

Vascular effects of the *Mangifera indica* L. extract (Vimang)

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Abstract

The effects of the *Mangifera indica* L. (Vimang) extract, and mangiferin (a C-glucosylxanthone of Vimang) on the inducible isoforms of cyclooxygenase (cyclooxygenase-2) and nitric oxide synthase (iNOS) expression and on vasoconstrictor responses were investigated in vascular smooth muscle cells and mesenteric resistance arteries, respectively, from Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Vimang (0.5–0.1 mg/ml) and mangiferin (0.025 mg/ml) inhibited the interleukin-1 β (1 ng/ml)-induced iNOS expression more in SHR than in WKY, and cyclooxygenase-2 expression more in WKY than in SHR. Vimang (0.25–1 mg/ml) reduced noradrenaline (0.1–30 μ M)- and U46619 (1 nM–30 μ M)- but not KCl (15–70 mM)-induced contractions. Mangiferin (0.05 mg/ml) did not affect noradrenaline-induced contraction. In conclusion, the antiinflammatory action of Vimang would be related with the inhibition of iNOS and cyclooxygenase-2 expression, but not with its effect on vasoconstrictor responses. Alterations in the regulation of both enzymes in hypertension would explain the differences observed in the Vimang effect.

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1. Introduction

Vimang is an aqueous extract obtained from the stem bark of *Mangifera indica* L. (Anacardiaceae). In Cuba, this extract is used to improve the quality of life in patients suffering from increased stress (Guevara et al., 1998). The extract contains a defined and standardised mixture of components such as polyphenols, terpenoids, steroids, fatty acids and microelements (Núñez-Selles et al., 2002), mangiferin being the main polyphenol present. These components have been reported to have cytotoxic, anti-neoplastic (Muanza et al., 1995; Guha et al., 1996), antioxidant (Born et al., 1996; Leiro et al., 2003), and antiinflammatory activities (Garrido et al., 2001).

The inflammatory processes involve a broad spectrum of chemical mediators, these include nitric oxide (NO) and

prostanoids, synthesized by the inducible isoforms of NO synthase (iNOS) and cyclooxygenase (cyclooxygenase-2). Vascular events associated with an inflammatory reaction include dilatation of the small arterioles, resulting in increased blood flow and increased permeability. In healthy blood vessels, the NO and prostanoids are predominantly formed by the constitutive isoforms of NOS and cyclooxygenase, respectively. Recently, some authors have found that vascular smooth muscle expresses cyclooxygenase, and produces prostanoids that could be involved in the modulation of vascular responses (Swierkosz et al., 1995; Cirino et al., 1996; Briones et al., 2002b).

Hypertension is considered a chronic inflammatory disease (Virdis and Schiffrin, 2003) with elevated proinflammatory cytokine blood levels (Wu et al., 1994; Chou et al., 1998). In addition, experimental hypertension has been associated with alterations in the function and expression of cyclooxygenase-2 (Henrion et al., 1997; Garcia-Cohen et al., 2000; Briones et al., 2002b) and of different isoforms of

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NOS (Wu et al., 1994; Chou et al., 1998; Pacheco et al., 2000; Briones et al., 2002a).

In the present study, we investigated the effects of Vimang and mangiferin (a C-glucosylxanthone present in the extract) on cyclooxygenase-2 and iNOS expression in vascular smooth muscle cells from mesenteric arteries from normotensive, Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats. In addition, the effects on vasoconstrictor responses induced by noradrenaline in mesenteric resistance arteries from both strains were evaluated.

2. Material and methods

Six-month-old male WKY and SHR rats were obtained from colonies maintained at the Animal Quarters of the “Facultad de Medicina” of the “Universidad Autónoma de Madrid”. Systolic blood pressure was recorded with an automatic sphygmomanometer using a tail-cuff method device placed on the tail of pretrained rats, which had spent 1 h in a warm chamber at 37 °C, and were restrained. Measurements of blood pressure were repeated at least three times, and the average systolic blood pressure was calculated: 146 ± 4 for WKY, $n=6$, and 220 ± 5 for SHR, $n=6$ (means \pm S.E.M., in mm Hg; $P<0.05$). For the experiments, the rats were killed by decapitation. The mesenteric vascular bed was removed and placed in cold (4 °C) Krebs–Henseleit solution (KHS) bubbled with a 95% O₂–5% CO₂ mixture. The superior mesenteric artery and its fourth branch were dissected for cell culture and reactivity experiments, respectively.

2.1. Cell culture

Primary cultures of rat vascular smooth muscle cells were obtained according to the method described elsewhere (Redondo et al., 1995). Briefly, mesenteric arteries from four WKY or SHR were cut into small pieces, placed in Dulbecco’s modified Eagle medium (DMEM, Gibco, Grand Island, NY, USA) with 0.1% bovine serum albumin (Sigma, St. Louis, MO, USA) and 4 mg/ml of collagenase (type II, Sigma), and incubated for 90 min at 37 °C in a humidified atmosphere of CO₂ (5%) and air (95%). The resulting cell suspension was washed three times by centrifugation and then resuspended in DMEM supplemented with 10% fetal calf serum (Gibco), 100 U/ml of penicillin, 100 µg/ml of streptomycin, and 2.5 µg/ml of amphotericin B (Sigma). Cells were characterized as smooth muscle by immunocytochemical staining with smooth muscle-specific monoclonal antibody anti- α -actin. Cells were incubated for 24 h with or without 1 ng/ml interleukin-1 β in the presence and the absence of Vimang (0.1, 0.2 and 0.5 mg/ml) or mangiferin (0.025 mg/ml). Cultures from identically treated lines between passages 4 and 5 were used.

