Mechanisms of Vasorelaxation to Gamma-Mangostin in the Rat Aorta

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Objective: *To investigate the effects of gamma-mangostin on vascular tone and its mechanisms in the isolated rat aorta.*
Material and Method: *Aortic rings from male Wistar rats were precontracted with methoxamine. Changes in tension were measured using an isometric force transducer and recorded on the MacLab recording system. Vasorelaxant effects of gamma-mangostin were studied in the presence of 300 microM N^G-nitro L-arginine methyl ester (L-NAME), 10 microM 1H-[1,2,4] oxadiazolo-[4,3-a] quinoxalin-1-one (ODQ), 10 microM indomethacin, 60 mM KCl, 5 mM tetraethylammonium (TEA), 10 microM glibenclamide, 1 mM 4-aminopyridine (4-AP) or 30 microM barium chloride ($BaCl_2$). Moreover,the effects of gamma-mangostin on contraction to $CaCl_2$ were evaluated.*
Results: *Gamma-mangostin (1-100 microM) induced a concentration-dependent vasorelaxation in rat aortic rings precontracted with methoxamine. This effect was significantly reduced after removal of the endothelium and after pre-treatment of the rings with L-NAME, ODQ, high KCl solution, or TEA. However, vasorelaxant responses to gamma-mangostin were not altered by indomethacin, 4-AP, $BaCl_2$ or glibenclamide. Moreover, contractions to $CaCl_2$ (10 mM-30 mM) were reduced by pre-treatment with gamma-mangostin (10 and 100 microM).*
Conclusion: *Gamma-mangostin causes vasorelaxation which is mediated via the NO-cGMP pathway. Moreover, activation of K^+ channels and inhibition of extracellular Ca^{2+} influx from the extracellular space are largely involved in the relaxant effects of gamma-mangostin. These data suggest that gamma-mangostin may acts as an antihypertensive agent.*

Keywords: *Gamma-mangostin, Vasorelaxation, K^+ channels, Ca^{2+} influx, Rat aorta*

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Garcinia mangostana L. (GM), known as the queen of fruits, belongs to the family of Clusiaceae. It is wildly cultivated in the Southeast Asian, such as Thailand, Indonesia and Philippines. GM has been used in traditional medicine to treat abdominal pain, diarrhea and infected wound[1]. The fruit hull (pericarp) of GM is rich in prenylated xanthones; for example, alpha-, beta- and gamma-mangostins, mangostenol, mangostenone and garcinones[1-3]. Pharmacological studies have shown that xanthones, isolated from mangosteen, act as anti-oxidants[4] and antitumoral agents[5,6]. Moreover, alpha- and gamma-mangostins also have anti-inflammatory property[7].

Concerning the vascular effects of the GM extract, previous studies in the rabbit aorta have demonstrated that alpha-mangostin inhibit contractions induced by histamine H_1 receptor in the rabbit aorta[8]. In addition, gamma-mangostin acts as an antagonist of 5-hydroxytryptamine$_{2A}$ receptors in the rabbit aorta[9]. However, the vascular effects of gamma-mangostin are still unclear. The present study aimed to investigate the effects of gamma-mangostin on vascular tone. The role of the endothelium and endothelium-derived relaxing factor in vasorelaxation to gamma-mangostin were also studied. In addition, the effects of a high concentration of KCl and K^+ channel inhibitors on relaxant responses to gamma-mangostin were examined. Finally, the effects of gamma-mangostin on extracellular Ca^{2+} influx were also investigated in Ca^{2+} free and high KCl solution.

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Material and Method

Extraction of gamma-mangostin

Mangosteen fruit (*G. mangostana*) was collected from Kombang District, Chantaburi Province, Thailand in April, 2008. A voucher specimen (Porntip

Wongnapa No. 002) is deposited at the Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand and was identified by Nopporn Damrongsiri. The dried and pulverized fruit hull of *G. mangostana* (0.5 kg) was thoroughly extracted with EtOAc at 50°C. The combined extract after filtration was concentrated under reduced pressure to yield the extract as a yellowish solid (285 g). A portion the extract (40 g) was subjected to repeated column chromatography over silica gel using a gradient of hexane/acetone (5% increment of polar solvent for 500 ml of each proportion) yielded the pure gamma-mangostin (0.9 g), including other xanthones. Purity of gamma-mangostin exceeded 98% as determined by LC analysis[10]. NMR and MS spectroscopic data of gamma-mangostin were consistent with the reported values[5].

