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Specific inhibition of p38 mitogen-activated protein kinase with FR167653 attenuates vascular proliferation in monocrotaline-induced pulmonary hypertension in rats

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Objectives: p38 mitogen-activated protein kinase is associated with many clinical entities characterized by inflammation. We postulated that inhibition of p38 mitogen-activated protein kinase with FR167653 attenuates inflammation and the development of pulmonary hypertension in monocrotaline-treated rats.

Methods: Rats were divided into 4 groups: (1) the control group (daily 0.9% saline), (2) the FR group (daily FR167653, 2 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}), (3) the MCT group (daily 0.9% saline the day after a single monocrotaline dose, 60 mg/kg), and (4) the MCT+FR group (daily FR167653, 2 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}, the day after a single MCT dose). Body weight, pulmonary artery pressure, and morphometric changes of the pulmonary artery with the histopathologic method were observed weekly for 4 weeks. Also, p38 mitogen-activated protein kinase activity and inflammatory cytokine expression in the lung were measured.

Results: Four weeks after monocrotaline administration, mean pulmonary artery pressure in the MCT+FR group was lower than in the MCT group (MCT+FR vs MCT: 24.7 $\pm$ 1.9 vs 36.5 $\pm$ 2.1 mm Hg; $P < .05$). In morphometric analysis the percentage of medial wall thickness and the percentage of muscularization in the MCT+FR group were reduced compared with those in the MCT group after 4 weeks ($P < .05$); however, the number of macrophages was not significantly different. p38 mitogen-activated protein kinase activity was significantly attenuated in the MCT+FR group compared with in the MCT group (7.2 $\pm$ 0.52 vs 2.1 $\pm$ 0.23 fold-increase, $P < .05$, at 1 week). Although mRNA levels of tumor necrosis factor α and interleukin 1β were reduced in the MCT+FR group compared with in the MCT group (tumor necrosis factor α: 1.18 $\pm$ 0.36 vs 3.05 $\pm$ 1.12 fold-increase, $P < .05$, at 2 weeks; interleukin 1β: 2.2 $\pm$ 0.34 vs 4.4 $\pm$ 1.09 fold-increase, $P < .05$, at 1 week), FR167653 did not suppress increased monocyte chemotactic protein 1

mRNA expression induced by monocrotaline (3.2 ± 0.62 vs 3.1 ± 0.42 fold-increase, at 1 week).

Conclusion: FR167653 significantly attenuates the expression of inflammatory cytokines, ultimately preventing the progression of pulmonary hypertension. These results suggest that p38 mitogen-activated protein kinase might play a central role in the molecular events that underlie the development and progression of pulmonary hypertension.

Primary pulmonary hypertension (PPH) is a rapidly progressive disease without an identifiable cause characterized by a sustained increase in pulmonary artery pressure (PAP) and right heart failure. Occurring in approximately 2 per million individuals, the persistent and progressive nature of PPH ultimately leads to death, with a median survival of 2.5 years.[1] Secondary pulmonary hypertension is associated with collagen vascular disease, portal hypertension, congenital systemic-to-pulmonary shunts, and HIV infection. PPH and secondary pulmonary hypertension have been grouped by the World Health Organization (Evian, France, 1998) as group I (pulmonary arterial hypertension) because of similar pulmonary histopathologic findings and responses to pharmacologic therapy. The histopathologic features of PPH in small- and medium-sized arterioles include endothelial cell proliferation with subsequent intimal fibrosis, smooth muscle cell proliferation leading to medial hypertrophy, adventitial thickening, perivascular inflammatory infiltrates, and in situ thrombosis.[2] In PPH monoclonal endothelial cell proliferation and smooth muscle cell migration produce obliterative intimal lesions called plexiform lesions that characterize the disease.[3]

The pathogenesis of PPH also includes an inflammatory response. In addition to perivascular infiltration of macrophages and lymphocytes, increased expression of inflammatory mediators, such as interleukin (IL) 1β, IL-6, and platelet-derived growth factor A have been shown in pulmonary hypertension (PH).[4-6] Furthermore, tumor necrosis factor (TNF) α, IL-1β, IL-6, and transforming growth factor (TGF) β induce pulmonary vascular remodeling.[7] These findings suggest that inflammation plays a significant role in the pathogenesis of PH. However, the molecular mechanisms responsible for the inflammatory response and vascular remodeling in PH remain largely undefined.

Proteins of the mitogen-activated protein kinase (MAPK) family are activated in a variety of physiologic and pathophysiologic states.[8,9] MAPKs are activated by distinct signaling pathway that induce MAPK activity in response to extracellular stimuli. MAPK activity results in increased transcription of genes that subserve growth, differentiation, and inflammation. p38 MAPK exists in 4 different isoforms that have different cellular functions; p38α is most often associated with inflammation.[10] Of interest, in a rat model of PH induced by chronic hypoxia, c-Jun NH_2-terminal kinase, extracellular signal–regulated kinase (JNK), and p38 MAPK have been demonstrated to be constitutively expressed in fibroblasts explanted from the pulmonary artery (PA)[11,12]; however, MAPK activities in other experimental forms of PH or in human PPH have not been reported. The goal of our study is to examine p38 MAPK inhibition of cytokine expression in a model of PH that includes an inflammatory component. More importantly, we were able to obtain a level of reproducibility in our results with the monocrotaline (MCT)–induced PH model that likely exceeds the degree of precision we would have obtained with the chronic hypoxia-induced model. We therefore evaluated p38 MAPK activity and the effect of long-term inhibition of p38 MAPK with FR167653,* a recently described specific inhibitor of p38 MAPK,[13] on PH in a rat model of PH induced by MCT.

