Pollutant Particles Produce Vasoconstriction and Enhance MAPK Signaling via Angiotensin Type I Receptor

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Exposure to particulate matter (PM) is associated with acute cardiovascular mortality and morbidity, but the mechanisms are not entirely clear. In this study, we hypothesized that PM may activate the angiotensin type 1 receptor (AT_1R), a G protein-coupled receptor that regulates inflammation and vascular function. We investigated the acute effects of St. Louis, Missouri, urban particles (UPs; Standard Reference Material 1648) on the constriction of isolated rat pulmonary artery rings and the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) and p38 mitogen-activated protein kinases (MAPKs) in human pulmonary artery endothelial cells with or without losartan, an antagonist of AT_1R. UPs at 1–100 μg/mL induced acute vasoconstriction in pulmonary artery. UPs also produced a time- and dose-dependent increase in phosphorylation of ERK1/2 and p38 MAPK. Losartan pretreatment inhibited both the vasoconstriction and the activation of ERK1/2 and p38. The water-soluble fraction of UPs was sufficient for inducing ERK1/2 and p38 phosphorylation, which was also losartan inhibitable. Copper and vanadium, two soluble transition metals contained in UPs, induced pulmonary vasoconstriction and phosphorylation of ERK1/2 and p38, but only the phosphorylation of p38 was inhibited by losartan. The UP-induced activation of ERK1/2 and p38 was attenuated by captopril, an angiotensin-converting enzyme inhibitor. These results indicate that activation of the local renin–angiotensin system may play an important role in cardiovascular effects induced by PM. *Key words:* air pollutant, angiotensin II, angiotensin-converting enzyme, copper, ERK, p38, vanadium. *Environ Health Perspect* 113:1009–1014 (2005). doi:10.1289/ehp.7736 available via *http://dx.doi.org/* [Online 15 April 2005]

Over the last decade, a growing body of epidemiologic and clinical evidence has raised the possibility of the potentially deleterious effects of ambient pollutant particles on cardiovascular health. Exposure to particulate matter (PM) has consistently been associated with increased hospitalization and mortality due to cardiovascular diseases (Pope et al. 2004). It has been estimated that for each 10-μg/m^3 increase in PM_{10} (PM < 10 μm in aerodynamic diameter), the daily cardiopulmonary mortality increased by 0.3% (Dominici et al. 2003). The risk is especially high in patients with congestive heart failure, frequent arrhythmias, or both (Goldberg et al. 2001; Mann et al. 2002), and the catastrophic cardiac events may occur as early as hours after PM exposure (Peters et al. 2001). The mechanisms for the acute increase in cardiovascular events are not entirely clear. Several hypotheses have been proposed, including imbalance in autonomic systems, increases in procoagulant activities, and systemic release of inflammatory mediators (Brook et al. 2004; Donaldson et al. 2001).

Recent *in vivo* and *in vitro* evidence also indicate that PM may cause endothelial dysfunction and vasoconstriction. Exposure to concentrated ambient particles (CAPs; median, 182.75 μg/m^3) for 5 hr/day for 3 days decreased the lumen:wall area ratio of small pulmonary arteries in rats, indicating increased pulmonary vascular resistance (Batalha et al. 2002). Motorcycle exhaust particulate enhanced constriction of rat aortic rings induced by phenylephrine (Tzeng et al. 2003). Exposure to PM for 4 weeks increased atherosclerotic plaque formation in rabbits (Suwa et al. 2002). Inhalation of CAPs (~ 150 μg/m^3) and ozone (120 ppb) for 2 hr causes acute constriction of the brachial artery in healthy adults (Brook et al. 2002). An air pollution episode in Germany was associated with increases in systemic blood pressure by as much as 8 mm Hg (Ibald-Mulli et al. 2001). Various vasoconstrictor mechanisms have been demonstrated, including the release of endothelins (Bouthillier et al. 1998; Thomson et al. 2004), activation of the epithelial growth factor receptor (EGFR; Huang et al. 2002), and inhibition of nitric oxide production (Bai et al. 2001; Bouthillier et al. 1998; Huang et al. 2002; Ikeda et al. 1995). These mechanisms, however, could not completely explain the epidemiologic findings of the acute effects of PM on cardiovascular events, which have a lag time of hours. The endothelins are potent vasoconstrictors, but the increased release occurs 24 hr after PM exposure. Vasoconstriction caused by the activation of EGFR is relatively weak, and the inhibition of NO production results in a loss of vasodilator activity.

