Journal of Traditional and Complementary Medicine 9 (2019) 297–303

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Endothelium-independent and calcium channel-dependent relaxation of the porcine cerebral artery by different species and strains of turmeric

Jesmin Akter [a, b], Md Zahorul Islam [b, c], Md Amzad Hossain [a, b, *], Shinsuke Kawabata [d], Kensaku Takara [a, b], Ha Thi Thanh Nguyen [e], De-Xing Hou [a, f], Atsushi Miyamoto [e]

[a] United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, 890-0065, Japan
[b] Faculty of Agriculture, University of the Ryukyus, Okinawa, 903-0213, Japan
[c] Dept. of Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, 2202, Bangladesh
[d] Okinawa Educational Publishing Co. Ltd, Makishi1-2-24, Naha City, Okinawa, 900-0013, Japan
[e] Department of Veterinary Pharmacology, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21- 24 Korimoto, Kagoshima, 890-0065, Japan
[f] Faculty of Agriculture, Kagoshima University, 1-21- 24 Korimoto, Kagoshima, 890-0065, Japan

ARTICLE INFO

Article history:
Received 11 April 2018
Received in revised form
29 July 2018
Accepted 7 August 2018
Available online 18 September 2018

Keywords:
Turmeric
Cerebral artery
Vasorelaxation
Ca^{2+}-channel
β-adrenergic receptor

ABSTRACT

Objective: To clarify the underlying mechanism of turmeric, which is traditionally used as a medicinal plant for the treatment of cardiovascular disorders, such as hypertension, and palpitations.
Methods: Methanol extracts of different turmeric were used. A tissue-organ-bath system was used to investigate the vasoactive effects of methanol extracts from 5 kinds of turmeric on isolated porcine basilar arteries. The arterial rings were suspended in physiological solution that was maintained at 37 °C temperature with a continuous supply of 95% O_2 and 5% CO_2.
Results: All turmeric extracts (20–800 μg/mL) induced concentration-dependent relaxation of the isolated porcine basilar artery pre-contracted with U46619 ($1\text{-}5 \times 10^{-9}$ M) in arterial rings with or without endothelium. There were no significant differences in the relaxation induced by different turmeric or between the endothelium-intact and denuded arteries. In depolarized, Ca^{2+}-free medium, the turmeric extracts inhibited $CaCl_2$-induced contractions and caused a concentration-dependent rightward shift of the response curves. In addition, propranolol (a non-specific β-adrenoceptor antagonist) slightly inhibited the relaxation induced by turmeric. In contrast, Nω-nitro-L-arginine, indomethacin, tetraethylammonium, glibenclamide and 4-aminopyridine did not affect turmeric-induced relaxation.
Conclusion: These results demonstrated that turmeric induced endothelium-independent relaxation of the porcine basilar artery, which may be due to the inhibition of extracellular and intracellular Ca^{2+} receptors and the partial inhibition of β-adrenergic receptors in vascular smooth muscle cells.

1. Introduction

Currently, vascular diseases, such as hypertension, atherosclerosis, subarachnoid hemorrhage, stroke, and Alzheimer's disease, have become public health challenges; therefore, it is necessary to develop modulators that control vascular tone to treat these diseases.[1,2] Vascular reactivity is an important factor for the treatment of cardiovascular diseases because it affects blood flow and pressure. The efficacy of synthetic antihypertensive drugs increases in a dose-dependent manner, which leads to various adverse effects.[3] In addition to synthetic drugs, the use of herbs or herbal extracts is increasing in China, Japan, and Korea.[4] Many plants used in traditional medicine have been investigated for treating cardiovascular disease.[5] The effect of curcumin and its parent plant *Curcuma* spp. (Family: Zingiberaceae) on cardiovascular systems has recently received much attention.

Turmeric, especially, *Curcuma longa*, has been extensively

* Corresponding author. Subtropical Field Science Center, Faculty of Agriculture, University of the Ryukyus, Senbaru-1, Nishihara, 903-0213, Japan.
E-mail address: amzad@agr.u-ryukyu.ac.jp (M.A. Hossain).
Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

https://doi.org/10.1016/j.jtcme.2018.08.002

studied for its various biological activities both *in vivo* and *in vitro*, including anti-inflammatory,[6–8] anti-diabetic,[9] wound healing,[10] anti-hemolytic,[11] antioxidant,[8,19] and anti-carcinogenic[12] effects, as well as protection against gastrointestinal and respiratory disorders.[13] Curcuma drugs have been traditionally used for "Oketsu" (syndromes caused by the obstruction of blood circulation such as psychataxia, arthralgia and dysmenorrhea) in the Chinese medicine system.[14] It has been reported that *C. longa* lowers arterial blood pressure and heart rate in rats[15,16] and induces endothelium-independent vasorelaxation in isolated rat aorta[14] and superior mesenteric arteries.[15,16] The genus *Curcuma* comprises approximately 80 species,[17] and some species have multiple cultivars/varieties, for example, *C. longa* comprises approximately 70 varieties in India.[18] The most important component of turmeric responsible for its biological activities is curcumin, which has been reported to have anti-hypertensive[19] neuroprotective and anti-ischemic,[6] anti-angiogenic,[20] hepatoprotective[8] and vasorelaxation effects in isolated rabbit basilar[21] and porcine coronary arteries.[22] Curcumin also reverses the pathogenesis of cerebral ischemia and stroke.[6] Tetrahydrocurcumin and hexahydrocurcumin, biotransformed products of curcumin, have hypotensive effects and induce vasorelaxation of isolated rat thoracic aorta, respectively.[19,23] We found that there are significant differences in the curcumin, demethoxycurcumin and bisdemethoxycurcumin content of different species and strains of turmeric.[24] Therefore, we hypothesized that different species and strains of turmeric might vary in their vasomotional effects in porcine basilar arteries. This study was designed to compare the vascular reactivity and underlying mechanisms of different species and strains of turmeric in isolated porcine basilar arteries.

