Tetramethylpyrazine Inhibits Angiotensin II-Increased NAD(P)H Oxidase Activity and Subsequent Proliferation in Rat Aortic Smooth Muscle Cells

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Abstract: Tetramethylpyrazine (TMP) is the major component extracted from the Chinese herb, Chuanxiong, which is widely used in China for the treatment of cardiovascular problems. The aims of this study were to examine whether TMP may alter angiotensin II (Ang II)-induced proliferation and to identify the putative underlying signaling pathways in rat aortic smooth muscle cells. Cultured rat aortic smooth muscle cells were preincubated with TMP and then stimulated with Ang II, [³H]-thymidine incorporation and the ET-1 expression was examined. Ang II increased DNA synthesis which was inhibited by TMP (1–100 µM). TMP inhibited the Ang II-induced ET-1 mRNA levels and ET-1 secretion. TMP also inhibited Ang II-increased NAD(P)H oxidase activity, intracellular reactive oxygen species (ROS) levels, and the ERK phosphorylation. Furthermore, TMP and antioxidants such as Trolox and diphenylene iodonium decreased Ang II-induced ERK phosphorylation, and activator protein-1 reporter activity. In summary, we demonstrate for the first time that TMP inhibits Ang II-induced proliferation and ET-1, partially by interfering with the ERK pathway via attenuation of Ang II-increased NAD(P)H oxidase and ROS generation. Thus, this study delivers important new
insight in the molecular pathways that may contribute to the proposed beneficial effects of TMP in cardiovascular disease.

**Keywords:** Endothelin-1; Tetramethylpyrazine; Angiotensin II; Smooth Muscle Cells; Reactive Oxygen Species; Extracellular Signal-Regulated Kinase.

**Introduction**

There is increasing evidence that the rennin-angiotensin system may contribute to the pathogenesis of chronic vascular disease. Angiotensin II (Ang II) is a vasoactive peptide which is an important component of the rennin-angiotensin system (Vaughan, 2000). Endothelin-1 (ET-1) was shown to mediate the growth-promoting effect of Ang II and thus play an important role in cardiovascular disease and vascular remodeling [(Hahn et al., 1990; Ito et al., 1993; Sung et al., 1994) (for review, see Ref. (Rossi et al., 1999)). Ang II has also been shown to stimulate membrane-bound NAD(P)H oxidase, which generates reactive oxygen species (ROS) in vascular smooth muscle cells (Seshiah et al., 2002; Touyz and Schiffirin, 2001). ROS including superoxide anions and hydrogen peroxide (H$_2$O$_2$) are recognized as important signaling molecules for cardiovascular tissues. Excess ROS generation is considered to be a likely initiator of atherosclerotic events, resulting in the increased synthesis of numerous mitogenic factors that contribute to the hyperproliferation of vascular smooth muscle cells and vascular plaque formation (Berliner and Heinecke, 1996). ROS may also act as second messengers that regulate various intracellular signal transduction cascades and the activity of various transcription factors such as activator protein-1 (AP-1) (Sen and Packer, 2000; Wung et al., 1997). We previously reported that ROS mediate Ang II-induced ET-1 gene induction within vascular smooth muscle cells (Hong et al., 2004).

Tetramethylpyrazine (TMP) is a biologically active ingredient isolated from the traditional herbal medicine *Ligusticum chuanxiong* Hort, which is widely used in China for the treatment of cardiovascular problems (Guo et al., 1983). Many studies have demonstrated that TMP is effective in the treatment of cardiovascular diseases. For example, studies have revealed that the coronary, pulmonary and bronchial arteries are relaxed by TMP (Dai and Bache, 1985; Liu et al., 1990; Tsai et al., 2002; Wu et al., 1989). In addition, TMP has been reported to greatly suppress the vasoconstriction produced by ET-1 and induced a significant decrease in plasma ET-1 levels in animal model (Cao et al., 1998; Zeng et al., 1998). Recently, several reports further indicate that TMP might prevent and even scavenge the intracellular ROS generation (Shih et al., 2002; Zhang et al., 2003). Although the significance of Ang II-induced ET-1 gene expression in vascular tissues is determined (Hahn et al., 1990; Moreau et al., 1997), whether TMP via attenuation of ROS generation inhibits Ang II-induced proliferation and ET-1 expression in rat aortic smooth muscle cells remains to be determined.