2.2. Western blot analysis

After appropriate treatments, vascular smooth muscle cells were washed twice in ice-cold buffer (25 mmol/l Tris–HCl, 1 mmol/l EDTA, pH 7.4). The cells were scraped and lysed in the following homogenization buffer: 1 mM sodium vanadate, 1% sodium dodecyl sulfate (SDS), 10 mM Tris–HCl, pH 7.4. Proteins from the whole cell lysates (12 µg) were separated on a 7.5% SDS-polyacrylamide gel and electrophoretically transferred to polyvinylidene difluoride membranes in Tris–Glycine transfer buffer with 20% methanol in a Bio-Rad Trans-Blot Cell (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were blocked for 90 min at room temperature with 5% non-fat dry milk in Tris-buffered saline (in mM): 10 Tris, 100 NaCl, pH 7.5 containing 0.1% Tween 20, and incubated with mouse monoclonal antibody anti-iNOS (1:10,000; Transduction Laboratories, Lexington, UK), rabbit polyclonal antibody anti-cyclooxygenase-2 (1:250; Cayman Chemical, Ann Arbor, MI, USA) or antibody anti-cyclooxygenase-1 (1:100; Santa Cruz Biotechnology) for 75 min at room temperature. The membranes were thoroughly washed and incubated with horseradish peroxidase-coupled anti-mouse (1:2,000; Transduction Laboratories) or anti-rabbit (1:2,000; Transduction Laboratories) immunoglobulin G antibody for 1 h. After thorough washing, the bound proteins were visualized by enhanced chemiluminescence (ECL) (Amersham International, Little Chalfont, Germany) and exposure to Kodak X-OMAT film. Signals on the immunoblot were quantified by using a computer program (NIH Image V1.56). The same membranes were used to determine α -actin expression using a mouse monoclonal antibody (1:3,000,000, Boehringer Mannheim, Mannheim, Germany) to correct for the amount of protein loaded per lane.

Stimulated mouse macrophages homogenates were used as the positive control for iNOS and cyclooxygenase-2 and rat platelets for cyclooxygenase-1.

2.3. Reactivity experiments

Ring segments, 2 mm in length, of mesenteric resistance arteries were mounted in a small vessel dual chamber myograph for measurement of isometric tension. Two tungsten wires (40 µm diameter) were introduced through the lumen of the segments and mounted according to the method described by Mulvany and Halpern (1977).

After a 30-min equilibration period in oxygenated KHS at 37 °C and pH=7.4, segments were stretched to their optimal lumen diameter for active tension development. This was determined based on the internal circumference-wall tension ratio of the segments by setting their internal circumference, L_0 , to 90% of what the vessels would have if they were exposed to a passive tension equivalent to that produced by a transmural pressure of 100 mm Hg (Mulvany

and Halpern, 1977). The effective lumen diameter, calculated as L_0/π , was lower ($P<0.05$) in segments from hypertensive ($235.0\pm 4.2\ \mu\text{m}$, $n=12$) than normotensive rats ($256.0\pm 5.0\ \mu\text{m}$, $n=11$).

Afterwards, segments were washed three times with KHS and left to equilibrate for 30 min; segment contractility was then tested by an initial exposure to a high- K^+ solution (120 mM K^+ -KHS that was identical to KHS except that NaCl was replaced by KCl on an equimolar basis).

The presence of endothelium was determined by the ability of 10 μM acetylcholine to induce relaxation of at least 60% and 30% of the contraction induced by noradrenaline at a concentration that produces close to 50% of the maximum contraction induced by 75 mM KCl in WKY and SHR segments, respectively (Briones et al., 2000). Once the presence or absence of endothelium was determined, concentration–response curves for noradrenaline (0.1–30 μM), U46619 (1 nM–30 μM) or KCl (7.5–70 mM) were performed by its cumulative addition. Two consecutive curves were performed at 1-h intervals; the curves for noradrenaline and U46619 were similar, while the second curve to KCl was greater than the first one. To investigate the effect of Vimang and mangiferin on vasoconstrictor responses, paired (noradrenaline or U46619) or unpaired (KCl) experiments were performed and 0.25, 0.5 or 1 mg/ml of Vimang or 0.05 mg/ml of mangiferin was added 30 min

before the second concentration–response curve to noradrenaline, U46619 or KCl.

In some experiments, the effect of Vimang (0.25–1 mg/ml) on segments precontracted with noradrenaline at a concentration that produces close to 50% of the maximum contraction induced by 75 mM KCl was analyzed.

At the end of the experiment, the segments were washed and KHS was replaced by 120 mM K^+ -KHS; once the contraction was stabilized, 0.1 mM papaverine was added. The maximum response of the arteries (2.1 ± 0.1 and 2.8 ± 0.1 mN/mm for WKY and SHR respectively, $n=11$ –12, $P<0.05$) was determined from the difference between the tone generated by the first 120 mM K^+ administration and that produced by 0.1 mM papaverine.

2.4. Data analysis and statistics

Vasoconstrictor responses were calculated as active wall tension (calculated as the increase in vessel wall force above the resting level divided by twice the vessel segment length) and expressed as a percentage of the maximum response. The maximum response (E_{max}) and the negative logarithm of concentrations of noradrenaline producing 50% of the maximum response (pD_2 values), were calculated from each individual concentration–response curve using GraphPad Prism Software (San Diego, CA, USA).

