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Relationship between vasodilation capacity and phenolic content of Spanish wines

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Abstract

We aimed to determine: 1) the concentration of polyphenols in Spanish red wines, 2) the vasodilatory properties of those wines in relation with their polyphenol concentrations and 3) the vasodilation induced by some of these polyphenols in rat aortic rings. In the wines studied the concentration of rutin and kaempferol was high compared with other polyphenols. All wines relaxed precontracted rat aortic rings and this effect was directly related with the concentration of myricetin and kaempferol in the wines. Kaempferol and rutin also induced endothelium-dependent and independent relaxation, kaempferol was more potent. This relaxation was not inhibited by the estrogen receptor α antagonist ICI 182,760. Kaempferol also potentiated the endothelium-dependent relaxation induced by acetylcholine, which was reversed by Nw-nitro-L-arginine methyl ester (L-NAME). These findings show a good correlation between the concentration of polyphenols (especially kaempferol) of Spanish red wines and the vasodilatory effect, which may confer on them unique features in the prevention of cardiovascular disease.

Keywords: Spanish red wine; Composition; Polyphenol; Vasodilation; Rat aorta; Endothelium

1. Introduction

Coronary heart disease is the main cause of death in developed countries. In this context, the Mediterranean diet has been considered beneficial in the prevention of cardiovascular diseases (Lorgeril de et al., 1996). Interestingly, it has been described that despite a similar distribution of some coronary risk factors such as high blood pressure or serum cholesterol levels, the mortality in France due to coronary heart disease was lower compared to other Western countries. Renaud and De Lorgeril (1992) explained this paradox by the fact that in France, the wine consumption is higher compared to other Western countries. This was named as the French Paradox and it opened a wide range of studies testing the beneficial effect of wine, and other alcoholic beverages in general, on the prevention of coronary heart disease. There is some evidence of the desirable effects of a moderate wine intake, such as: an increase in the production of high density lipoproteins (HDL) (Castelli et al., 1997; Gaziano et al., 1993), a reduction of oxidized low density lipoproteins (LDL), a vasodilatory effect of some wine components as quercetin (Perez-Vizcaino et al., 2002) and tannic acid (Flesch et al., 1998) and also an antioxidative effect due to the wine's content of polyphenols (Frankel et al., 1993a,b).

Polyphenols are among the most promising compounds found in wine. Red wine contains a large number of phenolic compounds that originate from grapes, which are removed during vinification of white wine (Singleton, 1982). Among the dietary phenolic compounds, flavonoids have been the most studied. The bioavailability of flavonols, especially quercetin and glycosylate derivatives, has been widely studied and it is well known that polyphenols present in wines have high solubility and

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hence the bioavailability of these compounds as found in wine is high. Flavonoids, and polyphenols in general, have received considerable public, media and scientific interest in the possibility that increased intake of polyphenols may protect against chronic diseases such as cancer and cardiovascular diseases mainly due to their antioxidant properties. Other polyphenols, such as resveratrol, a compound that is structurally similar to the phenylpropanoids phenolics, have been found to protect against certain types of cancer (Jang et al., 1997) and also to induce vascular relaxation (Orallo et al., 2002).

This study aims firstly to investigate, the concentration of certain antioxidant polyphenols in a wide range of Spanish wines and secondly, to study the relaxant properties of these wines in relation with their composition in polyphenols. The most relevant findings of this work are the increased concentrations of certain flavonoids, such as kaempferol or rutin, in the Spanish wines compared with other wines (Tsanova-Sanova and Rivarova, 2002; Wang and Huang, 2004; Burns et al., 2000) and also that the vasorelaxation induced directly by the wines is related to the content of some polyphenols such as kaempferol and myricetin.

2. Materials and methods

2.1. Analysis of polyphenols by High Performance Liquid Chromatography (HPLC)

In this work 21 young red wines (1999–2001) from different regions of Spain were analyzed. Wine samples (100 ml) were dry-concentrated and resuspended in a defined volume of methanol. Samples were then filtered in a 0.45 μm nylon syringe filter. A 20 μl aliquot of filtered sample was injected into the HPLC system using a Varian chromatograph comprising an Inert 9010 pump and a Dynamax AL-200 autoinjector. Polyphenols were detected using a Polychrom 9065 detector at 254 nm. Elution of polyphenols from a Nova Pak 4 μm C18 column was achieved using the following mobile phase: acetonitrile : acetic acid (95 : 5) and water : acetic acid (95 : 5) and a flow rate of 1 ml/min was maintained throughout. Peak areas were quantified with a chromatography computing integrator working with external standards. The standards were analyzed by injecting commercially available dilutions (1 mg/ml) of the following polyphenols: (-)epicatechin, rutin, myricetin, quercetrin, quercetin, *trans*-resveratrol and kaempferol.