Materials and Methods

Animals and Experimental Design

Male Sprague-Dawley rats (Charles River Japan, Inc, Yokohama, Japan), 6 to 8 weeks of age and weighing 250 to 300 g, were divided into 4 groups: (1) the control group (daily 0.9% saline, 1 mL administered intramuscularly); (2) the FR group (daily FR167653 [Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan], 2 mg $\cdot$ $kg^{-1} \cdot d^{-1}$ in 1 mL of saline administered intramuscularly); (3) the MCT group (daily 0.9% saline, 1 mL administered intramuscularly the day after a single MCT dose of 60 mg/kg in 1 mL of saline administered subcutaneously); and (4) the MCT+FR group (daily FR167653, 2 mg $\cdot$ $kg^{-1} \cdot d^{-1}$, in 1 mL of saline administered intramuscularly the day after a single MCT dose).

Body weight (BW) was measured on a weekly basis for 4 weeks. At each week of the study, rats were intubated and started on mechanical ventilation after general anesthesia was achieved with pentobarbital sodium (30 mg/kg administered intramuscularly). After determining the PAP with a small needle cannulated

*1-[7-(4-Fluorophenyl)-1,2,3,4-tetrahydro-8 (4-pyridyl) pyrazoro [5-1-c][1,2,4].

into the main PA, heart and lung blocks were quickly explanted. The lung tissues were frozen in liquid N_2 and maintained at −80°C before use for molecular analysis or distended and fixed by means of perfusion through the tracheal tube with 10% formalin at 20 cm H_2O pressure for 48 hours for histopathologic observation. After removing the atria, heart tissues were further dissected and separated into the right ventricle (RV) and the left ventricle plus septum (LV+S), and the dissected tissues were weighed individually. The RV/(LV+S) ratio by weight was determined.

To evaluate the effect of long-term treatment with FR167653, we collected blood samples and measured the serum levels of glutamic oxaloacetic transaminase, glutamic pyruvate transaminase, creatine phosphokinase, alkaline phosphatase, blood urea nitrogen, and creatinine in a separate series of rats treated with FR167653 daily (2 and 10 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} administered intramuscularly) on a weekly basis for 18 weeks by means of enzyme-linked immunosorbent assay. Histopathologic findings in the brain, heart, lung, liver, and kidney were assessed in rats killed each week.

All animals were maintained in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health and also with the Guideline for Animal Experiments in Mie University School of Medicine.

p38 MAPK Activity Assay

Whole-cell protein, extracted from frozen lung tissue with an ice-cold lysis buffer (Cell Signaling Technology, Inc, Beverly, Mass), was stored at −80°C until the time of assay. The p38 MAPK activity was measured with a p38 MAPK assay kit (Cell Signaling Technology, Inc). In brief, a selective immunoprecipitate of active (phosphorylated) p38 MAPK was obtained from whole-cell protein by using immobilized phospho-p38 MAPK (Thr180/Tyr182) monoclonal antibody. The resulting immunoprecipitate was then incubated with activating transcription factor (ATF) 2 fusion protein and cold adenosine triphosphate (ATP); this allows immunoprecipitated active p38 MAPK to phosphorylate ATF-2. The results were expressed as the immunoreactivity of phosphorylated ATF-2 (Thr71) detected with enhanced chemiluminescence and determined by means of densitometry (NIH Image 1.62). Results are expressed as fold activation over a control.

Quantitative Reverse Transcriptase–Polymerase Chain Reaction

Total RNA was isolated from frozen lung tissues by means of the guanidium thiocyanate-phenol-chloroform method and stored at −80°C until the time of the assay. Quantitative reverse transcriptase–polymerase chain reaction measurement used the LightCycler System (Roche Diagnostics GmbH, Mannheim, Germany). Sense and antisense primers for rat TNF-α, IL-1β, monocyte chemotactic protein (MCP) 1, and glyceraldehyde-3-phosphate dehydrogenase were synthesized by using published cDNA sequences.[14-17] Amounts of TNF-α, IL-1β, and MCP-1 cDNA were expressed relatively, being divided by the amount of cDNA for glyceraldehyde-3-phosphate dehydrogenase to correct for the varying efficiency of cDNA synthesis.

Histopathologic and Immunohistochemical Analysis

Morphometric analyses of PA were conducted exactly as previously described.[18] For histopathologic observations in lung tissues, particularly percentage of medial wall thickness (MWT) or percentage of muscularization of the PA, the sections from the mid-portion of the formalin-fixed lung tissues were stained with hematoxylin-eosin and elastica van Gieson stains. All arteries with external diameters of greater than 15 μm were assessed at 400× magnification. The structural type of each artery was determined as muscular (ie, with a complete medial coat of muscle), partially muscular (ie, with only a crescent of muscle), or nonmuscular (ie, no apparent muscle). The external diameter and medial thickness (ie, the distance between the external and internal elastic laminae) were noted for all muscular and partially muscular arteries, and the percentage of MWT was calculated by using the following formula: *%MWT = 2 × Medial wall thickness/External diameter × 100*.