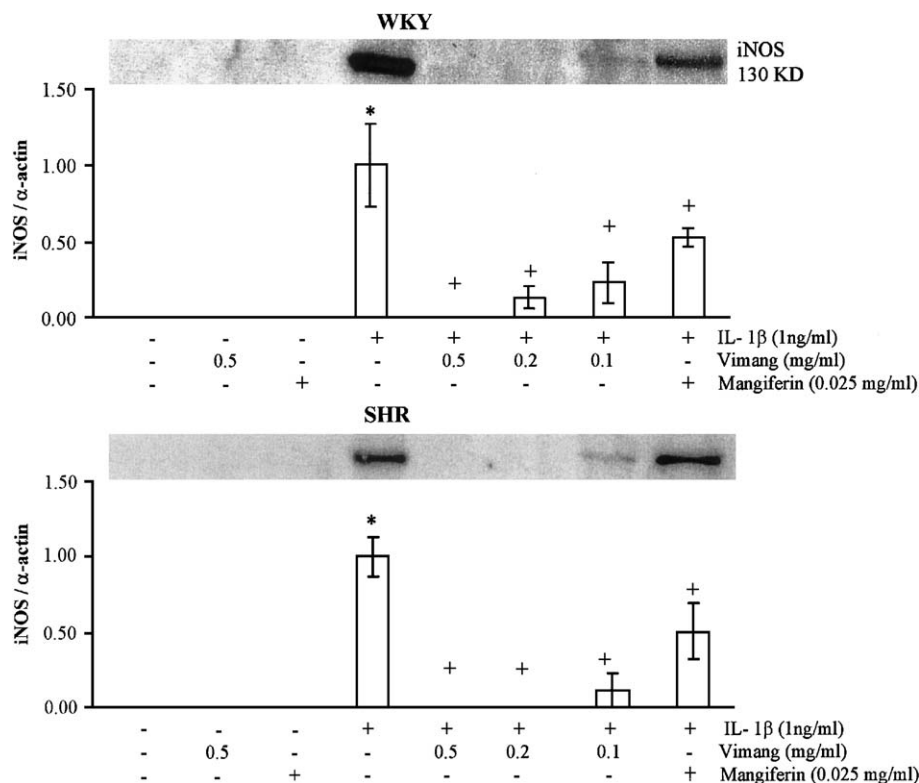


Fig. 1. Effect of incubation with Vimang or mangiferin on iNOS protein expression in vascular smooth muscle cells from normotensive (WKY) and hypertensive (SHR) rats in the absence (control) or the presence of interleukin (IL)-1 β ($n=3$ –4). Results (means \pm S.E.M.) are expressed as the ratio between the signal for the iNOS protein and the signal for α -actin. * $P<0.05$ vs. control, + $P<0.05$ vs. IL-1 β . Representative blots are also shown.

In order to compare the effect of the different drugs on the noradrenaline or U46619 response in arteries from both strains, some results are expressed as “difference of the area under the concentration–response curve” (dAUC) for noradrenaline, in the control and experimental situations. AUC were calculated from the individual concentration–response curve plot; the differences were expressed as a percentage of AUC of the control situation.

Data for protein expression are expressed as the ratio between signals on the immunoblot corresponding to the protein studied and the α -actin signals.

All values are expressed as means \pm S.E.M. of the number of rats indicated in each case. Statistically significant differences between means were calculated by unpaired Student's *t*-test. Differences between concentration–response curves were analysed by two-way analysis of variance (ANOVA) followed by the Bonferroni *t*-test. A probability value of less than 5% ($P < 0.05$) was considered significant.

2.5. Drugs and chemicals

KHS contained (mM): 115 NaCl, 25 NaHCO₃, 4.7 KCl, 1.2 MgSO₄·7H₂O, 2.5 CaCl₂, 1.2 KH₂PO₄, 11.1 glucose and 0.01 Na₂EDTA. Drugs used were: noradrenaline hydrochloride, acetylcholine chloride, papaverine hydrochloride, sodium metavanadate, dithiothreitol, interleukin-1 β recombinant mouse (Sigma); Tween 20, Tris, SDS and acrylamide (Bio-Rad Laboratories), U46619 (Calbiochem-Novabiochem, Bad Soden, Germany), methanol (Merck KGaA, Darmstadt, Germany). Vimang and mangiferin were supplied by the Centre of Pharmaceutical Chemistry (Cuba). Drug solutions were made in bidistilled water except for noradrenaline, which was dissolved in saline (0.9%-NaCl)-ascorbic acid (0.01% w/v) solution. Stock solutions were kept at -20°C , and appropriate dilutions were made on the day of the experiment.