2.2. Assay of vasodilation: general procedure

Wistar male rats weighing 253 ± 5.1 g were used for the study. Rats were housed in cages in an air-conditioned room with a 12 h light/dark cycle. Rats were fed with a standard rat chow and allowed access to water ad libitum. The animals were anaesthetized with ethyl ether and killed by exsanguination. The thoracic aorta was rapidly removed and placed in Krebs–Henseleit solution having the following composition (mM): NaCl 119, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 1.0, glucose 11.1; KH_2PO_4 1.2 and $CaCl_2$ 2.5.

2.3. Aortic rings

Adherent fat and surrounding tissue were cleaned off and the arteries were cut into rings approximately 2–3 mm wide. The rings were then suspended between two stainless steel hooks in organ

Table 1
Polyphenols content (μg/ml) Spanish wines analyzed by HPLC

Wine	Grape, region (year)	(-) Epicatechin	Rutin	Quercetrin	Myricetin	*trans*-Resveratrol	Quercetin	Kaempferol
1	Negramoll, La Palma (2001)	15.6 ± 0.5	22.8 ± 1.1	n.d.	4.1 ± 0.1	0.71 ± 0.01	13.32 ± 0.05	1.053 ± 0.08
2	Monistrell, Somontano (1999)	5.3 ± 0.2	4.0 ± 0.1	0.302 ± 0.005	3.43 ± 0.05	0.26 ± 0.06	3.32 ± 0.31	0.054 ± 0.003
3	Garnacha, La Mancha (2001)	n.d.	40.9 ± 1.7	0.70 ± 0.03	11.8 ± 0.9	4.0 ± 0.1	11.28 ± 0.1	1.25 ± 0.02
4	Bobal, Utiel-Requena (2001)	n.d.	28.3 ± 1.6	0.505 ± 0.001	7.1 ± 0.1	0.30 ± 0.01	8.4 ± 0.4	0.888 ± 0.007
5	Monistrell, Murcia (2000)	12.8 ± 0.5	56.6 ± 1.9	0.48 ± 0.02	12.5 ± 0.5	0.12 ± 0.01	19.98 ± 0.6	2.36 ± 0.02
6	Tinta Fina, Ribera de Duero (1999)	8.9 ± 0.3	13.9 ± 0.4	0.48 ± 0.01	8.1 ± 0.2	n.d.	4.8 ± 0.2	4.3 ± 0.2
7	Cencibel, La Mancha (2001)	7.2 ± 0.1	24.4 ± 0.4	0.505 ± 0.003	4.1 ± 0.2	n.d.	1.11 ± 0.03	0.260 ± 0.005
8	Merlot, La Mancha (2001)	13.0 ± 0.7	34.8 ± 1.0	3.1 ± 0.1	5.1 ± 0.3	0.15 ± 0.01	2.79 ± 0.04	0.562 ± 0.008
9	Cabernet Sauvignon, La Mancha (2001)	20.1 ± 1.0	21.6 ± 0.8	0.84 ± 0.06	3.3 ± 0.1	0.066 ± 0.003	1.05 ± 0.02	0.140 ± 0.001
10	Mencía, Bierzo (2001)	10.9 ± 0.4	5.5 ± 0.2	0.403 ± 0.007	15.3 ± 0.1	0.060 ± 0.002	6.60 ± 0.05	0.576 ± 0.006
11	Listan Negra-Negranoll, Tacoronte-Acentejo (2000)	n.d.	53.0 ± 0.9	0.29 ± 0.06	7.02 ± 0.08	n.d.	17.0 ± 0.1	2.27 ± 0.06
12	Listan Negra-Negranoll, Tacoronte-Acentejo (2000)	n.d.	14.0 ± 1.0	0.42 ± 0.01	5.8 ± 0.5	0.110 ± 0.006	7.9 ± 0.2	0.22 ± 0.02
13	Tinta de Toro, Toro (2001)	n.d.	5.9 ± 0.7	0.59 ± 0.01	3.7 ± 0.5	n.d.	0.43 ± 0.04	n.d.
14	Mencía, Ribeiro (2001)	16.0 ± 1.0	2.80 ± 0.07	0.82 ± 0.01	4.59 ± 0.09	0.098 ± 0.006	0.518 ± 0.001	n.d.
15	Cariñena, Cataluña (2001)	17.0 ± 1.0	23.82 ± 0.05	0.65 ± 0.02	8.88 ± 0.06	n.d.	14.8 ± 0.1	1.63 ± 0.04
16	Garnacha, Cataluña (2001)	28.0 ± 1.0	18.4 ± 0.5	0.45 ± 0.03	9.5 ± 0.4	0.115 ± 0.003	17.0 ± 0.3	1.16 ± 0.02
17	Tempranillo, Cataluña (2001)	n.d.	14.0 ± 1.0	0.49 ± 0.04	8.7 ± 0.2	0.113 ± 0.009	11.4 ± 0.3	1.15 ± 0.001
18	Garnacha, Cariñena(2001)	46.0 ± 3.0	n.d.	0.93 ± 0.10	6.16 ± 0.13	1.01 ± 0.13	17.0 ± 0.04	2.65 ± 0.02
19	Mazuelo, Cariñena (2001)	42.0 ± 2.0	n.d.	1.01 ± 0.05	14.71 ± 0.02	1.28 ± 0.06	20.81 ± 0.36	2.93 ± 0.02
20	Graciano, Rioja (2000)	34.0 ± 1.0	n.d.	0.51 ± 0.03	11.67 ± 0.22	1.15 ± 0.09	14.62 ± 0.03	0.87 ± 0.01
21	Tempranillo-Garnacha, Rioja (2001)	10.0 ± 0.5	n.d.	2.30 ± 0.04	12.95 ± 0.10	0.43 ± 0.02	7.39 ± 0.04	0.755 ± 0.004