For the immunohistochemical analysis of macrophages or localization of phosphorylated p38 MAPK, the sections were stained with specific antibodies (macrophage: ED3, Bachem AG, Bubendorf, Switzerland; phospho-p38 MAPK: 28B10, Cell Signaling Technology, Inc). The number of macrophages was determined by counting immunostained cells in 3 sections per rat and 250 × 250–μm portions of 20 fields at 400× magnification per section.

Statistical Analysis

All data are expressed as means ± SD. The significance of the difference between group means was analyzed by means of 1-way analysis of variance (ANOVA) with post hoc comparisons by using the Fisher protected least-significant-difference test. The effect of FR167653 was tested by means of 2-factor ANOVA for repeated measures, and differences at specific time points between the groups were assessed by means of 1-factor ANOVA with post hoc comparisons by using the Fisher protected least-significant-difference test. Relationships between 2 variables were tested by using linear regression analysis. All statistical analyses were done with StatView 6.0 (SAS Institute, Inc, Cary, NC).

Results

Hemodynamic Assessments and Right Ventricular Hypertrophy

Over the course of the 4-week study period, rats treated with MCT alone had retardation of growth (as measured on the basis of percentage BW gain) compared with that of saline-treated control animals (6.1% ± 5.1% vs 56.4% ± 4.4%, $P < .005$). FR167653 administered daily over the 4-week study period attenuated MCT-induced growth retardation; MCT+FR167653-treated animals gained just as much BW as control rats treated with only saline (51.8% ± 8.8%; Figure 1, *A*).

Four weeks after MCT administration, the MCT-treated rats exhibited significant PH with a mean PAP of 36.5 ± 2.1 mm Hg, which is increased compared with that seen in control animals (17.5 ± 1.6 mm Hg, $P < .0001$). FR167653 treatment attenuated the development of PH, reducing the MCT-induced mean PAP increase to 24.7 ± 1.9 mm Hg ($P < .001$; Figure 1, *B*). Furthermore, the development of

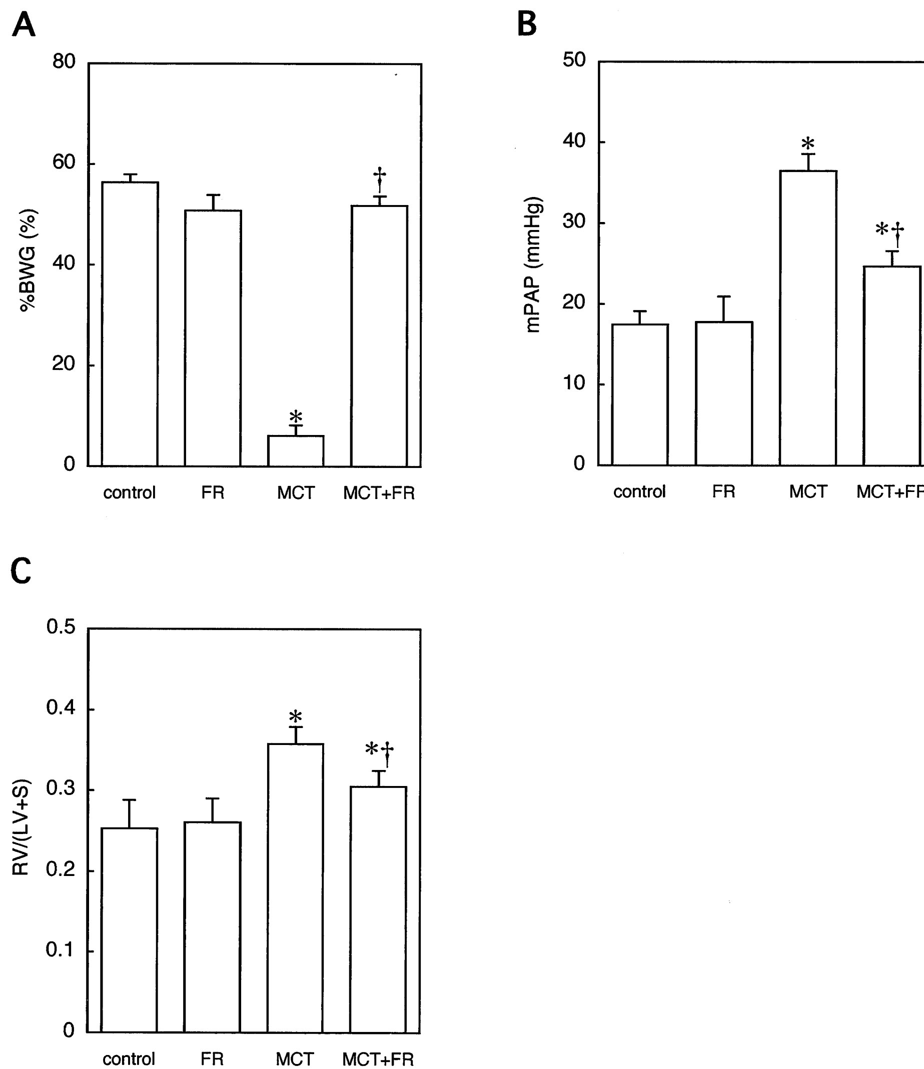

Figure 1. Effects of FR167653 on hemodynamic assessments in rats with MCT-induced PH. Shown are measurements of percentage BW gain (*%BWG*; A), mean PAP (B), and RV/(LV+S) ratio as RVH (C) in the control, FR, MCT, and MCT+FR groups at 4 weeks after MCT or saline administration. Values represent means ± SD of 5 animals: *P < .05 versus control group at 4 weeks; †P < .05 versus MCT group at 4 weeks.

chronic PH resulted in a compensatory right ventricular hypertrophy (RVH), as measured on the basis of an increased RV/(LV+S) ratio. MCT-treated rats had RVH with a higher RV/(LV+S) ratio when compared with values seen in control animals ($P < .05$). FR167653 treatment reduced RVH ($P < .05$; Figure 1, *C*).