Our study was conducted to examine whether TMP down-regulates the Ang II-induced ET-1 expression and to identify signaling protein kinase cascades that may be responsible
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for the putative effect of TMP. In the present study, we clearly demonstrate that TMP inhibits Ang II-induced proliferation, ET-1 expression, activator protein-1 (AP-1) reporter activity and ERK phosphorylation via attenuation of Ang II-induced ROS generation in rat aortic smooth muscle cells. Thus, this study delivers an important new insight in the molecular pathways that may contribute to the proposed beneficial effects of TMP in cardiovascular disease.

Methods

Materials

Dulbecco’s modified Eagle’s medium (DMEM), fetal calf serum, and tissue culture reagents were purchased from Life Technologies, Inc. A rat ET-1 cDNA probe (accession No. M64711) was obtained as previously described (Cheng et al., 2003b). 2′,7′-Dichlorofluorescin diacetate (DCF-DA) was obtained from Molecular Probes (Eugene, OR, USA). H$_2$O$_2$ was purchased from Acros Organics (Pittsburgh, PA, USA). The plasmid AP-1-Luc containing the firefly luciferase reporter gene driven by a basic promoter element (TATA box) joined to tandem repeats of AP-1 binding element were obtained from Stratagene (La Jolla, CA, USA). Ang II and all other chemicals were purchased from Sigma (St. Louis, MO, USA). Tetramethylyrazine (TMP) was purchased from Aldrich Chemical Co. (Milwaukee, MI, USA).

Culture of Rat Aortic Smooth Muscle Cells

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and approved by the Institutional Animal Care and Use Committee of Taipei Medical University. Thoracic aortae from male Sprague-Dawley rats were excised rapidly and immersed in DMEM containing penicillin (100 U/ml) and streptomycin (100 µg/ml). Connective tissue and adherent fat were cleaned away from the specimens. Isolated arteries were cut open, and the endothelium was removed by gently rubbing off the intimal surface with a pair of sharp scissors. Denuded aortae were cut into ~3-mm pieces and placed with the intimal face down into three 35-mm culture dishes (Iwaki, Osaka, Japan). DMEM containing 10% fetal calf serum and penicillin/streptomycin was gently added to the dishes to cover the tissues without disturbing the orientation of the explants. Vascular smooth muscle cells were allowed to proliferate from the tissue (7–10 days), and the tissues were removed using sterilized fine forceps and washed with culture medium. After reaching confluence in three 35-mm dishes, cells were harvested by brief trypsinization and subsequently cultured in T-75 flasks (Iwaki) (passage 1). Cells were routinely propagated in culture dishes to 75–95% confluence, and used between passages 3–12. The purity of smooth muscle cells was evaluated by staining the cells with monoclonal antibodies to α-smooth muscle actin. Vascular smooth muscle cells were grown in DMEM without
phenol red containing antibiotics and 10% fetal calf serum until 24 hours prior to the experimentation, at which time cells were in a defined serum-free medium containing insulin (0.5 µM) and transferrin (5 mg/ml) for all experiments. Cells were then preincubated with different concentration of TMP for 30 min and then with or without Ang II (100 nM) for different incubation times as indicated, followed by harvesting. Cellular viability under all treatment conditions was determined by cell count, morphology, and trypan blue exclusion.

**DNA Synthesis**

To measure synthesis of new DNA, cells (1 × 10^5/well) were plated in six-well (35-mm) dishes 24 hours before experiments as previously described (Hong et al., 2004). Cells were incubated with [³H]-thymidine (5 µCi/ml). After addition of indicated agent, cells were harvested by incubation at 4°C with trichloroacetic acid (5%) followed by solubilization in 0.1 N NaOH, and radioactivity was determined by scintillation counting. Data are presented as the mean ± SEM of 9–12 determinations in six different cell preparations and normalized to the untreated sample × 100 (i.e. percentage of control).

