Effects of Morelloflavone from *Garcinia dulcis* on Vasorelaxation of Isolated Rat Thoracic Aorta

Jarunet Lamai, Wilawan Mahabusarakam, Thanaporn Ratithammatorn, Siriphun Hiranyachattada

Abstract

Morelloflavone is a flavonoid isolated from *Garcinia dulcis*, Kurz., an Asian medicinal plant used to treat a sore throat, scurvy and cough. Previous *in vitro* experiments have shown that morelloflavone possesses anti-inflammatory, anti-oxidant and anti-bacterial properties while other pharmacological effects have never been researched. This work aimed to investigate the action and mechanism(s) of morelloflavone from *G. dulcis* on relaxation of isolated rat thoracic aorta precontracted with norepinephrine (1 g resting tension). Cumulative addition of 10^{-9}-10^{-5} M morelloflavone significantly relaxed the precontracted aortic rings in a dose-dependent manner with a maximal relaxation of 77.5±5.4% and an EC50 of approximately 10^{-7} M. In denuded rings, this effect was abolished. Pre-incubation of endothelium-intact aortic rings with 10^{-6} M L-NOARG, a nitric oxide synthase inhibitor, significantly abolished morelloflavone-induced vasorelaxation, while the cyclooxygenase inhibitor indomethacin (10^{-6} M) had no effect. Morevover, either 10^{-5} M glibenclamide (an ATP-sensitive K^+ channel inhibitor) or 10^{-3} M TEA (a non-selective Ca^{2+}-activated K^+ channel blocker) could partially inhibited morelloflavone-induced vasorelaxation. Therefore, the vasorelaxation mechanisms of morelloflavone was endothelium-dependent which involved nitric oxide signaling pathway and, partly, ATP-sensitive and Ca^{2+}-activated K^+ channels.

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One of the alternative means of hypertension treatment is using medicinal plants to relax resistant blood vessels. It has been widely reported that various plant flavonoids such as quercetin, dioclein and galangin possess vasorelaxation activity.[1-3] Morelloflavone is a bioactive bioflavonoid from *Garcinia dulcis* Kurz, a plant that belongs to the Guttiferae family which is widely distributed in Thailand and other Southeast Asian countries. This plant is also known as Maphuut (Thailand) and Mundu (Indonesia and Malaysia).[4]

G. dulcis has been known as an Asian medicinal plant used in folk medicines. Its stem bark has been used in as an antiseptic. The fruit juice has been used as an antiscurvy and expectorant for the relief of cough and sore throat, as a mild laxative and as a decongestant. Its root extract is also used as an antipyretic and antitoxin agent. In Indonesia, the leaves and seeds have been used for the treatment of lymphatitis, parotitis and struma.[5] All parts of this plant have been reported to contain an abundance of morelloflavone.

Previous *in vitro* experiments have shown that morelloflavone inhibits human secretory phospholipase A_2. This study was performed by measuring the radioactivity of [^{3}H] oleate-labeled membranes of *Escherichia coli* after incubation with phospholipase A_2 in the presence and absence of morelloflavone. In addition, morelloflavone also inhibits 12-O-tetradecanoylphorbol-13-acetate-induced ear inflammation and carrageenan-induced paw edema in mice.[6] Morelloflavone was reported to exhibit strong antioxidation effects in both Fe^{2+}-mediated and non-metal-induced human low-density lipoprotein (LDL) oxidations.[7] However, the effect of morelloflavone on vascular smooth muscle relaxation has never been investigated.