2. Materials and methods

2.1. Reagents

Bradykinin (BK) acetate salt, N′-tetraacetic acid (EGTA), nifedipine, Nω-nitro-L-arginine (L-NA), sodium nitroprusside (SNP), tetraethylammonium (TEA; Sigma-Aldrich, St, Louis, MO, USA) and U46619 (Cayman Chemical Co., Ann Arbor, MI, USA) were used. All Krebs salts and other chemicals were general-purpose or analytical grade and purchased from Nakalai Tesque (Kyoto, Japan) or Wako (Osaka, Japan). Stock solutions were dissolved in distilled water, except nifedipine, which was dissolved in ethanol. The solutions were prepared fresh on the day of the experiment.

2.2. Plant material and extraction

Seven different species and strains of turmeric, including *C. longa* (strain: Ryudai gold, Okinawa ukon and BK2), *C. xanthorrhiza*, *C. aromatica, C. amada* and *C. zedoaria*, were studied in this experiment. Fresh, healthy rhizomes were harvested from the field of the University of the Ryukyus. Botanical identification and authentication of the turmeric samples were confirmed by qualified taxonomists of the Subtropical Field Science Center, Faculty of Agriculture, University of the Ryukyus, Japan. The rhizomes were washed, sliced and oven-dried at 60 °C for 72 h. Then, turmeric powder was prepared and passed through a sieve with a nominal mesh size of 2 mm. The extractions were carried out by dissolving the different turmeric powders in methanol at room temperature (27 °C) and atmospheric pressure for 2 days with shaking. The solvent-soluble compounds were filtered using double filter paper (Whatman™). Fresh solvent was added into the used plant material, and the process was repeated three times. The filtered solutions containing plant compounds were dried by rotary evaporator. All extracts were kept in a refrigerator at 4 °C for experimental analyses. Prior to the experiments, the methanol extracts were dissolved in DMSO (1%) and further diluted in distilled water to give the desired concentration. The final concentration of DMSO in the organ-bath was less than 0.1%.

2.3. Tissue preparation

Basilar arteries were obtained from freshly slaughtered pigs (both sexes, approximately 6–7 months old, LWD cross-breed) at a local slaughter house and transferred to our laboratory in ice-cold physiological saline solution (119 mM NaCl, 4.7 mM KCl, 1.6 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4 and 10 mM glucose, pH 7.4) aerated with carbogen (95% (v/v) O_2, 5% (v/v) CO_2). After the adherent tissues had been carefully removed, several rings approximately 4 mm long were cut from each artery. When required, the endothelium was removed by gently rubbing the intimal space with a stainless-steel rod with a diameter equivalent to the lumen of the artery. Arterial rings were mounted vertically between two L-shaped stainless steel holders, with the upper part fixed to an isometric force transducer (TB-611 T, Nihon Kohden Kogyo, Tokyo, Japan), and immersed in a 5-ml water-jacketed organ bath containing oxygenated salt solution at 37 °C (pH 7.4). Each suspended ring was left to equilibrate under a resting tension of 0.75 g, which allowed us to induce maximum contractions in the artery. KCl (60 mM) was applied to stimulate the artery. After the contraction reached the maximum, the artery was washed, re-equilibrated and again stimulated with 60 mM KCl. This process was continued until the contraction amplitude reached a constant value. The isometric tension was recorded with an amplifier (AP-621 g, Nihon Kohden Kogyo, Tokyo, Japan), digitized with an analog-digital converter (PowerLab/8SP, ADInstruments Co., Castle Hill, NSW, Australia) and stored on the hard disc of a personal computer. The presence of endothelial cells was confirmed pharmacologically by testing the relaxant response to BK under pre-contracted conditions with U46619 (this response was abolished by endothelial denudation).[25]

2.4. Turmeric-induced relaxation of basilar arteries pre-contracted with U46619

A thromboxane analog (U46619; $1\text{-}5 \times 10^{-9}$ M) was used to induce stable pre-contraction of arterial rings with the endothelium intact or denuded, and turmeric extract was added cumulatively (20–800 μg/mL) to obtain the concentration response curve (CRC). At the end of the experiments, 10^{-4} M SNP (sodium nitroprusside) was added, and the resulting relaxation was taken as 100%. Turmeric-induced relaxation was calculated as a percentage relative to the response elicited by 10^{-4} M SNP. The test for the Ca^{2+} influx-inhibitive effect of turmeric was modified from Khan and Gilani (2009).[26] Endothelium-denuded arterial segments were allowed to stabilize in normal physiological saline (PSS), which was then replaced with Ca^{2+}-free PSS containing EGTA (2 mM) for 30 min to remove extracellular Ca^{2+} from the tissues. This solution was finally replaced with Ca^{2+}-free and K^+-rich (60 mM) PSS. After an incubation period of 30 min, the CRC was obtained by addition of extracellular $CaCl_2$ (extracellular Ca^{2+} CRC) to the bath fluid. After the extracellular Ca^{2+} CRCs were found to be superimposable (after 2 cycles), the arterial segment was pre-treated with different concentrations of turmeric or nifedipine for 30 min, and the next extracellular Ca^{2+} CRC was constructed in the presence of these agents. The concentration-dependent inhibitory effect of turmeric was tested. The change in extracellular Ca^{2+} CRC induced by turmeric or nifedipine was used to estimate the Ca^{2+} influx-inhibitory effect.

