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INHIBITION OF CYCLIC STRAIN-INDUCED ENDOTHELIN-1 SECRETION BY TETRAMETHYLPYRAZINE

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SUMMARY

1. *Chuanxiong* is a Chinese herb that has been used widely in China to treat vascular disorders. 2,3,5,6-Tetramethylpyrazine (TMP) is one of the major components purified from *chuanxiong*. Many studies have demonstrated that TMP is effective in the treatment of cardiovascular diseases. However, the mechanism of action by which TMP exerts relaxation in vascular vessels remains unclear.

2. Endothelin (ET)-1 is a potent vasopressor synthesised by endothelial cells both in culture and *in vivo*. The aims of the present study were to test the hypothesis that TMP may alter strain-induced ET-1 secretion and to identify the putative underlying signalling pathways in endothelial cells.

3. We showed that TMP inhibits strain-induced ET-1 secretion. 2,3,5,6-Tetramethylpyrazine also inhibits the strain-induced formation of reactive oxygen species (ROS) and phosphorylation of extracellular signal-regulated kinases (ERK) 1/2. Furthermore, pretreating cells with TMP or the anti-oxidant *N*-acetyl-cysteine decreased strain-induced increases in ET-1 secretion and ERK1/2 phosphorylation. Using a reporter gene assay, TMP and *N*-acetyl-cysteine were demonstrated to also attenuate the strain-induced activity of the activator protein-1 reporter.

4. In summary, we have demonstrated, for the first time, that TMP inhibits strain-induced ET-1 gene expression, in part by interfering with the ERK1/2 pathway via attenuation of ROS formation. Thus, the present study provides important new insights into the molecular pathways that may contribute to the proposed beneficial effects of TMP in the vascular system.

Key words: endothelial cells, endothelin-1, extracellular signal-regulated kinases 1/ 2, reactive oxygen species, strain, tetramethylpyrazine.

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INTRODUCTION

Chuanxiong is a Chinese herb that has been used widely in China to treat vascular disorders. 2,3,5,6-Tetramethylpyrazine (TMP) is one of the major components purified from *chuanxiong*. Many studies have demonstrated that TMP is effective in the treatment of cardiovascular diseases. 2,3,5,6-Tetramethylpyrazine has been shown to increase coronary blood flow in dogs[1] and to serve as a powerful vasodilator.[2,3] In addition, TMP was demonstrated to produce antihypertensive effects in rats with portal hypertension.[4] Direct relaxation of vessels by TMP has been demonstrated in vascular tissue and smooth muscle cells.[2,5,6] However, the mechanism of action by which TMP exerts relaxation in vascular vessels remains unclear.

Among the earliest indications of vascular dysfunction in atherosclerosis is an impaired regulation of vasomotion, representing disturbed homeostasis of vascular cells.[7] Key regulators of vasomotor function are the vasodilator nitric oxide and the vasoconstrictor endothelin (ET)-1. Among the endogenous mediators of cardiovascular disorders, ET-1, a 21 amino acid peptide, is a primary antecedent in coronary heart disease.[8–11] Such effects are mediated by extremely potent vasopressors and mitogenic responses to ET-1 in the vasculature.[12,13] Results from preclinical studies in humans, as well as from animals studies, have shown that plasma ET-1 levels are consistently elevated in many spasm-related cardiovascular diseases[13,14] and that antagonists of ET receptors can substantially alleviate complications of such diseases.[14,15]

Endothelin-1 was originally isolated from a culture of porcine endothelial cells.[15] Endothelial cells are constantly under the influence of mechanical forces, including cyclic strain, as a consequence of vessel contraction and relaxation. Recently, numerous studies have shown that oxidative stress, represented by reactive oxygen species (ROS), is capable of significantly altering vascular function.[16–18] Thus, oxidative stress appears to be causally linked to the pathogenesis of atherosclerosis.[16–18] Previous studies have demonstrated that intracellular ROS levels are elevated in endothelial cells after cyclic strain treatment.[19,20] One previous study demonstrated that intracellular ROS mediate cyclic strain-induced ET-1 expression via the Ras/Raf/extracellular signal-regulated kinases (ERK) 1/2 signalling pathway.[21] However, no studies exist that address the effects of TMP on ET-1 gene

induction in vascular endothelial cells. The aim of the present study was to investigate the effect of TMP on strain-induced ET-1 gene expression and to identify signalling protein kinase cascades that may be responsible for the putative effect of TMP.

