Family Medicine and Community Health
ORIGINAL RESEARCH

Induction of resistance vessel dilation by ginger root juice, possibly through extracellular signal-regulated kinase 1/2 and endothelial nitric oxide synthase activation in endothelial cells

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Abstract

Objective: To investigate the effects of ginger root juice on contractibility of resistance blood vessels from mice and on activation of extracellular signal-regulated kinase 1/2 (ERK1/2) and endothelial nitric oxide synthase (eNOS) in human endothelial cells.

Methods: Juice was prepared from semi-dry ginger roots. Mesenteric artery rings were dissected from healthy adult C57BL/J6 mice. Primary human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cords of normal full-term babies. The contractibility of the dissected vessel rings in the presence or absence of ginger root juice at 0.1% (v/v) after potassium (100 mM KCL) stimulation was measured by wire myography. The phosphorylation levels of ERK1/2 and eNOS in the presence of ginger root juice in the culture medium at 0, 0.025%, 0.05%, 0.1%, and 0.2% (v/v) in HUVECs were assessed by western blotting analysis.

Results: An immediate sharp increase in the contractile activity was observed in mesenteric artery rings in response to KCL stimulation. Ginger root juice effectively attenuated the KCL-mediated vessel contraction. Moreover, ginger root juice significantly increased phosphorylation of ERK1/2 and eNOS in a dose-dependent manner.

Conclusions: Ginger root juice is capable of relaxing resistance blood vessels. Activation of ERK1/2 and eNOS through phosphorylation in endothelial cells may be a mechanism underlying the vasodilator activity of ginger root.

Keywords: Ginger, Resistance blood vessels, Vasodilation, ERK1/2, eNOS, Phosphorylation, Myography, Endothelial cell

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Introduction

Herbal medicine is being increasingly used in primary health care throughout the world [1–4]. Ginger (*Zingiber officinale*), a perennial plant originally native to China and India but now cultivated widely in almost all tropical and subtropical continents, is one of the most important medicinal plants. The underground stem of ginger, commonly known as ginger root or rhizome, has been used in herbal medicine for thousands of years [5]. Ginger root has been documented to have anti-inflammatory, anti-emetic, and gastroprotective properties [6]. In addition, ginger root also possesses vasodilator activity; crude ginger root extracts have been demonstrated

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to reduce arterial blood pressure in a dose-dependent manner in animal studies [7, 8]. Nevertheless, more definitive evidence is needed to establish a direct vasodilator effect for ginger root. Moreover, the cellular and molecular mechanisms underlying the blood pressure-lowering activity of ginger root have yet to be elucidated.

The vascular endothelium, a continuous monolayer of cells lining the vessel lumen, is known to play a critical role in the regulation of vascular smooth muscle relaxation [9, 10]. Nitric oxide (NO) [11], originally known as "endothelium-derived relaxing factor" (EDRF) at the time of its discovery [12], is one of the best-known mediators of endothelium-dependent vasodilation [13, 14]. As a small diatomic gas, NO is synthesized from substrates (L-arginine and molecular oxygen) by the following three NO synthases in mammals: nNOS (neuronal or type I NOS); iNOS (inducible or type II NOS); and eNOS (endothelial or type III NOS) [15]. eNOS is the major enzyme responsible for NO production in the endothelium. Although eNOS was initially regarded as a constitutive enzyme, it is now generally accepted that eNOS expression is also subject to regulation at the transcriptional and post-transcriptional levels [16, 17]. Recent studies have demonstrated that post-transcriptional modification of eNOS involves various mitogen-activated protein kinase (MAPK)-dependent signaling pathways [18, 19]. However, to the best of our knowledge, no information is currently available regarding the effect of ginger root on eNOS activity in endothelial cells.

We hypothesized that ginger root may relax artery vessels through activation of extracellular signal-regulated kinase 1/2 (ERK1/2) and eNOS in endothelial cells. The present study was conducted to test this hypothesis by myographic analyses of mesenteric arteries from mice and *in vitro* laboratory assays using human endothelial cells.