The serum levels of glutamic oxaloacetic transaminase, glutamic pyruvate transaminase, creatine phosphokinase, alkaline phosphatase, blood urea nitrogen, and creatinine in rats treated with FR167653 daily (2 and 10 $mg \cdot kg^{-1} \cdot d^{-1}$ administered intramuscularly) for up to 18 weeks stayed within normal limits. Also, we did not detect any abnormal findings in histopathologic assessment of the brain, heart, lung, liver, and kidney (data not shown). Thus chronic administration of FR167653 to rats has no apparent toxicity.

Morphometric Analysis of the PA

Representative sections, including small and large PAs from all groups at 4 weeks after initial treatment with MCT, were stained for elastin to reveal the inner elastic lamina (Figure 2, *A*). The percentage of MWT in the MCT-treated rats was increased compared with that seen in the control rats ($P < .005$). FR167653 treatment in the MCT+FR group reduced this increase in the percentage of MWT ($P < .05$; Figure 2, *B*). The degree of medial hypertrophy in the MCT-treated animals at the end of the 4-week period increased signifi-

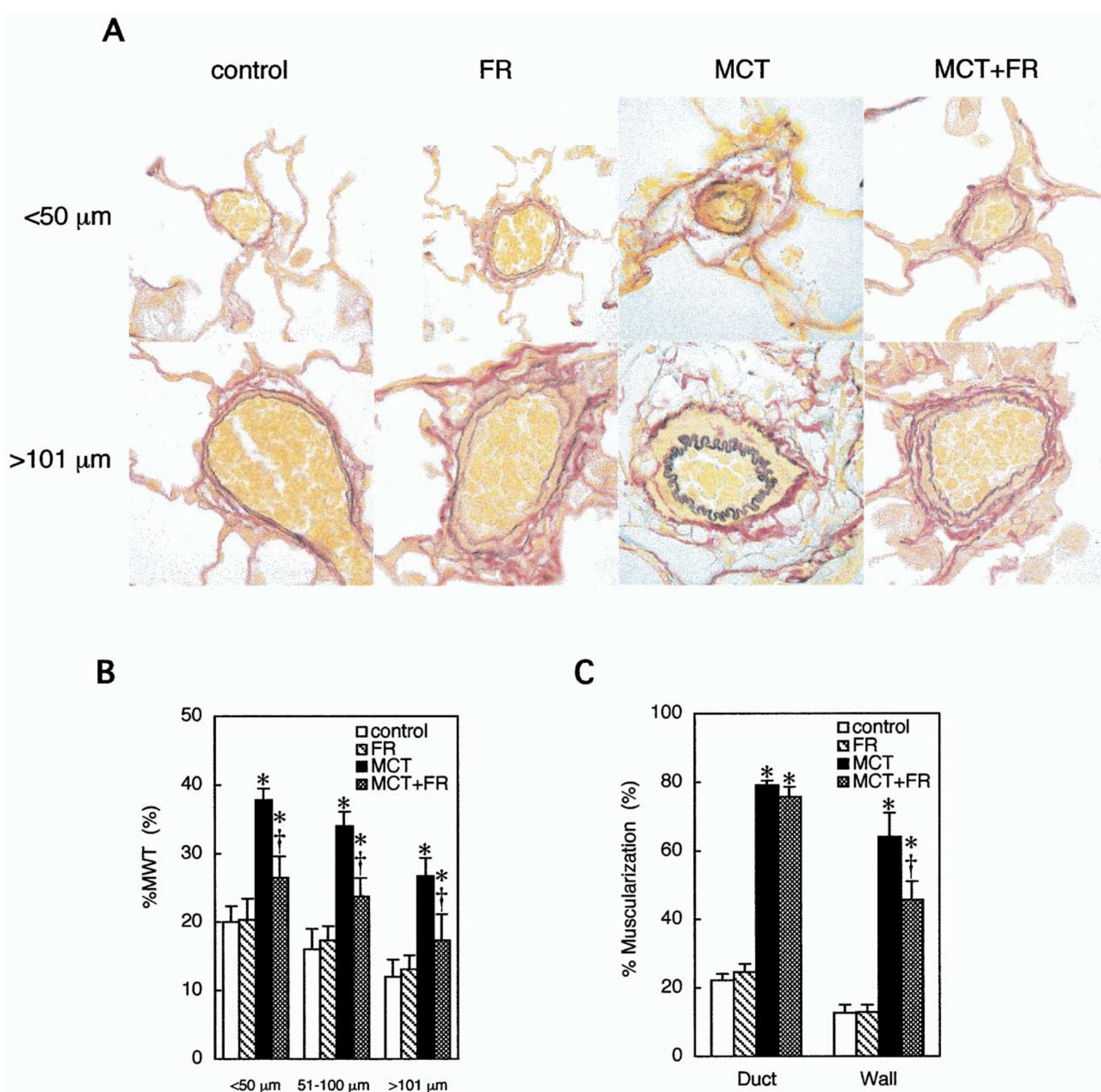

Figure 2. Effects of FR167653 on morphometric analysis of PA in rats with MCT-induced PH. Shown are histopathologic findings of PAs with external diameters of less than 50 μm and greater than 101 μm (elastica van Gieson, original magnification 400×; A); measurements of percentage of medial wall thickness of muscular PA *(%MWT)* with external diameters of less than 50 μm, 51 to 100 μm, and greater than 101 μm (B); and percentage of muscularized PA *(% Muscularization)* in normally nonmuscular peripheral PA at the alveolar duct and alveolar wall levels (C) in the control, FR, MCT, and MCT+FR groups at 4 weeks after MCT administration. Values represent means ± SD of 4 animals: *$P < .05$ versus period-matched control animals; †$P < .05$ versus period-matched MCT-treated animals.

