Short-Term Administration of a Cell-Permeable Caveolin-1 Peptide Prevents the Development of Monocrotaline-Induced Pulmonary Hypertension and Right Ventricular Hypertrophy

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Background—Caveolins (Cavs), the principal structural proteins of caveolar microdomains, have been implicated in the development of pulmonary hypertension (PH). Mice with homozygous deletion of the Cav-1 gene develop PH and right ventricular hypertrophy (RVH). Reductions in pulmonary Cav-1 expression have been shown in several animal models of PH and in patients with severe PH. Whether in vivo modulation of Cav-1 expression could affect the development of PH and RVH remains unknown. Therefore, we investigated the effect of in vivo administration of a Cav-1 mimetic peptide on the development of monocrotaline (MCT)-induced PH.

Methods and Results—Thirty minutes after injection of saline or 60 mg/kg MCT, rats were assigned to receive a daily injection of saline, a peptide corresponding to the homeodomain of the Drosophila transcription factor antennapedia (AP; 2.5 mg · kg⁻¹ · d⁻¹), or a peptide consisting of the Cav-1–scaffolding domain coupled to AP (AP-Cav; 2.5 mg · kg⁻¹ · d⁻¹) for 2 weeks. MCT and MCT+AP rats developed PH with respective right ventricular systolic pressures of 40.2±1.5 and 39.6±1.5 mm Hg. Administration of AP-Cav to MCT rats significantly reduced the right ventricular systolic pressure to 30.1±1.3 mm Hg. MCT and MCT+AP rats also developed pulmonary artery medial hypertrophy and RVH, which was normalized by administration of AP-Cav. Mechanistically, the development of PH was associated with reduced expression of pulmonary Cav-1 and Cav-2, hyperactivation of the STAT3 signaling cascade, and upregulation of cyclin D1 and D3 protein levels, all of which were prevented by administration of AP-Cav.

Conclusions—Short-term administration of a Cav-based cell-permeable peptide to MCT rats prevents the development of pulmonary artery medial hypertrophy, PH, and RVH. (Circulation. 2006;114:912-920.)

Key Words: caveolin ▪ hypotrophy ▪ hypertension, pulmonary ▪ remodeling

Caveolae are vesicular organelles that are abundant in cells of the cardiopulmonary system, including endothelial cells, smooth muscle cells, epithelial cells, fibroblasts, and cardiomyocytes.1–3 In these cell types, caveolae function in protein trafficking, cholesterol homeostasis, and signal transduction.4–6 Caveolins (Cavs) are the structural proteins that are both necessary and sufficient for the formation of caveolae.7,8 Interestingly, Cav-1 and Cav-2 are coexpressed in most cell types, whereas the expression of Cav-3 is muscle specific.1–3 Therefore, endothelial cells, epithelial cells, and fibroblasts are rich in Cav-1 and Cav-2, whereas cardiomyocytes express Cav-3.1–3 On the other hand, smooth muscle cells express all 3 Cavs.1,2

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Cav proteins recently have been implicated in the development of pulmonary hypertension (PH) and the structural remodeling of the lungs.9–12 Indeed, Cav-1−/− and Cav-2−/− deficient mice [Cav-1−/− and Cav-2−/−] show abnormalities in pulmonary structure and function as demonstrated by hypercellularity, interstitial fibrosis, thickening of the alveolar septa, and reduced exercise tolerance.9,10 Cav-1−/− mice were further shown to develop PH and right ventricular (RV) hypertrophy.12 Interestingly, we recently demonstrated a marked decrease in both Cav-1 and Cav-2 protein levels in the lungs of rats with myocardial infarction (MI)–induced PH.11 This decreased expression of pulmonary Cav was associated with increased tyrosine phosphorylation of the signal transducer and activator of transcription-3 (STAT3), as well as an upregulation of cyclin D1 and D3 protein levels.11 A reduction in pulmonary Cav-1 expression was later reported in rats with monocrotaline (MCT)- and 3-[2,4 dimethylpyrrol-5-yl]methyldienyl]-indolin-2-one (SU5419)–induced PH.13,14 Importantly, decreases in both Cav-1 and Cav-2 protein levels also were recently demonstrated in...
plexiform lesions of patients with severe PH.\textsuperscript{14} As previously suggested,\textsuperscript{11} downmodulation of pulmonary Cav protein expression could thus represent an initiating mechanism leading to the development of PH and lung remodeling. Whether in vivo modulation of Cav protein levels could prevent the development of PH remains unknown.