Data are expressed as mean ± S.E.M. (n = 3). (n.d.) not detected.

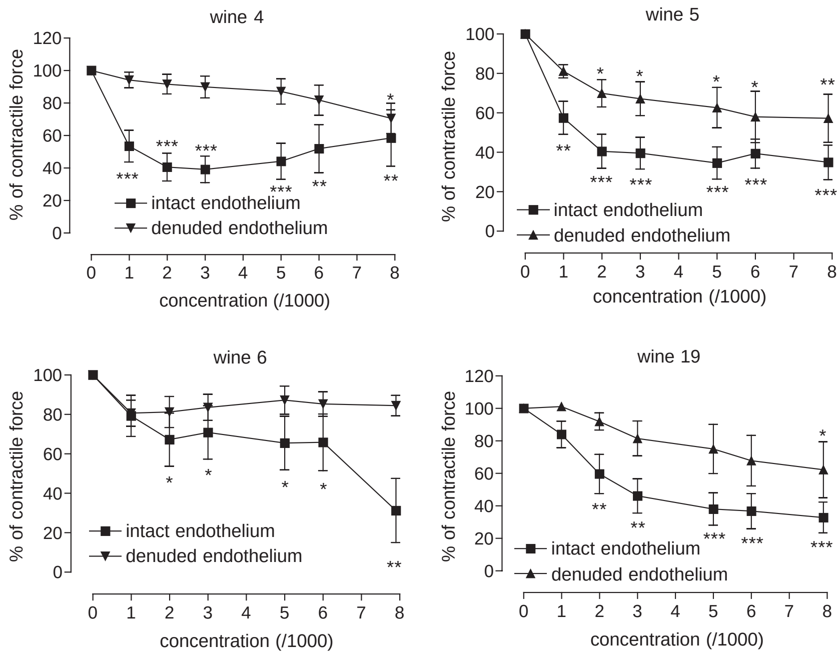

Fig. 1. Relaxation induced by the wines studied in noradrenaline-precontracted rat aortic rings. Arteries were separated in two groups; endothelium intact and endothelium denuded and precontracted with noradrenaline according to methods. Aliquots of the wines were added when the plateau was reached. Each data shows the mean ± S.E.M. of $n = 8$ animals. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (one way ANOVA).

baths containing 5 ml of Krebs–Henseleit solution. The solution was kept at 37 ± 0.5 °C and gassed continuously with a 95% O_2–5% CO_2 gas mixture. Aortic rings were mounted under 1 g of resting tension. Each preparation was allowed to equilibrate for 45–60 min. Contractile responses were measured isometrically by means of force-displacement transducers (Grass FT 03) and were recorded on a Grass polygraph as previously described (Tejerina et al., 1988).The isometric force was also digitalized by a MacLab A/D converter (Chart v3.2, A.D. Instruments Pty. Ltd., Castle Hill, Australia) and stored and displayed on a Mackintosh computer (Ruiz and Tejerina, 1998).