cantly in PA segments at the alveolar wall and duct levels ($P < .05$ vs control). FR167653 treatment reduced medial hypertrophy at the alveolar wall level ($P < .05$), but there was no effect on the midlayer at the alveolar duct level (Figure 2, *C*).

p38 MAPK Activity in the Lung

p38 MAPK activity was increased at 1 week in the rats treated with MCT alone ($P < .005$). Thereafter, the fold increase of p38 MAPK activity in the MCT-treated group compared with that in the control group remained increased

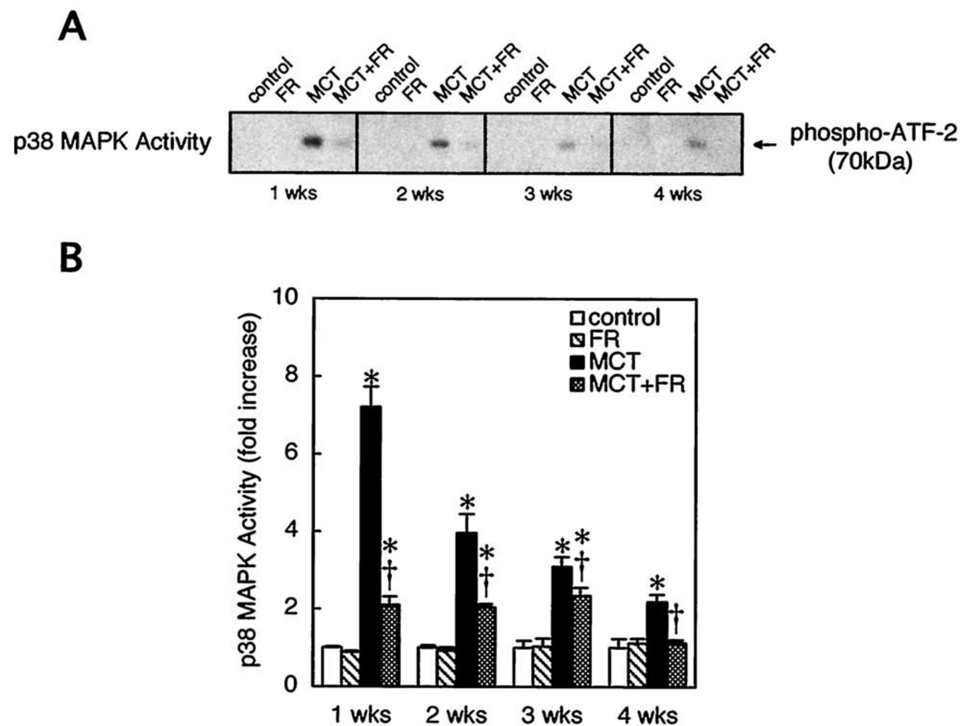

Figure 3. Effects of FR167653 on p38 MAPK activity in the lungs of rats with MCT-induced PH: A, Western blotting analysis of phospho-ATF-2 expression after immunoprecipitation and kinase assay as p38 MAPK activity; B, p38 MAPK activities denoted as fold activation over those of a period-matched control animal. Values represent means ± SD of 8 animals: *$P < .01$ versus period-matched control animals; †$P < .05$ versus period-matched MCT-treated animals.

but decreased progressively over the 4 weeks of the study. In the FR167653-treated group, however, p38 MAPK activity was attenuated at 1, 2, and 3 weeks after MCT treatment and completely blocked by 4 weeks ($P < .05$ vs MCT; Figure 3).

Immunohistochemical analyses of lung tissue from MCT-treated rats at 1 week after treatment demonstrated increased phospho-p38 MAPK staining localized in the endothelial and subendothelial layers of the PA. Phospho-p38 MAPK also localized in alveolar macrophages surrounding the intra-acinar PA and alveolar spaces. In contrast, FR167653 treatment reduced phospho-p38 MAPK in MCT-treated rats to a level comparable with that in lung tissue from control rats not treated with MCT (Figure 4).