Materials and methods

Ethical statements

This study involved the use of human placentas and laboratory animals. One protocol for the use of mice was approved by the Animal Care and Use Committee of the University of Wisconsin-Madison after a full review. Another protocol for the use of human placenta, classified as clinically discarded specimens without identifiable information, was approved by the Institutional Review Board of the University of Wisconsin-Madison and Meriter Hospital after an expedited review.

Ginger root juice preparation

Semi-dried ginger roots were obtained from a local grocery store. The skin was peeled off, and the ginger roots were then chopped into small slices, from which fresh juice was squeezed out and collected. The juice prepared from different batches of ginger roots was 'semi-standardized' based on the optical density at a wavelength of 400 nm using a spectrophotometer.

Wire myography

Healthy adult C57BL/J6 mice from the Jackson Laboratory (Bar Harbor, ME, USA) were sacrificed by CO_2 euthanasia. Rings of mesenteric arteries (150–200 μm in diameter) were dissected under a stereomicroscope and mounted onto the jaws of a 4-chamber wire myograph (Model 610M; Danish Myo Technology-USA, Inc., Ann Arbor, MI, USA) using a 25-μm tungsten wire. After equilibration in a physiologic saline solution ([PSS] 118.99 mM NaCl, 4.69 mM KCl, 1.17 mM $MgSO_4$, and 1.18 mM KH_2PO_4), pre-heated to 30°C and aerated with a mixture of 95% O_2 and 5% CO_2, passive tension was adjusted to optimize the detection of active force using the DMT normalization module. The vasoreactivity of the rings in response to 100 mM KCl was recorded. After washout and a 30-min recovery, the changes in the tension of the vessel rings were recorded for 1 h, during which 0.1% ginger root juice was added 5 min after KCl stimulation.

Preparation of primary umbilical vein endothelial cell cultures

Normal, full-term placentas were obtained within 30 min after vaginal or cesarean delivery from the Birthing Center at Meriter Hospital in Madison, Wisconsin. Umbilical cords were collected and transported in cold HBSS with antibiotics on ice to the laboratory. Human umbilical vein endothelial cells (HUVECs) were isolated by collagenase infusion following a well-established method [20]. For maintenance, isolated HUVECs were cultured in RPMI-1640 (Clontech Laboratories Inc., Mountain View, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Clontech Laboratories

Inc.), 0.375 mg/mL endothelial cell growth supplement (BD Biosciences, San Jose, CA, USA), 0.1 mg/mL heparin (Sigma, St. Louis, MO, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin, and cultured at 37°C in a 5% CO_2 humidified atmosphere.

Western blotting analysis

HUVECs were plated at 2.5×10^6 cells/dish in 10-cm dishes in RPMI-1640 supplemented with 10% FBS and endothelial cell growth supplement. At ~90% confluence, cells were serum-starved in FBS-free RPMI-1640 supplemented with 1% bovine serum albumin (BSA) for 16 h. The cells were then cultured in serum-free RPMI containing 0, 0.025%, 0.05%, 0.1%, and 0.2% ginger root juice for 15 min. An additional group was included in which the cells were cultured in 0.1% ginger juice in the presence of 20 µm PD98059 (Cell Signaling Technology, Danvers, MA, USA). Whole cell lysates were prepared and analyzed to detect levels of phosphorylated eNOS and phosphorylated ERK1/2, and subsequently for levels of total eNOS and total ERK1/2 by western blotting analyses. The density ratios of the phosphorylated protein bands over the total protein bands were calculated and analyzed. Antibodies against human total ERK1/2 (i.e., p44/p42 MAPK) and phospho-ERK1/2 (i.e., phosphor-p44/p42 MAPK at Thr202/Tyr204) were purchased from Cell Signaling Technology. Antibodies against human total eNOS and phosphor-eNOS at Ser1179 were purchased from Transduction Laboratories (Lexington, KY, USA).