The circulating and local renin–angiotensin systems have been known to play a key role in the pathogenesis of cardiovascular diseases (Dzau 1988). The end product of this pathway, angiotensin II, is one of the most potent vasoconstrictors, and its effects are mediated primarily by the G protein-coupled angiotensin type 1 receptor (AT_1R; Daugherty and Cassis 2004). Agonist binding of AT_1R activates mitogen-activated protein kinases (MAPKs; Touyz and Schiffrin 2000), a common early signaling event induced by PM exposure (Roberts et al. 2003; Silbajoris et al. 2000). In preliminary experiments, we found that pulmonary vasoconstriction induced by St. Louis, Missouri, urban particles [UPs; Standard Reference Material (SRM) 1648] could be inhibited by losartan, an AT_1R antagonist. In the present study, we characterized the role of AT_1R in UP-induced vasoconstriction and MAPK activation. The study was performed in the isolated pulmonary artery ring system and human pulmonary artery endothelial cells (HPAECs).

Materials and Methods

Reagents and chemicals. We obtained HPAECs from Cell Applications, Inc. (San Diego, CA). We purchased endothelial growth medium (EGM-2) and supplements from Clonetics (Bio Whittaker Inc., Walkersville, MD), and vanadyl sulfate ($VOSO_4$) and copper sulfate ($CuSO_4$) from Johnson Matthey Co. (Ward Hill, MA). We obtained Captopril from Sigma Chemical Co. (St. Louis, MO); SB203580, a p38 MAPK inhibitor, and PD98059 an ERK1/2 MAPK inhibitor, from Calbiochem-Novabiochem Corp. (San Diego, CA); and

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losartan potassium from Merck & Co., Inc. (West Point, PA). Monoclonal antibodies against phospho-p38, total extracellular signal-regulated kinases 1 and 2 (ERK1/2), total p38, and phospho-ERK1/2 were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit and goat anti-mouse IgG were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). We purchased molecular mass standards, polyacrylamide, and buffers from Bio-Rad (Richmond, CA). The enhanced chemiluminescent (ECL) blotting detection reagents were purchased from Amersham Biosciences Corp. (Piscataway, NJ).

Urban particles. We purchased St. Louis UPs (SRM 1648) from the National Institute of Standards and Technology (Gaithersburg, MD). They were prepared from urban PM collected in the St. Louis, Missouri, area in a baghouse over a period of > 12 months. The material was removed from the filter bags, combined in a single lot, screened through a fine mesh sieve to remove extraneous materials, and thoroughly blended in a v-blender. The material was then packaged into sequentially numbered bottles. The major constituent elements are (mass fraction) aluminum, 3.4%; iron, 3.9%; and potassium, 1.1%; the minor constituent elements are sodium, 0.43%; lead, 0.66%; and zinc, 0.48%. There are also trace constituents (nanograms per milligram), including arsenic (115), cadmium (75), chromium (403), copper (609), manganese (786), nickel (82), selinium (27), uranium (5.5), and vanadium (127). UPs were suspended in distilled deionized water for use in experiments.

Preparation of the water-soluble and -insoluble fraction of UP. The water suspension of UPs was centrifuged for 10 min at 14,000 rpm; we then collected the supernatant and centrifuged it again. We repeated this procedure several times until no sediments could be seen in the supernatant. This final supernatant was designated as the water-soluble fraction of UP. The pellets from the first centrifuge were washed using deionized distilled water for three times and then resuspended in appropriate volume of water. We designated this as the water-insoluble fraction of UP.

Isolated perfused rat pulmonary artery ring model. We removed segments of the right and left main pulmonary arteries of Sprague-Dawley rats (250–350 g) measuring approximately 2–3 mm and placed them in the Krebs-Henseleit (KH) buffer as described previously (Li et al. 2004). The artery segments were then suspended in the Radnoti four-unit tissue organ bath system (Glass Technology Inc., Monrovia, CA). The reservoirs held

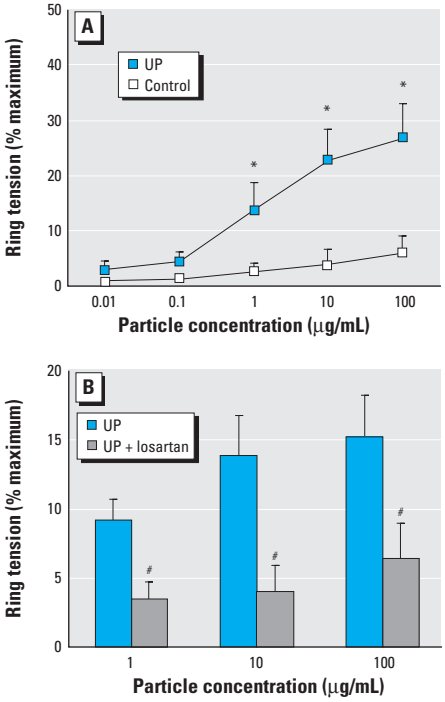

Figure 1. Constriction of isolated rat pulmonary artery induced by UPs (*A*) and the effects of losartan, an angiotensin II receptor subtype 1 antagonist, on UP-induced vasoconstriction (*B*). The constriction was measured by ring tension and expressed as percentage of maximum tension induced by 1 μM phenylephrine. $n = 6–8$ rings from different animals.
*$p < 0.05$ compared with 0.01 μg/mL; #$p < 0.05$ compared with UP.