Cytokine Expression in the Lung

TNF-α mRNA expression increased at 1, 2, and 3 weeks after MCT administration compared with that seen in age-matched control animals ($P < .005$). By 4 weeks, TNF-α mRNA levels returned to baseline. FR167653 completely inhibited TNF-α mRNA expression at all time points after MCT treatment ($P < .05$ vs MCT; Figure 5, *A*). IL-1β mRNA expression was also upregulated in rats treated with MCT administration compared with that seen in age-matched control animals ($P < .005$), and FR167653 blocked IL-1β mRNA expression (1 week, $P < .005$ vs MCT; 2, 3, and 4 weeks, $P < .05$; Figure 5, *B*). Similar to TNF-α and IL-1β, MCP-1 mRNA expression in the MCT and MCT+FR groups at 1 week after MCT administration was greater than that seen in period-matched control rats ($P < .001$). Thereafter, MCP-1 expression decreased to the same levels as that of period-matched control rats. FR167653 treatment had no effect on either the increase or decrease in MCP-1 expression induced by MCT (Figure 5, *C*).

Macrophage Infiltration in the Lung

The number of macrophages in the lung tissue, particularly in the alveolar spaces and surrounding PA, increased in rats treated with MCT and in MCT-treated rats also treated with FR167653 compared with that seen in period-matched control animals ($P < .01$). FR167653 treatment showed a trend toward decreased macrophage infiltration; however, this did not reach statistical significance (Figure 6).

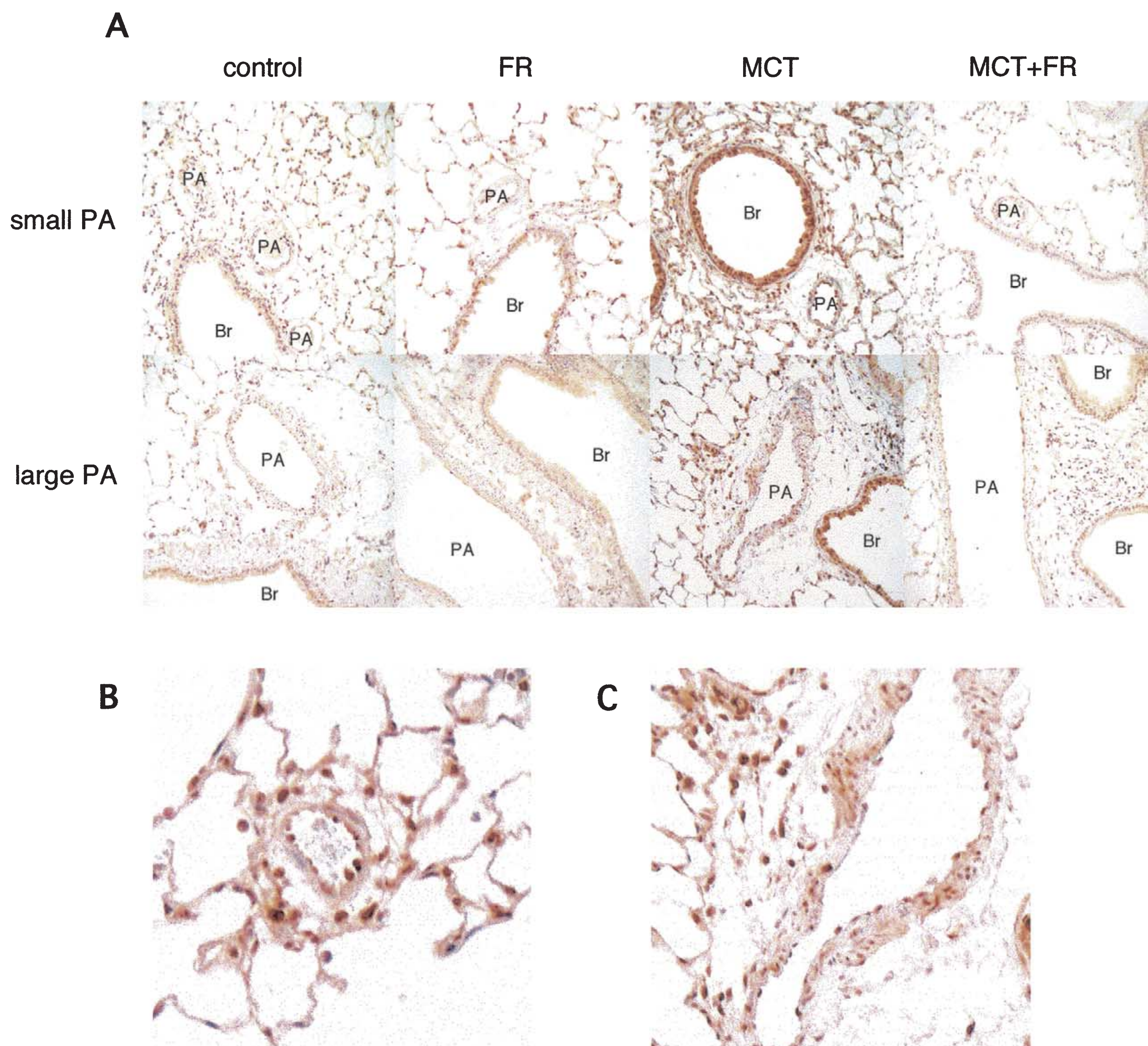

Figure 4. Immunohistochemical analyses for phospho-p38 MAPK localization *(dark brown)* in large and small PAs at 1 week after initial treatment (A; original magnification 400×). High-power resolution in small (B) and large (C) PAs in MCT-treated rats (original magnification 800×). Phospho-p38 MAPK–positive macrophages surrounding the intra-acinar PA in MCT-treated rats. *PA,* Pulmonary artery; *Br,* bronchiole.