**NADPH Oxidase Activity Assay**

NADPH oxidase was measured as described previously (Juan et al., 2004). In brief, cells were scraped into ice-cold phosphate-buffered saline buffer containing 1 mM EGTA and centrifuged for 10 min with 750 × g at 4°C. The pellet was resuspended in lysis buffer (20 mM potassium phosphate, 1 mM EGTA, 10 mM aprotinin, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM leupeptin, pH 7.0) and sonicated. The protein concentration was adjusted to 2 mg/ml. Total cell suspension with a volume of 250 µl was mixed with 250 µl of Hanks buffered salt solution (HBSS: 136.6 mM NaCl, 5.4 mM KCl, 4.2 mM NaHCO₃, 2.7 mM Na₂HPO₄, 1 mM CaCl₂, 0.5 mM MgCl₂, 0.44 mM KH₂PO₄, 0.41 mM MgSO₄, pH 7.8) containing 500 µM lucigenin and kept at 37°C for 10 min. NADPH oxidase activity assay was initiated by adding 10 µl of NADPH (100 µM) as a substrate. The photon emission was measured using a microplate scintillation counter (Topcount, Packard Instrument Co., Meriden, CT), and the respective background counts were subtracted. Neither the cellular fraction alone nor NADPH alone evoked any lucigenin chemiluminescence signal.

**Chemiluminescence Assay of Superoxide Production**

Superoxide was measured by lucigenin-amplified chemiluminescence (Liu et al., 2003). In brief, superoxide production by cells was measured using lucigenin-enhanced chemiluminescence in a microplate scintillation counter (Topcount, Packard Instrument Co.). A low lucigenin concentration (5 µM) was employed to minimize artifactual superoxide production owing to redox cycling. Briefly, proteins were diluted in modified
HEPES buffer (NaCl 140 mM, KCl 5 mM, MgCl₂ 0.8 mM, CaCl₂ 1.8 mM, Na₂HPO₄ 1 mM, HEPES 25 mM, and 1% glucose, pH 7.2), and distributed (100 µg/well) onto a 96-well microplate. NADPH (100 µM) and dark-adapted lucigenin were added to wells just before reading. Superoxide production was expressed as arbitrary light units over 20 min. All studies were performed in triplicate.

Assay of Intracellular ROS

ROS were measured using a previously described method (Cheng et al., 2001). Prior to the chemical or Ang II treatment, rat aortic smooth muscle cells were incubated in culture medium containing a fluorescent dye, 2',7'-dichlorofluorescin diacetate (DCF-DA) (Molecular Probes, Eugene, OR, USA) of 30 µM for 1 hour to establish a stable intracellular level of the probe. The concentration of DCF-DA was maintained during the chemical or Ang II treatment. Subsequently, the cells were washed with PBS, removed from Petri dishes by brief trypsinization, and measured for 2',7'-dichlorofluorescein (DCF) fluorescence intensity. The DCF fluorescence intensity of the cells is an index of intracellular levels of ROS; and it can be determined by fluorescence spectrophotometry with excitation and emission wavelengths at 475 and 525 nm, respectively. For counting cell numbers, cells were harvested and counted in an automatic cell counter (S.ST.II/ZM, Coulter Electronics Ltd., Miami, FL, USA). The cell number in each sample was counted and utilized to normalize the DCF fluorescence intensity.

RNA Isolation and Northern Blot Analysis

Total RNA was isolated from cells by the guanidine isothiocyanate/phenol chloroform method as previously described (Cheng et al., 2001). The RNA (10 g/lane) was separated by electrophoresis on a 1% agarose formaldehyde gel and transferred to a nylon membrane (Nyttran, Schleicher & Schuell, Inc, Germany) by a vacuum blotting system (VacuGene XL, Pharmacia, Sweden). After hybridization with the ³²P-labeled ET-1 cDNA probes, the membrane was washed with 0.1× SSC containing 1% SDS at 42°C for 30 min and then exposed to X-ray film at 70°C. Blots of specific mRNA bands were detected by autoradiography and analyzed with a densitometer (Computing Densitometer 300S, Molecular Dynamics). Blots were stripped and reprobed for 18S cDNA (obtained from American Type Culture Collection) for the control. The expression of ET-1 mRNA was quantitated and normalized to the 18S signal.

Assay of ET-1 Peptide Secretion

ET-1 levels were measured in culture medium using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Amersham-Pharmacia, Amersham, UK). Results were normalized to cellular protein content in all experiments and expressed as a percentage relative to the cells incubated with the vehicle.
Western Blot Analysis

Rabbit polyclonal anti-phospho-specific ERK antibodies were purchased from New England Biolabs (Beverly, MA, USA). Anti-ERK antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Western blot analysis was performed as previously described (Cheng et al., 2001).

