′,4′,5,7- pentahydroxy flavone	Quercetin	20.6 ± 3.8	116.7 ± 4.0*	Moderate
5		3,3′,4′,5,5′,7- hexahydroxy flavone	Myricetin	22.4 ± 3.1	55.8 ± 6.9*	Weak
6		3′,4′,5,7-tetrahydroxy-3-O-rutinose flavone	Rutin	29.7 ± 3.5	73.6 ± 6.9*	Weak
7	Flavanon(ol)es	4,5,7- trihydroxy flavanone	Naringenin	75.2 ± 4.4*	108.4 ± 1.2*	Moderate
8		3,3′,4′,5,7- pentahydroxy flavanone	Taxifolin	45.5 ± 7.0	80.6 ± 4.4*	Weak
9	Flavan(ol)es	(+)-trans-3,3′,4′,5,7- pentahydroxy flavane	(+)-catechin	31.9 ± 7.9	56.2 ± 7.4*	Weak
10		cis-3,3′,4′,5,7- pentahydroxy flavane	(−)-epicatechin	34.0 ± 8.7	66.8 ± 7.6*	Weak
11	Chalcones	2′,4′,6′-trihydroxy-3(4-hydroxyphenyl)propiophenone	Phloretin	68.2 ± 6.9*	105.8 ± 1.8*	Moderate
12	Anthocyanidins	3,4′,5,7- tetrahydroxy flavylium	Pelargonidin	54.8 ± 9.5*	87.9 ± 5.3*	Moderate
13	Isoflavones	3,4′,5,7- tetrahydroxy isoflavone	Genistein	95.4 ± 3.2*	106.2 ± 3.7*	Good
14		3,4′,5,7- tetrahydroxy isoflavone 7-glucoside	Genistin	24.4 ± 1.1	25.1 ± 2.4	None
15		4′,7-dihydroxy-8-β-D-glucose isoflavone	Puerarin	25.3 ± 4.8	45.0 ± 5.5*	Weak
16		4′,7-dihydroxy isoflavone	Daidzein	77.3 ± 3.2*	102.9 ± 0.6*	Moderate
17		4′,7-dihydroxy-6-methoxy isoflavone	Glycetein	46.7 ± 4.3*	93.1 ± 1.7*	Moderate

Data represent mean ± S.E.M.
* $P < 0.05$ vs the relevant solvent control group.

calculated by density functional theory. The vascular effects of flavonoids in porcine coronary arteries were also investigated.

2. Results and discussion

2.1. In vitro vascular relaxation activity

In recent years, flavonoids have gained a tremendous amount of interest with regard to their potential in cardiovascular protection (Harborne and Williams, 2000; Knekt et al., 2002; Jenkins et al., 2003). Until now, the mechanism(s) for their action and SAR have not been fully understood. The investigation of flavonoids by the SAR method is hampered by their vast structural diversity. Flavonoids are based on the structure of a phenylbenzopyrone and differ from one another in terms of hydroxyl or glycosylated substitutents, the position of the benzenoide substituent, the degree of unsaturation and the types of sugar attached.