Discussion

The pathogenesis of PH involves vasoconstriction, pulmonary vascular remodeling, in situ thrombosis, and inflammation. The inflammatory hypothesis of PH is supported by multiple experimental studies demonstrating a perivascular inflammatory cell infiltrate, abnormal TGF-β signaling (in both familial and sporadic PPH), increased expression of adhesion molecules (vascular cell adhesion molecule 1, intracellular adhesion molecule 1, and E-selectin), and upregulation of chemokines (eg, macrophage inflammatory protein 1α).[4,6,8,19] In addition, inflammatory mediators, such as TNF-α, IL-1β, IL-6, TGF-β, vascular endothelial growth factor, and platelet-derived growth factor, have been shown to play a central role in pulmonary vascular remodeling.[7] The role of inflammation in PH is further supported by the clinical improvement observed in a subset of patients with PH after steroid treatment or immunosuppressive therapy.[20,21] Multiple studies suggest that the initial inciting event in the development of PH might be endothelial cell injury by an unknown mechanism.[22] Injured endothelium can be activated to express adhesion molecules, vasoactive substances (eg, endothelin), cytokines, chemokines, and

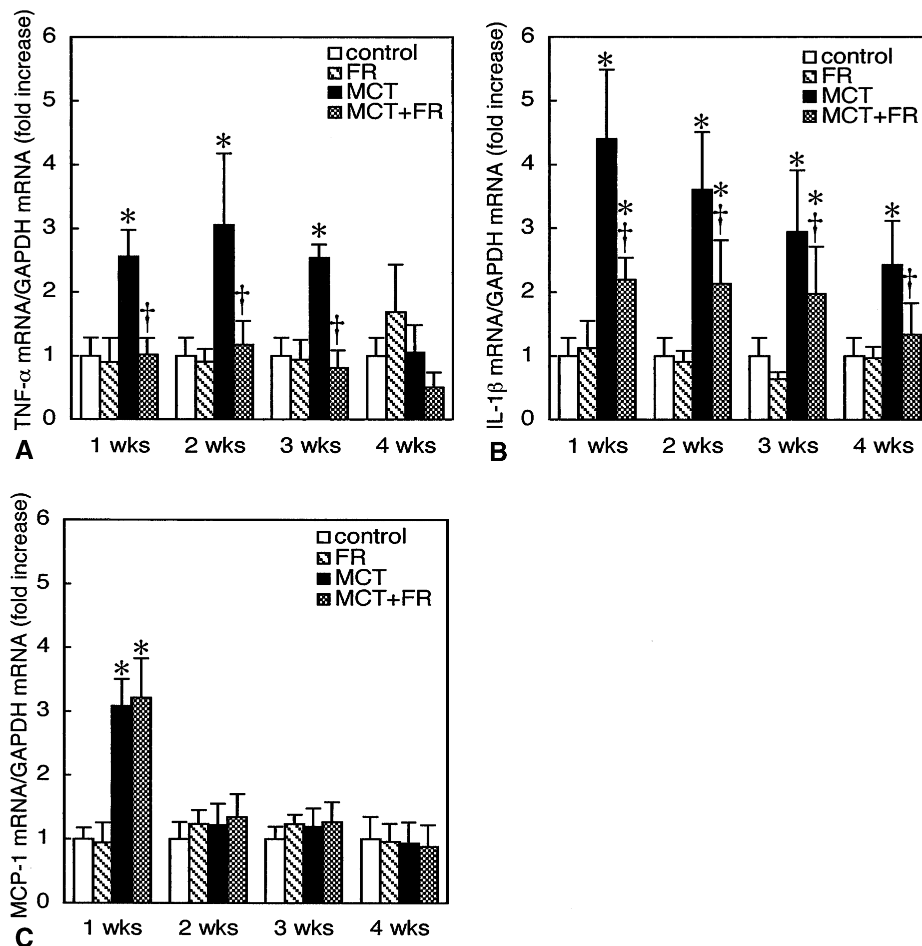

Figure 5. Effects of FR167653 on quantitative reverse transcriptase–polymerase chain reaction analysis of TNF-α (A), IL-1β (B), and MCP-1 (C) mRNA in the lungs of rats with MCT-induced PH. The *vertical axis* denotes a fold increase over values in period-matched control animals after the amount of mRNA for each cytokine was normalized to that of glyceraldehyde-3-phosphate dehydrogenase *(GAPDH)* mRNA. Values represent means ± SD of 8 animals: *$P < .05$ versus period-matched control animals; †$P < .05$ versus period-matched MCT-treated animals.

growth factors.[23] The subsequent inflammatory and procoagulant responses are thought to promote smooth muscle cell proliferation, vasoconstriction, and in situ thrombosis, resulting in pulmonary vascular remodeling and PH and ultimately right heart failure. To clarify the molecular mechanism underlying the development and progression of inflammation in PH, we examined p38 MAPK activation and inflammatory cytokine expression in MCT-induced PH in rats. p38 MAPK activity is associated with inflammation in a variety of systems, and we believe MCT-induced PH and many forms of human PH are, in part, inflammatory conditions. In addition, p38 MAPK is involved in the vessel wall response to mechanical stress, which might also contribute to PH in a subgroup of causes.[24]