Vascular relaxing factors are nitric oxide (NO), prostacyclin (PGI_2), and endothelium-derived hyperpolarizing factors (EDHF).[8] NO is biosynthesized endogenously from L-arginine, oxygen and NADPH by nitric oxide synthase (NOS) enzymes and diffuses to vascular smooth muscle cells to activate soluble guanylate cyclase (sGC), which leads to an increased production of cyclic guanosine 3',5'-monophosphate (cGMP) and therefore, relaxes the underlying vessels.[9] PGI_2 is an endothelium-derived relaxing factor, generated through the sequential activities of cyclooxygenases (COX) and activates adenylate cyclase, leading to an increased production of cyclic AMP (cAMP), which causes the relaxation of vascular smooth muscle. EDHF is generated via

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endothelium-dependent agonists activating the endothelial cell receptors, leading to the influx of extracellular and the release of intracellular Ca^{2+} and synthesis of EDHF. Along with the synthesis of EDHF, hyperpolarization of endothelial cells also occurs because Ca^{2+} activates K^+ channels (K_{Ca} channel) and induces K^+ efflux. EDHF, then, diffuses to the vascular smooth muscle cells, activates K_{Ca} channels, and causes endothelium-dependent hyperpolarization and relaxation.

This study aimed to investigate the action and mechanism(s) of morelloflavone from *G. dulcis* on relaxation of isolated rat thoracic aortic rings precontracted with norepinephrine and their mechanisms of action using specific endothelial-derived vasorelaxation inhibitors (N^G-nitro-L-arginine; a nitric oxide synthase inhibitor and indomethacin; a cyclooxygenase inhibitor) and K^+ channels blockers (glibenclamide; an ATP-sensitive K^+ channel inhibitor and tetraethylammonium chloride; a non-selective calcium activated K^+ channel blocker).

Materials and methods

Extraction of compounds from *G. dulcis* fruits

Fruits of *G. dulcis* were collected from Amphur Meuang, Songkhla province, Thailand. The voucher specimen (Collection No 02, Herbarium No 0012652) has been deposited at the Herbarium of Faculty of Science, Prince of Songkla University, Thailand. The fruits of *G. dulcis* were extracted with acetone and the extract was subjected to solvent partitioning, chromatography and crystallization to give morelloflavone as previously described.[5]

Animals

Male Wistar rats weighing 200-300 g obtained from Southern Laboratory Animal Facility (Prince of Songkla University, Songkhla, Thailand). All animals were housed in cages under controlled conditions (temperature 23-25 °C, relative humidity 50-55% and 12 h light/dark cycle). They were given a commercial animal feed (S.W.T., Thailand) and free access to tap water. All experimental rats were maintained and handled according to the approval of the Prince of Songkla University Animal Ethics Committee (Project license number MOE 0521.11/051).

Reagents

Acetylcholine (ACh) chloride, norepinephrine (NE), N^G-nitro-L-arginine (L-NOARG), indomethacin, glibenclamide, tetraethylammonium (TEA) and dimethylsulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). ACh, NE, L-NOARG, glibenclamide and TEA were dissolved in distilled water, whereas indomethacin were dissolved in 0.1% sodium bicarbonate and morelloflavone were dissolved in DMSO before experimentation.

Preparation of isolated thoracic aortic rings

All experimental rats were sacrificed by decapitation. Abdominal aorta was dissected, and cut into four ring segments approximately 2-3 mm in length. Aortic rings were mounted in 20 ml organ baths containing 37 °C Krebs Henseleit solution which was composed of (mM) 118.41 NaCl, 4.6 KCl, 1.12 $MgSO_4.7H_2O$, 1.18 KH_2PO_4, 1.9 $CaCl_2$, 25.0 $NaHCO_3$ and 11.66 D-glucose. The pH of solution was maintained at 7.4 by continuous aeration in the bath with 95% O_2 and 5% CO_2. All aortic rings were then set with a resting tension of 1 g using force displacement transducer connected to a MacLab (Model 4/20, ADInstruments, Australia). Precontraction of these rings was induced by addition of 10^{-7} M NE. Endothelium function was tested using 10^{-5} M ACh and was accepted at 80% relaxation.

Concentration-response curve of morelloflavone

Precontraction of aortic rings were performed by addition of 10^{-7} M NE into the bath. When maximal contraction developed, either each dose of morelloflavone or vehicle (0.1% DMSO) was added. Morelloflavone was added cumulatively, allowing the final concentration at 10^{-10}-10^{-5} M, respectively. Subsequent concentrations were added after the maximal response by the previous concentration.