Results

Ginger root juice-induced relaxation of mesenteric arteries

Fig. 1 shows the myographic recordings of contractile forces in an isolated mouse mesenteric artery ring in a representative experiment. Within seconds of stimulation of the ring with 100 mM KCL, the recorded tension increased robustly and sharply, and this increase was sustained during the entire test period of 60 min (only the first 15 min of recording is shown in Fig. 1A). When ginger root juice was added to the myograph chamber at a dose of 0.1% (v/v) 5 min after KCL stimulation, the tension of the ring decreased instantly and continuously in a time-dependent manner (Fig. 1B).

Effect of ginger root juice on eNOS phosphorylation in HUVECs

Fig. 2 shows the effect of ginger root juice on eNOS phosphorylation in endothelial cells, based on western blotting analysis. Fifteen minutes after treatment with ginger root juice, the ratio of phosphorylated eNOS over total eNOS protein in primary HUVECs increased in a dose-dependent manner ($P<0.05$). PD98059, a highly selective inhibitor of ERK signaling-specific pathway, effectively attenuated the increase in eNOS phosphorylation induced by ginger root juice at a dose of 0.1% ($P<0.05$).

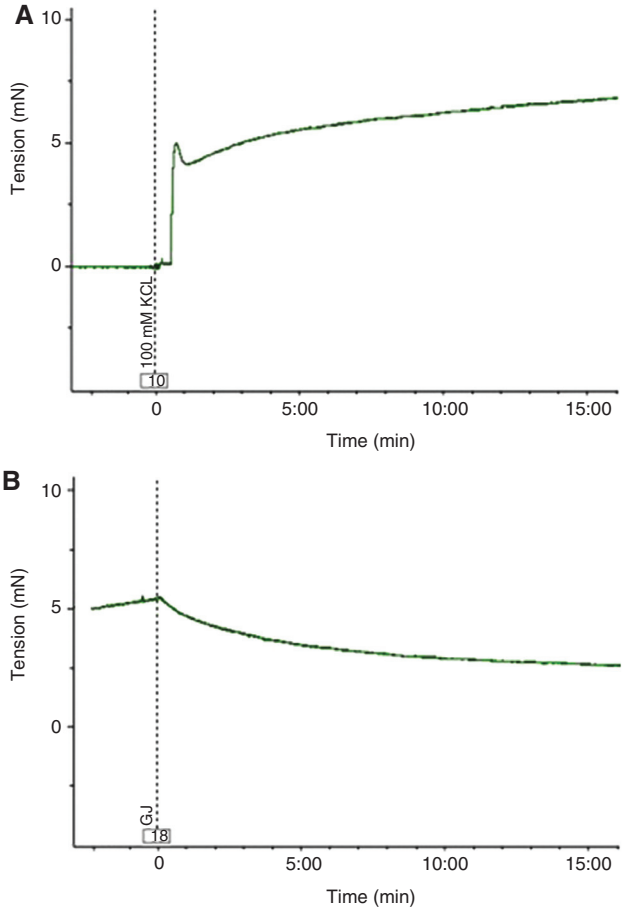

Fig. 1. Myographic recordings of isometric contractile force in isolated mouse mesenteric artery rings contracted by 100 mM KCL in the absence (A) or presence of ginger root juice (0.1%, B). The passive tension before the recording was calibrated to zero.