Interestingly, the coupling of molecules to a 16–amino acid peptide corresponding to the homeodomain of the Drosophila transcription factor antennapedia (AP or penetratin) has been shown to facilitate their uptake into cultured mammalian cells through a nonendocytic and nondegradative pathway.\textsuperscript{15,16} Accordingly, coupling of the Cav–1–scaffolding domain to the AP peptide (AP-Cav or cavitatin\textsuperscript{17}) was recently shown to facilitate its translocation across the cell membranes and to reduce inflammation, microvascular hyperpermeability, and tumor progression in mice.\textsuperscript{17,18} Furthermore, perfusion of a Cav–1 peptide was shown to exert cardioprotective effects in myocardial ischemia–reperfusion experiments.\textsuperscript{19}

Therefore, the present study was designed to determine the efficacy of the in vivo administration of a cell-permeable Cav–1–derived peptide on the development of MCT-induced PH and RV hypertrophy.

**Methods**

**Materials**

Biotinylated peptides corresponding to AP [(biotin)-\textit{RQPKWFPNRKKWK}-(OH)] and AP-Cav [(biotin)-\textit{RQPKWFPNRKKWK}-DIGWKAASFTFTVTKYWFR-(OH)] were custom synthesized (at the Tufts University Core Facility). MCT and a mouse monoclonal antibody (mAb) to β-actin were purchased from Sigma-Aldrich (St Louis, Mo). Cav-1 and -2 mAbs were the generous gifts of Dr Roberto Campos-Gonzalez (BD-Pharmingen, San Diego, Calif). A rabbit polyclonal antibody to von Willebrand Factor (vWF), a mouse mAb to STAT3, a mouse mAb to phospho-tyrosine (PY)-STAT3, a mouse mAb to endothelial nitric oxide synthase (eNOS), and rabbit and mouse horseradish peroxidase–conjugated secondary antibodies were all purchased from BD-Pharmingen. Mouse mAbs to cyclin D1 and cyclin D3 were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, Calif). Rabbit and mouse fluorescein (FITC) and rhodamine (TRITC)-conjugated secondary antibodies were purchased from Jackson ImmunoResearch (West Grove, Pa).

**Animal Studies**

This study was conducted according to the guidelines of the National Institute of Health and the Thomas Jefferson University Institute for Animal Studies.

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass) weighing between 250 and 300g received a single intraperitoneal injection of either 0.5 mL 0.9% NaCl or 0.5 mL 60 mg/kg MCT. Thirty minutes later, rats were randomly assigned to receive a daily intraperitoneal injection of either 0.5 mL 0.9% NaCl, 0.5 mL AP (2.5 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}) or 0.5 mL AP-Cav (2.5 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}) for 2 weeks. This resulted in the following 5 groups: control (n = 17), control+AP-Cav (n = 10), MCT (n = 25), MCT+AP (n = 19), and MCT+AP-Cav (n = 24).

At 2 weeks, rats were anesthetized with xylazine (10 mg/kg)-ketamine (50 mg/kg) followed by 2000 U heparin (Sigma-Aldrich). The right jugular vein and carotid artery were then isolated and incised, and Millar catheters (SPR-249, Millar Instruments, Houston, Tex) were advanced into the RV and left ventricle (LV) for hemodynamic measurements. The RV and LV pressures were recorded with the Ponemah P3-Data acquisition system (LDS Test and Measurement, Middleton, Wis).

Afterward, the lower lobe of the right lung and the heart were dissected and weighed to determine pulmonary edema and RV hypertrophy, respectively, as previously described.\textsuperscript{11} The remaining lobes of the right lung were submerged in liquid nitrogen and frozen at −80°C. The left pulmonary artery was then cannulated and perfused with 4% paraformaldehyde for 2 minutes. This was followed by perfusion of the airways with 4% paraformaldehyde for 2 minutes. The left lung was then immersed in 4% paraformaldehyde for 24 hours.