Effect of morelloflavone on relaxation of denuded thoracic aortic rings

To investigate the role of intact endothelium on relaxation of aortic rings, mechanical removal of aortic ring endothelium was demonstrated by the disappearance of relaxation induced by 10^{-5} M ACh. Precontraction of aortic rings were then performed by addition of 10^{-7} M NE into the bath, after which the procedure for studying the concentration-response curve above was repeated.

Effect of inhibitors on relaxation response of morelloflavone in thoracic aortic rings

To investigate the mechanism(s) of morelloflavone induced vasorelaxation, the effects of pretreatment with specific inhibitors before NE precontraction and cumulative addition of morelloflavone: were observed. The incubation periods and inhibitors used were 30 min 10^{-6} M L-NOARG, 20 min of 10^{-6} M indomethacin, 20 min of 10^{-5} M glibenclamide and 20 min of 10^{-3} M TEA.

Statistical analysis

The tension of thoracic aortic ring relaxant responses are expressed as a percentage relaxation from NE (10^{-7} M) precontraction levels. All data were expressed as mean ± SEM. The number of animals used in each treatment was indicated as n. Significant difference between the group means was determined using one-way analysis of variance (ANOVA), followed by Student-Newman Keul post hoc test. Statistical significance of the mean differences was accepted when P value < 0.05.

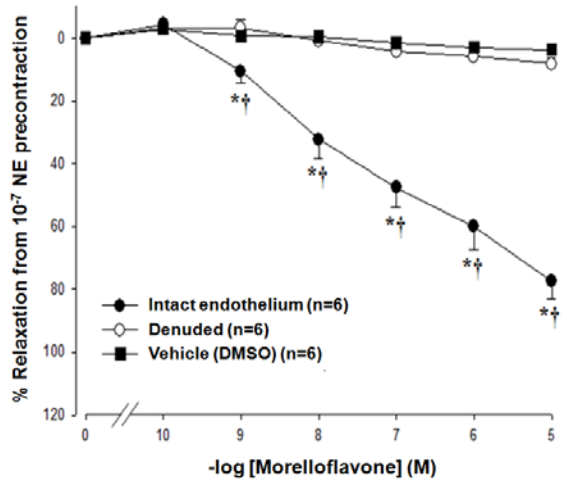

Figure 1 Effects of morelloflavone on thoracic aortic ring relaxation in the presence (●, n=6) and absence (○, n=6) of the endothelium. (■, n=6) vehicle control. Each value shows mean ± SEM in percentage relaxation from NE precontraction. $^{*\,\dagger}P < 0.05$ *vs* vehicle control and denuded, respectively.

Results

Effects of morelloflavone on thoracic aortic ring relaxation

As shown in Figure 1, it is found that the morelloflavone relaxed NE precontracted aortic rings with the endothelium-intact in a concentration-dependent manner (between 10-78% relaxation). The maximal relaxant effect of morelloflavone was 77.5±5.4% at the concentration of 10^{-5} M. The half maximal effective concentration (EC50) was approximately 10^{-7} M,. Denudation of the functional endothelium abolished morelloflavone-induced vasorelaxation. In vehicle control experiment using 0.1% DMSO, no significant effect on vascular tone was found. This finding indicated that morelloflavone induces vasorelaxation via endothelium-dependent signaling.

Effects of L-NOARG and indomethacin on morelloflavone-induced vasorelaxation

To evaluate the involvement of endothelium-derived relaxing factors in the morelloflavone-induced vasorelaxation, the effects of L-NOARG (10^{-6} M), and indomethacin (10^{-6} M) were examined. As shown in Figure 2, pretreatment of the endothelium-intact aortic rings with L-NOARG significantly abolished morelloflavone-induced vasorelaxation. In contrast, indomethacin had no effect on the morelloflavone-induced vasorelaxation. In the presence of this cyclooxygenase inhibitor, the EC50 of morelloflavone was between 10^{-7}-10^{-6} M suggesting the unlikely prostanoids involvement in this vasorelaxation mechanism. These findings suggested that morelloflavone induces vasorelaxation occuring via nitric oxide signaling pathway rather than cyclooxygenase-induction pathway.