METHODS

Materials

Imubind ET-1 ELISA kits were purchased from Amersham-Pharmacia (Amersham, UK). 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) was obtained from Serva (Heidelberg, Germany). Hydrogen peroxide (H_2O_2) was purchased from Acros Organics (Pittsburgh, PA, USA). 2,3,5,6-Tetramethylpyrazine was purchased from Aldrich (Milwaukee, WI, USA). *N*-acetyl-cysteine (NAC) and all other chemicals of reagent grade were obtained from Sigma (St Louis, MO, USA).

Endothelial cell culture

Human umbilical vein endothelial cells (HUVEC) were isolated from human umbilical cords, as described previously.[21] All procedures involving human samples were conducted according to the Guidelines for Animal and Human Experimentation of Taipei Medical University. The transformed human cell line ECV304 (ATCC CRL-1998) was purchased from the American Type Culture Collection (Bethesda, MD, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, penicillin (100 U/mL) and streptomycin (100 μg/mL) before subculture.

In vitro cyclic strain of cultured endothelial cells

Endothelial cells cultured on a flexible membrane base were subjected to cyclic strain produced by computer-controlled application of sinusoidal negative pressure, as described previously.[21]

Measurement of ET-1 concentrations

Endothelin-1 levels were measured in the culture medium using a commercial ELISA kit. Results were normalized to cellular protein content in all experiments and are expressed as a percentage relative to cells incubated with vehicle (dimethylsulphoxide).

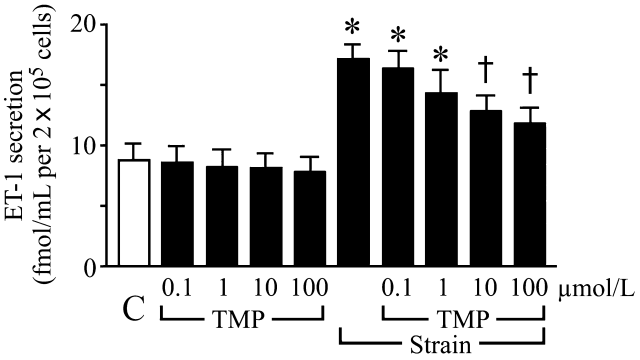

Fig. 1 2,3,5,6-Tetramethylpyrazine (TMP) inhibits strain-induced endothelin (ET)-1 secretion. Human umbilical vein endothelial cells were pretreated with TMP (0.1–100 μmol/L) 30 min prior to strain treatment. After strain (20%) for 24 h, the culture media were collected and the concentrations of ET-1 analysed by enzyme immunoassay. Results are the mean±SEM ($n = 6$). *$P < 0.05$ compared with unstrained control cells; †$P < 0.05$ compared with strained cells (ANOVA). C, control.

Detection of intracellular ROS

Measurement of intracellular ROS formation in HUVEC was recorded by monitoring changes in diclorofluorescein (DCF) fluorescence, as described previously.[21] A chemiluminescence assay of superoxide production was performed, as described previously.[19]

Western blot analysis

Rabbit polyclonal anti-phospho-specific ERK1/2 antibody was purchased from New England Biolabs (Beverly, MA, USA). The anti-ERK antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Western blot analysis was performed as described previously.[21]