2.4. Experimental procedure

Two groups of arteries were established; those with intact endothelium and those in which the endothelium was removed. Endothelium-denuded arteries were prepared mechanically by inserting a stainless-steel rod into the rings and rubbing the rings gently with our fingers. After the equilibration period, the arteries were contracted sub-maximally with noradrenaline 10^{-6} M and then exposed to acetylcholine (10^{-8}–10^{-4} M) to test the endothelium's functionality. Those arteries in which acetylcholine relaxed at least 50% of the precontracted force were considered endothelium-holding whereas arteries in which the relaxation was less than 10% of the precontracted force were considered endothelium-lacking. When the relaxation achieved by acetylcholine was between 50% and 10%, the artery was discarded.

After the washing and following equilibration period, the arteries were contracted sub-maximally with noradrenaline 10^{-6} M and once a stable plateau had been reached, increased concentrations of each wine (or vehicle; ethanol) were added cumulatively using various dilutions from 1/1000 to 8/1000 to both groups of arteries. Only one wine was used for each ring.

3. Results

3.1. Analysis of the polyphenol content by HPLC

Table 1 shows the polyphenol content (μg/ml) in the wines tested. We found that epicatechin and rutin were the polyphenols more abundant, although epicatechin was not found in all the wines tested. The wine with the highest concentration of epicatechin was Wine 18 (Garnacha, Cariñena) while rutin was found at 56.6 μg/ml in Wine 5 (Monistrell, Murcia, 2000). In contrast, quercetrin and *trans*-resveratrol seem to be the less concentrated. Generally, quercetin is thought to be the major contributor to the antioxidant activity of red wine. We found it in a similar range of concentration as in wines from other countries. On the other hand, total

Table 2
Maximal relaxation induced by the wines with the highest concentration in kaempferol in rat aortic rings precontracted with noradrenaline

	Maximal	Relaxation
Wine	Intact endothelium	Denuded endothelium
1	45.0 ± 12.6	11.0 ± 3.7*
3	45.1 ± 11.4	49.7 ± 13.0
4	60.8 ± 8.1	29.4 ± 9.3*
5	68.8 ± 16.2	42.8 ± 12.2
6	65.4 ± 8.1	15.6 ± 5.2*
11	50.6 ± 12.4	22.1 ± 11.3*
15	39.4 ± 4.7	28.4 ± 11.0
16	24.8 ± 15.9	30.5 ± 9.2
18	49.4 ± 11.9	15.0 ± 5.4*
19	78.0 ± 14.5	27.4 ± 16.6*

Each data shows the mean ± S.E.M. of $n = 8$ animals. *$P < 0.05$ respect to intact endothelium (one way ANOVA).

kaempferol, which is not present in elevated concentration in wines, was found in increasing concentration in the wine tested compared with wines from other countries (Tsanova-Sanova and Rivarova, 2002; Wang and Huang, 2004). The wine with the highest concentration of kaempferol was Wine 6 (Tinta Fina, Ribera de Duero, 1999), where the concentration of kaempferol was 4.3 μg/ml (equivalent to 15 μM). The average in the concentration of kaempferol was 2 μg/ml (equivalent to 7.1 μM).

3.2. Relaxation induced by the different wines

Since kaempferol was found in increasing concentrations in the wines studied, we determined the relaxant effects of 10 wines with the highest amount of kaempferol. Aliquots of the wines tested were added to the organ chamber of precontracted rat aortic rings. All wines tested caused a concentration-dependent relaxation to different extents; thus, in terms of the maximum relaxation achieved, the decrescent order was: wine 19>wine 5>wine 6>wine