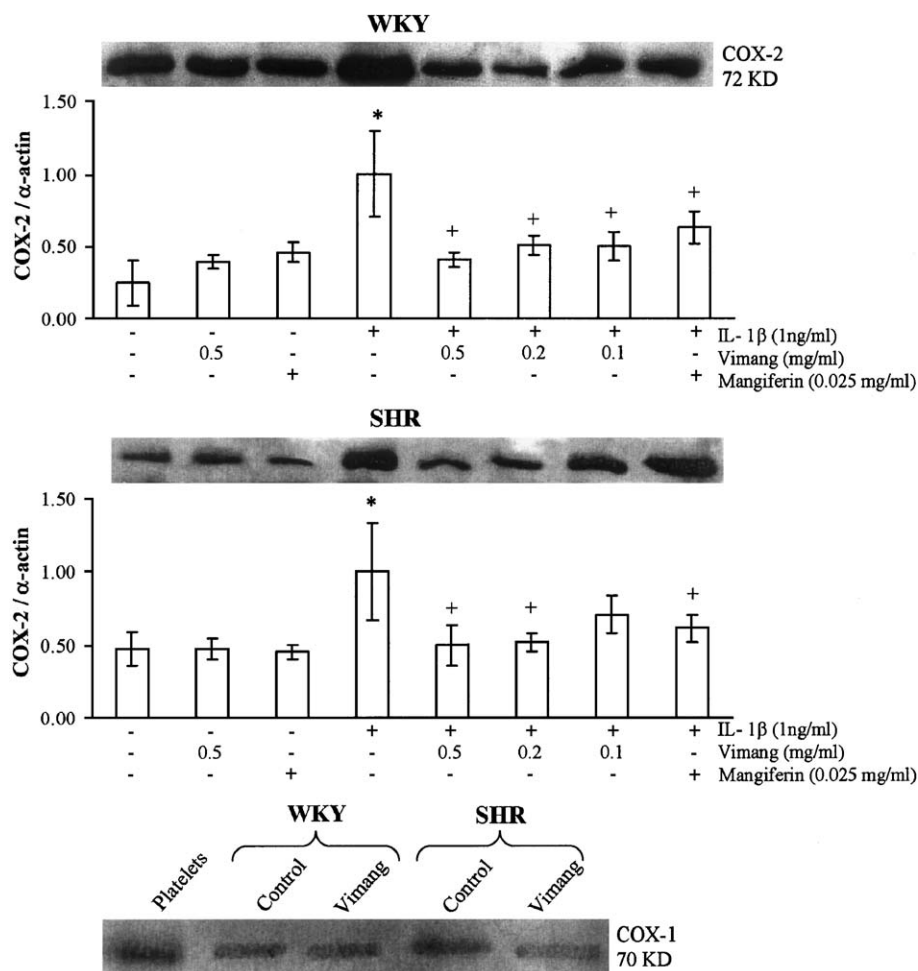


Fig. 2. (A) Effect of Vimang or mangiferin on cyclooxygenase-2 protein expression in vascular smooth muscle cells from normotensive (WKY) and hypertensive (SHR) rats in the absence (control) or the presence of interleukin (IL)-1 β ($n=4$). Results (means \pm S.E.M.) are expressed as the ratio between the signal for the cyclooxygenase-2 (COX-2) protein and the signal for α -actin. * $P < 0.05$ vs. control, $^+P < 0.05$ vs. IL-1 β . Representative blots are also shown. (B) Representative blot showing the effect of Vimang (5 mg/ml) on cyclooxygenase-1 (COX-1) protein expression in vascular smooth muscle cells from normotensive (WKY) and hypertensive (SHR) rats ($n=3$).

3. Results

3.1. Effect of Vimang and mangiferin on iNOS and cyclooxygenase-2 expression

iNOS expression was not detected in unstimulated vascular smooth muscle cells from both strains in the absence or the presence of 0.5 mg/ml Vimang or 0.025 mg/ml mangiferin (Fig. 1). The incubation of cells with interleukin-1 β (1 ng/ml) for 24 h caused a similar increase in iNOS protein levels in vascular smooth muscle cells from both strains. This increase was inhibited by Vimang (0.5, 0.2 and 0.1 mg/ml) in a concentration-dependent way and by mangiferin (0.025 mg/ml). The inhibitory effect of Vimang on iNOS protein expression was greater in vascular

smooth muscle cells from SHR than from WKY (Fig. 1). Thus, 0.5 and 0.2 mg/ml abolished iNOS expression in vascular smooth muscle cells from SHR while in those from WKY, iNOS expression was only abolished at 0.5 mg/ml.

Basal cyclooxygenase-2 expression was detected in vascular smooth muscle cells from both WKY and SHR; addition of 0.5 mg/ml Vimang or 0.025 mg/ml mangiferin alone to the medium did not modify cyclooxygenase-2 expression (Fig. 2). The incubation of cells with interleukin-1 β (1 ng/ml) for 24 h increased cyclooxygenase-2 protein levels in vascular smooth muscle cells from both strains. In vascular smooth muscle cells from WKY, this increase was inhibited by Vimang (0.5, 0.2 and 0.1 mg/ml) and by mangiferin (0.025 mg/ml). In vascular smooth muscle cells from hypertensive rats, the increase in cyclooxygenase-2