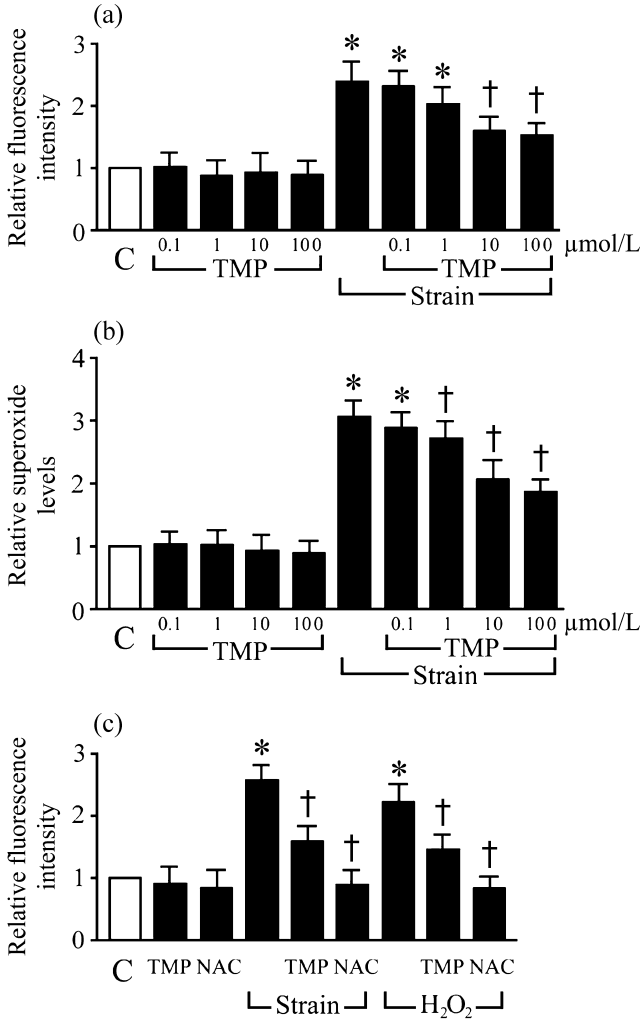

Fig. 2 Effects of 2,3,5,6-tetramethylpyrazine (TMP) on strain-increased reactive oxygen species (ROS) formation. (a) Effect of TMP (0.1–100 μmol/L) on strain-induced ROS generation. Strain-induced increases in intracellular ROS were revealed by fluorescent intensities of 2′,7′-dichlorofluorescin (DCF). (b) Effect of TMP (0.1–100 μmol/L) on strain-induced superoxide formation. Human umbilical vein endothelial cells (HUVEC), after cyclic strain, were lysed, which was followed immediately by the superoxide assay using the lucigenin method. (c) Data for control (C) HUVEC or those treated with cyclic strain or H_2O_2 (25 μmol/L) in the presence of 100 μmol/L TMP or 10 mmol/L *N*-acetyl-cysteine (NAC) for 1 h. Fluorescence intensities of cells are shown as the relative intensity of experimental groups compared with untreated control cells. Data are the mean±SEM ($n = 6$). *$P < 0.05$ compared with control; †$P < 0.05$ compared with strain (or H_2O_2)-treated cells (ANOVA).

Luciferase assay

The ECV304 cells, plated on 3 cm diameter culture dishes, were transfected with the luciferase reporter construct possessing consensus activator protein-1 (AP-1) binding sites (AP-1-Luc; Stratgene, La Jolla, CA, USA) using the calcium phosphate method, as described previously.[21] After incubation for 24 h in 2% serum DMEM, ECV304 were cultured under different treatments with TMP and NAC for 48 h. The ECV304 cells were assayed for luciferase activity with a luciferase reporter assay kit (Strategene). The firefly luciferase activities at AP-1 transcriptional activity were normalized for transfection efficiency to the respective β-galactosidase activity and expressed as relative activity to control.

Lactate dehydrogenase assay

Cytotoxicity was assessed by measuring lactate dehydrogenase (LDH) in the culture medium spectrophotometrically. Lactate dehydrogenase release was standardized using a cell injury index defined as $(A - B)/(C - B) \times 100$, where A is LDH activity in the test sample, B is LDH activity measured in media with no cells (0% control) and C is LDH activity in samples from wells in which cells were lysed with Triton X-100 (100% control).