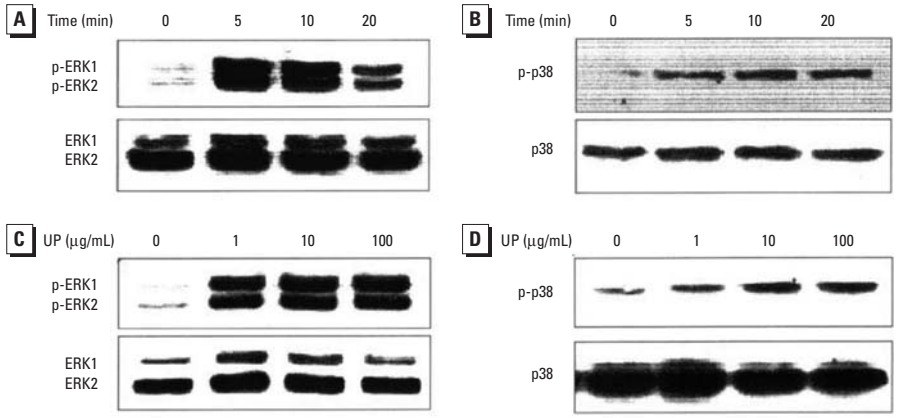

Figure 2. Effects of UPs on phosphorylation of p38 and ERK MAPKs. Time-dependent increase in phospho-ERK1/2 (p-ERK1/2; *A*) and phospho-p38 (p-p38; *B*); confluent cells were treated with 10 μg/mL UPs for up to 20 min. Dose-dependent increase in p-ERK1/2 (*C*) and p-p38 (*D*); cells were treated with 1, 10, and 100 μg/mL UPs for 10 min. Cell lysates (50 μg) were separated by 10% SDS-PAGE gel and immunoblotted with monoclonal antibodies against p-p38 or ERK1/2 as described in "Materials and Methods." Results are representative of three independent experiments.

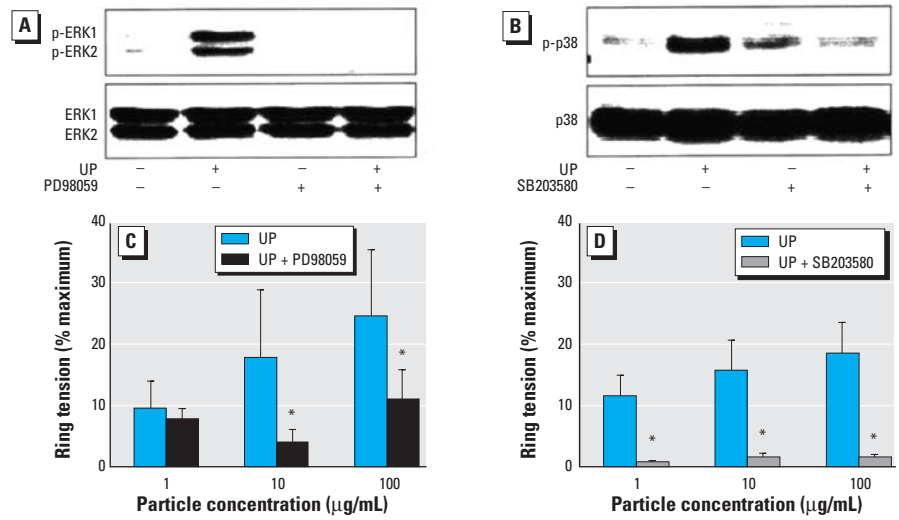

Figure 3. Effects of PD98059 (*A*) and SB203580 (*B*) on UP-induced phosphorylation of ERK1/2 and p38, respectively. Quiescent cells were pretreated with PD98059 (30 μM) and SB203580 (10 μM) for 30 min and then incubated with 10 μg/mL UPs for 10 min; phosphorylation of p38 (p-p38) and ERK (p-ERK1/2) in cell lysates was measured by Western blotting. Effects of PD98059 (30 μM; *C*) and SB203580 (10 μM; *D*) on UP-induced pulmonary vasoconstriction (*C*) and (*D*), respectively. $n = 4–6$ rings from different animals; results are representative of three independent experiments.
*$p < 0.05$ compared with UP.