2.5. Statistical analysis

The contraction response was expressed as a percentage of the response obtained with SNP (10^{-4} M). The relaxation response was expressed as a percentage of the response obtained with 10^{-4} M SNP. The results are expressed as the means ± standard error mean (SEM). Statistical analyses were performed by paired *t*-test or the Bonferroni test after one-way analysis of variance (one-way ANOVA). Significance was established when the probability level was equal to or less than 5%.

3. Results

3.1. Effect of different species and strains of turmeric on isolated porcine basilar arteries pre-contracted with U46619

Methanol extracts of different species and strains of turmeric (20–800 μg/mL) induced relaxation of the porcine basilar artery rings pre-contracted with U46619 in a concentration-dependent manner. There were no significant differences observed among the different species and strains of turmeric-induced relaxation of the isolated porcine basilar artery (Fig. 1).

3.2. Effect of endothelium denudation, L-NA and indomethacin on turmeric-induced relaxation in porcine basilar arteries pre-contracted with U46619

Because a similar pattern of vascular reactivity for the different species and strains of turmeric was observed, we took Ryudai gold (containing curcumin, demethoxycurcumin and bisdemethoxycurcumin) and *C. amada* (does not contain curcumin, demethoxycurcumin or bisdemethoxycurcumin) for the graphical presentation of the subsequent experiments. Endothelium denudation, pretreatment of endothelium-intact arteries with L-NA (a nitric oxide synthase inhibitor) and indomethacin (a non-selective cyclooxygenase inhibitor) for 30 min had no significant effect on the turmeric-induced relaxation of the pre-contracted porcine basilar artery (Fig. 2).

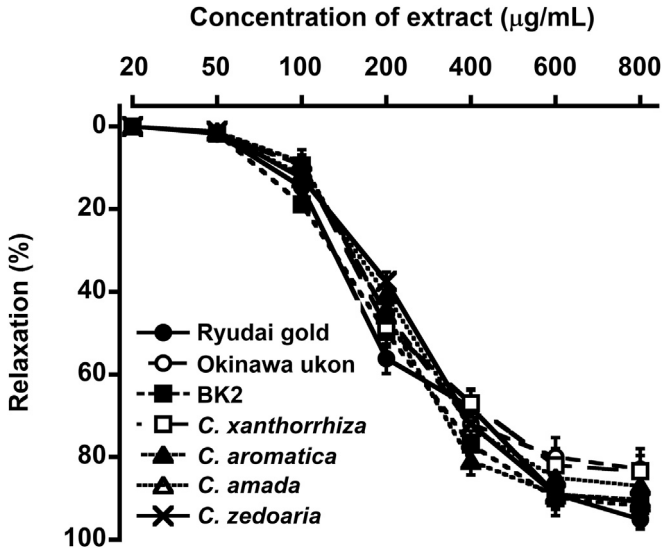

Fig. 1. Effect of methanol extracts from different species and strains of turmeric on isolated porcine basilar arteries. The results are expressed as the mean ± SEM (n = 7–9 pigs).

3.3. Role of K^+-channel on turmeric-induced relaxation of the pre-contracted arterial rings

To test the involvement of K^+-channels in turmeric-induced relaxation of endothelium-denuded arterial rings, the arteries were pre-treated with K^+-channel antagonists, such as tetraethylammonium (TEA; a non-selective K^+-channel inhibitor, 10 mmol/L),[26] glibenclamide (a non-specific ATP-sensitive K^+-channel antagonist, 10 μmol/L)[27] and 4-aminopyridine (4-AP; a voltage-dependent K^+-channel antagonist, 1 mmol/L)[28] for 30 min before pre-contraction with U46619 ($1\text{-}5 \times 10^{-9}$ M). As shown in Fig. 3, pretreatment with K^+-channel antagonists had no significant effect on turmeric-induced relaxation.

3.4. Effect of propranolol (a non-selective β-adrenergic receptor antagonist) on turmeric-induced relaxation in pre-contracted rings

To test the involvement of the β-adrenergic receptor in turmeric-induced relaxation in endothelium-denuded porcine basilar arteries, we pre-treated arterial rings with propranolol (1 μM), a non-selective β-adrenergic receptor antagonist, for 30 min. Propranolol significantly decreased the maximal relaxation from 93.1 ± 2.7% and 91.0 ± 3.4 to 81.3 ± 3.9 and 80.3 ± 3.6 for Ryudai gold and *C. amada*, respectively. Propranolol significantly inhibited the relaxation when the turmeric concentration was 600 μg/mL and 800 μg/mL (Fig. 4).

3.5. Effect of turmeric extracts on $CaCl_2$-induced contraction in isolated porcine basilar arteries

Pre-treatment with turmeric extract at 100, 200 and 400 μg/mL and nifedipine (10^{-4} M) for 30 min significantly attenuated the $CaCl_2$-induced contraction of the endothelium-denuded arterial rings exposed to Ca^{2+}-free medium containing high K^+ in a concentration-dependent manner (Fig. 5). Pre-treatment with turmeric extracts and nifedipine shifted the concentration response curve for $CaCl_2$ to the right.