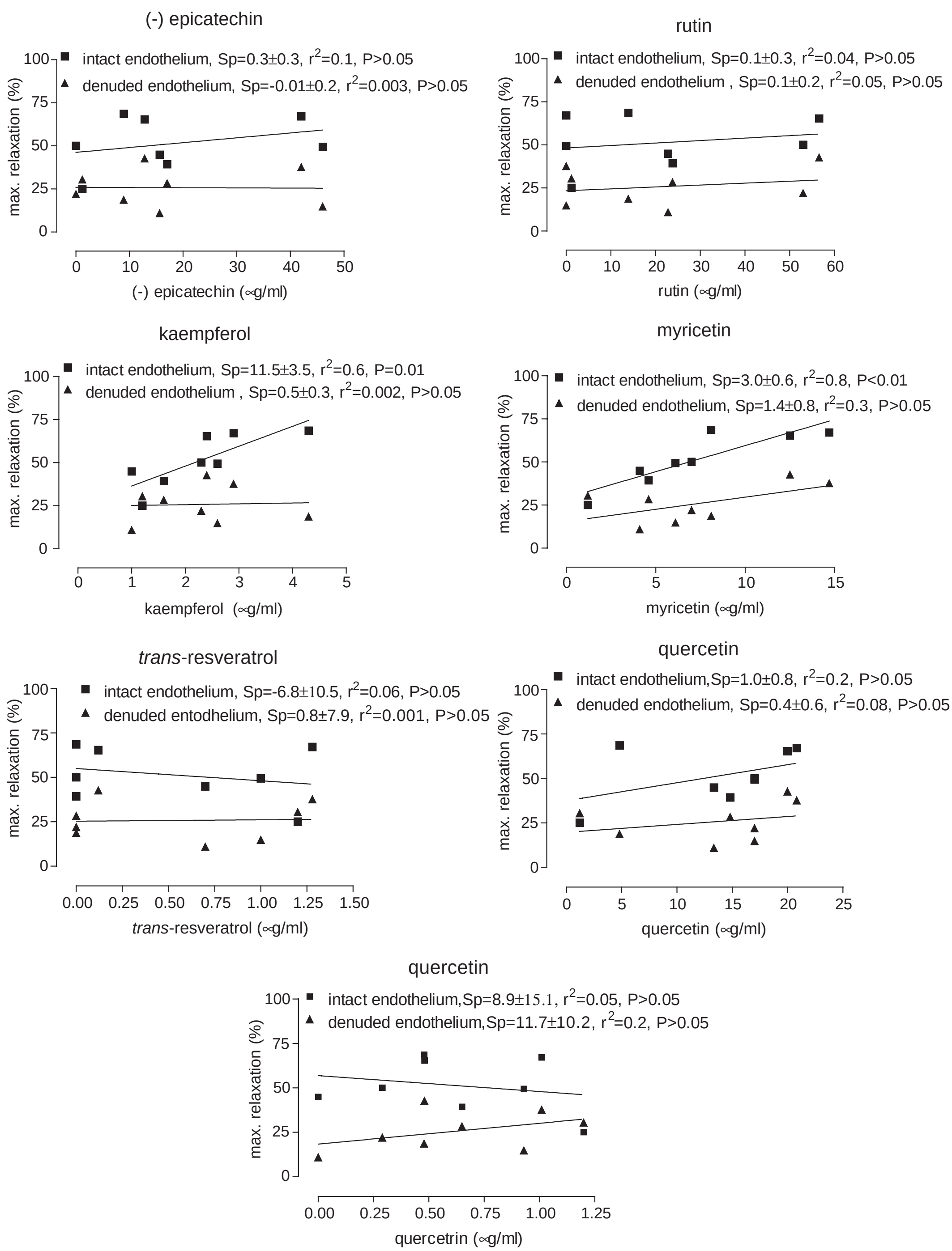

Fig. 2. Relationship between the content in polyphenols and the maximal relaxation. Wine polyphenols were analyzed by HPLC according to methods and are expressed in microgram per milliliter.