20 mL KH buffer and were bubbled constantly with 21% O_2/5% CO_2 gas. After a 10–15 min stabilization period, the baseline tension of the rings was adjusted to 1 g before all experiments. We exposed the artery rings to increasing doses of particles from 1 to 100 μg/mL, and the rings were washed with buffer between the two doses of particles. We recorded the maximum tension within 5 min after each dose of particles.

Cultured HPAECs. HPAECs were grown in endothelial growth medium (EGM-2) supplemented with 2% fetal bovine serum, 0.04% hydrocortisone, 0.4% human fibroblast growth factor-B, 0.1% vascular endothelial growth factor, 0.1% R^3-insulin growth factor-1, 0.1% ascorbic acid, 0.1% human epithelial growth factor, 0.1% GA-1000, and 0.1% heparin. We used cells at passages 4–9 grown to 80% confluence in six-well plates for the experiments. The cells were exposed to particles at 1–100 μg/mL for up to 20 min.

Western blot analysis. After the exposure, the cells were washed once with ice-cold phosphate-buffered saline (PBS) and then lysed with RIPA buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS in PBS, pH 7.4) containing 0.1 mM $VOSO_4$ and protease inhibitors (0.5 mg/mL aprotinin, 0.5 mg/mL E-64, 0.5 mg/mL pepstatin, 0.5 mg/mL bestatin, 10 mg/mL chymostatin, and 0.1 ng/mL leupeptin). We then centrifuged the cell lysates at 3,000*g* for 10 min at 4°C. Protein concentration of supernatant was measured with Bio-Rad protein assay reagent. Cellular proteins were separated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane. The blot was blocked with 5% milk in PBS with 0.05% Tween-20 for 1 hr at room temperature, washed briefly, and then probed with primary antibodies against phospho-p38 or phospho-ERK1/2 overnight at 4°C. This was followed by incubation with HRP-conjugated secondary antibodies. We detected bands using ECL and films. We then stripped the blot and reprobed with antibodies against total p38 or total ERK1/2 and appropriate HRP-conjugated secondary antibodies.

Statistical analysis. Data are reported as mean ± SE. Data from the artery ring experiments were analyzed by the repeat-measures analysis of variance (ANOVA). Data from the cell experiments were analyzed by ANOVA followed by the Scheffe's test for post hoc comparisons. The statistical analysis was performed using commercially available software (Statview, version 5.0.1; SAS Institute Inc., Cary, NC). We considered a p-value < 0.05 as statistically significant.

Results

Effects of losartan on UP-induced pulmonary artery constriction. Treatment of isolated rat pulmonary artery rings with UPs produced a dose-dependent increase in vasoconstriction (Figure 1A). At 100 μg/mL, the ring tension was approximately 25% of that produced by 1 μM phenylephrine. UP-induced pulmonary vasoconstriction was inhibited by losartan (0.2 μM), an AT_1R receptor antagonist (Figure 1B).

Effects of UPs on ERK1/2 and p38 phosphorylation. Figure 2A shows the time-dependent increase in phospho-ERK1/2 induced by UPs. The intensity of phosphorylated ERK1/2 peaked at 5 min and gradually decreased. The phosphorylation of ERK1/2 was enhanced significantly by 1–100 μg/mL UPs (Figure 2B). The phosphorylated p38 increased with time after UP treatment (Figure 2C). There was also a dose-dependent increase in the intensity of phospho-p38 (Figure 2D). The UP-induced phosphorylation of ERK1/2 and p38 was completely inhibited by PD98059, an ERK1/2 MAPK inhibitor, and SB203580, a p38 MAPK inhibitor (Figure 3A,B). The UP-induced constriction of rat pulmonary artery was also attenuated by PD98059 and SB203580 (Figure 3C,D), indicating that activation of ERK1/2 and p38 may mediate UP-induced pulmonary vasoconstriction.

Effects of losartan on UP-induced ERK1/2 and p38 phosphorylation. We then investigated the role of losartan in UP-induced ERK1/2 and p38 phosphorylation. HPAECs were pretreated with losartan (0.2 μM) for 30 min before incubation with 10 μg/mL UPs for 10 min. Figure 4 shows that the UP-induced ERK1/2 and p38 phosphorylation was completely inhibited by losartan, indicating that AT_1R mediates the activation of ERK1/2 and p38 MAPK induced by UPs.