Luciferase Assay

Cells plated on 6-well (35-mm) dishes were transfected with the luciferase reporter construct possessing consensus AP-1 binding sites (AP-1-Luc) (Stratagene, La Jolla, CA, USA). After incubation for 24 hours in serum-free DMEM, rat aortic smooth muscle cells were cultured under different treatments as indicated for 48 hours. Rat aortic smooth muscle cells were assayed for luciferase activity with a luciferase reporter assay kit (Stratagene). The firefly luciferase activities as AP-1 transcriptional activity were normalized for transfection efficiency to its respective β-galactosidase activity and expressed as relative activity to control.

Statistical Analysis

Results are expressed as mean ± SEM of at least 3 experiments unless designated otherwise. Statistical analysis was performed using student’s t-test and analysis of variance (ANOVA) followed by a Dunnett multiple comparison test using GraphPad Prism (GraphPad Software, San Diego, CA, USA). A value of p < 0.05 was considered to be statistically significant.

Results

Effects of Tetramethylpyrazine on Ang II-Induced Proliferation of Rat Aortic Smooth Muscle Cells

Effects of tetramethylpyrazine (TMP) on Ang II-stimulated rat aortic smooth muscle cell proliferation was assessed by analyzing DNA synthesis with [3H]-thymidine incorporation. Smooth muscle cells were preincubation with TMP for 30 min and then treated with Ang II (100 nM) for 24 hours, TMP (100 µM) significantly decreased Ang II-induced rat aortic smooth muscle cell proliferation (Fig. 1). These data indicate that TMP inhibits Ang II-induced proliferation in rat aortic smooth muscle cells.

Effects of Tetramethylpyrazine on Ang II-induced ET-1 Expression in Rat Aortic Smooth Muscle Cells

To examine whether TMP inhibits Ang II-increased ET-1 mRNA levels in rat aortic smooth muscle cells, we performed Northern blot analysis (Fig. 2A). Rat aortic smooth muscle
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cells were preincubated with TMP (1–100 µM) for 30 min and then treated with Ang II (100 nM) for 6 hours, TMP (100 µM) significantly inhibited Ang II-induced ET-1 mRNA expression. Effect of TMP on Ang II-induced ET-1 secretion had also been performed at the protein level (Fig. 2B). Smooth muscle cells exposed to Ang II (100 nM) for 24 hours significantly increased ET-1 peptide secretion (Fig. 2B). Preincubated with TMP (100 µM) also significantly inhibited Ang II-induced ET-1 peptide secretion. These data show that TMP down-regulates Ang II-induced ET-1 expression in rat aortic smooth muscle cells.

Effects of Tetramethylpyrazine on Ang II-Increased NAD(P)H Oxidase Activity and Intracellular ROS Levels

We next examined whether TMP prevents the Ang II-increased intracellular ROS generation. Rat aortic smooth muscle cells were pretreated with TMP (1–100 µM) for 30 min and then treated with Ang II (100 nM). The addition of TMP (10 and 100 µM) to cultured rat aortic smooth muscle cells significantly inhibited Ang II-increased NAD(P)H oxidase activity and superoxide production as measured after one hour Ang II treatment (Figs. 3A and 3B). The preincubation of TMP (10 and 100 µM) to cultured rat aortic smooth muscle cells also significantly inhibited Ang II-induced ROS levels as measured after one hour Ang II treatment (Fig. 3C). The pretreatment of TMP (100 µM), Trolox (200 µM), or DPI (10 µM) to cultured smooth muscle cells significantly inhibited Ang II-induced ROS levels (Fig. 3D). These findings clearly demonstrate that TMP inhibits Ang II-increased NAD(P)H oxidase activity and intracellular ROS levels in rat aortic smooth muscle cells.

Figure 1. Effects of tetramethylpyrazine (TMP) on Ang II-induced proliferation in smooth muscle cells. All experiments were performed by using the incorporation of [3H]-thymidine into DNA. Increases in [3H]-thymidine incorporation are expressed relative to the [3H] content (100%) in their respective controls (C). Cells were preincubated with TMP (1–100 µM, 30 min) and then treated with Ang II (100 nM) for 24 hours, and [3H]-thymidine incorporation was assayed. All data are shown as the mean ± SEM for triplicate determinations in 6 cell preparations. *p < 0.05 versus control. #p < 0.05 versus Ang II alone.
Figure 2. Tetramethylpyrazine (TMP) down-regulates Ang II-induced ET-1 expression in smooth muscle cells. The results are shown as the mean ± SEM (n = 6). *p < 0.05 versus control. **p < 0.05 versus Ang II alone.