**Immunoblot Analysis**

Lung samples from control (n = 17), control+AP-Cav (n = 10), MCT (n = 25), MCT+AP (n = 19), and MCT+AP-Cav (n = 24) groups were homogenized in a RIPA lysis buffer containing protease and phosphatase inhibitors. Proteins were then separated by SDS-PAGE (12% acrylamide) and transferred to nitrocellulose membranes. The membranes were placed in blocking solution for 30 minutes. Afterward, the membranes were washed and incubated with a given primary antibody for 1 hour (Cav-1, Cav-2, and β-actin) or 3 hours (eNOS, STAT3, PY-STAT3, cyclin D1, and cyclin D3). Finally, horseradish peroxidase–conjugated secondary antibodies were used to detect bound primary antibody using the SuperSignal chemiluminescence substrate (Pierce Biotechnology, Rockford, Ill). Western blots for Cav-1, Cav-2, eNOS, PY-STAT3, cyclin D1, and cyclin D3 were subsequently quantified with the NIH Image J software (using the mean gray value for each band).

**Immunofluorescence Analysis**

As mentioned, the left lung of control (n = 17), control+AP-Cav (n = 10), MCT (n = 25), MCT+AP (n = 19), and MCT+AP-Cav
(n=24) rats was dissected and perfused-fixed. Transverse sections were obtained and embedded with paraffin. Sections of 10 μm were cut and stained with hematoxylin and eosin. Paraffin from 10-μm-thick sections was removed by immersion in xylene. These sections were then rehydrated with graded alcohol to water and blocked overnight. The sections were subsequently incubated with a given primary antibody for 3 hours. FITC- and TRITC-conjugated secondary antibodies were then added to the sections after a 15-minute wash in PBS. After 1 hour of incubation with the secondary antibodies, the sections were washed in PBS and mounted with the Prolong Gold antifade reagent (Molecular Probes, Carlsbad, Calif).

Lung Vascular Morphometry

Hematoxylin and eosin sections of the left lung (n=10 for each group) were microscopically assessed for the medial wall thickness of pulmonary arteries. Measurements of the luminal diameter and the medial thickness on either side were obtained with the Image J software. Measurements were made on 30 muscular arteries (<50-, 51- to 100-, and 100- to 150-μm external diameter) per lung section. The medial wall thickness was then related to the external diameter and expressed as percent wall thickness as previously described.20

Statistical Analysis

Hemodynamic and morphometric variables and the mean gray value of each Western blot are expressed as mean±SEM. Differences between the 5 groups were evaluated by ANOVA, followed by Tukey’s multiple-group comparisons test. Statistical significance was assumed at P<0.05.

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

Results

Hemodynamic and Morphological Effects of AP-Cav

The MCT and MCT+AP rats developed PH with respective RV systolic pressures of 40.2±1.5 and 39.6±1.5 mm Hg compared with 26.0±0.9 mm Hg in the control rats (P<0.01; Figure 1A). Interestingly, the MCT+AP-Cav rats demonstrated a significant reduction in RV systolic pressures to 30.1±1.3 mm Hg (P<0.01 versus MCT and MCT+AP; Figure 1A). The central venous pressures and RV end-diastolic pressures behaved similarly, with significant increases in both MCT and MCT+AP rats that were normalized in the MCT+AP-Cav rats (P<0.05; the Table). The MCT and MCT+AP rats also developed RV hypertrophy with respective RV/LV+septum weight ratios of 31.1±0.6% and 30.6±0.6% compared with 25.3±0.5% in the control rats (P<0.01; Figure 1B). Interestingly, the RV/LV+septum weight ratio was normalized to 24.6±0.5% in the MCT+AP-Cav rats (P<0.01; Figure 1B). The lung weights of the MCT and MCT+AP rats were significantly increased in the absence of significant edema formation (P<0.05; the Table). Administration of AP-Cav to MCT rats significantly reduced the lung weight (P<0.05; the Table). LV function remained unchanged in all experimental groups (the Table). All other hemodynamic and morphometric variables are summarized in the Table. Administration of AP-Cav to the control group did not affect any of the hemodynamic and morphometric variables compared with the control group receiving 0.9% NaCl alone (Table).