In the present work, 17 compounds (**1**–**17**) from different subgroups of flavonoids were investigated for their vasorelaxant activities in porcine coronary arteries with and without an intact endothelium, and their molecular structures are showed in Fig. 1. The chemical name and compound name of the 17 flavonoids are presented in Table 1 according to their subgroup classification. The biological evaluation of flavonoids examined in this study were expressed as a percentage relaxation of the initial contraction in the presence of the tested compound. In endothelium-intact porcine coronary artery that were contracted with U46619 (30 nM), all tested flavonoids except genistin (**14**) showed significant vasorelaxant activities at 100 μM compared with the respective solvent control groups (Table 1). According to their ability to induce vasorelaxation, it is possible to classify these flavonoids into three categories taken into account of their potency (the concentration to achieve 50% relaxation) and efficacy (the maximal relaxation). The flavonoids that induce almost 100% relaxation at 30 μM are considered to be good relaxing agents, and this group includes apigenin (**1**), luteolin (**2**), kaempferol (**3**) and genistein (**13**). Flavonoids inducing 50% relaxation at concentration around 30 μM and good relaxation (from 85% to >100%) at the maximum concentration tested, i.e. 100 μM, are moderate relaxing agents, whereas those with little or no relaxation at 30 μM but some effect (45% to 80%) at 100 μM are weak vasodilators (Table 1). Concentration–relaxation curves of some representative flavonoids in these three categories are shown in Fig. 2. In spite of the differences in the type and species of the blood vessels, our results are consistent with the findings of Duarte et al. (1993), who reported that the order of potency for flavonoids to relax rat aorta was flavon(ol)es > flavanols.

In porcine coronary artery without an intact endothelium, flavonoids also induced significant relaxation. Using