MCT-induced PH in rats and human PH are similar in the following respects. First, early pathologic changes include PA endothelial swelling and blebbing and alveolar inflammatory infiltrates similar to those seen in clinical PH. Later changes include progressive vascular remodeling characterized in part by increased elastin and collagen protein synthesis and deposition. Second, histologically, both show medial hypertrophy involving the PAs. Third, tropoelastin and type I collagen staining are positive in both. Fourth, cytokine expression occurs in both and might be causally related in both. Finally, neointimal formation in response to injury occurs in both, although to a more pronounced degree in human PH.[25,26] However, so-called plexiform lesions, which are present in human PH, are not detected consistently in MCT-induced PH in rats.[27] MCT is a pyrrolizidine alkaloid that induces pulmonary vascular injury. Infiltration of mononuclear cells, particularly macrophages, is a prominent pathologic feature of MCT-in-

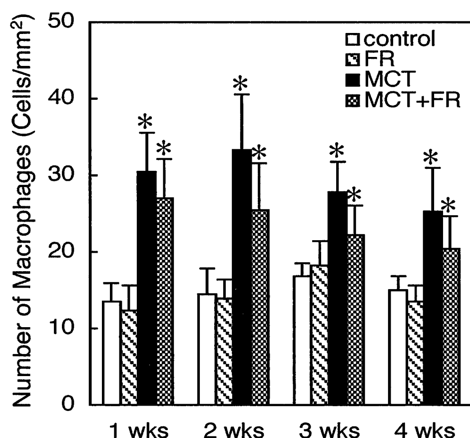

Figure 6. Effects of FR167653 on numbers of interstitial macrophages in the lungs of rats with MCT-induced PH. Values represent means ± SD of 8 animals: $^*P < .05$ versus period-matched control animals.

duced PH in the early phase.[28] Recent studies suggest that inflammatory cytokines, such as IL-1β and IL-6, which are produced by infiltrating macrophages, play a role in not only MCT-induced PH in rats[7,29] but also a variety of human diseases, including PPH.[4] Also, MCP-1 expression is associated with the development of MCT-induced PH in rats.[30,31]

We initially included TNF-α assays as a negative control because of a report showing normal circulating serum levels of TNF-α in patients admitted with PH for lung transplantation.[5] We were therefore surprised by increased levels of TNF-α mRNA in lung tissue in MCT-treated rats. It is quite possible that TNF-α released from resident mononuclear phagocytes is released and acts locally and would not be detected systemically. It is also possible that TNF-α transcription is upregulated in PH, but translation of TNF-α mRNA does not occur. These issues deserve further study. Furthermore, we are aware that although TNF-α induces p38 MAPK activity, p38 MAPK might signal TNF-α expression.[32]

p38 MAPK activation is involved in a variety of cellular responses, including inflammatory cytokine production, cell growth, cellular differentiation, and apoptosis.[33] p38 MAPK activation is induced in vascular smooth muscle cells and endothelial cells by means of atherogenic stimuli, such as mechanical stress, TNF-α, IL-1β, or oxidative stress.[34] Moreover, p38 MAPK is activated by chronic hypoxic stress, causing proliferation of the PA fibroblasts required for remodeling of the pulmonary vasculature in hypoxia-induced PH.[11,12] In this study we find an increase in p38MAPK activity in MCT-treated rat lungs, and we demonstrate that the production and action of many of the potential inflammatory mediators of PPH are associated with activation of the p38 MAPK signaling pathway.[33] Other signaling pathways, however, acting in concert with p38 MAPK activation might also be involved in the development of PH.[35]The use of specific p38 MAPK inhibitors for the treatment of inflammatory diseases has recently been examined.[36] In this study we have demonstrated that FR167653, a selective p38 MAPK inhibitor, decreased the expression of TNF-α and IL-1β in the PA and also prevented progression of PH in MCT-induced rats with PH. Although the expression of TNF-α mRNA appeared to be disassociated from p38 MAPK activity, likely this was due to the time needed for transcription and translocation of TNF-α after these events were initiated by the p38 MAPK pathway. The attenuation of inflammatory-mediated PH by FR167653 occurred without a decrease in expression of MCP-1, a potent chemotactic factor for macrophages, or in the number of interstitial macrophages migrating into the vessel wall. We therefore infer that MCP-1 secretion from activated human mononuclear phagocytes in PH is not dependent on p38 MAPK activity.[37]

MCT is converted in the liver by cytochrome P450 mono-oxygenases to monocrotaline pyrrole (MCTP) and other pyrrolic metabolites, which then travel to the pulmonary circulation. The chemical nature of MCTP suggests that it is an important pneumotoxic metabolite because MCTP is among the more stable toxic primary pyrrolizidine alkaloid pyrroles. MCTP binds to tissue components and initiates toxicity, beginning within the first 24 hours of MCT treatment in rats.[38,39] Therefore FR167653 treatment beginning the day after MCT administration in our model does not effect the bioactivation of MCT.

Most previous studies that have examined the roles of p38 MAPK in cellular function have used SB203580 to inhibit p38 MAPK. SB203580 activity is not confined to inhibition of p38 MAPK. SB203580 has also been reported to inhibit thromboxane synthase,[40] COX-1 and -2,[40] and JNKs.[41] This nonspecific action of SB203580 might explain contradictory mechanistic conclusions regarding the role of p38 MAPK. FR167653 is specific for p38 MAPK inhibitor, with no measurable affect on COXs or other MAPKs.[13]

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