Statistical analysis

Data are presented as the mean±SEM. Statistical analysis was performed using Student's *t*-test and analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using Prism version 3.00 for Windows (GraphPad Software, San Diego, CA, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Effect of TMP on strain-induced ET-1 secretion in endothelial cells

Human umbilical vein endothelial cells cultured on flexible membrane bases were subjected to deformation to produce an average strain of 20%. Endothelin-1 released into the culture media was measured. Human umbilical vein endothelial cells under cyclic strain for 24 h demonstrated a twofold increase in the secretion of ET-1 into the culture medium (Fig. 1) compared with unstrained endothelial cells. The effect of TMP on strain-induced increases in ET-1 secretion was then investigated. As shown in Fig. 1, treatment with cyclic strain for 24 h increased ET-1 secretion, but 1–100 μmol/L TMP significantly inhibited this strain-induced increase in ET-1 secretion (Fig. 1). These data indicate that TMP inhibits strain-induced increases in ET-1 secretion by endothelial cells.

To verify that the TMP-mediated effects on vascular endothelial cells are not a consequence of cytotoxicity, we determined lactate dehydrogenase (LDH) activity as a parameter of necrosis in the supernatants of TMP (1–100 μmol/L)- and strain-treated HUVEC. Consistent with light microscopic observations, LDH activity in the supernatants of treated and untreated cells remained unchanged (data not shown).

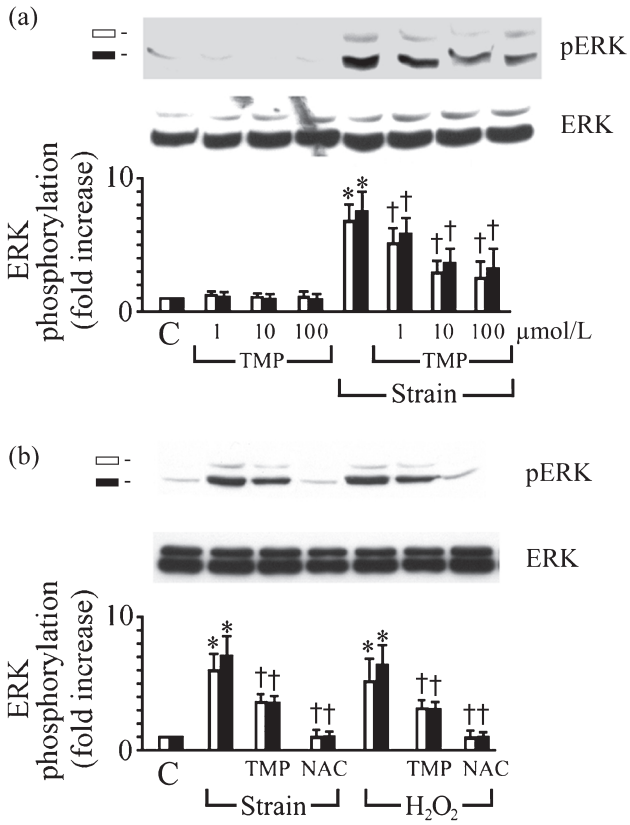

Fig. 3 Inhibitory effects of 2,3,5,6-tetramethylpyrazine (TMP) on strain-increased extracellular signal-regulated kinase (ERK)1/2 phosphorylation. (a) Effect of TMP (1–100 μmol/L) on strain-activated ERK1/2 phosphorylation. (b) Effect of TMP on strain- or H_2O_2-induced phosphorylation of ERK1/2. Human umbilical vein endothelial cells were pre-incubated with either TMP (100 μmol/L) or *N*-acetyl-cysteine (NAC; 10 mmol/L) for 30 min and stimulated with cyclic strain or H_2O_2 (25 μmol/L) for 30 min. Phosphorylation of ERK1/2 was detected by western blotting using anti-phospho-ERK1/2 antibody. Both TMP and NAC inhibited strain-induced activation of ERK1/2. Phosphorylation of ERK1/2 was detected and densitometric analyses were performed. (□), degree of ERK1 phosphorylation; (■), degree of ERK2 phosphorylation. Data are the fold increase relative to control groups and show the mean±SEM ($n = 6$). $^*P < 0.05$ compared with control; $^\dagger P < 0.05$ compared with strain (or H_2O_2) alone (ANOVA). C, control; pERK, phosphorylated ERK.