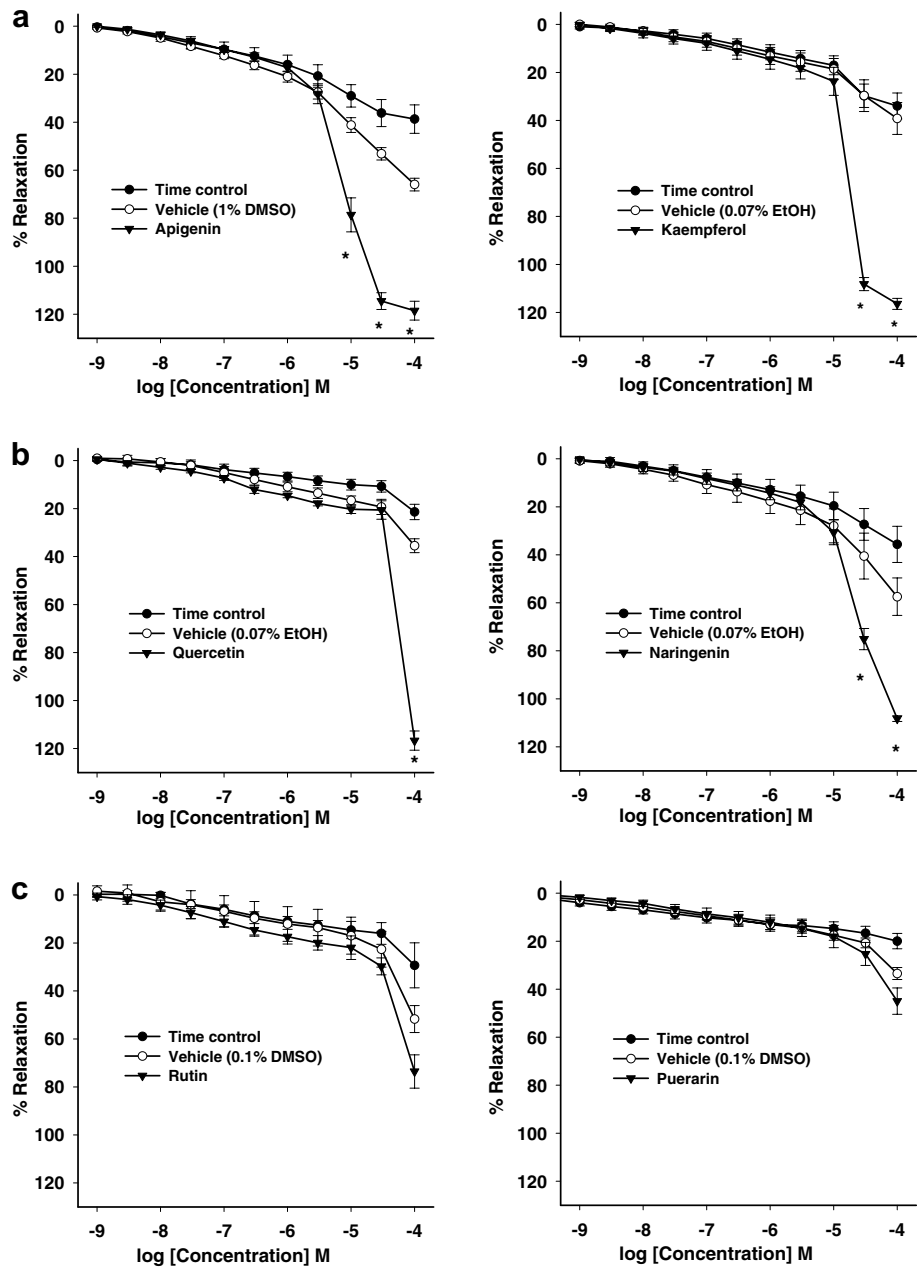


Fig. 2. Effects of flavonoids on relaxation in porcine coronary artery rings. Rings were contracted with U46619 (30 nM). Flavonoids were added cumulatively to achieve the appropriate concentrations. Results are expressed as means ± standard error of mean in terms of percentage relaxation of the contraction to U46619 ($n = 6$–8). (a) Flavonoids with good relaxation effect; (b) flavonoids with moderate relaxation effect; and (c) flavonoids with weak relaxation effect.

a similar classification approach with respect to their ability to induce relaxation, three categories can be identified and the concentration–relaxation curves of the representative flavonoids in these three categories in endothelium-disrupted rings are shown in Fig. 3. On comparison of the relaxing effect in the artery with and without an intact endothelium (Figs. 2 and 3), it appears that flavonoids induced relaxation mainly through direct action on the vascular smooth muscle. Our data are thus in agreement with the findings from other studies (Mishra et al., 2000; Zhang et al., 2002; Ajar et al., 2003). The independence of the endothelium suggests that mechanisms other than the stimulation of release of endothelium-derived relaxing factors such as nitric oxide and endothelium-derived hyperpolarizing factor are largely responsible for the vasorelaxing effect of flavonoids. Since the maximal relaxation in arteries that were contracted with U46619 (30 nM) and potassium chloride (50 mM) were not significantly different (Fig. 4), it is unlikely that the flavonoids induce relaxation through antagonism of thromboxane A_2 receptor or through hyperpolarization with activation of potassium channels. While the exact mechanism of action for flavonoids to elicit relax-