(A) Down-regulation of Ang II-induced ET-1 mRNA by TMP. Cells were preincubated with TMP (1–100 µM, 30 min) and then stimulated with or without Ang II (100 nM) for 6 hours. Total RNA was extracted and Northern hybridization was performed with 32P-labeled ET-1 as the probe. 18S RNA was used to normalize the RNA applied in each lane. Data was presented as percentage changes of experimental groups compared to untreated controls.

(B) TMP inhibits Ang II-induced ET-1 peptide secretion. Cells were pretreated with TMP (1–100 µM) 30 min prior to Ang II (100 nM) treatment. Results are presented as mean ± SEM (n = 6).
Effects of Tetramethylpyrazine on Ang II-Activated ERK Phosphorylation in Rat Aortic Smooth Muscle Cells

We recently reported that ROS were involved in the activation of ERK pathway, which culminated in ET-1 gene expression (Hong et al., 2004). To gain insight into the mechanism of action of TMP, we examined whether TMP affects Ang II-activated ERK pathway in rat aortic smooth muscle cells. We examined the effect of TMP and antioxidants on Ang II-induced ERK phosphorylation. As shown in Fig. 4, rat aortic smooth muscle cells exposure to Ang II (100 nM) for 30 min rapidly activated phosphorylation of ERK. However, smooth muscle cells pretreated with TMP (1–100 µM) for 30 min and then stimulated with or without Ang II (100 nM) for one hour. TMP (1–100 µM), the antioxidants Trolox (200 µM) or DPI (1 µM) and then stimulated with or without Ang II (100 nM) for one hour. H_{2}O_{2} (100 µM) was used as a positive control.

Effects of Tetramethylpyrazine on Ang II-Increased AP-1 Reporter Activity in Rat Aortic Smooth Muscle Cells

We recently reported that ROS were involved in the activation of ERK pathway, which culminated in ET-1 gene expression (Hong et al., 2004). To gain insight into the mechanism of action of TMP, we examined whether TMP affects Ang II-activated ERK pathway in rat aortic smooth muscle cells. We examined the effect of TMP and antioxidants on Ang II-induced ERK phosphorylation. As shown in Fig. 4, rat aortic smooth muscle cells exposure to Ang II (100 nM) for 30 min rapidly activated phosphorylation of ERK. However, smooth muscle cells pretreated with TMP (100 µM) showed significantly decreased Ang II-induced ERK phosphorylation. Rat aortic smooth muscle cells pretreated with antioxidants Trolox (200 µM) or DPI (10 µM) also significantly decreased Ang II-induced ERK phosphorylation. These findings imply that TMP inhibits Ang II-activated ERK signaling pathway via attenuation of Ang II-induced ROS generation in rat aortic smooth muscle cells.

Effects of Tetramethylpyrazine on Ang II-Increased AP-1 Reporter Activity in Rat Aortic Smooth Muscle Cells

To evaluate the effect of TMP on Ang II-increased AP-1 activation which is involved in ET-1 gene induction (Cheng et al., 2003a; Cheng et al., 1999; Hirotani et al., 2002). The
effects of TMP on Ang II-induced AP-1 functional activity were assessed in a reporter gene assay. Preincubation with TMP for 30 min and then treated with Ang II (100 nM) for 24 hours, TMP (100 µM) significantly decreased Ang II–increased AP-1-luciferase activities (Fig. 5A). We examined the effects of TMP, Trolox, and DPI on AP-1 reporter activity also. TMP (100 µM), Trolox (200 µM), or DPI (10 µM) significantly attenuated Ang II–induced AP-1 reporter activation (Fig. 5B). These results indicate that TMP inhibits Ang II-increased AP-1 activation.