3.6. Effect of the main active compounds of turmeric (curcumin, demethoxycurcumin and bisdemethoxycurcumin) on isolated porcine basilar arteries pre-contracted with U46619

We studied the effect of curcumin, demethoxycurcumin and bisdemethoxycurcumin on isolated porcine basilar arteries pre-contracted with U46619. All three compounds induced relaxation of the isolated porcine basilar artery. There were no significant differences at lower concentrations (10^{-9}–10^{-6} M) of curcuminoids. However, at higher concentrations (10^{-5} and 10^{-4} M), demthoxycurcumin induced significantly higher relaxation, followed by curcumin and bisdemethoxycurcumin (Fig. 6).

4. Discussion

Our results showed that, despite the variation in curcuminoid content, all turmeric induced strong relaxation of isolated porcine basilar arteries. There were no significant differences in relaxation effects among the different species and strains of turmeric. It is well known that, in addition to nitric oxide, the relaxation of vascular smooth muscles is induced by prostaglandin I_2, Ca^{2+} antagonists, and β-adrenoceptor agonists. Therefore, these pathways were evaluated to reveal the mechanism of vasorelaxation induced by turmeric. In this study, pretreatment of endothelium-intact arterial rings with L-NA (a nitric oxide synthase inhibitor) did not affect turmeric-induced relaxation. Mechanical removal of the endothelium also had no significant effect on turmeric-induced relaxation.

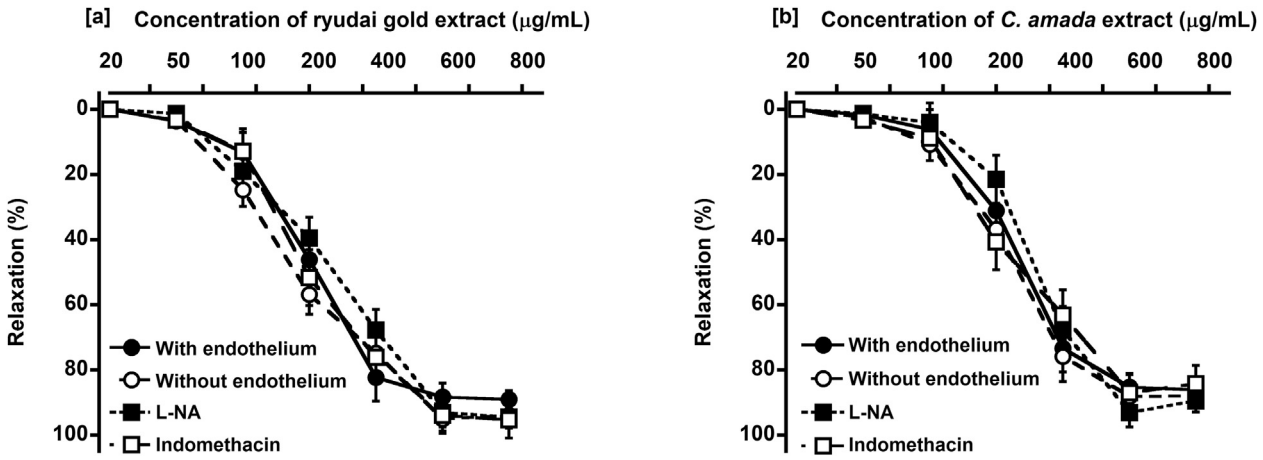

Fig. 2. Effects of endothelial denudation and pretreatment of endothelium-intact arterial rings for 30 min with L-NA (a nitric oxide synthase inhibitor) and indomethacin (a non-specific cyclooxygenase inhibitor) on vascular relaxation induced by Ryudai gold [a] and *C. amada* [b] in isolated porcine basilar arteries. The results are expressed as the mean ± SEM (n = 5–7 pigs).