Expression of Cav Proteins and eNOS in the Lungs of PH Rats

As demonstrated in Figure 2, immunoblot analysis showed a marked decrease in Cav-1 (≈3 fold; P<0.01) and Cav-2 (≈2 fold; P<0.01) protein levels in the lungs of MCT and MCT+AP rats. Administration of AP-Cav to MCT rats significantly prevented the reduction of pulmonary Cav-1 and Cav-2 protein levels (P<0.05; Figure 2). Similarly, dual-label immunofluorescence analysis demonstrated marked reductions in both Cav-1 (Figure 3) and Cav-2 (Figure 4) expression in pulmonary arteries of MCT and
MCT/H11001 AP rats, which were prevented by administration of AP-Cav. Immunoblot analysis of eNOS protein levels did not reveal any significant differences among all groups (P/NS; Figure 5).

STAT3 Signaling Cascade in the Lungs of PH Rats

Immunoblot analysis demonstrated increased levels of PY-STAT3 (≈5 fold; P<0.05) in the lungs of MCT and MCT+AP rats (Figure 6). Administration of AP-Cav to MCT rats prevented the hyperactivation of the STAT3 signaling cascade (Figure 6). Importantly, pulmonary expression of total STAT3 was similar in all groups.

Expression of Cyclin D1 and D3 in the Lungs of PH Rats

As demonstrated in Figure 7, immunoblot analysis showed marked increases in cyclin D1 (≈4 fold; P<0.01) and cyclin D3 (≈4 fold; P<0.01) protein levels in the lungs of MCT and MCT+AP rats. Interestingly, administration of AP-Cav to MCT rats prevented the upregulation of pulmonary cyclin D1 and cyclin D3 expression (P<0.01; Figure 7).

Pulmonary Artery Remodeling

Pulmonary arteries (<50, 51 to 100, and 101 to 150 μm) of MCT and MCT+AP rats showed increased percent medial wall thickness (≈2- to 3-fold; P<0.05) compared with control rats (Figure 8). Administration of AP-Cav to MCT rats significantly reduced the percent medial wall thickness (P<0.05; Figure 8). Administration of AP-Cav to control rats had no effect on the pulmonary artery percent medial wall thickness (data not shown).

Discussion

These results demonstrate a marked decrease in Cav-1 and Cav-2 protein levels in the lungs of rats with MCT-induced PH. This decrease is associated with the hyperactivation of the STAT3 signaling cascade and the upregulation of cyclin D1 and cyclin D3 protein levels. Importantly, our results also demonstrate for the first time that short-term administration
of a cell-permeable Cav-1 peptide prevents the development of pulmonary artery medial hypertrophy, PH, and RV hypertrophy in MCT rats. Mechanistically, administration of a Cav-1–derived peptide prevented the decreased expression of Cav-1 and Cav-2, the phosphorylation of STAT3, and the upregulation of cyclin D1 and cyclin D3 protein levels in the lungs of MCT rats.

**Cav Protein and eNOS Expression in PH Rats**

Cav proteins have been suggested to function as key regulators of the development of PH and lung remodeling. Indeed, the lungs of Cav-1<sup>−/−</sup> and Cav-2<sup>−/−</sup> mice showed hypercellularity, fibrosis, and thickened alveolar septa. Cav-1<sup>−/−</sup> mice were further shown to develop PH and RV hypertrophy. Interestingly, we and others recently reported the decreased expression of pulmonary Cavs in several animal models of PH such as the MCT, MI, and SU5419 rat models. Importantly, these reports and our present results appear to be relevant to human PH in that decreases in both Cav-1 gene and protein expression have been reported in patients with severe PH. Our present results validate those of Mathew et al, who demonstrated a decrease in Cav-1 protein levels in the lungs of MCT rats. As we previously reported in the MI rat model of PH, our present results further demonstrate the downregulation of Cav-2 protein levels in the lungs of MCT rats. Moreover, our dual-label immunofluorescence analysis also validate the results of Mathew et al, who showed a reduction in Cav-1 expression in pulmonary artery endothelial cells of MCT rat lungs. Interestingly, our dual-label immunofluorescence analysis also demonstrated a reduction in Cav-2 expression in pulmonary artery endothelial cells of MCT rat lungs.

**Figure 4.** Dual-label immunofluorescence analysis of Cav-2 (A, D, G, J) and vWF (B, E, H, K) expression shows a marked decrease in Cav-2 expression in the pulmonary arteries of MCT (D, E, F) and MCT + AP (G, H, I) rats vs control rats (A, B, C). Administration of AP-Cav to MCT rats prevented the reduction in Cav-2 in pulmonary arteries (J, K, L). Merged images (yellow) of Cav-2 (red) and vWF (green). All pictures were taken at the same magnification of x40 and are representative of 15 fields per animal (n=10 to 25 for each group).