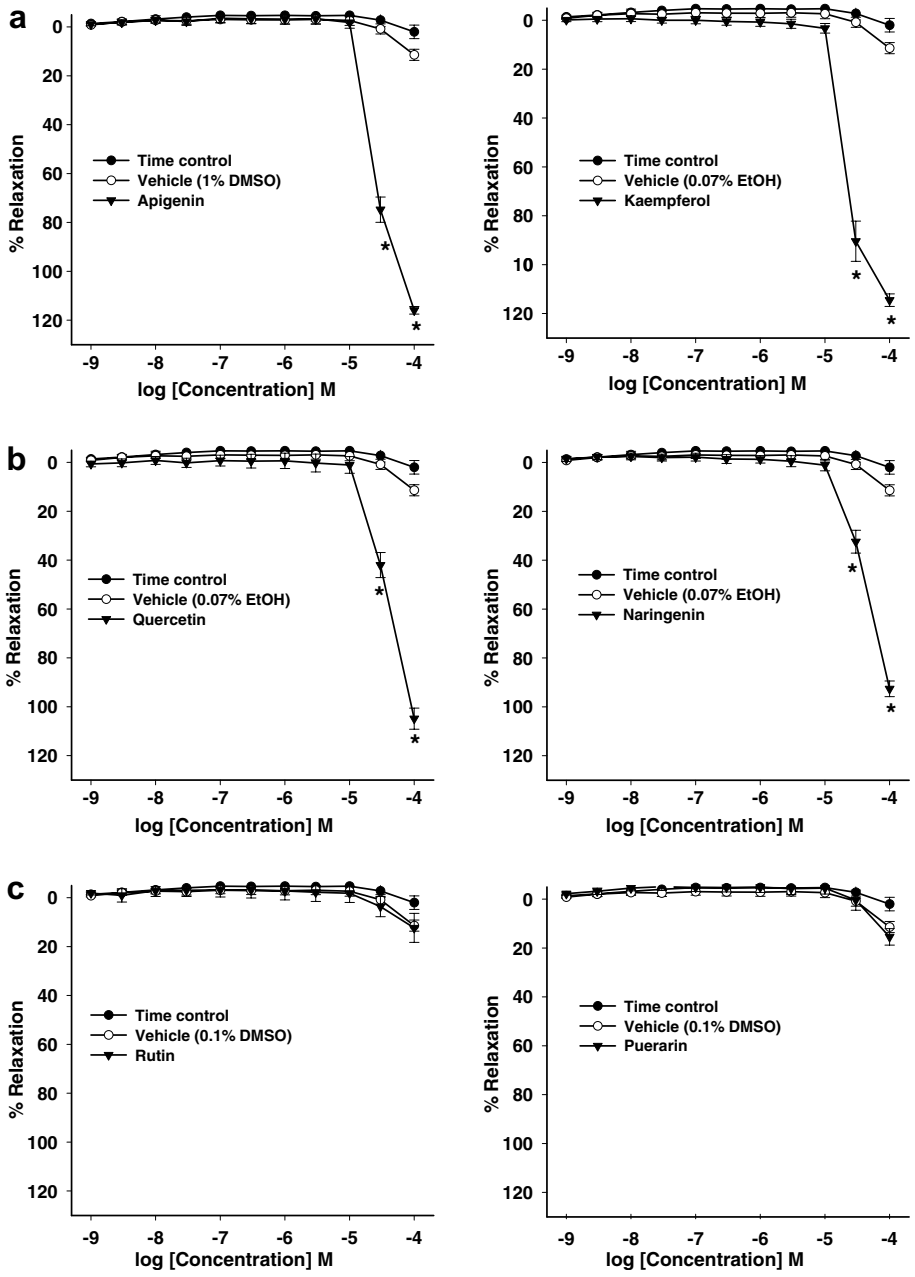


Fig. 3. Effects of flavonoids on relaxation in porcine coronary artery rings with disrupted endothelium. Rings were contracted with potassium chloride (50 mM). Flavonoids were added cumulatively to achieve the appropriate concentrations. Results are expressed as means ± standard error of mean in terms of percentage relaxation of the contraction to potassium chloride ($n = 6$–8). (a) Flavonoids with good relaxation effect; (b) flavonoids with moderate relaxation effect; and (c) flavonoids with weak relaxation effect.

ation remains to be determined, the present findings demonstrate that the vascular action of these flavonoids possess common characteristics. Together with the structural similarities, it is reasonable to postulate a common signaling cascade for the flavonoids to exert vascular actions. Indeed, the ability of these flavonoids to interact with the same biological receptor as the female sex hormone, estrogen, has been reported previously (Kuiper et al., 1998; Boue et al., 2003; Schmidt et al., 2005). However, it is unlikely that this genomic estrogen receptor is involved in the vascular effects of flavonoids, in view of the fast onset of the relaxation, which occurs within 30 min following exposure of porcine coronary artery to flavonoids.

2.2. Relationship between chemical structures and potency of relaxation

Kaempferol (**3**), quercetin (**4**) and myricetin (**5**) belong to the flavonol class of compounds which have an OH group at the C3 position. Kaempferol (**3**) has a single B-ring OH group (4′ position) while quercetin (**4**) has 2 B-ring OH groups and myricetin (**5**) has 3 B-ring OH groups.