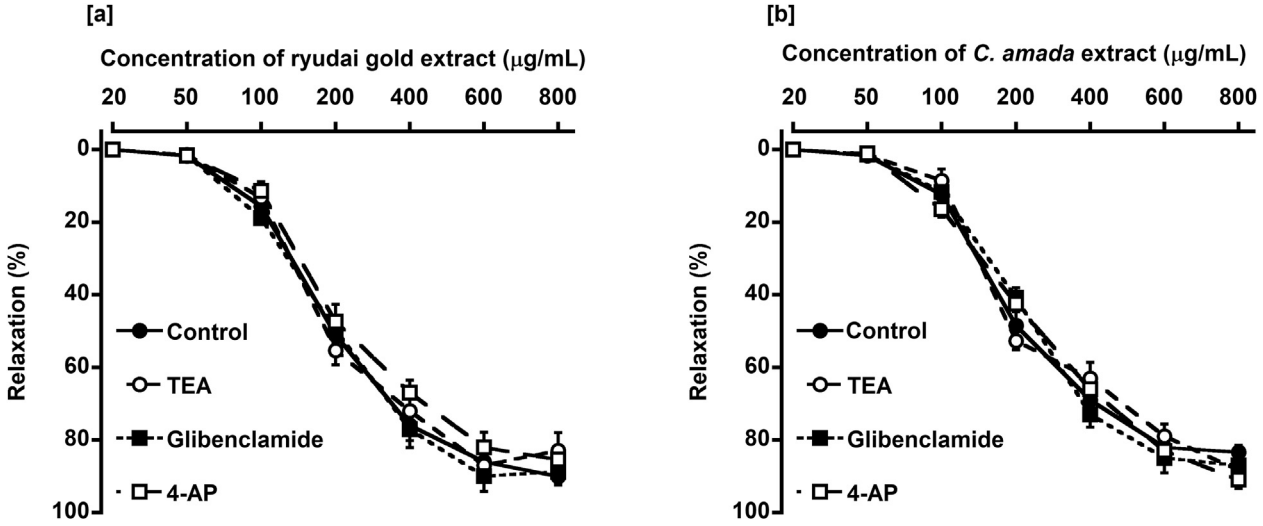

Fig. 3. Effect of K^+-channel antagonists on vascular relaxation induced by Ryudai gold [a] and *C. amada* [b] in endothelium-denuded porcine basilar arterial rings pre-contracted with U46619. The arterial rings were pre-incubated with tetraethylammonium (TEA; 10 mM), glibenclamide (10 μM) and 4-aminopyridine (4-AP; 1 mM) for 30 min. The results are expressed as the mean ± SEM (n = 6–8 pigs).

These results suggested that turmeric induces NO and endothelium-independent relaxation and directly acts through smooth muscle cells of the porcine basilar artery. These results are in accordance with the findings of Sasaki,[14] who reported the effectiveness of curcuma drugs as vasorelaxant agents in the vascular smooth muscle of rat aortas. It has also been reported that methanol extracts of *C. longa* induce endothelium-independent relaxation in isolated rat aorta[14] and superior mesenteric arteries.[15,16] Similarly, the major active compounds of turmeric such as curcumin, demethoxycurcmin and bisdemethoxycurmin induce endothelium-independent relaxation of isolated rat pulmonary artereis.[29] However, one study reported that curcumin induces partial endothelium and NO-dependent relaxation of porcine coronary arteries.[22] This difference might be due to the difference in the vascular bed studied. Indomethacin (a non-selective cyclooxygenase inhibitor) did not affect turmeric-induced relaxation in endothelium-intact porcine basilar arteries, which indicates that the release of vasodilator prostanoids is not involved in turmeric-induced relaxation of the porcine basilar artery. Similarly, vasodilator prostanoids are not involved in the curcumin-induced relaxation of porcine coronary arteries.[22]

It is well known that K^+-channels play a substantial role in the regulation of vascular contractility and tone.[30] Direct activation of K^+-channels in arterial smooth muscle cells leads to membrane hyperpolarization, inhibits Ca^{2+} influx through voltage operated Ca^{2+}-channel and suppresses smooth muscle contraction and subsequent vasodilation.[30] Therefore, we evaluated the influence of K^+-channel inhibitors on the vasorelaxant response induced by turmeric. However, TEA (a Ca^{2+}-activated K^+-channel antagonist), glibenclamide (a non-specific ATP-sensitive K^+-channel antagonist) and 4-AP (a voltage-dependent K^+-channel antagonist) had no significant effect on turmeric-induced relaxation. These results suggested that opening of the K^+-channels was not involved in the mechanism of the vasomotional action of turmeric. Similar results were observed in *C. longa*-induced vasorelaxation in rat thoracic aorta.[16]

KCl induces smooth muscle contraction via the activation of voltage-dependent Ca^{2+}-channels and subsequent release of Ca^{2+}

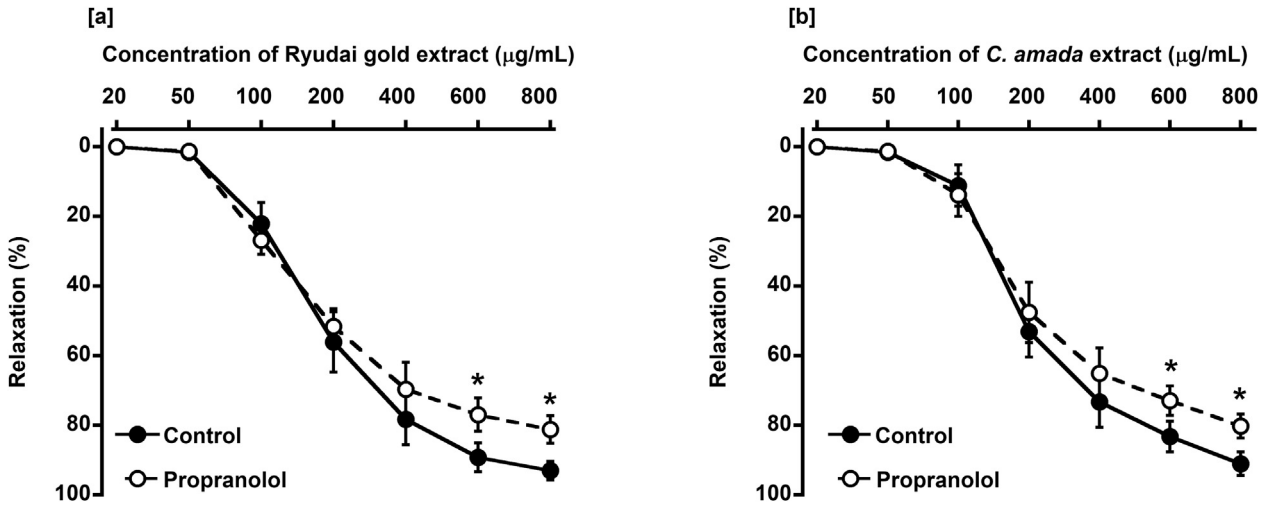

Fig. 4. Effect of propranolol (a nonselective β-adrenergic receptor antagonist, 1 μM) on vasorelaxation induced by Ryudai gold [a] and *C. amada* [b] in endothelium-denuded porcine basilar artery rings. The arteries were pre-contracted with U46619 (10^{-9} M). The results are expressed as the mean ± SEM (n = 6 pigs). $^{*}P < 0.05$ vs. control.