Tissue preparation

Experiments were performed using aorta obtained from male Wistar rats (300-350 g) bred and kept by the National Laboratory Animal Center, Mahidol University, Thailand. All experiments were reviewed and approved by the Animal Research Ethics Committee of the Faculty of Medicine, Srinakharinwirot University.

The rats were anaesthetized with zolitil 50 mg/kg (tiletamine chloridrate and zolazepan chloridrate) i.m. into quadriceps muscle[11] and killed by cervical dislocation. Following a thoracotomy, the thoracic aorta was dissected from the rat. The aorta was cleaned of fat and connective tissue and cut into 4-5 mm ring segments. Each ring was mounted between two stainless wires and then transferred to a jacketed organ bath filled with 20 ml of modified Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2, D-glucose 10, pH 7.4. The solution was maintained at 37°C and gassed with 95% O_2 and 5% CO_2 mixture. The solution in the organ bath was exchanged every 15 min for 1 h. Then, the rings were stretched to an optimal passive tension of about 1 g and then allowed to equilibrate for 60 min before experiments were started. Tension was measured by isometric force transducers (MLT 0210, New South Wales, Australia) and recorded on a MacLab recording system (AD instruments, New South Wales, Australia).

Experimental protocol

After equilibration, methoxamine (10-100 microM, an $alpha_1$-adrenoceptor agonist) was added to increase tension about 0.5-1 g above baseline. Gamma-mangostin (1-100 mM) was added cumulatively to the bathing solution. To characterize the mechanisms involved in gamma-mangostin vasorelaxation to aortic rings were incubated with each inhibitor added to the bath for 30 min before methoxamine was added to increase tone.

In order to investigate the involvement of the endothelium in vasorelaxation to gamma-mangostin, the endothelium was removed using a cocktail stick to rub the luminal surface. The preparation was considered to be endothelium-denuded if vasorelaxation to 10 microM carbachol was less than 10% of induced tone. To verify the participation of endothelium-derived substances in the relaxant effects of gamma-mangostin, the experiments were performed after pretreatment with indomethacin (10 microM), a cyclooxygenase (COX) inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 300 microM), a nitric oxide synthase (NOS) inhibitor. In addition, the involvement of the GC/cGMP-dependent pathway was investigated using 1H-[1,2,4] oxadiazolo-[4,3-a] quinoxalin-1-one (ODQ, 10 microM), a guanylyl cyclase inhibitor.

To investigate the role of K^+ channels, vasorelaxation to gamma-mangostin was performed in aortic rings pre-contracted with a high K^+ (60 mM) Krebs solution, which was prepared by replacing an equimolar concentration of NaCl with KCl[11]. Concentration-response curves of gamma-mangostin were also performed in the presence of tetraethylammonium (TEA, 5 mM), a non-specific K^+ channel inhibitor, 4-aminopyridine (4-AP, 1 mM), a K_V channel inhibitor, glibenclamide (10 microM), a K_{ATP} inhibitor, or barium chloride ($BaCl_2$, 30 microM), a K_{IR} channel inhibitor, to identify the types of K^+ channels involved in vasorelaxation induced by gamma-mangostin.

The effects of gamma-mangostin on extracellular Ca^{2+} influx was evaluated by examining concentration-dependent responses to $CaCl_2$ (10 microM-30 mM) in the absence or presence of gamma-mangostin (10 or 100 mM). In this set of experiment, aortic rings were rinsed three times and allowed to equilibrate at 1 g tension in a Ca^{2+}-free Krebs solution. Then, the rings were bathed with Ca^{2+}-free, high KCl (100 mM) Krebs solution[12]. After 30 min incubation with gamma-mangostin, concentration-response curves for the contractile responses to $CaCl_2$ were constructed. In vehicle-control experiments, DMSO was added in the same volume as that used in the experiments with gamma-mangostin.

Statistical analysis

The results were expressed as mean $\pm$ SEM.

The concentration of vasorelaxant giving half-maximal relaxation (EC_{50}) and maximal responses (R_{max}) were obtained from the concentration-response curve fitted to a sigmoidal logistic equation using the GraphPad Prism package[13]. In some experiments, the relaxant effects of KPE were presented as the percentage reduction of the initial tone in each ring precontracted with methoxamine as data could not be fitted to any sigmoidal dose-response curves. Statistical comparisons between groups were compared by analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. When p-value was less than 0.05, the results were considered statistically significant. The number of animals in each group is represented by n.