Effects of inhibitors of K^+ channels on morelloflavone-induced vasorelaxation

To evaluate the role of K^+ channels in morelloflavone-induced vasorelaxation, pretreatment of thoracic aortic rings with glibenclamide (10^{-5} M), or TEA (10^{-3} M), were examined. It is found that either of these two blockers partially inhibited morelloflavone-induced vasorelaxation as shown in Figure 3. In the presence of either glibenclamide or TEA, the vasorelaxation effect caused by 10^{-7} M morelloflavone were found to be 20 and 30%, respectively. These findings suggested that the activation of K^+ channels may be partially involved in morelloflavone-induced vasorelaxation.

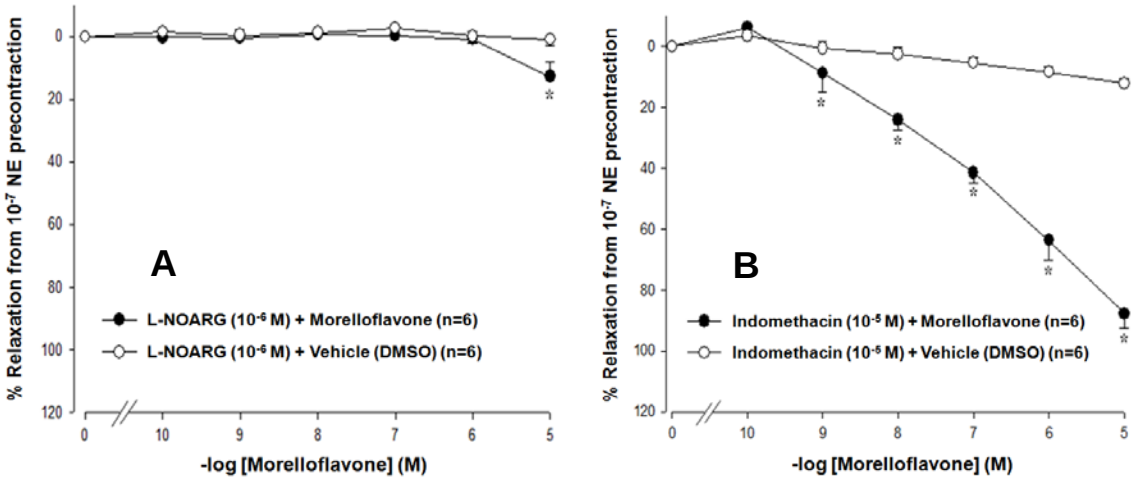

Figure 2 Effects of N^G-nitro-L-arginine (L-NOARG, 10^{-6} M) and indomethacin (10^{-6} M) on morelloflavone-induced vasorelaxation in thoracic aortic rings. (**A**) Effects of L-NOARG on morelloflavone-induced vasorelaxation. (●, n=6), L -NOARG + morelloflavone;○(n=6) L -NOARG + vehicle. (**B**) Effects of indomethacin on morelloflavone-induced vasorelaxation. (●, n=6) indomethacin + morelloflavone; (○, n=6) indomethacin + vehicle. Each value shows mean ± SEM in percentage relaxation of NE pre-contraction. $^{*}P < 0.05$ *vs* vehicle respectively.