Effects of particle components on ERK1/2 and p38 phosphorylation. Because the soluble fraction of the particles may better penetrate the alveolar–capillary membrane and be in contact with the pulmonary vessels than the whole particles when inhaled, we determined whether the soluble fraction of UPs could activate ERK1/2 and p38 MAPK. The water-soluble fraction of UPs was capable of

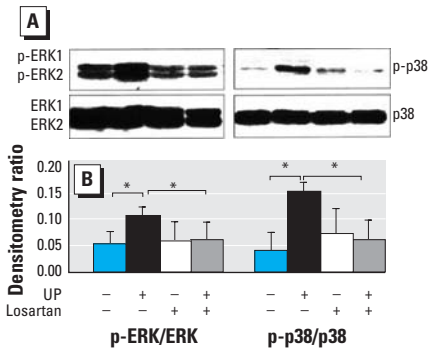

Figure 4. Effects of losartan on UP-induced phosphorylation of p38 and ERK shown by a representative Western blot (*A*) and the densitometry results (*B*). Quiescent cells were pretreated with losartan (0.2 μM) for 30 min and then incubated with 10 μg/mL UPs for 10 min. Phosphorylation of p38 (p-p38) and ERK (p-ERK1/2) in cell lysates was measured by Western blotting. Data are mean ± SE; n = 4 independent experiments.
*p < 0.05.

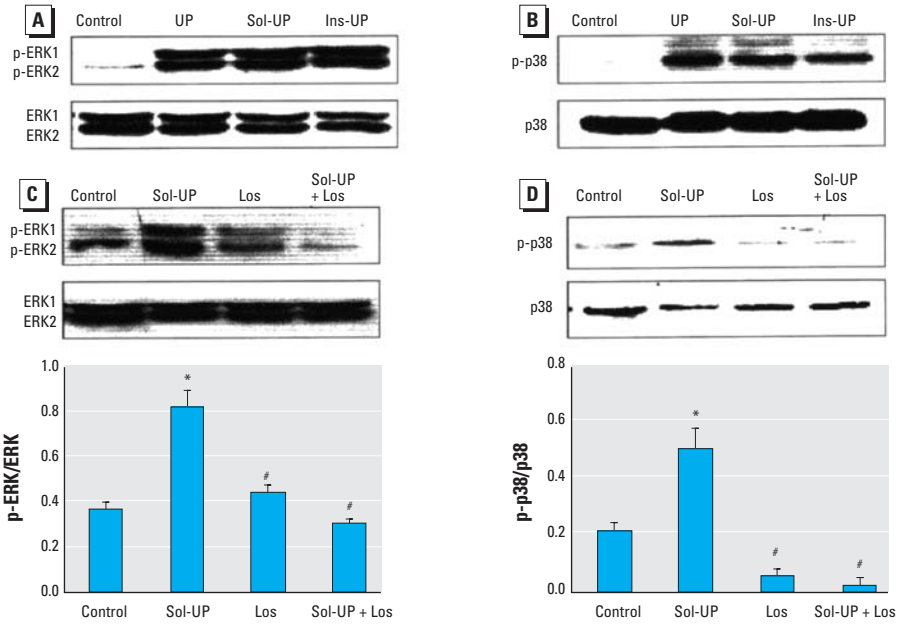

Figure 5. Phosphorylation of ERK (p-ERK1/2; *A*) and p38 (p-p38; *B*) by the components of UPs; cells were treated with the whole UPs (10 μg/mL), and the water-soluble fraction (Sol-UP) and water-insoluble fraction (Ins-UP) were extracted from 10 μg/mL UPs for 10 min. Effects of losartan (Los) on phosphorylation of ERK (*C*) and p38 (*D*) induced by Sol-UP. Results are from a representative Western blot from three independent experiments; n = 3 independent experiments.
*p < 0.05 compared with the control. #p < 0.05 compared with Sol-UP.

increasing the phosphorylation of ERK1/2 and p38 (Figure 5A,B). The insoluble fraction was equally effective. Similar to the whole UP, the soluble-fraction–induced activation of ERK1/2 and p38 was inhibited by losartan (Figure 5C,D).

Effects of UP-associated soluble metals on pulmonary artery constriction. To determine which water-soluble metal components of UPs may be responsible for the activation of ERK1/2 and p38 MAPK, we tested the vasoconstrictor effects of soluble metals contained in UPs. We found that Cu and V induced significant vasoconstriction (Figure 6). Other metals, including Ni, Fe, Mn, Zn, and Al, produced no or weak vasoconstriction (data not shown).

Effect of Cu and V on ERK1/2 and p38 phosphorylation. Because only Cu and V showed significant vasoconstrictor activity, we determined whether or not Cu and V could activate ERK1/2 and p38 MAPK. Figure 7 shows that $CuSO_4$ and $VOSO_4$ increased phosphorylation of ERK1/2 and p38 in a dose-dependent manner.