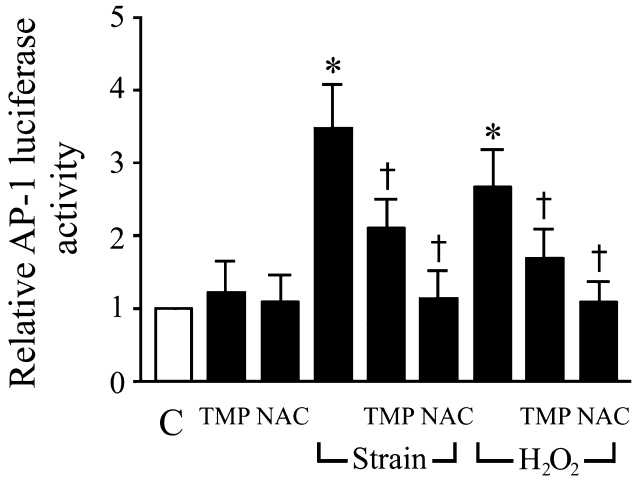

Fig. 4 2,3,5,6-Tetramethylpyrazine (TMP) attenuates the strain-stimulated activator protein-1 (AP-1) reporter activity in endothelial cells. ECV304, transfected with AP-1-Luc, were incubated for 24 h with either no drug, 100 μmol/L TMP or 10 mmol/L *N*-acetyl-cysteine (NAC) in the absence or presence of strain treatment or H_2O_2 (25 μmol/L). Data are the mean±SEM for six experiments performed in triplicate. $^*P < 0.05$ compared with untreated; $^\dagger P < 0.05$ compared with strain (or H_2O_2) alone (ANOVA). C, control.

2,3,5,6-Tetramethylpyrazine inhibits strain-increased ROS formation

Previous studies have shown that cyclic strain increases ROS formation in endothelial cells,[19,20,22] which is involved in ET-1 induction.[21] We next examined whether TMP prevents strain-increased ROS formation. Human umbilical vein endothelial cells were treated with TMP (0.1–100 μmol/L) in the absence or presence of strain treatment. The addition of TMP (1–100 μmol/L) to cultured HUVEC significantly inhibited strain-induced ROS formation measured after strain treatment for 1 h (Fig. 2a,b). Pretreatment of cultured HUVEC with TMP (100 μmol/L) or *N*-acetyl-cysteine (NAC; 10 mmol/L) also significantly inhibited strain- or H_2O_2-induced ROS formation (Fig. 2c). These findings clearly demonstrate that TMP inhibits strain-induced increases in intracellular ROS levels in endothelial cells.

2,3,5,6-Tetramethylpyrazine inhibits strain-activated ERK1/2 phosphorylation in endothelial cells

To gain an insight into the mechanism of action of TMP, we examined whether TMP affects intracellular protein kinase signalling pathways. Given that the ERK1/2 signalling pathway is involved in strain-induced ET-1 expression,[21] we investigated whether TMP inhibits the ERK1/2 pathway in strain-treated endothelial cells. We examined the phosphorylation of ERK1/2 in HUVEC exposed to TMP (1–100 μmol/L) in the absence or presence of strain treatment. As shown in Fig. 3, HUVEC exposure to strain treatment for 30 min rapidly activated the phosphorylation of ERK1/2. However, treatment of HUVEC with 100 μmol/L TMP resulted in significantly decreased strain-induced ERK1/2 phosphorylation. Moreover, treatment of HUVEC with 25 μmol/L H_2O_2 resulted in increased ERK1/2 phosphorylation (Fig. 3). Human umbilical vein endothelial cells pretreated with 100 μmol/L TMP or NAC 10 mmol/L showed significantly decreased strain- or H_2O_2-induced ERK1/2 phosphorylation. These findings imply that TMP inhibits the strain-activated ERK1/2 signalling pathway via attenuation of ROS formation in endothelial cells.