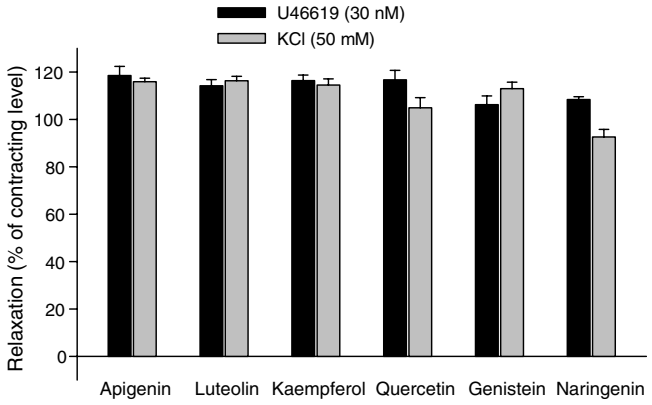


Fig. 4. Relaxation induced by different flavonoids at the maximum concentration tested (100 μM) in porcine coronary artery rings. Rings were contracted with the stable thromboxane A_2 analogue, U46619 (30 nM) or potassium chloride (KCl; 50 mM) before being exposed to various flavonoids. Results are expressed as means ± standard error of mean in terms of percentage relaxation of the respective contracting level ($n = 6$–8).

Quercetin (**4**) and myricetin (**5**) have reduced relaxation activity compared with kaempferol (**3**). Hence more OH groups in the B ring will affect the 4′-OH group, one of the pharmacophore in flavonoids. This will attenuate the vasorelaxation effect. Rutin (**6**), the 3-rutinose glycoside of quercetin (**4**), is also a flavonol with glycosylation at the 3-OH position. Its relaxation effect became very weak. This may be due to the presence of the bulky oligosaccharide rutinose causing steric hindrance or to the modification of the 3-OH group. Compared with apigenin (**1**) and luteolin (**2**), two examples of flavones without an OH group at the 3 position of the C-ring, the relaxation effects of the flavonols are slightly potentiated (Table 1). It seems that the presence of the 3-OH group in the C-ring or glycosylation of this OH group will attenuate the relaxation effect. It should be noted that good relaxing compounds (apigenin (**1**), luteolin (**2**), kaempferol (**3**) and genistein (**13**)) all possess a 5-OH, 7-OH, 4′-OH, C(2)=C(3) double bond and C(4)=O functionalities.

In the isoflavone class, the B-ring is connected at the 3-position to the C-ring. Genistein (**13**) has the strongest vascular effect because it also possesses a 5-OH, 7-OH, 4′-OH and a C(2)=C(3) double bond. Daidzein (**16**) lacks the 5-OH group and this may account for the smaller vascular relaxation effect than genistein (**13**). Genistin (**14**), which is glycosylated at the 7-OH position, has no relaxation effect compared with genistein (**13**). Puerarin (**15**) has a C-glycosyl group at C-8 and this strongly decreases vascular relaxation as compared with daidzein (**16**). Hence, glycosylation in isoflavones, like glycosylation in other flavonoids, also decrease the vascular relaxation property.

(+)-Catechin (**9**) and (−)-epicatechin (**10**), which have no C(4)=O and C(2)=C(3) double bonds, belong to the flavan(ol)es class. Their vascular relaxation effect is very weak. These results confirm that the presence of C(4)=O and C(2)=C(3) bonds are very important in producing vascular relaxation.