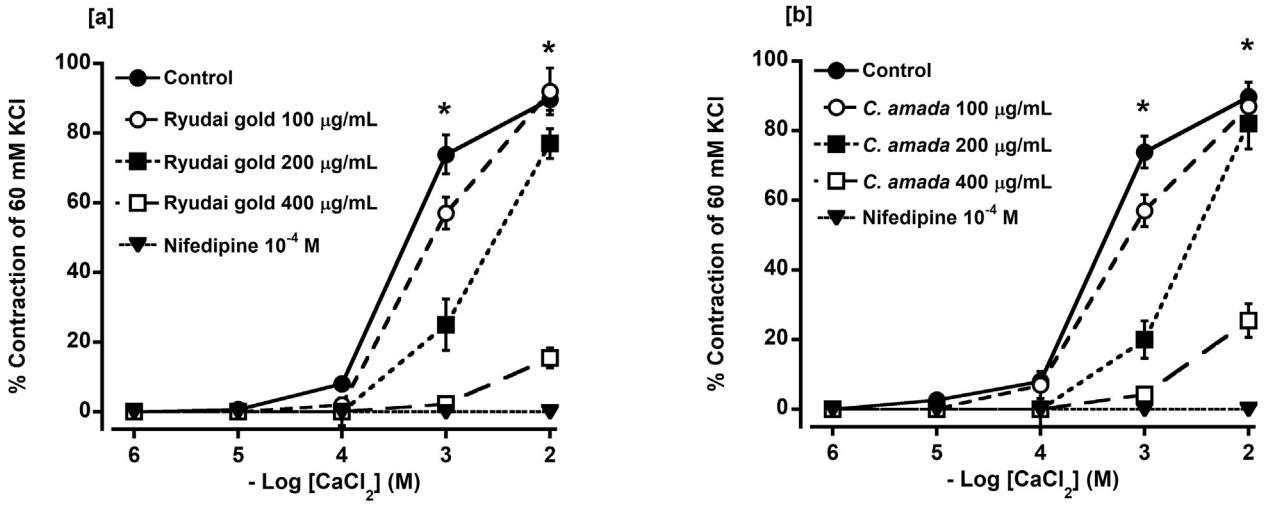

Fig. 5. Effect of Ryudai gold [a], *C. amada* [b] extracts and nifedipine on $CaCl_2$-induced contraction of endothelium-denuded porcine basilar arteries. Concentration–response curves were determined in Ca^{2+}-free solution after the depletion of extracellular Ca^{2+}, where the $CaCl_2$-contractile effect was dependent on Ca^{2+}-influx through voltage-operated Ca^{2+}-channels activated by KCl (60 mM). The curves were constructed in the absence of added substance (control) or after 30 min incubation in the presence of Ryudai gold, *C. amada* (100, 200 and 400 μg/mL) and nifedipine (10^{-4} M) or turmeric extract prior to the cumulative addition of $CaCl_2$. The results are expressed as the mean ± SEM (n = 7–9 pigs). $^{*}P < 0.05$ vs. control.

from the sarcoplasmic reticulum, causing a significant increase in the intracellular Ca^{2+} concentration.[31] Turmeric antagonism of the Ca^{2+}-channel was confirmed by our experiment in which extracellular Ca^{2+} removal (using 2 mM EGTA) suppressed KCl-induced contraction, and pretreatment with turmeric significantly inhibited Ca^{2+}-induced contraction of 60 mM KCl-depolarized arteries. In addition, the magnitude of the inhibitory effect of turmeric and nifedipine (an L-type Ca^{2+}-channel antagonist) was similar, which suggested the involvement of the L-type voltage-operated Ca^{2+}-channel. This observation is consistent with a study by Gilani et al.,[13] who concluded that Ca^{2+}-channel antagonism is the main mechanism of vasorelaxation elicited by curcuma drugs and forms the basis of traditional uses in hyperactive states of the gut, airway inflammation disorders, palpitation and hypertension. Similarly, curcumin analogs and synthesized curcumin mimics dilate rabbit basilar arteries via antagonism of the L-type Ca^{2+}-channel.[29,32]

Stimulation of vascular smooth muscle by β-adrenoreceptors results in vasorelaxation of the porcine basilar artery.[33] Our results showed that propranolol (a non-selective β-adrenergic receptor antagonist) inhibited the turmeric-induced relaxation when the concentrations were 600 μg/mL and 800 μg/mL, indicating that the high degree of turmeric-induced relaxation might be attributed to β-adrenoceptor activation. Our results consistent with the results of Xu[22] and Moohammadaree,[23] who demonstrated the involvement of β-adrenoceptors in curcumin and hexahydrocurcuminin-induced relaxation of porcine coronary arteries and rat thoracic aorta, respectively. It has been reported that noradrenaline-induced relaxation of the porcine basilar artery occurs via the stimulation of β-adrenoceptors on vascular smooth muscle cells, which is inhibited by propranolol.[33] We performed HPLC to detect the presence of noradrenaline in the turmeric extracts. However, noradrenaline was not detected in any of the tested samples (data not shown).