Drugs and chemicals

All drugs and chemicals were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA), but zoletil was purchased from Virbac (Carros Cedex, France). Glibenclamide and gamma-mangostin were dissolved in DMSO. Indomethacin was dissolved in ethanol. $BaCl_2$ and 4-AP were dissolved in distilled water. The remaining drugs were dissolved in the perfusion fluid. All drug solutions were freshly prepared on the day of the experiment.

Results

The effects of endothelial denudation, L-NAME and indomethacin on vasorelaxation to gamma-mangostin

Gamma-mangostin (1-100 microM) caused concentration-dependent vasorelaxation (Fig. 1). Endothelial denudation significantly reduced vasorelaxation to gamma-mangostin (Fig. 1). In addition, L-NAME (300 microM) and ODQ (10 microM) significantly reduced vascular responses to gamma-mangostin (Fig. 1). However, pre-treatment with indomethacin (10 microM) did not affect vasorelaxation induced by gamma-mangostin (data not shown).

The effects of high extracellular potassium and potassium channel inhibitors on vasorelaxation to gamma-mangostin

Vasorelaxant effects of gamma-mangostin at concentrations from 3 to 100 microM were reduced by 60 mM KCl (Fig. 2). Moreover, pretreatment with TEA (5 mM) reduced the effects of gamma-mangostin (10-100 mM) (Fig. 2). However, the effects of gamma-mangostin were not affected by pretreatment with 4-AP (1 mM), $BaCl_2$ (30 microM) or glibenclamide (10 microM) (Fig. 3).

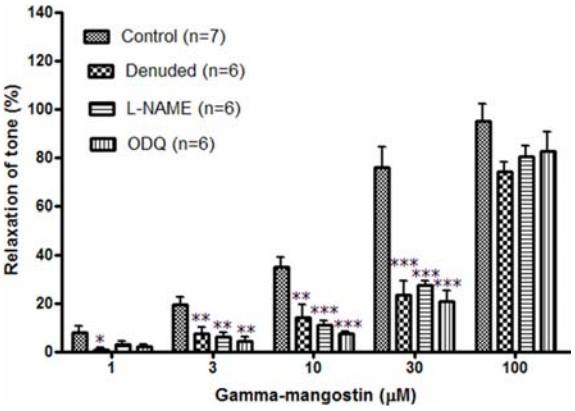

Fig. 1 Effects of removal of the endothelium, L-NAME (300 microM) and ODQ (10 microM) on vasorelaxation to gamma-mangostin in aortic rings precontracted with methoxamine. Data were shown as mean ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.001 versus control

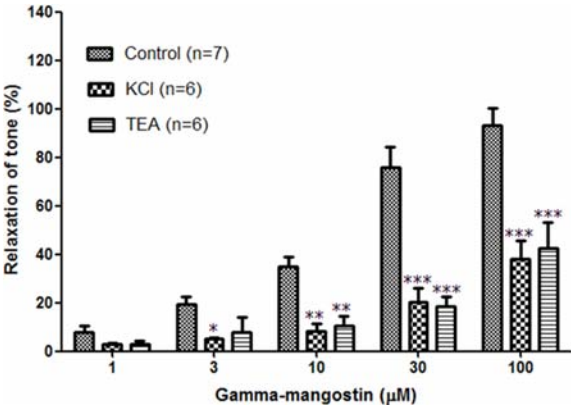

Fig. 2 Effects of 60 mM KCl and TEA (5 mM) on vasorelaxation to gamma-mangostin in aortic rings precontracted with methoxamine. Data were shown as mean ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.001 versus control

The effects of gamma-mangostin on $CaCl_2$-induced contraction in rat aortic rings

In aortic rings in calcium-free buffer depolarized by 100 mM KCl, $CaCl_2$ (10 mM-30 mM) induced concentration-dependent contractions. (pEC_{50} = 4.07 ± 0.06, with R_{max} = 1.26 ± 0.03 g, n = 7, Fig. 4). Pretreatment with gamma-mangostin (10 and 100 microM) significantly (p < 0.001) reduced contractile responses to $CaCl_2$ (10 microM gamma-mangostin: pEC_{50} = 3.97 ± 0.08, with R_{max} = 0.87 ± 0.03 g, n = 7; 100 microM gamma-mangostin: pEC_{50} = 3.83 ± 0.12, with R_{max} = 0.61 ± 0.04 g, n = 7, Fig. 4).