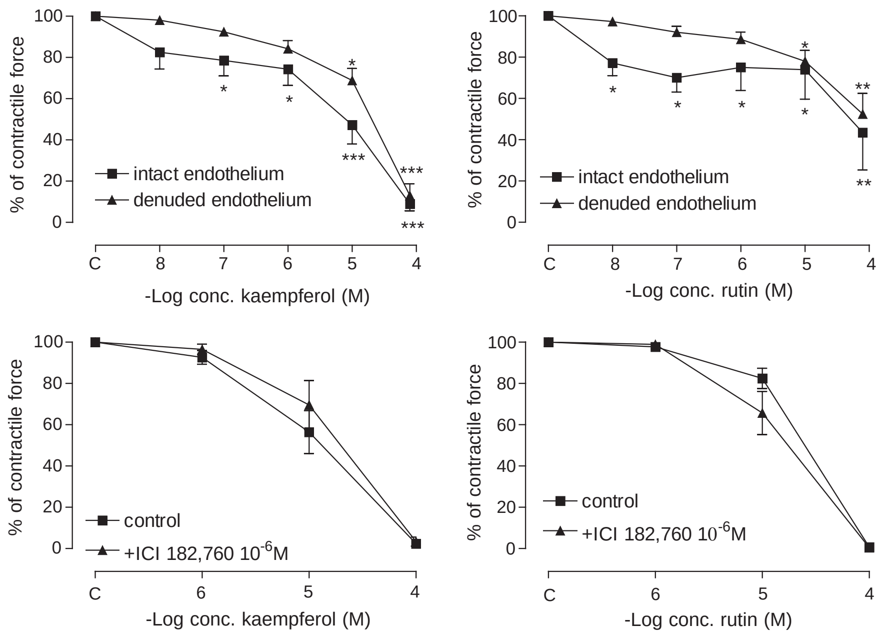

Fig. 3. Upper panels: relaxation induced by kaempferol and rutin in noradrenaline-precontracted arteries. Lower panels: effect of the estrogen receptor α antagonist ICI 182,760 on the relaxation induced by kaempferol and rutin. Each data shows the mean ± S.E.M. of $n=8$ animals. *$P<0.05$, **$P<0.01$, ***$P<0.001$ (one way ANOVA).

4>wine 11>wine 18>wine 3>wine 1>wine 15>wine 16. Fig. 1 shows the relaxation induced by wines 4, 5, 6 and 19 whereas the relaxation induced by other wines is shown in Table 2. The majority of wines with high concentration of kaempferol showed a significant decrease in their relaxant effect in arteries with denuded endothelium, as shown in Table 2. However, since a percentage of relaxation still remained in arteries with denuded endothelium, one can argue that there is also an endothelium independent component in the relaxation induced by these wines.

3.3. Relationship between the polyphenol content and the relaxant responses

Since wine polyphenols seem to be important in the cardiovascular effect of wines, we analyzed the polyphenol content in the different wines. Fig. 2 shows the relationship between the maximal relaxation and the content of polyphenols (in microgram per milliliter). We did not find any significant relationship between the content of epicatechin, rutin, quercetrin

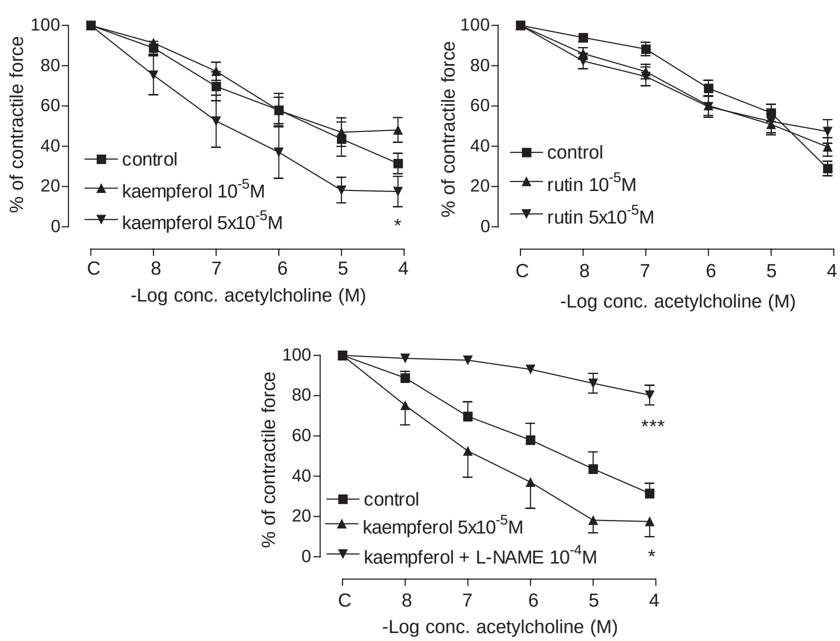

Fig. 4. Upper panels: effect of kaempferol and rutin on the endothelium-dependent relaxation induced by acetylcholine in noradrenaline-precontracted arteries. Lower panel: the nitric oxide synthase antagonist L-NAME reversed the effect of kaempferol on the endothelium-dependent relaxation. Each data shows the mean ± S.E.M. of $n=8$ animals. *$P<0.05$, ***$P<0.001$ (one way ANOVA).

and *trans*-resveratrol and the maximal relaxant responses obtained. On the other hand, the content of myricetin and kaempferol in the different wines shows a direct relationship with the maximum relaxation. Moreover, when the endothelium was removed, the relaxation diminished significantly.