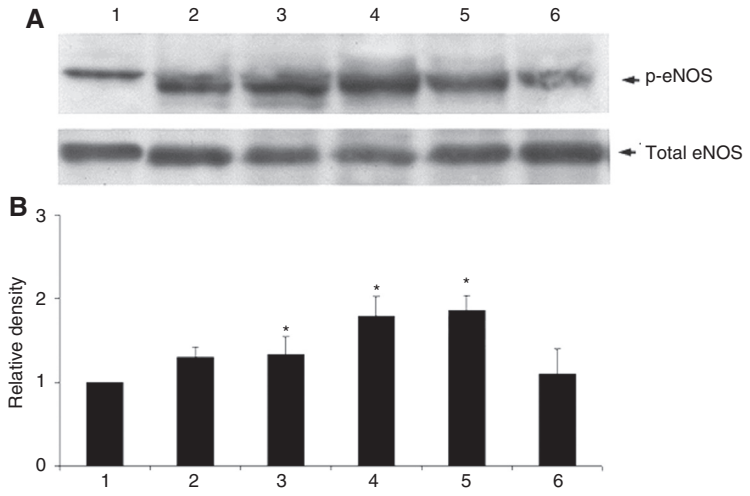

Fig. 2. Changes in phosphorylation of eNOS at serine 1177 in HUVECs. (A) Representative western blot showing phosphorylated and total eNOS protein bands. (B) Bar graph showing fold changes in the adjusted level of eNOS/S1177 in HUVECs treated for 15 min with 0.025% (2), 0.05% (3), 0.1% (4), or 0.2% (5) ginger juice or 0.1% ginger juice + 20 μM PD 98059 (6) compared with control (arbitrarily defined as 1) (n=3, *P<0.05).

Effect of ginger root juice on ERK1/2 phosphorylation in HUVECs

The effect of ginger root juice on phosphorylation of ERK1/2 in endothelial cells was valuated by western blotting analysis. As shown in Fig. 3, ginger root juice also increased the ratio of phosphorylated ERK1/2 over total ERK1/2 protein in primary HUVECs in a dose-dependent manner. This increase was significantly attenuated by PD98059 (P<0.05).

Discussion

Hypertension is the most common medical disorder diagnosed in general practice worldwide [21]. Hypertension poses

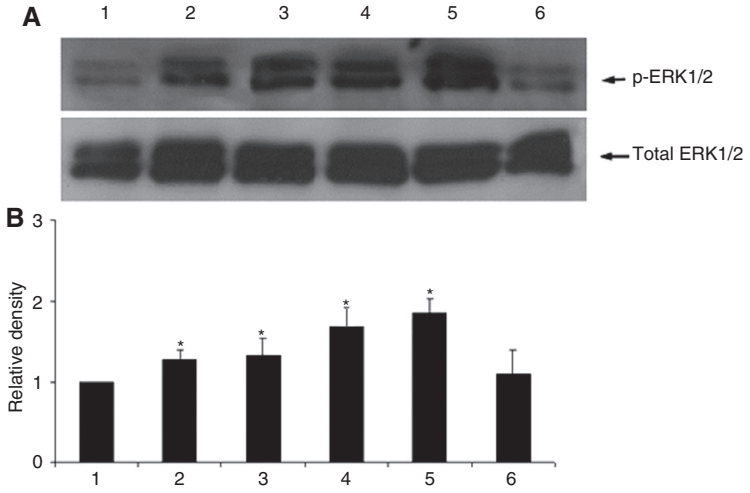

Fig. 3. Changes in ERK1/2 phosphorylation in HUVECs. (A) Representative western blot, showing phosphorylated and total ERK1/2 protein bands. (B) Bar graph showing fold changes in the adjusted level of ERK1/2 phosphorylation in HUVECs treated for 15 min with 0.025% (2), 0.05% (3), 0.1% (4), or 0.2% (5) ginger juice or 0.1% ginger juice + 20 μM PD 98059 (6) compared with the control (arbitrarily defined as 1) (n=3, *P<0.05).

a significant management challenge in primary care settings [22], particularly in developing countries [23]. As an initial effort towards developing novel alternative strategies to manage hypertension, we first evaluated the potential vasodilator activity of ginger root derivatives by wire myography, a technique commonly used to measure active and passive contractile activities under isometric conditions *in vitro* [24]. Given that proximal resistance vessels contribute substantially to peripheral resistance, and the subsequent development of hypertension, and that mesenteric arteries between 100 and 400 μm in diameter are typical proximal resistance vessels, we used mouse mesenteric arteries for wire myographic analyses. The results clearly demonstrated that freshly-prepared ginger root juice effectively relaxed mouse mesenteric arteries pre-contracted by KCL (Fig. 1).