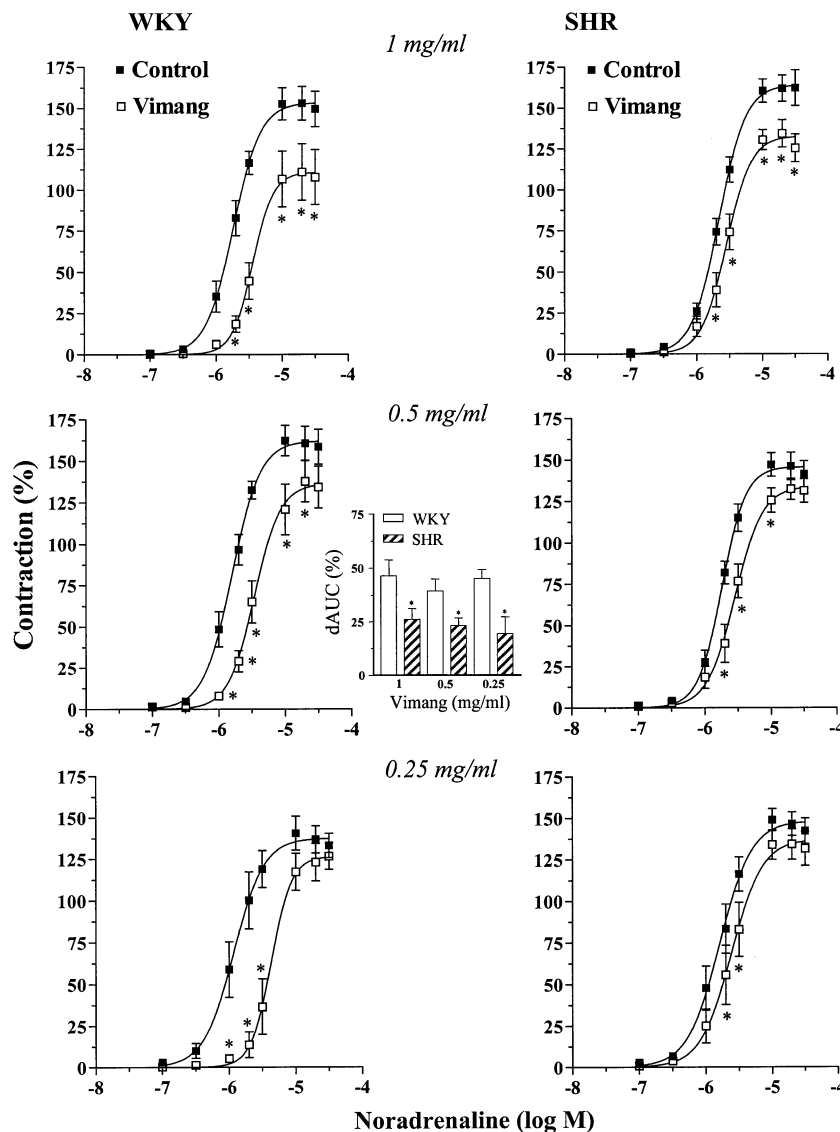


Fig. 3. Effect of Vimang on the concentration–response curve for noradrenaline in segments of mesenteric arteries from normotensive (WKY, $n=6-13$) and hypertensive (SHR, $n=9-11$) rats. Results (means \pm S.E.M.) are expressed as a percentage of the maximum response. * $P<0.05$ vs. control. Insert shows differences of area under the concentration–response curve (dAUC) for noradrenaline in the absence (control) and the presence of Vimang in segments of mesenteric arteries from WKY and SHR rats. Results (means \pm S.E.M.) are expressed as a percentage of AUC in the control situation. * $P<0.05$ vs. WKY.

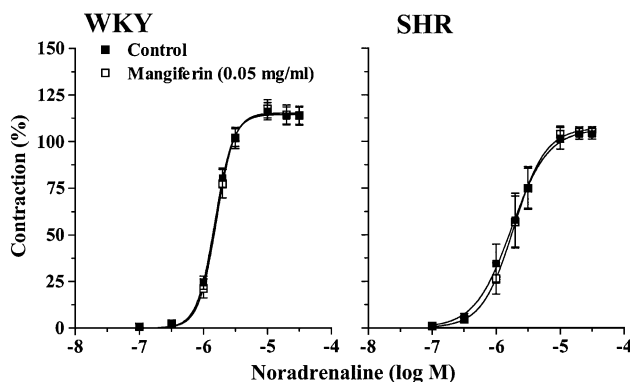


Fig. 4. Effect of mangiferin on the concentration–response curve for noradrenaline in segments of mesenteric arteries from normotensive (WKY, $n=4$) and hypertensive (SHR, $n=4$) rats. Results (means \pm S.E.M.) are expressed as a percentage of the maximum response.

expression was inhibited by mangiferin (0.025 mg/ml) and by 0.5 and 0.2 mg/ml, but not by 0.1 mg/ml, of Vimang.

Vimang (0.5 mg/ml) did not alter clooxygenase-1 protein expression in vascular smooth muscle cells from both strains (Fig. 2).

3.2. Effect of Vimang and mangiferin on the contractile responses

Noradrenaline (0.1–30 μ M) induced concentration-dependent vasoconstrictor responses that were similar in segments of mesenteric resistance arteries from both strains

(E_{\max} : $147.8\pm11.5\%$ and $144.3\pm6.6\%$ of maximum response; pD_2 : 5.79 ± 0.06 and 5.78 ± 0.05 for WKY and SHR, respectively, $n=10-14$, $P>0.05$). The incubation with Vimang (1, 0.5 and 0.25 mg/ml) induced a reduction of the contraction elicited by noradrenaline, both in WKY and SHR (Fig. 3). This inhibitory effect, that was similar for the different concentration of Vimang used, was greater in arteries from WKY than in those from SHR, as can be deduced from the comparison of dAUC (Fig. 3). In contrast to that found with Vimang, mangiferin (0.05 mg/ml) did not change noradrenaline contraction in arteries from both strains (Fig. 4). The effect of Vimang on noradrenaline-induced contraction was not a toxic effect because the NA responses were reproducible after washing Vimang (results not shown). On the other hand, in segments precontracted with noradrenaline, Vimang (0.25–1 mg/ml) did not induce any effect in either WKY or SHR (results not shown).

U46619 (1 nM–30 μ M) induced contractile responses that were greater in segments from SHR than from WKY (E_{\max} : $66.46\pm9.73\%$ and $94.93\pm7.86\%$ of maximum response, $P<0.05$; pD_2 : -6.83 ± 0.18 and -6.58 ± 0.19 , $P>0.05$, for WKY and SHR, respectively, $n=12-13$). The incubation with Vimang (1 mg/ml) induced a reduction of the contraction elicited by U46619 in both WKY and SHR (Fig. 5). The inhibitory effect induced by Vimang was similar in segments from both strains (Fig. 5). The contraction induced by KCl (15–70 mM) was similar in both strains and remained unmodified after Vimang incubation (1 mg/ml) (Fig. 5).