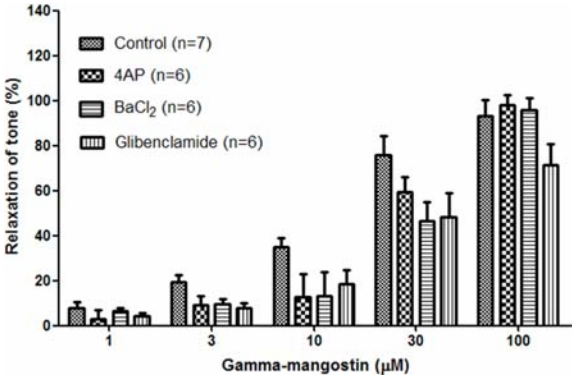

Fig. 3 Effects of 4AP (1 mM), BaCl2 (30 microM) and glibenclamide (10 microM) on vasorelaxation to gamma-mangostin in aortic rings precontracted with methoxamine. Data were shown as mean ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.001 versus control

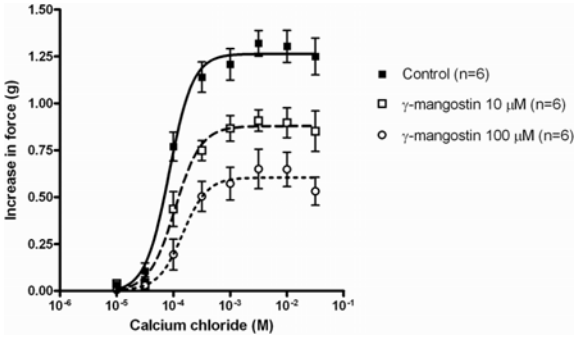

Fig. 4 Effects of gamma-mangostin (10 and 100 mM) on $CaCl_2$-induced contraction in aortic rings depolarized by 100 mM KCl. Data were shown as mean ± SEM

Discussion

The present study showed that gamma-mangostin caused vasorelaxation in a concentration-dependent manner. The possible mechanisms involved in vasorelaxation induced by gamma-mangostin were then investigated. As the vascular endothelium plays an important role in regulating vascular tone via the production of endothelium-derived relaxing factors (EDRFs), mainly NO and prostacyclin[13], the authors sought to investigate the role of the endothelium and EDRFs in vasorelaxation to gamma-mangostin. The present findings showed that removal of the functional endothelium and L-NAME, a NOS inhibitor, reduced the effects of gamma-mangostin. Moreover, the inhibitory effects of L-NAME on gamma-mangostin-induced responses were the same as responses found in endothelium-denuded rings. Taken together, it is indicated that NO is largely involved in endothelium-dependent vasorelaxations induced by gamma-mangostin. However, vasorelaxant responses to gamma-mangostin were not affected by indomethacin, a COX inhibitor. These results suggest that vasodilator prostanoids is not involved in the effects of gamma-mangostin.

NO induced relaxation of vascular smooth muscle cells via several mechanisms, including activation of guanylyl cyclase. An increased cGMP levels causes vasorelaxation[14,15]. In the present study, ODQ was used to investigate the involvement of cGMP in relaxant responses to gamma-mangostin. It was found that ODQ reduced vasorelaxation to gamma-mangostin. These findings suggest that the responses to gamma-mangostin are mediated via cGMP-dependent pathway.

The opening of K^+ channels in vascular smooth muscle cells causes membrane hyperpolarization, leading to closure of voltage-gated Ca^{2+} channels and subsequently relaxation of vascular smooth muscle cells. Blockade of K^+ channels promote Ca^{2+} influx through voltage-sensitive Ca^{2+} channels and increases vascular tone[16,17]. The present findings demonstrated that vasorelaxation to gamma-mangostin was found after endothelial denudation. These results suggest that gamma-mangostin also has a direct effect on vascular smooth muscle cells, likely activation of K^+ channels. Then, the role of K^+ channels in vasorelaxation to gamma-mangostin was investigated. It was found that the responses to gamma-mangostin involve activation of K^+ channels as the responses to gamma-mangostin were reduced when aortic rings were contracted with a high K^+ (60 mM). Then, aortic rings were incubated with different types of K^+ channel inhibitors to further investigate which types of K^+ that gamma-mangostin might selectively acted on. It was found that vasorelaxation to gamma-mangostin was reduced by TEA, a K^+ channel inhibitor. However, 4-AP, a K_V channel inhibitor, $BaCl_2$, a K_{IR} channel inhibitor, or glibenclamide, a K_{ATP} channel inhibitor had no effects on vascular responses to gamma-mangostin. These results suggest that vasorelaxation to gamma-mangostin was mediated by increasing K^+ efflux, at least in part, through K_{Ca} channels.