Naringenin (**7**) and taxifolin (**8**) belong to the flavanon(ol)es class that have a single bond between the C-2 and C-3 positions in the C-ring. Taxifolin (**8**) is a flavanonol that is structurally similar to quercetin (**4**) but lacks the C(2)=C(3) double bond in the C-ring. It is not effective in causing vascular relaxation. Naringenin (**7**) is a flavanone that is structurally similar to apigenin (**1**) but also lacks the C(2)=C(3) double bond in the C-ring. It has slight vascular relaxation effect as compared with apigenin (**1**). With a double bond between C-2 and C-3, the B-ring is coplanar with the A-ring and C-ring. This suggests that a planar flavonoid structure is important for increasing the relaxation effect. Comparing naringenin (**7**) and taxifolin (**8**), there are two extra OH groups in 3-position in the C-ring and in the 3′-position of the B-ring of taxifolin. Our results showed taxifolin (**8**) has a weaker relaxation effect than naringenin (**7**). This further supports the hypothesis that the 3-OH position of the C-ring decreases the vascular effect in porcine coronary arteries, as noted in the previous case when comparing flavones and flavonols (apigenin (**1**) and luteolin (**2**) vs. kaempferol (**3**) and quercetin (**4**), respectively) and that with 3′-OH position in the B-ring, the vascular effect in porcine coronary arteries were decreased as the case for flavones (apigenin (**1**) vs. luteolin (**2**)) or flavonols (kaempferol (**3**) vs. quercetin (**4**)).

Phloretin (**11**) is a chalcone compound while pelargonidin (**12**) is an anthocyanidin compound. Our results showed that both compounds had a moderate relaxation effect. For the former, it has a broken C-ring and this attenuates coplanarity of the A- and B-rings. The latter compound has positive charges at the 1-position in the C-ring. Both compounds also lack a C(2)=C(3) double bond. These properties may account for the fact that these compounds do not have a strong vascular action when tested.

2.3. Analysis of the atomic and molecular descriptors (chemical parameters)

SAR analysis has been carried out using structural parameters in our experiments. The values of the calculated chemical parameters are presented in Table 2. These results suggested that not all the chemical parameters are essential for biological activity. The hardness (η), Mulliken electronegativity (X) and dipole moment (μ) were not significant for determining relaxation activities of the compounds studied. The two most important chemical parameters are the total energy (E) and molecular volume (V_m). The most effective relaxing agents such as apigenin (**1**), luteolin (**2**), kaempferol (**3**) and genistein (**13**) exhibit lower energy and small molecular volume size. For weak relaxant compounds, such as genistin (**14**), puerarin (**15**) and rutin (**6**), a large molecular volume and high total energy were found. Therefore, it appears that a small molecular volume and low total energy are favourable for a given flavonoid to become active. A small volume may combine suitably with the biological receptors (Souza et al., 2003). As total energy is a descriptor that represents electronic features, a low

Table 2
Chemical parameters representing electronic and steric features of 17 flavonoids

Number	Compound name	Hardness (η)	Mulliken electronegativity (X)	Dipole moment (μ)	Energy (E) (Kcal/mol)	Molecular volume (Vm)($Å^3$)
1	apigenin	0.07705	0.1413	6.4583	−34.44	223.98
2	luteolin	0.07618	0.1416	5.2081	0.07	232.79
3	kaempferol	0.07041	0.1126	3.1417	43.76	232.58
4	quercetin	0.06735	0.1356	2.8218	75.60	241.32
5	myricetin	0.06979	0.1402	5.8383	86.86	249.56
6	rutin	0.07024	0.1366	3.4293	263.86	499.07
7	naringenin	0.08774	0.1239	5.1160	−51.92	231.09
8	taxifolin	0.07213	0.1381	1.3004	49.68	248.36
9	(+)-catechin	0.09932	0.1073	5.7922	39.95	246.67
10	(-)-epicatechin	0.09827	0.1051	5.1630	47.00	246.76
11	phloretin	0.08814	0.1250	2.5418	−22.87	242.04
12	pelargonidin	0.05311	0.2927	2.5643	27.61	224.03
13	genistein	0.08158	0.1216	3.4673	−20.06	224.37
14	genistin	0.07650	0.1384	5.1264	82.77	357.00
15	puerarin	0.07785	0.1389	4.0522	148.40	348.03
16	daidzein	0.07878	0.1287	3.1793	13.47	215.75
17	glycetein	0.07909	0.1251	2.2100	69.14	241.93

total energy may affect the possibility of charge transfer reactions. It should be noticed that these two chemical parameters represent two distinct classes of interactions between the compounds and the biological receptors, namely, steric and electronic interactions. The behavior of these chemical parameters may be useful when trying to predict and obtain flavonoids with better relaxation effects.