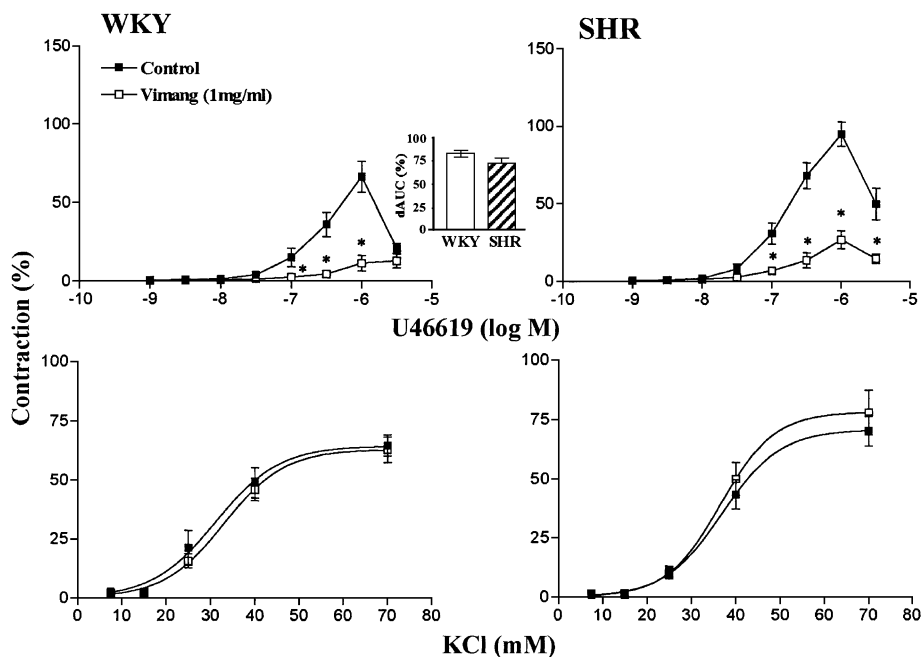


Fig. 5. Effect of Vimang on the concentration–response curves for U46619 and KCl in segments of mesenteric arteries from normotensive (WKY, $n=12$) and hypertensive (SHR, $n=13$) rats. Results (means \pm S.E.M.) are expressed as a percentage of the maximum response. * $P<0.05$ vs. control. Insert shows differences of area under the concentration–response curve (dAUC) to U46619 in the absence (control) and the presence of Vimang in segments of mesenteric arteries from WKY and SHR rats. Results (means \pm S.E.M.) are expressed as a percentage of AUC in the control situation.

4. Discussion

Vimang is an aqueous extract of the stem bark of *M. indica* L. with antioxidant and antiinflammatory properties (Martínez et al., 2000); however, the underlying mechanisms involved in the Vimang effects are not clear. Recently, phytochemical analysis of this extract had led to the isolation of different polyphenols which include phenolic acids, phenolic esters, flavan-3-ols and a xanthone (mangiferin) (Núñez-Selles et al., 2002). Several polyphenols are biochemically active compounds with known antiinflammatory, anticarcinogenic and free radical-scavenging properties (Lin and Lin, 1997). In a previous investigation, it was shown that Vimang exhibits a potent action in the second phase of the formalin-induced pain test and that this effect was more potent than that of indomethacin, a known inhibitor of cyclooxygenase activity (Garrido et al., 2001). In the present study, we demonstrated that Vimang inhibits interleukin-1 β -induced iNOS and cyclooxygenase-2 expression in vascular smooth muscle cells, an effect that would be related with its potent antiinflammatory action.

According to the phytochemical analysis, it is quite possible that some active principles present in Vimang, known for their antioxidant and antiinflammatory activities (Arts et al., 2000; Lin et al., 2000), could be responsible for the inhibitory effect on cyclooxygenase-2 or iNOS expression observed in this study. Mangiferin, the main polyphenol present in the extract (approximately 7%), also inhibited cyclooxygenase-2 and iNOS expression in vascular smooth muscle cells from WKY rats, suggesting this compound would be implicated in the inhibitory effect exerted on the expression of these proteins by Vimang. In agreement with this, mangiferin decreased NO production or iNOS mRNA levels in activated macrophages (Garcia et al., 2002; Leiro et al., 2003). In addition, there is evidence supporting the antiinflammatory and antioxidant activity of mangiferin (Muruganandan et al., 2002; Garcia et al., 2002; Leiro et al., 2003). On the other hand, other reports have also given evidence that several flavonoids such as the (–)-epigallocatechin-3-gallate are able to suppress iNOS and cyclooxygenase-2 expression in LPS-activated macrophages by down-regulation of the nuclear factor- κ B (NF- κ B) (Lin and Lin, 1997; Liang et al., 1999). As previously mentioned, gallate and methyl-gallate are some of the derivative polyphenols present in Vimang. In agreement with the above findings, a study from our laboratory using Hela cell line has demonstrated that Vimang inhibits NF- κ B activation (Garrido, personal communication). It is well known that NF- κ B factor plays an important role in the induction of the promoter of both cyclooxygenase-2 and iNOS genes (Barnes and Karin, 1997). The inhibition of NF- κ B activation could thus be involved in the molecular mechanisms of Vimang.

Overexpression of either cyclooxygenase-2 or iNOS has been implicated in the pathogenesis of many diseases (Kroncke et al., 1998; Dubois et al., 1998). Hypertension is considered a chronic inflammatory disease (Virdis and

Schiffrin, 2003) with elevated proinflammatory cytokines blood levels. Abnormal regulation of vascular protein expression of iNOS and cyclooxygenase-2 has been described in hypertension (Henrion et al., 1997; Chou et al., 1998; García-Cohen et al., 2000; Briones et al., 2002a,b). In a previous study, there was found a higher increase in iNOS protein expression induced by interleukin-1 β in vascular smooth muscle cells from hypertensive rats than in such cells from normotensive rats (Pacheco et al., 2000). In contrast, in the present study, interleukin-1 β induced a similar iNOS and cyclooxygenase-2 expression in vascular smooth muscle cells from both strains. Differences in the vascular bed (aorta or mesenteric) used to obtain vascular smooth muscle cells in the two studies would explain this discrepancy. Similar to what observed in vascular smooth muscle cells from normotensive rats, Vimang inhibited both iNOS and cyclooxygenase-2 expression in SHR; however, the effect on iNOS expression was greater whereas that on cyclooxygenase-2 expression was lower in vascular smooth muscle cells from SHR compared to WKY. In addition, mangiferin also inhibited both cyclooxygenase-2 and iNOS expression. It is possible that the alterations in the regulation of both enzymes subsequent to hypertension would explain these differences, although further studies are necessary in order to elucidate the differences in the Vimang effects.