Like the effect of the turmeric extract, pure curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) induced relaxation of the isolated porcine basilar artery. A previous study

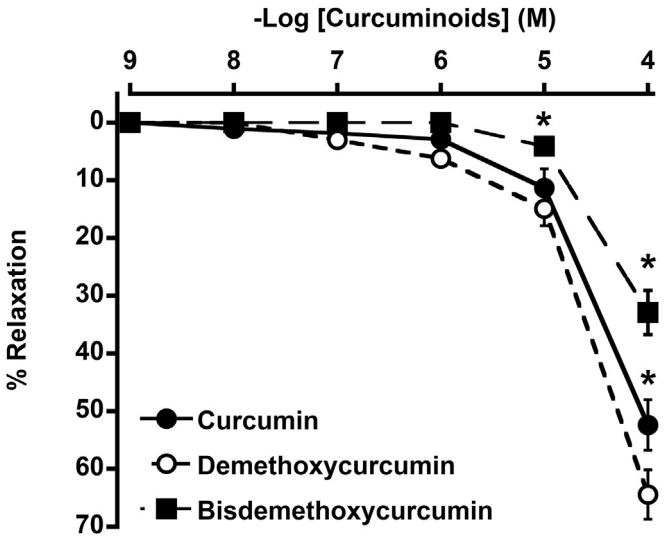

Fig. 6. Effect of curcumin, demethoxycurcumin and bisdemethoxycurcumin on porcine basilar artery rings. The arteries were pre-contracted with U46619 (10^{-9} M). The results are expressed as the mean ± SEM (n = 6 pigs). $^{*}P < 0.05$ vs. demethoxycurcumin.

also reported that these three curcuminods induce endothelial-independent and Ca^{2+}-channel-dependent relaxation of rat pulmonary arteries.[29] Demethoxycurcumin induced significantly more relaxation, followed by curcumin and bisdemethoxycurcumin at higher concentrations. However, the relaxation induced by the turmeric extract showed no significant differences. This might be due to the difference in the concentration and ratio of the active compounds present in the turmeric extract. Moreover, we reported previously that among the different species and strains of turmeric, *C. amada* and *C. zedoaria* do not contain curcumin, demethoxycurcumin and bisdemethoxycurcumin[24] but still induce relaxation. Therefore, vascular relaxation induced by *C. amada* and *C. zedoaria* indicated that there are other vasoactive compounds besides curcuminoids present in turmeric, which is consistent with the results of Sasaki,[14] who isolated curcumin and eight sesquiterpenes that induced NO-independent relaxation with almost the same intensities.

In conclusion, our results suggested that different species and strains of turmeric induce nitric oxide- and endothelium-independent relaxation of the porcine basilar artery. Possible mechanisms of vasorelaxation include partial activation of β-adrenoceptors and inhibition of Ca^{2+} from both extracellular and intracellular sources. Turmeric, its active compounds and/or synthetic curcuminoids may be used as novel vasodilation agents for the treatment of cardiovascular diseases such as atherosclerosis, essential hypertension, pulmonary artery hypertension, coronary artery disease, stroke, and diabetic complications of vasculature. However, additional work is required to investigate the vasoactive compounds and molecular mechanisms of the vasorelaxation induced by turmeric.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgements

We are grateful to Okinawa Educational Publishing Co. Ltd, Naha City, Japan for financial support to this work.