High KCl induces vasoconstriction by increasing Ca^{2+} influx via voltage-gated Ca^{2+} channels whereas methoxamine, an $alpha_1$-adrenoceptor agonist, causes contraction via stimulating Ca^{2+} entry through receptor-operated Ca^{2+} channels[17-20]. The present study demonstrated that contractions to methoxamine and 60 mM KCl were reduced by gamma-mangostin,

suggesting that the effects of gamma-mangostin are likely mediated via inhibition of extracellular Ca^{2+} influx into smooth muscle cells. Then, the effect of gamma-mangostin on extracellular Ca^{2+} influx in a Ca^{2+}-free, high KCl (100 mM) Krebs solution were examined. It was found that gamma-mangostin inhibited contractile responses to $CaCl_2$. These results suggest that inhibition of extracellular Ca^{2+} influx is involved in the relaxant responses to gamma-mangostin.

In conclusion, the present findings have clearly shown for the first time that gamma-mangostin causes vasorelaxation through endothelium-derived NO. The relaxant responses to gamma-mangostin are largely mediated via activation of K^+ channels and inhibition of extracellular Ca^{2+} influx. Importantly, the present study provides pharmacological evidence to support the use of gamma-mangostin acts as a vasodilator.

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Potential conflicts of interest

None.

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กลไกการคลายตัวของหลอดเลือดโดยสาร gamma-mangostin ในเอออร์ตาของหนูแรท

พัชรินทร์ เทพอารีนันท์, สุนิตย์ สุขสำราญ

วัตถุประสงค์*: เพื่อศึกษาผลของ gamma-mangostin ต่อความตึงตัวของหลอดเลือดและกลไกที่เกี่ยวข้องในเอออร์ตาของหนูแรทที่ตัดแยกออกมา*

วัสดุและวิธีการ*: เอออร์ตาจากหนูแรทพันธ์ Wistar เพศผู้ถูกทำให้หดตัวโดยสาร methoxamine การเปลี่ยนแปลงความตึงตัวของหลอดเลือดวัดโดย isometric force transducer และบันทึกด้วยเครื่อง MacLab ผลการคลายตัวของหลอดเลือดโดยสาร gamma-mangostin ถูกศึกษาเมื่อมีสาร 300 microM N^G-nitro L-arginine methyl ester (L-NAME), 10 microM 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ), indomethacin, 60 mM KCl, 5 mM tetraethylammonium (TEA), 10 microM glibenclamide, 1 mM 4-aminopyridine (4-AP) หรือ 30 microM barium chloride ($BaCl_2$) นอกจากนี้ยังศึกษาผลของ gamma-mangostin ต่อการหดตัวโดย $CaCl_2$*

ผลการศึกษา*: Gamma-mangostin (1-100 microM) ทำให้เกิดการคลายตัวของหลอดเลือดเอออร์ตาของหนูแรทที่หดตัวโดยสาร methoxamine ซึ่งผลนี้ถูกยับยั้งเมื่อลอกชั้นเยื่อบุหลอดเลือดออกและเมื่อได้รับสาร L-NAME, ODQ, KCl หรือ TEA อย่างไรก็ตามการคลายตัวของหลอดเลือดโดย gamma-mangostin ไม่เปลี่ยนแปลงโดย 4-AP, $BaCl_2$ หรือ glibenclamide นอกจากนี้การหดตัวของหลอดเลือดโดย $CaCl_2$ (10 microM-30 mM) ถูกยับยั้งโดย gamma-mangostin ที่ความเข้มข้น 10 และ 100 microM*

สรุป*: Gamma-mangostin ทำให้หลอดเลือดแดงคลายตัวโดยมีกลไกที่เกี่ยวข้องกับเส้นทางของ NO และ cGMP นอกจากนี้ยังเกี่ยวข้องกับการกระตุ้น K^+ channel และการยับยั้งการผ่านของ Ca^{2+}จากภายนอกเซลล์เข้าสู่เซลล์ ข้อมูลนี้สนับสนุนว่า gamma-mangostin อาจเป็นสารช่วยลดความดันเลือดแดง*