Although in healthy vessels NO and prostanoids are mainly generated by the constitutive isoforms of NOS and cyclooxygenase, it has also been found that vascular smooth muscle expresses cyclooxygenase-2 and the prostanoids produced could be involved in the modulation of vascular responses (Swierkosz et al., 1995; Cirino et al., 1996; Briones et al., 2002b). In addition to the inhibitory effect of Vimang on iNOS and cyclooxygenase-2 expression, this compound also reduced the vasoconstrictor responses induced by noradrenaline and by the thromboxane A₂ analogue, U46619, but not by KCl in rings of mesenteric resistance arteries from WKY and SHR. These results suggest that the effect of Vimang on vasoconstrictor responses is specific for receptor signal transduction mechanisms. We have previously reported that the noradrenaline response in this type of artery is mediated by contractile prostanoids derived from cyclooxygenase-2 (Briones et al., 2002b). The inhibitory effect of Vimang on vasoconstrictor responses could thus be related with its ability to reduce cyclooxygenase-2 expression; however, the fact that mangiferin inhibited cyclooxygenase-2 expression but not the response to noradrenaline and that Vimang did not affect the basal expression of this isoform in vascular smooth muscle cells eliminates this possibility. In addition, these results suggest that the effects of Vimang on vasoconstrictor responses and on cyclooxygenase-2 and iNOS expression are probably mediated by different compounds. It has been described that flavonoids can inhibit both 5-lipoxygenase/cyclooxygenase pathways of eicosanoid synthesis (Laughton et al., 1991) and a recent report has shown

that Vimang inhibits the production of leukotriene B₄ and prostaglandin E₂ in calcium ionophore and lipopolysaccharide-stimulated macrophages, respectively (Delgado et al., 2001). On the other hand, the strong inhibitory effect of Vimang on the contraction induced by U46619 and the fact that contractile prostanoids participate in the noradrenaline contraction in these arteries (Briones et al., 2002b) suggest that some of the compounds present in the extract would have properties of non-competitive antagonists of TP receptors. All these findings could explain the inhibitory effect of Vimang on vasoconstrictor responses to noradrenaline. The effect of Vimang on the response to noradrenaline was less in mesenteric resistance segments from hypertensive rats than in segments from normotensive rats. A lower participation of contractile prostanoids in the noradrenaline response described in mesenteric resistance arteries from SHR (Briones et al., 2002b) could explain the differences in the Vimang effect.

In conclusion, Vimang and mangiferin inhibited iNOS and cyclooxygenase-2 expression in vascular smooth muscle cells. These results could help to explain the antiinflammatory properties of Vimang and suggest that mangiferin is an active antiinflammatory component of Vimang. However, Vimang also reduced vasoconstrictor responses, an effect that does not support its antiinflammatory properties. The fact that mangiferin did not show a vasodepressor effect suggests that the inhibitory effect of Vimang on vasoconstrictor responses and on cyclooxygenase-2 and iNOS expression would be mediated by different compounds. Different polyphenols, such as gallic acid, catechins and gallate derivatives, present in the extracts might participate in the Vimang effect. However, the possible relationship between properties of polyphenols and their antiinflammatory activities deserves further investigation.

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References

- Arts, I.C., van de Putte, B., Hollman, P.C., 2000. Catechin contents of foods commonly consumed in The Netherlands: Part I. Fruits, vegetables, staple foods, and processed foods. *J. Agric. Food Chem.* 48, 1746–1751.
- Barnes, P.J., Karin, M., 1997. Nuclear factor- κ B: a pivotal transcription factor in chronic inflammatory diseases. *N. Engl. J. Med.* 336, 1066–1071.
- Born, M., Carrupt, P.A., Zini, R., Brée, F., Tillement, J.P., Hostettmann, K., Testa, B., 1996. Electrochemical behavior and antioxidant activity of some natural polyphenols. *Helv. Chim. Acta* 79, 1147–1158.
- Briones, A., Alonso, M.J., Marín, J., Balfagón, G., Salaices, M., 2000. Influence of hypertension on nitric oxide synthase expression and vascular effects of lipopolysaccharide in rat mesenteric arteries. *Br. J. Pharmacol.* 131, 185–194.
- Briones, A.M., Alonso, M.J., Hernanz, R., Miguel, M., Salaices, M., 2002a. Alterations of the nitric oxide pathway in cerebral arteries from spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol.* 39, 378–388.
- Briones, A.M., Alonso, M.J., Hernanz, R., Tovar, S., Vila, E., Salaices, M., 2002b. Hypertension alters the participation of contractile prostanoids and superoxide anions in lipopolysaccharide effects on small mesenteric arteries. *Life Sci.* 71, 1997–2014.
- Chou, T.C., Yen, M.H., Li, C.Y., Ding, Y.A., 1998. Alterations of nitric oxide synthase expression with aging and hypertension in rats. *Hypertension* 31, 643–648.
- Cirino, G., Sorrentino, R., Cicala, C., Sorrentino, L., Pinto, A., 1996. Indomethacin and thromboxane A₂/prostaglandin H₂ antagonist SQ 29,548 impair in vitro contractions of aortic rings of ex vivo-treated lipopolysaccharide rats. *J. Lipid Mediators Cell Signal.* 13, 177–187.
- Delgado, R., Garrido, G., González, D., Herrera, A., Beltrán, A., Lemus, Y., Rodríguez, J., Quintero, G., Lodeiro, L., Tamayo, D., Sironi, M., Ledon, N., Romay, C., García, D., Nuñez, A.J., 2001. *Mangifera indica* L. extract (Vimang) as a natural antioxidant with antinociceptive and anti-inflammatory properties. *Minerva Med.* 92, 98–102.
- Dubois, R.N., Abramson, S.B., Crofford, L., Gupta, R.A., Simon, L.S., van de Putte, L.B., Lipsky, P.E., 1998. Cyclooxygenase in biology and disease. *FASEB J.* 12, 1063–1073.
- García, D., Delgado, R., Ubeira, F.M., Leiro, J., 2002. Modulation of rat macrophage function by the *Mangifera indica* L. extracts Vimang and mangiferin. *Int. Immunopharmacol.* 2, 797–806.
- García-Cohen, E.C., Marín, J., Díez-Picazo, L.D., Baena, A.B., Salaices, M., Rodríguez-Martínez, M.A., 2000. Oxidative stress induced by *tert*-butyl hydroperoxide causes vasoconstriction in the aorta from hypertensive and aged rats: role of cyclooxygenase-2 isoform. *J. Pharmacol. Exp. Ther.* 293, 75–81.
- Garrido, G., Gonzalez, D., Delporte, C., Backhouse, N., Quintero, G., Nuñez-Selles, A.J., Morales, M.A., 2001. Analgesic and anti-inflammatory effects of *Mangifera indica* L. extract (Vimang). *Phytother. Res.* 15, 18–21.
- Guevara, G.M., Tamayo, B.D., González, L.S., Paez, B.E., Oceguera, J., Nuñez-Sellés, A.J., 1998. Ethnopharmacologic study of *Mangifera indica* L. extract (QF-808). Abstract of 1st International Congress Pharmacology '98, Ed. ORTOP, La Habana, 19–23 October 1998, p. 45.
- Guha, S., Ghosal, S., Chattopadhyay, U., 1996. Antitumor, immunomodulatory and anti-HIV effect of mangiferin, a naturally occurring glucosylxanthone. *Chemotherapy* 42, 443–451.
- Henrion, D., Dechaux, E., Dowell, F.J., MacIour, J., Samuel, J.L., Levy, B.I., Michel, J.B., 1997. Alteration of flow-induced dilatation in mesenteric resistance arteries of L-NAME treated rats and its partial association with induction of cyclooxygenase-2. *Br. J. Pharmacol.* 121, 83–90.
- Kroncke, K.D., Fehsel, K., Kolb-Bachofen, V., 1998. Inducible nitric oxide synthase in human diseases. *Clin. Exp. Immunol.* 113, 147–156.
- Laughton, M.J., Evans, P.J., Moroney, M.A., Hoult, J.R., Halliwell, B., 1991. Inhibition of mammalian 5-lipoxygenase and cyclooxygenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion-reducing ability. *Biochem. Pharmacol.* 42, 1673–1681.
- Leiro, J.M., Alvarez, E., Arranz, A., González-Siso, I., Orallo, F., 2003. In vitro effects of mangiferin on superoxide concentrations and expression of the inducible nitric oxide synthase, tumor necrosis factor- α and transforming growth factor- β genes. *Biochem. Pharmacol.* 65, 1361–1371.
- Liang, Y.C., Huang, Y.T., Tsai, S.H., Lin-Shiau, S.Y., Chen, C.F., Lin, J.K., 1999. Suppression of inducible cyclooxygenase and nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. *Carcinogenesis* 20, 1945–1952.