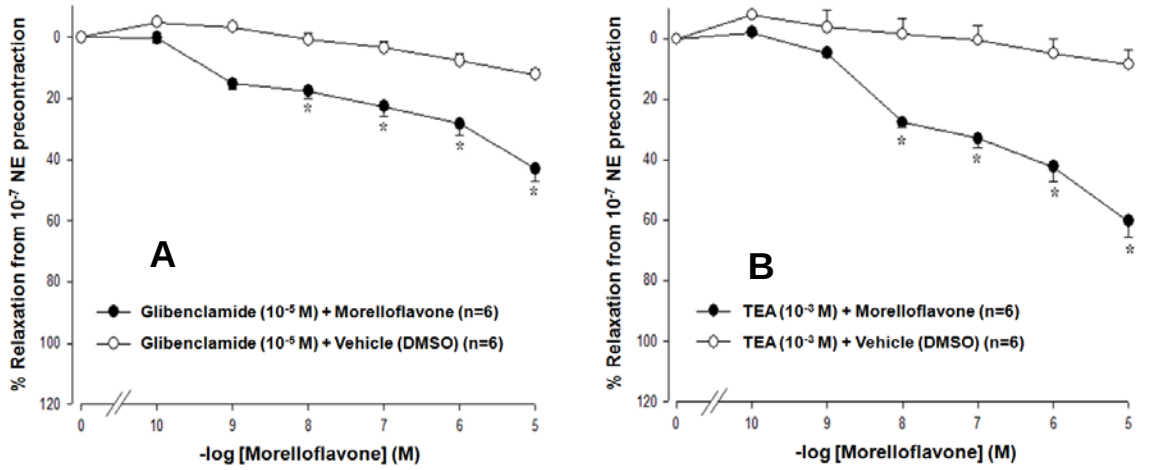

Figure 3 Effects of glibenclamide (10^{-5} M) and tetraethylammonium (TEA, 10^{-3} M) on morelloflavone-induced vasorelaxation in thoracic aortic rings. (**A**) Effects of glibenclamide on morelloflavone-induced vasorelaxation. (●, n=6) glibenclamide + morelloflavone; (○, n=6) glibenclamide + vehicle (DMSO). (**B**) Effects of TEA on morelloflavone-induced vasorelaxation. (●, n=6) TEA + morelloflavone; (○, n=6) TEA + DMSO. Each value shows mean ± SEM in percentage relaxation of NE pre-contraction. $^{*}P < 0.05$ *vs* vehicle control respectively.

Discussion

It has been reported widely that, flavonoids isolated from herbal plants have been recognized as compounds with potent biological activities that may be active in prevention of cardiovascular disease. Various flavonoids have been found to exhibit vasodilator effects in isolated vascular preparation and in animal models. The present study was the first to show the potent vasorelaxant effect of morelloflavone. The effective concentrations of morelloflavone in relaxing isolated rat thoracic aortic ring with functional endothelium were found between 10^{-9}-10^{-5}M. This effect is comparable to other plant flavonoids such as chrysin, diadzein, epigallocatechin gallate, fisetin, flavone, naringenin, quercetin, dioclein and galangin reported in the dose-dependently relax the precontracted rat thoracic aorta.[1-3] Since we have found the vasorelaxant effect was blunted in denuded aortic rings and in the pre-incubated rings with L-NOARG, it is proposed that the mechanisms of morelloflavone action on vascular smooth muscle happen via endothelium-dependent and mainly involve nitric oxide signaling pathway. However, at the pharmacological or maximal concentration (10^{-5} M) used in this study, morelloflavone could not completely abolish the precontracted tension induced by 10^{-7} M NE suggesting possible involvement of other vasorelaxing pathways. In addition, our experiments showed that in aortic rings treatment with indomethacin had no effect on the morelloflavone-induced vasorelaxation. This finding demonstrated that prostanoids may not be involved in vasodilatation induced by morelloflavone.

Some vasodilators are known to cause membrane hyperpolarization by activating K^+ channels in the vascular smooth muscle. Previous study has shown that natural products of plant origin can induce vasodilation via K^+ channels activation (10). To evaluate the participation of K^+ channels activation in the vasorelaxant activity by morelloflavone, aortic rings were treated with either of TEA, or glibenclamide. It is found that these two particular blockers partially attenuated the morelloflavone-induced vasorelaxation. This finding suggested that morelloflavone-induced vascular relaxation is closely related with the activation of K_{Ca} and K_{ATP} channel, which in turn, could hyperpolarize vascular smooth muscle cells and lead to vasorelaxation.

Conclusion

The present study demonstrated that morello-flavone vasorelaxant mechanism of action is endothelium-dependent and mainly involve nitric oxide signaling pathway. It is likely that the ATP-sensitive K^+ channel and calcium activated K^+ channel also participate in the vasorelaxant effect of morelloflavone. This may provide the alternative use of plant flavonoids in treatment of hypertension. However, further studies in other resistant vessels or *in vivo* experiment should be investigated.

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Conflict of Interest

None to declare.

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