It has been well-established that endothelium-dependent vasodilation is mainly attributed to NO synthesized through an enzymatic reaction catalyzed by NOS [25]. There is increasing evidence to indicate that the enzyme activity of NOS can be modified through post-translational phosphorylation or dephosphorylation [26]. To elucidate the molecular mechanisms underlying the vasodilator activity of ginger roots, we also evaluated the effect of freshly-prepared ginger root juice on the phosphorylation of eNOS in HUVECs, which are widely used as a model of endothelium in vascular research *in vitro*. We demonstrated that freshly-prepared ginger root juice effectively increased the phosphorylation of eNOS at ser1177 (Fig. 2) in a dose-dependent manner. To our knowledge, this observation provides the first experimental evidence of an effect of ginger root on eNOS activation in endothelial cells.

Various MAPKs have been implicated in NOS phosphorylation. To study the possible involvement of MAPK signaling pathway(s) in ginger-induced eNOS activation, we determined changes in ERK1/2 phosphorylation in primary HUVECs after treatment with various doses of ginger root juice. In a pattern similar to that for eNOS phosphorylation, ERK1/2 phosphorylation increased significantly in response to ginger root juice in a dose-dependent manner (Fig. 3). This observation is in agreement with previous findings on the involvement of MAPKs in eNOS activation reviewed by But and Sultan [27].

We investigated the involvement of ERK1/2 activation in ginger root-induced elevation in eNOS phosphorylation in endothelial cells using the ERK1/2 signaling pathway-specific inhibitor PD98059. PD98059 significantly attenuated ginger root juice-induced increases in phosphorylation of eNOS and ERK1/2. Given that PD98059 has no direct effect on eNOS phosphorylation, our observations strongly suggest that the effect of ginger root on eNOS phosphorylation must be achieved through an ERK-dependent signaling pathway.

In summary, the results of this preliminary study demonstrated that ginger root may dilate resistance blood vessels through ERK1/2 signaling-dependent activation of eNOS in endothelial cells. Our findings suggest the possibility of developing a novel ginger root-based treatment for hypertensive disorders. Such a novel therapy would be of a particular significance for patients with the pregnancy-specific hypertensive disorder pre-eclampsia. Pre-eclampsia is characterized by elevated blood pressure, proteinuria and, in its severe form, edema with eclamptic seizures and coma in the third trimester of gestation [28], and affects 3–5% of human pregnancies. It is one of the major reasons for induced preterm delivery and the leading cause of maternal and perinatal morbidity and mortality [29], thereby representing a common and significant obstetric problem worldwide. Although numerous synthetic anti-hypertensive drugs have been developed over the last few decades, few can be safely used for the benefit of pre-eclamptic women, without any potential risk to the fetus. As shown in a population-based retrospective cohort study, only a small proportion (0.65%) of 1,964 pregnant women who had chronic hypertension were exposed to at least one conventional anti-hypertensive drugs, and these patients had significantly higher rates of intrauterine growth restriction (7.2% vs. 2.1%, respectively; adjusted odds ratio [OR], 4.37; 95% confidence interval [CI], 3.00–6.36; $P<0.001$), small for gestational age (3% vs. 1.7%, respectively; adjusted OR, 2.23; 95% CI, 1.27–3.92; $P=0.005$), and preterm deliveries (<37 weeks, 22.9% vs. 8.0%, respectively; adjusted OR, 3.69; 95% CI, 2.90–4.69; $P<0.001$), compared with those who were not exposed to anti-hypertensive drugs [30].

Conflict of interest

The authors declare no conflict of interests.

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