3.4. Relaxation induced by kaempferol and rutin

We chose kaempferol and rutin because they were found in elevated concentrations in the Spanish wines studied. Rat aortic rings were precontracted with noradrenaline and cumulative concentrations of kaempferol or rutin (10^{-8}–10^{-4}M) were added when a steady plateau was reached. Fig. 3 shows the relaxant effect of kaempferol or rutin. Kaempferol induced vasorelaxation in arteries with intact endothelium from concentrations as low as 10^{-7} M. This relaxant effect was significantly diminished in arteries with denuded endothelium. Rutin also exerted a relaxant effect, although less potent than that of kaempferol. The endothelium removal also affected negatively the relaxant effect of rutin.

We also investigated the estrogen receptor alpha on the relaxation induced by kaempferol and rutin. The estrogen receptor alpha antagonist ICI 182,760 was incubated 30 min prior to precontracting the arteries. Fig. 3 (lower panels) shows that ICI 182,760 had no effect on the relaxation induced by kaempferol (lower left panel) or rutin (lower right panel) either in endothelium-intact or endothelium-denuded arteries.

3.5. Effect of kaempferol and rutin on the endothelium-dependent relaxation

Since previous studies show that polyphenols increase the endothelium-dependent relaxation in precontracted arteries in vitro, we investigated the effect of kaempferol and rutin on the relaxation induced by acetylcholine in noradrenaline-precontracted aortic rings. Fig. 4 shows that preincubation with kaempferol 5×10^{-5} M for 30 min, but not with rutin, increased the endothelium-dependent relaxation induced by acetylcholine. Moreover, to test the role of nitric oxide on the effect of kaempferol, we incubated the arteries with a known nitric oxide synthase inhibitor (L-NAME) prior to kaempferol, which completely abolished the effect of kaempferol on the endothelium-dependent relaxation (Fig. 4, lower panel).

4. Discussion

There is now a significant body of evidence supporting the idea that most vasodilatory compounds extracted from plants, including red wine, are skin-derived, supported by the low vasodilatation activity of white wine, for which there is extensive grape skin extraction, compared to that of red wine. Numerous epidemiological studies have shown an inverse association between moderate alcohol intake and coronary heart disease, especially with red wine intake compared to other alcoholic beverages. This phenomenon has been attributable to the wine's polyphenolic compounds.

Spain is one of the largest wine-makers in the world. As part of the Mediterranean diet, there is a widespread red wine intake in Spain; however, there are few works about the composition of polyphenols in Spanish red wines and about the possible relation of the composition of these wines to their vasodilator activity. For that reason we aimed to analyze the composition of 21 Spanish red wines from different regions and made from different types of grapes, focusing in the concentration of flavonoids and of the stilbene *trans*-resveratrol. Wines were selected from a wide range of wines all over Spain. We have chosen wines from different geographical and climatological conditions. Flesch et al. (1998) showed that wines matured in barrique caused a stronger relaxation than those matured in steel tanks likely due to the effect of tannic acid. For that reason, we have chosen wines no more than 1 year old after the harvest to avoid the effect of oak on their composition and in their vasodilatory effects. Thus these wines range from 1999 to 2001 and none of them were oak matured at the beginning of the study.

As shown in Table 1, the polyphenol found in highest concentration was rutin, followed by epicatechin and by quercetin. Quercetin is thought to be the major contributor to the antioxidant activity of red wine (Maxwell, 1997). Although other members of the flavonol family, such as kaempferol or myricetin, exhibit a strong antioxidant activity (Rice-Evans et al., 1995) they are probably not present in sufficient quantities in wines. A clear example of that is kaempferol which is barely detectable in some wines (Vourinen et al., 2000) or found in very low concentrations (Tsanova-Sanova and Rivarova, 2002; Wang and Huang, 2004; Burns et al., 2000). Surprisingly, we have found that in the majority of the wines studied, the concentration of kaempferol was far higher than in wines from other countries. The wine with the highest concentration of kaempferol was wine 6 (from Ribera de Duero), where the concentration of kaempferol was 4.3 μg/ml (equivalent to 15 μM). All in all, the average of the concentration of kaempferol was 2 μg/ml (equivalent to 7.1 μM). Burns et al. (2000) showed that the composition of free kaempferol of 16 wines analyzed ranged from 1.1 to 4.5 μM, with an average of 2.1 μM.

It has also been proposed that the stilbene resveratrol plays a key role in the beneficial effects of red wine (Soleas et al., 1997) by preventing platelet aggregation (Pace-Asciak et al., 1995), by inhibiting the oxidation of LDL (Frankel et al., 1993a,b), and by reducing the risk of certain types of cancer (Jang et al., 1997). We have also analyzed the concentration of *trans*-resveratrol in our wines and found that the average of concentration was lower than the concentration of *trans*-resveratrol in wines from other countries. In some wines, *trans*-resveratrol was not even detected.