Figure 4. Inhibitory effect of tetramethylpyrazine (TMP) on Ang II-increased ERK phosphorylation in smooth muscle cells. Cells were preincubated with TMP (100 µM), Trolox (200 µM), or DPI (1 µM) and then stimulated with Ang II (100 nM) for 30 min. TMP, Trolox, and DPI inhibited Ang II-induced phosphorylation of ERK. H$_2$O$_2$ (100 µM) was used as a positive control. Phosphorylation of ERK was detected by Western blotting using anti-phospho-ERK antibody and densitometric analyses were performed. Data are represented as fold increase relative to the control groups. The results are shown as the mean ± SEM (n = 6). *p < 0.05 versus control. #p < 0.05 versus Ang II alone.
Discussion

It is clear that the proliferation of vascular smooth muscle cells contributes to the pathophysiology of hypertension, atherosclerosis, coronary artery restenosis after...
angioplasty, and stent placement (Ross, 1993). In addition, the production of ROS in the vessel wall is increased in models associated with vascular remodeling such as hypercholesterolemia, hypertension, diabetes, and balloon injury to the coronary arteries (Grunfeld et al., 1995; Langenstroer and Pieper, 1992; Ohara et al., 1993). Thus an inhibition of ROS-mediated mechanisms may lead to additional treatments for such diseases. In the present study, we demonstrated that TMP modulates Ang II-induced proliferation and ET-1 expression via attenuation of ROS generation in smooth muscle cells. Ang II has been shown to stimulate both hyperplasia and hypertrophy in vascular smooth muscle cells (Touyz et al., 1999). Moreau et al. (1997) reported that Ang II infusion in intact animals increases blood pressure and induces vascular hypertrophy together with increased vascular ET-1 levels. In addition, Chen et al. (1995) reported that Ang II-induced vasoconstriction in some blood vessel preparations in vitro, can be blocked by an ET receptor antagonist. Similarly, ET receptor antagonism prevents Ang II-induced increase of total peripheral and renal vascular resistance as well as urinary protein excretion in vivo (Herizi et al., 1998). In keeping with these observations, our findings clearly indicate the inhibitory effect of TMP on Ang II-induced proliferation and ET-1 gene expression in smooth muscle cells which gains benefit in modulation of the Ang II-attributed pathological effects that may occur in the vascular system.

Recent studies have shown that a major source of ROS in vascular tissues is NAD(P)H oxidase, and the activity of NAD(P)H oxidase is increased by angiotensin II (Griendling et al., 2000). Thus, the source of ROS in the present study can be Ang II-stimulated NAD(P)H oxidase. Elevated ROS levels are involved in cell proliferation and ET-1 induction which can be attenuated by antioxidant pretreatment of cells (Cheng et al., 2003a; Cheng et al., 2001). Zhang et al. (2003) reported that TMP scavenges ROS generation in human polymorphonuclear leukocytes. The results of our present study further demonstrated that TMP reduced the Ang II-induced ROS generation in the smooth muscle cells, suggesting reduction of intracellular ROS generation due to Ang II treatment. In particular, it has been demonstrated that activation of ERK is redox-sensitive (Sano et al., 2001; Shih et al., 2001; Tanaka et al., 2001) and that suppression of ROS inhibits ET-1 gene expression (Cheng et al., 2003a; 2001). The possible explanation for the inhibitory effect of TMP on Ang II-induced proliferation and ET-1 gene expression may thus be its ability to attenuate ROS formation in smooth muscle cells.

The ET-1 promoter contains AP-1 element that could be activated by ROS (Lee et al., 1990). Several evidences suggest that ROS serve as messengers in AP-1 activation (Wung et al., 1997). The cis-acting AP-1 element binds the proto-oncogene products jun and fos (Paul et al., 1995) and it is well-known that the genes for jun and fos are activated by ROS (Guyton et al., 1996). Recently, we also found that the activation of AP-1 is redox-sensitive and might play a key role in ET-1 gene induction (Cheng et al., 2003a). Our present results indicate that TMP inhibits Ang II-induced AP-1 reporter activity. The inhibitory effect of the TMP on Ang II-induced AP-1 transcriptional activation suggested that attenuation of Ang II-induced ROS by TMP leads to inhibition of AP-1.

The present study delivers important new insights to the molecular mechanisms of action of TMP in vascular smooth muscle cells. It is plausible that the Ang II-activated...
signaling pathway consists of redox-sensitive steps and that TMP treatment could modulate the redox state of the cell. In summary, our data show that TMP inhibits Ang II-induced proliferation and ET-1 gene expression in part via attenuation of ROS formation and the suppression of ERK pathway in vascular smooth muscle cells. These findings support the proposed beneficial effects of TMP in cardiovascular disorder.

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References