**Figure 5.** Western blot analysis shows similar eNOS protein levels among the different groups (A) (3 rats are shown for each group). Quantification is shown (B; n=10 to 25 for each group). Immunoblotting with β-actin is shown as a control for equal protein loading.

**Figure 6.** Western blot analysis of MCT and MCT + AP rat lungs shows increased expression of PY-STAT3 vs control rat lungs (A). Administration of AP-Cav to MCT rats prevented the hyperactivation of the STAT3 signaling cascade (A) (3 rats are shown for each group). Quantification is shown (B). Immunoblotting with total STAT3 is shown as a control for equal protein loading. *P<0.05 vs control; †P<0.05 vs MCT; and ‡P<0.05 vs MCT + AP (n=10 to 25 for each group).
importantly, we further show that administration of AP-Cav to MCT rats prevents the reduction of pulmonary Cav-1 and Cav-2 protein levels.

Although eNOS protein levels appear slightly reduced in all MCT-treated rats, no significant differences were observed among all groups. However, previous studies reported decreased expression of pulmonary eNOS at 5 and 6 weeks after MCT.22,23 Therefore, it is likely that significant modulations of eNOS expression appear at a later stage in the development of MCT-induced PH. Interestingly, although Cav-1 is well recognized for its negative regulation of eNOS activity,24 administration of AP-Cav to both control and MCT rats did not have a significant effect on pulmonary eNOS expression. The lack of effect of the AP-Cav administration on any hemodynamic variables in control rats also supports the absence of effect of AP-Cav on eNOS activity.

STAT3 Activation and Cyclin Expression in PH Rats

Upregulations of PY-STAT3, cyclin D1, and cyclin D3 protein levels were reported in the lungs of Cav-1(-/-) and Cav-2(-/-) mice.11 Hyperactivation of the pulmonary STAT3 signaling cascade also was reported in the MI and MCT rat models of PH.11,13 An upregulation of both cyclin D1 and cyclin D3 expressions also was observed in the lungs of rats subjected to MI-induced PH.11 Our present results confirm the hyperactivation of the pulmonary STAT3 signaling cascade and further show marked increases in cyclin D1 and cyclin D3 protein levels in the lungs of MCT and MCT + AP rats. As we previously suggested,11 the downmodulation of Cav proteins may thus represent an initiating mechanism leading to the activation of the STAT3/cyclins pathway and ultimately to the development of PH. Accordingly, our present results confirm that administration of AP-Cav to MCT rats is sufficient to restore normal levels of pulmonary PY-STAT3, cyclin D1, and cyclin D3. The initiating role of Cav-1 is further supported by the observations of Mathew et al,13 who demonstrated that although pulmonary Cav-1 ex-
pression decreased as early as 48 hours after the MCT injection, increases in PY-STAT3 were perceptible only at 1 week after MCT.13

Effects of a Cav-1–Derived Peptide on MCT-Induced PH and RV Hypertrophy

Cav-1 is well known to interact with many signaling molecules through its Cav-scaffolding domain (residues 82 to 101). Indeed, the Cav-scaffolding domain recognizes and binds a specific motif within many known proteins such as eNOS, G-alpha subunits, protein kinase-C, and extracellular signal-regulated kinase-1/2 (ERK1/2).24–26 Interestingly, Cav-1 appears to negatively regulate many of these signaling proteins.24–26 For instance, a peptide corresponding to the Cav-1–scaffolding domain was previously shown to inhibit the in vitro activity of ERK1/2 and eNOS.24,26 Importantly, the generation of Cav-deficient mice also supports the Cav-1–mediated negative regulation of many proteins such as eNOS, ERK1/2, cyclins, and STAT3.9,11,27,28 For instance, Cav-1+/− mice display reduced vascular tone and microvascular hyperpermeability secondary to eNOS hyperactivation.9,27 Hearts of Cav-1+/− mice further display increased ERK1/2 phosphorylation.28 As mentioned, the lungs of Cav-1+/− mice also show hyperactivation of the STAT3 signaling cascade, as well as the upregulation of cyclin D1 and cyclin D3 protein levels.11 Interestingly, in vivo administration of a Cav-1–scaffolding domain peptide was shown to reduce microvascular hyperpermeability, inflammation, and tumor progression in mice.17,18 Moreover, perfusion of a Cav-1–peptide also was shown to exert cardioprotective effects in myocardial ischemia-reperfusion experiments by reducing polymorphonuclear neutrophil adherence and infiltration.19 However, whether in vivo administration of such a Cav-1–scaffolding domain peptide could complement the decreased expression of endogenous Cav-1 and prevent the development of PH remains unknown.