Effects of losartan on Cu- and V-induced phosphorylation of ERK1/2 and p38. We further determined whether or not the Cu- and V-induced activation of ERK1/2 and p38 was inhibitable by losartan. Figure 8 shows that Cu- and V-induced phosphorylation of p38 was inhibited by losartan. The phosphorylation of ERK1/2, however, was not inhibited by losartan (data not shown).

Effects of captopril on UP-induced ERK1/2 and p38 phosphorylation. Because angiotensin II, the ligand for AT_1R, is a metabolic product of angiotensin-converting enzyme (ACE), we further determined whether or not UP-induced AT_1R-mediated MAPK activation required ACE. We pretreated the cells with captopril (100 μM), an ACE inhibitor, for 30 min before the addition of UPs. Figure 9 shows that the UP-induced ERK1/2 and p38 phosphorylation was inhibited by captopril.

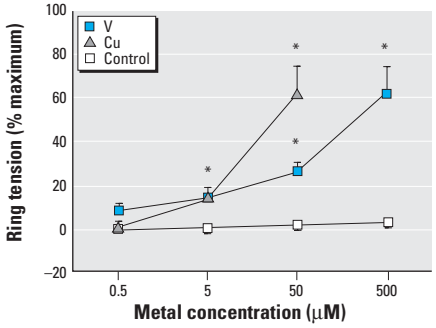

Figure 6. Effects of Cu and V on pulmonary vasoconstriction measured by ring tension and expressed as the percentage of maximum tension induced by 1 μM of phenylephrine. $n = 6–8$ rings from different animals.
*$p < 0.05$ compared with control.

Discussion

In this study, we have shown that UPs induced vasoconstriction in rat pulmonary arterial rings. Thus, UPs have joined a list of pollutant particles that have been shown to cause vasoconstriction, including CAPs (Batalha et al. 2002), residual oil fly ash (Huang et al. 2002), and diesel particles (Tzeng et al. 2003). We further have shown that the UP-induced pulmonary vasoconstriction could be inhibited by losartan, an AT_1R antagonist, indicating that the renin–angiotensin system may play an important role. PM has been shown to activate several vasoactive pathways, including the release of endothelins (Bouthillier et al. 1998; Thomson et al. 2004), the activation of EGFR (Huang et al. 2002), and the inhibition of NO production (Bai et al. 2001; Bouthillier et al. 1998; Huang et al. 2002; Ikeda et al. 1995). Compared with these vasoactive pathways, the angiotensin II–AT_1R pathway has the following advantages: *a*) the activation of AT_1R-mediated signaling by particles occurs earlier than that of endothelins; *b*) angiotensin II is a much more potent vasoconstrictor than EGF; and *c*) activation of AT_1R leads to vasoconstriction rather than the loss of vasodilator activity as a result of inhibition of NO production. In these aspects, the AT_1R pathway may be a more clinically relevant mechanism for the acute cardiovascular events associated with ambient PM demonstrated in the epidemiologic and clinical studies, especially in patients with compromised heart function.

Our study also showed that both the water-soluble and -insoluble fractions of UPs

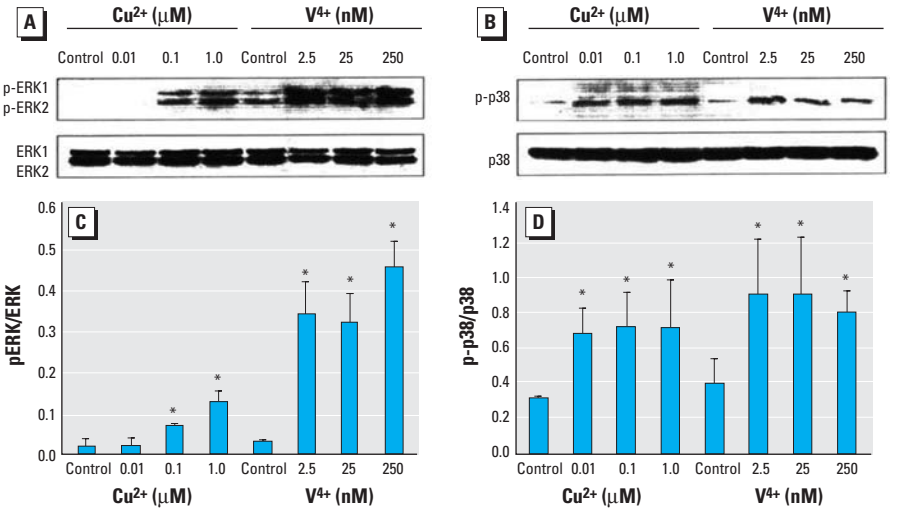

Figure 7. Effects of Cu (as $CuSO_4$) and V (as $VOSO_4$) on phosphorylation of ERK (p-ERK1/2; *A* and *C*) and p38 (p-p38; *B* and *D*). Results are shown as representative Western blots (*A* and *B*) and the densitometry results (*C* and *D*). $n = 4$ independent experiments.
*$p < 0.05$ compared with control.