3. Conclusions

Among the 17 flavonoids (**1**–**17**) examined in this study, the most effective relaxation agents are apigenin (**1**), luteolin (**2**), kaempferol (**3**) and genistein (**13**). It appears that for good vascular relaxation effects, the OH group at C-5 and C-7 positions in the A-ring, C-4′ position in the B-ring, a double bond between C-2 and C-3, and a C=O functionality at the C-4 position in the C-ring are important. The presence of an OH group at the C-3 position slightly decreased the vascular effect. Moreover, glycosylation of an OH group and C-glycosyl groups at C-8 position strongly decrease vascular relaxation. Addition of an OH group in the B-ring causes steric hindrance and weakens the relaxation action. The order of potency of vascular relaxation in our study was as follows: flavones (apigenin (**1**) and luteolin (**2**)) > flavonols (kaempferol (**3**) and quercetin (**4**)) > isoflavones (genistein (**13**) and daidzein (**16**)) > flavanon(ol)es (naringenin (**7**)) > chalcones (phloretin (**11**)) > anthocyanidins (pelargonidin (**12**)) > flavan(ol)es ((+)-catechin (**9**) and (−) epicatechin (**10**)). Chemical parameters including total energy and molecular volume are important for determining the relaxation effects of flavonoids. Since molecular volume represents steric interaction, this indicates that an increase in steric interactions decreases the vascular effect of flavonoids in porcine coronary arteries. The findings of the present study, thus, provide the information relating the chemical structure of flavonoids to their ability in relaxing blood vessels. This understanding would facilitate the design of chemical compounds acting through the same signaling cascade as these flavonoids but with higher potency to serve as potential vasodilating agents.

4. Experimental

4.1. Flavonoids materials

Apigenin (**1**), (+)-catechin (**9**), daidzein (**16**), (−)-epicatechin (**10**), genistein (**13**), genistin (**14**), glycetein (**17**), kaempferol (**3**), luteolin (**2**), myricetin (**5**), naringenin (**7**), pelargonidin (**12**), phloretin (**11**), puerarin (**15**), quercetin (**4**), rutin (**6**) and taxifolin (**8**) were obtained from Sigma (St. Louis, MO, USA). The flavonoid compounds were dissolved in dimethyl sulphoxide (DMSO; Sigma, St. Louis, MO, USA) and used within 1 week of preparation.

4.2. Determination of vascular relaxation activity in vitro

Pig hearts of either sex were collected from a local slaughter house after the pigs (50–70 kg) were killed in the early morning. Pigs were processed according to the regulations laid down by Food and Environmental Hygiene Department of the Government of Hong Kong SAR. The use of animals for this study had been approved by the Committee on the Use of Live Animals in Teaching and Research at the University of Hong Kong following guidelines as recommended by the Helsinki Declarations for use of experimental animals. Hearts were immersed in cold physiological solution (Krebs-Henseleit solution, 4 °C) for transportation to the research laboratory. Krebs-Henseleit solution (KHS) had a composition of the followings in mM: NaCl 120, KCl 4.76, $MgSO_4$

1.18, $CaCl_2$ 1.25, $NaHCO_3$ 25, NaH_2PO_4 1.18 and glucose 5.5.