References

1. Miller AA, Drummond GR, Sobey CG. Selective inhibition of NADPH-oxidase isoforms as a therapeutic strategy in hypertension. *Drug Discov Today Ther Strat*. 2005;2:187–192.
2. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet*. 2005;365:217–223.
3. Handler J. Quality of life and antihypertensive drug therapy. *J Clin Hypertens*. 2005;7:274–285.
4. Alaerts G, Merino-Arevalo M, Dumarey M, et al. Exploratory analysis of chromatographic finger prints to distinguish *rhizoma chuanxiong* and *rhizoma ligustici*. *J Chromatogr A*. 2010;1217:7706–7716.
5. Ibarra-Alvarado C, Rojas A, Mendoza S, et al. Vasoactive and antioxidant activities of plants used in mexican traditional medicine for the treatment of cardiovascular diseases. *Pharm Biol*. 2010;48:732–739.
6. de Alcântara GF, Simões-Neto E, da Cruz GM, et al. Curcumin reverses neurochemical, histological and immuno-histochemical alterations in the model of global brain ischemia. *J Tradit Complement Med*. 2016;7:14–23.
7. Verma S, Mundkinajeddu D, Agarwal A, Chatterjee SS, Kumar V. Effects of turmeric curcuminoids and metformin against central sensitivity to pain in mice. *J Tradit Complement Med*. 2016;7:145–151.
8. Salahshoor M, Mohamadian S, Kakabaraei S, Roshankhah S, Jalili C. Curcumin improves liver damage in male mice exposed to nicotine. *J Tradit Complement Med*. 2015;6:176–183.
9. Dehghan MH, Mirmiranpour H, Faghihi-Kashani S, et al. Inhibitory effect of curcumin on angiogenesis in a streptozotocin-induced diabetic rat model: an aortic ring assay. *J Tradit Complement Med*. 2016;6:437–441.
10. Sidhu GS, Singh AK, Thaloor D, et al. Enhancement of wound healing by curcumin in animals. *Wound Repair Regen*. 1998;6:167–177.
11. Mathuria N, Verma RJ. Aflatoxin induced hemolysis and its amelioration by turmeric extracts and curcuminin vitro. *Acta Pol Pharm*. 2007;64:165–168.
12. Aqqarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res*. 2003;23:363–398.
13. Gilani AH, Shah AJ, Ghayur MN, Majeed K. Pharmacological basis for the use of turmeric in gastrointestinal and respiratory disorders. *Life Sci*. 2005;76:3089–3105.
14. Sasaki Y, Goto H, Tohda C, et al. Effects of curcuma drugs on vasomotion in isolated rat aorta. *Biol Pharm Bull*. 2003;26:1135–1143.
15. Adaramoye OA, Medeiros IA. Involvement of $Na^{(+)}$–$Ca^{(2+)}$ exchanger in the endothelium-independent vasorelaxation induced by *Curcuma longa* L. in isolated rat superior mesenteric arteries. *J Smooth Muscle Res*. 2008;44:151–158.
16. Adaramoye OA, Anjos RM, Almeida MM, et al. Hypotensive and endothelium-independent vasorelaxant effects of methanolic extract from *Curcuma longa* L. in rats. *J Ethnopharmacol*. 2009;124:457–462.
17. Larsen K. Distribution patterns and diversity centres of Zingiberaceae in SE Asia. *Biol Skr*. 2005;55:219–228.
18. Sasikumar B. Genetic resources of Curcuma: diversity, characterization and utilization. *Plant Gen Resour*. 2005;3:230–251.
19. Nakmareong S, Kukongviriyapan U, Pakdeechote P, et al. Antioxidant and vascular protective effects of curcumin and tetrahydrocurcumin in rats with L-NAME-induced hypertension. *Naunyn-Schmiedeberg's Arch Pharmacol*. 2011;383:519–529.
20. Dehghan MH, Mirmiranpour H, Faghihi-Kashani S, et al. Inhibitory effect of curcumin on angiogenesis in a streptozotocin-induced diabetic rat model: an aortic ring assay. *J Tradit Complement Med*. 2016;6:437–441.
21. Ahn CM, Park BG, Woo HB, Ham J, Shin WS, Lee S. Synthesis of sulfonyl curcumin mimics exerting a vasodilatation on the basilar artery of rabbits. *Bioorg Med Chem Lett*. 2009;19:1481–1483.
22. Xu PH, Long Y, Dai F, Liu ZL. The relaxant effect of curcumin on porcine coronary arterial ring segments. *Vasc Pharmacol*. 2007;47:25–30.
23. Moohammadaree A, Changtam C, Wicha P, Suksamrarn A, Tocharus J, Tocharus C. Mechanisms of vasorelaxation induced by hexahydrocurcumin in isolated thoracic aorta. *Phytother Res*. 2015;29:1806–1813.
24. Akter J, Hossain MA, Sano A, Takara K, Islam MZ, Hou DX. Antifungal activity of various species and strains of turmeric (*curcuma* spp.) against *Fusarium Solani Sensu lato*. *Pharm Chem J*. 2018;52:320–325.
25. Miyamoto A, Ishiguro S, Nishio A. Stimulation of bradykinin B_2-receptors on endothelial cells induces relaxation and contraction in porcine basilar artery in vitro. *Br J Pharmacol*. 1999;128:241–247.
26. Khan AU, Gilani AH. Antispasmodic and bronchodilator activities of Artemisia vulgaris are mediated through dual blockade of muscarinic receptors and calcium influx. *J Ethnopharmacol*. 2009;126:480–486.
27. Zhang N, Zou H, Jin L, et al. Biphasic effects of sodium danshensu on vessel function in isolated rat aorta. *Acta Pharmacol Sin*. 2010;31:421–428.
28. Shen M, Zhao L, Wu R, Yue SQ, Pei JM. The vasorelaxing effects of resveratrol on abdominal aorta from rats and its underlying mechanism. *Vasc Pharmacol*. 2013;58:64–70.
29. Kruangtip O, Chootip K, Temkitthawon P, et al. Curcumin analogues inhibit phosphodiesterase-5 and dilate rat pulmonary arteries. *J Pharm Pharmacol*. 2015;67:87–95.
30. Jackson WF. Potassium channels in the peripheral microcirculation. *Microcirculation*. 2005;12:113–127.
31. Ratz PH, Berg KM, Urban NH, Miner AS. Regulation of smooth muscle calcium

sensitivity: KCl as a calcium-sensitizing stimulus. *Am J Physiol Cell Physiol*. 2005;288:769–783.
32. Park CB, Ahn CM, Oh S, et al. Synthesis of alkylsulfonyl and sunstituted benzenesulfonyl curcumin mimics as dual antagonist of L-type Ca^{2+} channel and endothelin A/B_2 receptor. *Bioorg Med Chem*. 2015;23:6673–6682.
33. Miyamoto A, Ito K, Nishio A. Characterization of β-adrenoceptors in pig basilar artery from functional and radioligand binding studies. *Jpn J Pharmacol*. 1993;61:93–99.