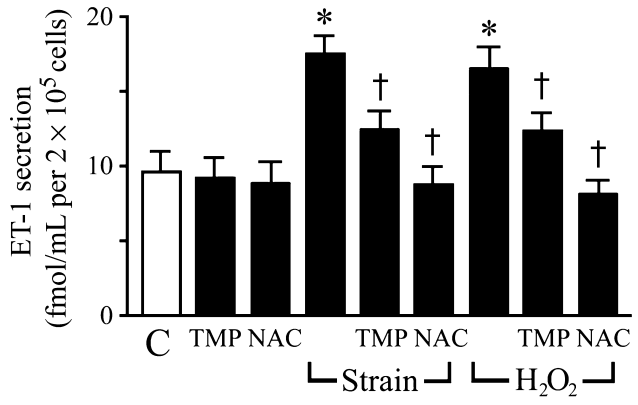

Fig. 5 2,3,5,6-Tetramethylpyrazine (TMP) modulates strain-induced endothelin (ET)-1 secretion, which is compatible with an anti-oxidant action. 2,3,5,6-Tetramethylpyrazine modulates strain (or H_2O_2)-increased ET-1 secretion. Some cells were pretreated with 10 mmol/L TMP or 100 μmol/L *N*-acetyl-cysteine (NAC) for 30 min. Endothelial cells were then treated with cyclic strain or H_2O_2 (25 μmol/L) for 24 h. Data are the mean±SEM ($n = 6$). *$P < 0.05$ compared with control; †$P < 0.05$ compared with strain (or H_2O_2) alone (ANOVA). C, control.

2,3,5,6-Tetramethylpyrazine inhibits strain-induced increases in AP-1 transcriptional activity in endothelial cells

The effect of TMP on AP-1 activation, which is involved in ET-1 gene induction,[21,23] was then evaluated. The effects of TMP on strain-induced AP-1 functional activity were assessed in a reporter gene assay. Both TMP (10 μmol/L) and NAC (10 mmol/L) significantly attenuated strain- or H_2O_2-induced AP-1 reporter activation (Fig. 4). These results indicate that TMP inhibits strain-induced increases in AP-1 transcriptional activation.

2,3,5,6-Tetramethylpyrazine inhibits strain-induced ET-1 secretion, which is compatible with an anti-oxidant action

To further determine whether TMP affects strain-induced ET-1 gene expression via attenuation of ROS formation, the effects of TMP on ET-1 gene induction were examined under cyclic strain or H_2O_2 stimulation. As shown in Fig. 5, neither TMP nor NAC alone had any effect on basal ET-1 secretion. However, HUVEC treated with either strain or H_2O_2 (25 μmol/L) for 24 h demonstrated significantly increased ET-1 secretion (Fig. 5). In the presence of TMP (or NAC), both strain- or H_2O_2-induced increases in ET-1 secretion were significantly inhibited. These data imply that TMP affects strain-induced ET-1 gene expression, which is compatible with an anti-oxidant action in endothelial cells.

DISCUSSION

The major new finding of the present study is that TMP inhibits strain-induced ET-1 secretion in endothelial cells. This is supported by the observations that TMP inhibits strain-induced ET-1 protein secretion by, in part, attenuation of ROS formation in endothelial cells. Previous studies have indicated that haemodynamic forces, including shear flow[24] and pressure-induced strain,[21,25] can stimulate ET-1 gene expression. Recent studies provide evidence that ROS may act as second messengers in cells exposed to various stimuli.[21,23] Previous studies have shown that cyclic strain treatment of endothelial cells can induce intracellular ROS generation.[19,20,26] This ROS generation is sustained at an elevated level as long as the mechanical forces are maintained and returns to basal levels following the removal of the mechanical forces. Elevated ROS levels are involved in the release of ET-1[21] and this gene induction can be attenuated by anti-oxidant pretreatment of cells.