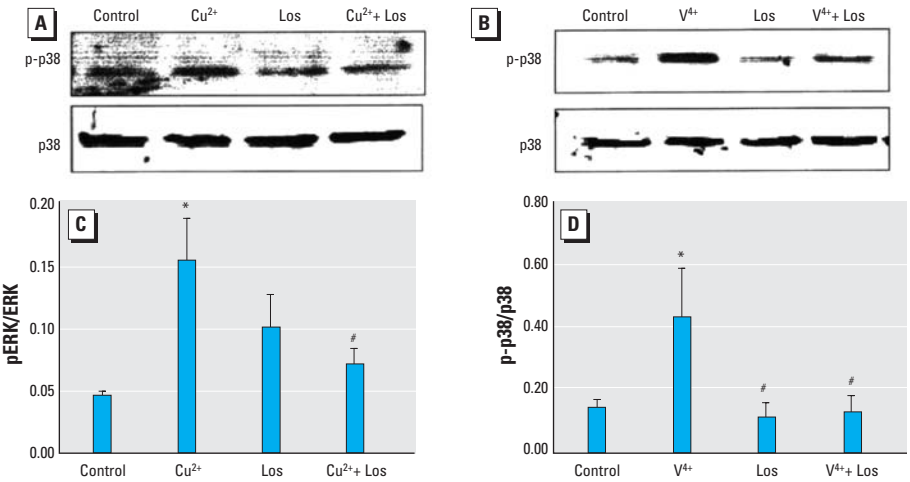

Figure 8. Effects of losartan on Cu-induced (*A* and *C*) and V-induced (*B* and *D*) p38 phosphorylation (p-p38). Cells were pretreated with losartan (Los; 0.2 μM) for 30 min before incubation with $CuSO_4$ (0.1 μM) or $VOSO_4$ (0.25 μM) for 10 min. Results shown are representative Western blots (*A* and *B*) and densitometry results (*C* and *D*). $n = 4$ independent experiments.
*$p < 0.05$ compared with control; #$p < 0.05$ compared with UPs. $n = 4$ each.

were equally effective in increasing ERK1/2 and p38 phosphorylation. The effects by the water-soluble fraction would be more biologically relevant because components in this fraction are more likely than the whole particles or the insoluble fraction to gain access to the pulmonary vessels quickly by diffusion after the particles are inhaled into the lung. Many components are present in the water-soluble fraction of UPs. Among them are transition metals. In this study, we found that Cu and V produced strong vasoconstriction and activated ERK1/2 and p38. Thus, Cu and V may be among the active components for PM-induced vasoconstriction. Residual oil fly ash, a pollutant dust containing abundant V, also produces vasoconstriction and MAPK activation (Huang et al. 2002; Silbajoris et al. 2000). Cu and V may reach the vasculature via specialized membrane transporters (Chasteen et al. 1986; Eisses and Kaplan 2002). V appeared to be a stronger inducer for ERK1/2 and p38 phosphorylation than did Cu, but its vasoconstrictor property was weaker. This indicated that V, a nonspecific protein tyrosine phosphatase inhibitor, may also activate other signaling proteins that counteract vasoconstriction. Our results do not exclude other mechanisms that are metal independent. Diesel particles, which contain little metal, impair endothelium-dependent vasorelaxation (Ikeda et al. 1995) possibly via the production of reactive oxygen species inactivating NO. Ultrafine particles (PM with a mass median aerodynamic diameter < 100 nm), which can more effectively penetrate the distal lung regions, may produce cardiovascular adverse effects via more intense lung inflammation (Brown et al. 2000; Utell and Frampton 2000). Previous studies have also shown that some components of ultrafine particles deposited in the respiratory tract could enter the circulation exerting their adverse effects on the cardiovascular system (Takenaka et al. 2001).