In the second part of the study we have determined the vasodilatory activity of the wines studied. It has been suggested that one of the mechanisms of cardioprotection exerted by wine might partly be due to the vasodilatory effect of its phenolic compounds (Fitzpatrick et al., 1993). We have tested the vasodilatory properties of those wines with a high concentration of kaempferol. We reported here that the majority of the wines studied caused a vasorelaxation of rat aortic rings precontracted with noradrenaline. The wine which showed a more potent vasorelaxant effect was wine 6. Other studies have shown that the vasodilatation induced by wine is mediated by endothelial generation and release of nitric oxide (Flesch et al., 1998). Nitric oxide is known as one of the more potent vasodilators; its effect is mediated by cGMP, leading to smooth muscle cell relaxation (Arnold et al., 1977). The majority of the wines studied showed a decrease in their vasodilatory activity when the endothelial layer was mechanically removed, which denotes that the presence of an intact and functional endothelium is important for the vasodilatory effect of the wines studied. Other studies have previously shown that the vasodilatory effect of wines is dependent on endothelium integrity and is reduced by the nitric oxide synthase inhibitor L-NAME (Flesch et al., 1998).

Vasorelaxation induced by wines was correlated with the concentrations of certain polyphenols. Interestingly, those wines which show higher concentrations of myricetin and kaempferol showed a greater vasorelaxation. We have also studied the direct vasodilatory effect of kaempferol and rutin on rat aortic rings precontracted with noradrenaline. We chose kaempferol because it is found in elevated concentrations in the Spanish wines studied and rutin because it is the polyphenol found in the highest concentrations in the wines studied. Moreover, the vascular properties of these two flavonoids, specially rutin, have been studied in a lesser extent.

Fig. 3 shows that both kaempferol and rutin relaxed aortic rings precontracted with noradrenaline, although the potency of kaempferol was higher. To understand why kaempferol has a more potent relaxant effect than rutin, we should look at their chemical structure. The chemical structure of kaempferol is quite similar to that of quercetin and hence, more lipophilic than rutin, which is bound to a glucoside. Having this in mind, it is reasonable to assume that kaempferol might be more easily uptaken by cells than rutin. The relaxation exerted by these two compounds is dependent on the integrity of the endothelial layer. When arteries were endothelium-denuded, the effect of kaempferol was slightly but significantly reduced; however the effect of rutin was only reduced when using a low concentration. Interestingly, kaempferol, at a concentration of 5×10^{-5} M, potentiated the endothelium-dependent relaxation induced by acetylcholine, which was reversed by L-NAME; however, rutin, at the same concentration, did not have any effect. This could mean that rutin has a concentration-dependent effect on the endothelium. It has been shown that flavonoids scavenge superoxide anion and might then protect nitric oxide degradation (Rice-Evans and Packer, 1998). This fact might explain why the effect of kaempferol is more potent when the endothelium-dependent relaxation was induced by an agonist than the endothelium-dependent relaxation induced by kaempferol itself. This phenomenon might explain the endothelium dependent effect of flavonoids. Interestingly, we have also found that there is a percentage of relaxation independent of endothelium integrity. Flavonoids, including kaempferol, are potent inhibitors of the myosin light chain kinase in vascular smooth muscle cells (Hagiwara et al., 1988), which might explain, at least in part, the endothelium-independent relaxation observed.

Polyphenols are also shown to act on the estrogen receptor, acting as phytoestrogen (Karamsetty et al., 2001). Activation of the estrogen receptor α in endothelial cells induces nitric oxide synthesis (Chen et al., 1999), whereas in vascular smooth muscle it induces direct relaxation (Freay et al., 1997). Since kaempferol seems to be a more potent relaxant, we determined whether the relaxation, endothelium-dependent, independent or both was due to the action of kaempferol as a phytoestrogen. Preincubation with the estrogen receptor α antagonist ICI 182,760 did not modify the relaxant effect of kaempferol either in endothelium-denuded or endothelium-intact arteries, showing that the effect of kaempferol as a phytoestrogen, if any, has no significant importance in this effect.

In summary, the main findings of this work are, firstly, that for the first time the composition of a wide variety of Spanish wines has been extensively examined and secondly that the concentration of kaempferol is directly related to the vasodilatory effect of these wines and may confer on them unique features in the prevention of cardiovascular disease.

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