Zhang *et al*. reported that TMP scavenges ROS generation in human polymorphonuclear leucocytes and cultured neurons.[27,28] The results of the present study further demonstrated that TMP reduced strain-induced ROS generation in the endothelial cells. In particular, it has been demonstrated that the activation of ERK1/2 is redox sensitive[21,29,30] and that suppression of ROS inhibits strain-induced ET-1 gene expression.[21] Thus, one possible explanation for the inhibitory effect of TMP on strain-induced ET-1 gene expression may be its ability to attenuate ROS formation. In a previous study, deletion mapping revealed constructs containing 143 bp of the ET-1 5′-flanking sequence allowing strain-induced transcrip-

tion and the presence of responsive elements for ROS- or strain-induced ET-1 expression located within 143 bp upstream of the transcription initiation site.[21] Recently, we also found that activation of AP-1 is redox sensitive and may play a key role in ET-1 gene induction.[23] The results of the present study indicate that TMP inhibits strain-induced AP-1 reporter activity. The inhibitory effect of TMP on strain-induced AP-1 transcriptional activation suggests that the attenuation of strain-induced ROS by TMP leads to inhibition of AP-1.

In conclusion, the data of the present study suggest that the TMP-induced suppression of cyclic strain-induced ET-1 expression can be considered as one of the mechanisms responsible for the protective effect of TMP in vascular vessels. The effects of TMP on endothelial cells observed in the present study (i.e. inhibition of ET-1 secretion, suppression of ROS formation and inhibition of ERK1/2 phosphorylation) all are compatible with its putative vasoprotective effect.