The right coronary arteries from the pig hearts were used. Excess fat and connective tissue were removed and the arteries were cut into 3-mm rings. For the experiments requiring rings with disrupted endothelium, the porcine coronary artery was perfused for 30 s with Triton X-100 solution (0.5% in KHS) at a rate of 1 ml/min before being cut into 3-mm segments. The rings were immediately placed in KHS-filled organ baths (5 ml) under a resting tension of 2 g. The preparations were allowed to equilibrate for 120 min in oxygenated condition (95% O_2: 5% CO_2) at 37 °C. The solution was changed every 25 min. Basal tension of 2 g was maintained continuously throughout the equilibration period.

After the equilibration period, a viability test was carried out. Rings were first incubated with indomethacin (10 μM) for 20 min (to block prostanoids production) and remained exposed to the drug for the rest of the experiment. Indomethacin (Sigma, St. Louis, MO, USA) was dissolved in sodium bicarbonate solution (1 mM). Rings were contracted with 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$ (U46619, 30 nM; a thromboxane A_2 analog) and relaxed with bradykinin (1 μM). U46619 (Biomol, Plymouth Meeting, PA, USA) was dissolved in ethanol whereas bradykinin (Sigma, St. Louis, MO, USA) was dissolved in distilled water. The final concentration of ethanol in each bath was maintained at $\leqslant$0.1%. Endothelium-intact rings that contracted more than 4 g to U46619 (30 nM) and relaxed more than 80% to bradykinin (1 μM) were regarded as viable. For porcine coronary artery exposed to triton X-100 perfusion, the successful disruption of the endothelium was confirmed by its lack of response to bradykinin (1 μM) such that rings with relaxation of more than 5% was not used. After the viability test, the rings were allowed to return to the basal tension by changing the KHS every 15 min before further experimentation. U46619 (30 nM) or potassium chloride (KCl; 50 mM) was then added to endothelium-intact or endothelium-disrupted rings until a stable and sustained contraction was achieved. Flavonoids were added cumulatively and concentration–relaxation curves were constructed. The final organ bath concentrations of ethanol or DMSO used had no significant effect on the results obtained when compared to the corresponding time control (without any treatment).

Rings from the same heart were used for one treatment only. Results are expressed as the means ± SEM and n represents the number of pig from which coronary arterial rings were isolated for use in the experiments. Relaxation was expressed as percentages of the U46619- or KCl-induced contraction. One-way analysis of variance (ANOVA) and Bonferroni's test were used to determine the significant differences among groups treated with flavonoids, vehicle control groups and time control groups (SPSS Inc., Chicago, IL, USA). A P-value less than 0.05 was considered as statistically significant.

4.3. Computer modeling of the flavonoids and calculation of the atomic and molecular descriptors

The molecular structures of the flavonoids were fully optimized using density functional theory B3L YP method with 6-31G(d) basis set (Chermette, 1999). It optimized the geometry obtained for each compound studied and represented the stable conformation assessed theoretically. Some conclusions have been drawn that, in the B3LYP/6-31G computation, the intramolecular hydrogen bonds are strong in regards to those obtained from other levels, and these strong bonds were supposed to contribute to giving the lowest energy values (Rahal-Sekkal et al., 2003). The software package Cerius (Accelrys, San Diego, CA, 2003) was used to generate the descriptor-based 3D-SAR functionality. Only those final structures which represented the most stable conformers for a given compound were used to obtain the molecular descriptors.

The following descriptors were calculated and utilized in this study:

- The energy of the HOMO (highest occupied molecular orbital energy) and LUMO (lowest unoccupied molecular orbital energy).
- Dipole moment (μ).
- Total energy of conformation (E).
- Hardness (η): obtained from the equation $\eta = (E_{LUMO} - E_{HOMO})/2$.
- Mulliken electronegativity (X): calculated from the equation $X = -(E_{LUMO} + E_{HOMO})/2$.
- Molecular volume (V_m).

The descriptors were selected as they represented electronic (HUMO, LUMO, μ, E, η, X) and steric (V_m) features of the compounds studied (Chattaraj et al., 2003). These features are considered to be important for biological activity (Chattaraj et al., 2003; Parthasarathi et al., 2004).

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