The UP-induced activation of ERK1/2 and p38 could be inhibited by an antagonist of AT_1R, losartan. Similar inhibition by losartan was also seen when activation was induced by the water-soluble fraction. This is the first demonstration that PM-induced vascular effects may be mediated by the angiotensin signaling. AT_1R is one of the four G protein-coupled receptors that mediates intracellular signaling induced by angiotensin II (Touyz and Berry 2002). AT_1R activation leads to cell growth, vascular contraction, inflammatory responses, and salt and water retention (Touyz and Berry 2002). These effects are regulated by complex intracellular signaling pathways including MAPKs, phospholipase C, protein kinase C, and phospholipase A2. Our results that inhibitors of ERK1/2 and p38 MAPKs attenuated UP-induced vasoconstriction support the established role of these MAPKs in angiotensin II–induced vasoconstriction (Ishihata et al. 2002; Massett et al. 2002; Meloche et al. 2000; Touyz et al. 1999). Phosphorylation of MAPKs may further activate downstream MAPK-activated protein kinase-2 and heat shock protein 27 with or without phosphorylation of myosin light chain (Meloche et al. 2000; Roberts 2004). Occupancy and activation of AT_1R also stimulate many intracellular nonreceptor tyrosine kinases, such as Src, Pyk2, p130Cas, FAK, and JAK/STAT (Eguchi and Inagami 2000). In the cardiovascular system, alterations of these highly regulated pathways underlie the pathologic processes such as hypertension and atherosclerosis, conditions that also have been linked to PM exposure (Ibald-Mulli et al. 2001; Suwa et al. 2002). That losartan only inhibited p38 activation induced by V and Cu indicates that other components of PM may also be involved in PM-induced ERK1/2 MAPK activation.

The mechanisms by which UPs activate AT_1R are unclear. Because the UP-induced phosphorylation of ERK1/2 and p38 could be inhibited by captopril, an ACE inhibitor, the ACE activity appeared required for these effects. The vascular endothelial cells contain a large amount of ACE and can synthesize angiotensins via the local renin–angiotensin system (Kifor and Dzau 1987). Such a local renin–angiotensin system is likely the more important source of angiotensin II in our cultured HPAECs than is the extracellular angiotensin I contained in the culture medium. One might also consider whether or not UPs may act as an AT_1R agonist. At least one nonpeptide agonist of AT_1R has been described: a biphenylimidazole derivative (L-162313), whose biologic effects could be inhibited by AT_1R antagonist (Perlman et al. 1995). Binding of nonpeptide agonists of AT_1R may lead to conformational modifications that affect the preferential binding of agonists or antagonists (Costa-Neto et al. 2002).

Conclusions

The renin–angiotensin system plays an important role in many types of inflammatory and cardiovascular diseases. Angiotensin II via AT_1R signaling has been shown to promote cell growth and regulate the expression of bioactive substances such as vasoconstrictor hormones, growth factors, cytokines, aldosterone, and extracellular matrix components (Jacoby and Rader 2003; Schiffrin and Touyz 2004). Our *in vitro* results indicate that the angiotensin–AT_1R signaling pathway may be important in mediating vascular effects induced by PM. Further studies are needed to determine the role of the circulating and local renin–angiotensin systems in PM-induced adverse cardiovascular effects.

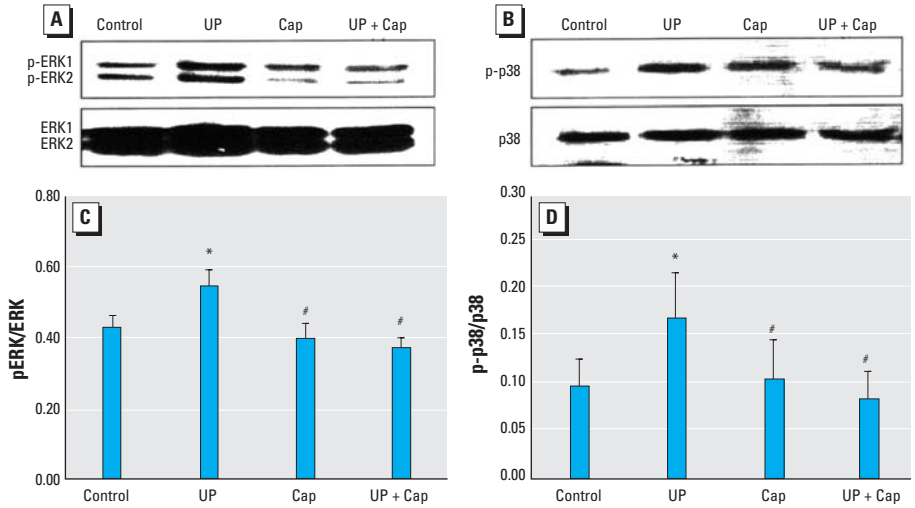

Figure 9. Effects of captopril (Cap) on UP-induced ERK1/2 (p-ERK1/2; *A* and *C*) and p38 phosphorylation (p-p38; *B* and *D*). Cells were pretreated with captopril (Cap; 100 µM) for 30 min before incubation with UPs (10 µg/mL) for 10 min. Results shown are representative Western blots and densitometry results. $n = 4$ independent experiments.

*$p < 0.05$ compared with control. #$p < 0.05$ compared with UPs.

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