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REFERENCES

1. Dai XZ, Bache RJ. Coronary and systemic hemodynamic effects of tetramethylpyrazine in the dog. *J. Cardiovasc. Pharmacol.* 1985; **7**: 841–9.
2. Wu CC, Chiou WF, Yen MH. A possible mechanism of action of tetramethylpyrazine on vascular smooth muscle in rat aorta. *Eur. J. Pharmacol.* 1989; **169**: 189–95.
3. Cai YN, Barer GR. Effect of ligustrazine on pulmonary vascular changes induced by chronic hypoxia in rats. *Clin. Sci.* 1989; **77**: 515–20.
4. Chang FC, Chen KJ, Lin JG, Hong CY, Huang YT. Effects of tetramethylpyrazine on portal hypertensive rats. *J. Pharm. Pharmacol.* 1998; **50**: 881–4.
5. Kwan CY. Plant-derived drugs acting on cellular Ca^{2+} mobilization in vascular smooth muscle: Tetramethylpyrazine and tetrandrine. *Stem Cells* 1994; **12**: 64–7.
6. Pang PK, Shan JJ, Chiu KW. Tetramethylpyrazine, a calcium antagonist. *Planta Med.* 1996; **62**: 431–5.
7. Gielen S, Schuler G, Hambrecht R. Exercise training in coronary artery disease and coronary vasomotion. *Circulation* 2001; **103** (Suppl.): E1–6.
8. Schiffrin EL. Role of endothelin-1 in hypertension and vascular disease. *Am. J. Hypertens.* 2001; **14** (Suppl.): S83–9.
9. Wenzel RR, Czyborra P, Luscher T, Philipp T. Endothelin in cardiovascular control: The role of endothelin antagonists. *Curr. Hypertens. Rep.* 1999; **1**: 79–87.
10. Mundhenke M, Schwartzkopff B, Kostering M, Deska U, Klein RM, Strauer BE. Endogenous plasma endothelin concentrations and coronary circulation in patients with mild dilated cardiomyopathy. *Heart* 1999; **81**: 278–84.
11. Schiffrin EL, Touyz RM. Vascular biology of endothelin. *J. Cardiovasc. Pharmacol.* 1998; **32** (Suppl. 3): S2–13.
12. Tonnessen T, Giaid A, Saleh D, Naess PA, Yanagisawa M, Christensen G. Increased *in vivo* expression and production of endothelin-1 by porcine cardiomyocytes subjected to ischemia. *Circ. Res.* 1995; **76**: 767–72.
13. Hoffmann E, Assennato P, Donatelli M, Colletti I, Valenti TM. Plasma endothelin-1 levels in patients with angina pectoris and normal coronary angiograms. *Am. Heart J.* 1998; **135**: 684–8.
14. Pernow J, Wang QD. Endothelin in myocardial ischaemia and reperfusion. *Cardiovasc. Res.* 1997; **33**: 518–26.
15. Webb DJ, Monge JC, Rabelink TJ, Yanagisawa M. Endothelin: New discoveries and rapid progress in the clinic. *Trends Pharmacol. Sci.* 1998; **19**: 5–8.
16. Witztum JL. The oxidation hypothesis of atherosclerosis. *Lancet* 1994; **344**: 793–5.
17. Drexler H. Endothelial dysfunction: Clinical implications. *Prog. Cardiovasc. Dis.* 1997; **39**: 287–324.
18. Griendling KK, Alexander RW. Oxidative stress and cardiovascular disease. *Circulation* 1997; **96**: 3264–5.
19. Wung BS, Cheng JJ, Hsieh HJ, Shy YJ, Wang DL. Cyclic strain-induced monocyte chemotactic protein-1 gene expression in endothelial cells involves reactive oxygen species activation of activator protein 1. *Circ. Res.* 1997; **81**: 1–7.
20. Cheng JJ, Wung BS, Chao YJ, Wang DL. Cyclic strain-induced reactive oxygen species involved in ICAM-1 gene induction in endothelial cells. *Hypertension* 1998; **31**: 125–30.
21. Cheng TH, Shih NL, Chen SY *et al*. Reactive oxygen species mediate cyclic strain-induced endothelin-1 gene expression via Ras/Raf/extracellular signal-regulated kinase pathway in endothelial cells. *J. Mol. Cell. Cardiol.* 2001; **33**: 1805–14.
22. Cheng JJ, Chao YJ, Wang DL. Cyclic strain activates redox-sensitive proline-rich tyrosine kinase 2 (PYK2) in endothelial cells. *J. Biol. Chem.* 2002; **277**: 48 152–7.
23. Cheng CM, Hong HJ, Liu JC *et al*. Crucial role of extracellular signal-regulated kinase pathway in reactive oxygen species-mediated endothelin-1 gene expression induced by endothelin-1 in rat cardiac fibroblasts. *Mol. Pharmacol.* 2003; **63**: 1002–11.
24. Ziegler T, Bouzourene K, Harrison VJ, Brunner HR, Hayoz D. Influence of oscillatory and unidirectional flow environments on the expression of endothelin and nitric oxide synthase in cultured endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 1998; **18**: 686–92.
25. Wang DL, Wung BS, Peng YC, Wang JJ. Mechanical strain increases endothelin-1 gene expression via protein kinase C pathway in human endothelial cells. *J. Cell. Physiol.* 1995; **163**: 400–6.
26. Matsushita H, Lee Kh K, Tsao PS. Cyclic strain induces reactive oxygen species production via an endothelial NAD(P)H oxidase. *J. Cell. Biochem.* 2001; **81**: 99–106.
27. Zhang Z, Wei T, Hou J, Li G, Yu S, Xin W. Tetramethylpyrazine scavenges superoxide anion and decreases nitric oxide production in human polymorphonuclear leukocytes. *Life Sci.* 2003; **72**: 2465–72.
28. Zhang Z, Wei T, Hou J, Li G, Yu S, Xin W. Iron-induced oxidative damage and apoptosis in cerebellar granule cells. Attenuation by tetramethylpyrazine and ferulic acid. *Eur. J. Pharmacol.* 2003; **467**: 41–7.
29. Sano M, Fukuda K, Sato T *et al*. ERK and p38 MAPK, but not NF-kappaB, are critically involved in reactive oxygen species-mediated induction of IL-6 by angiotensin II in cardiac fibroblasts. *Circ. Res.* 2001; **89**: 661–9.
30. Tanaka K, Honda M, Takabatake T. Redox regulation of MAPK pathways and cardiac hypertrophy in adult rat cardiac myocyte. *J. Am. Coll. Cardiol.* 2001; **37**: 676–85.