- Lin, Y.L., Lin, J.K., 1997. (–)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulation lipopolysaccharide-induced activity of transcription factor- κ B. *Mol. Pharmacol.* 52, 465–472.
- Lin, J.K., Chen, P.C., Ho, C.T., Lin-Shiau, S.Y., 2000. Inhibition of xanthine oxidase and suppression of intracellular reactive oxygen species in HL-60 cells by theaflavin-3-3' digallate, (–) epigallocatechin-3-gallate, and propylgallate. *J. Agric. Food Chem.* 48, 2736–2743.
- Martínez, G., Delgado, R., Pérez, G., Garrido, G., Núñez, A.J., León, O.S., 2000. Evaluation of the in vitro antioxidant activity of *Mangifera indica* L. extract (Vimang). *Phytother. Res.* 14, 424–427.
- Muanza, D.N., Euler, K.L., Williams, L., Newman, D.J., 1995. Screening for antitumor and anti-HIV activities of nine medicinal plants from Zaire. *Int. J. Pharmacogn.* 33, 98–106.
- Mulvany, M.J., Halpern, W., 1977. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.* 41, 19–26.
- Muruganandan, S., Gupta, S., Kataria, M., Lal, J., Gupta, P.K., 2002. Mangiferin protects the streptozotocin-induced oxidative damage to cardiac and renal tissues in rats. *Toxicology* 176, 165–173.
- Núñez-Selles, A.J., Vélez, H.T., Agüero, J., González, J., Naddeo, F., De Simone, F., Rastrelli, L., 2002. Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyols from Mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. *J. Agric. Food Chem.* 50, 762–766.
- Pacheco, M.E., Marín, J., Manso, A.M., Rodríguez-Martínez, M.A., Briones, A., Salaices, M., Redondo, J., 2000. Nitric oxide synthase induction by ouabain in vascular smooth muscle cells from normotensive and hypertensive rats. *J. Hypertens.* 18, 877–884.
- Redondo, J., Peiró, C., Rodríguez-Mañas, L., Salaices, M., Marín, J., Sánchez-Ferrer, C.F., 1995. Endothelial stimulation of sodium pump in cultured vascular smooth muscle. *Hypertension* 26, 177–185.
- Swierkosz, T.A., Mitchell, J.A., Warner, T.D., Blotting, R.M., Vane, J.R., 1995. Co-induction of nitric oxide synthase and cyclo-oxygenase: interactions between nitric oxide and prostanoids. *Br. J. Pharmacol.* 114, 1335–1342.
- Virdis, A., Schiffrin, E.L., 2003. Vascular inflammation: a role in vascular disease in hypertension? *Curr. Opin. Nephrol. Hypertens.* 12, 181–187.
- Wu, C.C., Szabó, C., Chen, S.J., Thiemeermann, C., Vane, J.R., 1994. Activation of soluble guanylyl cyclase by a factor other than nitric oxide or carbon monoxide contributes to the vascular hyporeactivity to vasoconstrictor agents in the aorta of rats treated with endotoxin. *Biochem. Biophys. Res. Commun.* 201, 436–444.