Our present results show that administration of a cell-permeable Cav-1 peptide to MCT rats prevents increases in pulmonary artery percent medial wall thickness, RV systolic pressures, and RV/LV+septum weight ratio. Mechanistically, we show that administration of AP-Cav to MCT rats prevents the reduction in Cav-1 and Cav-2 protein levels and the increases in pulmonary phosho-STAT3 and protein levels of cyclins. We hypothesize that the reduction in RV systolic pressures observed in MCT+AP-Cav rats could be ascribed, at least in part, to the reduction in pulmonary artery medial hypertrophy. Accordingly, we show that administration of AP-Cav to control rats has no effects on any of the hemodynamic variables. These results are consistent with previous reports that demonstrated that in vivo delivery of AP-Cav to mice had no effect on the systemic blood pressure, blood flow, and heart rate.17,18 Inhibition of the mitogenic STAT3/cyclins pathway observed in the lungs of MCT+AP-Cav rats also supports an essential role for AP-Cav treatment in the prevention of pulmonary artery medial hypertrophy development.

The functional role of Cav proteins in vascular remodeling also supports the effects of AP-Cav administration on the reduction in pulmonary artery medial hypertrophy. Indeed, reductions in Cav-1 and Cav-2 protein expression were previously shown in the in vitro model of serum-induced vascular smooth muscle cell proliferation.29 A decrease in Cav-1 expression also was observed in proliferating smooth muscle cells isolated from human atherosclerotic arteries.30 Interestingly, administration of a Cav-1–derived peptide to cultured rat vascular smooth muscle cells was shown to inhibit histamine- and norepinephrine-induced increases in intracellular calcium concentrations through inhibition of phospholipase-C and mitogen-activated protein kinase activation.31 Importantly, the generation of Cav-1+/− mice supports the key regulatory roles of Cav proteins in smooth muscle cell proliferation and vascular remodeling.32 Indeed, cultured aortic smooth muscle cells derived from Cav-1+/− mice display increases in proliferation and migration rates and upregulation of phosho-ERK1/2, cyclin D1, and the proliferating cell nuclear antigen protein levels.32 Collectively, these reports and our present results suggest that administration of a cell-permeable Cav-1 peptide might initially prevent the development of pulmonary artery medial hypertrophy that consequently could prevent the increases in pulmonary artery pressures and ultimately the development of RV hypertrophy. Accordingly, MCT injection has previously been shown to initially stimulate the appearance of muscle in normally nonmuscular arterioles, to increase the percent medial wall thickness, and to reduce the lumen diameter, which ultimately results in rises in the pulmonary vascular resistances and pulmonary artery pressures.33 However, a direct effect of AP-Cav on RV hypertrophy itself cannot be ruled out. Indeed, decreased expression of Cav proteins has been documented in the hypertrophic hearts of both spontaneously hypertensive rats and perinephritic hypertensive dogs.34,35 Accordingly, both Cav-1+/− and Cav-3+/− mice were shown to develop RV and LV hypertrophy.28,36 As previously suggested,28 because Cav-1 is normally not expressed in the cardiomyocytes themselves, the development of ventricular and individual cardiomyocyte hypertrophy observed in Cav-1+/− mice is most likely to be attributed to the release of autocrine and paracrine factors such as endothelin-1 and the transforming growth factor-β1. Thus, administration of AP-Cav could possibly affect such autocrine/paracrine mechanisms and alter the development of cardiac hypertrophy. Therefore, whether administration of a Cav-1 peptide can directly affect the development of ventricular hypertrophy remains to be clarified.

Study Limitations

Although our present results demonstrate a lack of effect of the AP-Cav administration on pulmonary eNOS expression, the precise effects of such a cell-permeable Cav-1 peptide on actual NO production remains to be determined. Accordingly, previous studies showed conflicting results concerning the effect of Cav-1 peptide administration on eNOS activity and NO release.17–19 Indeed, although delivery of a Cav-1 peptide was shown to reduce microvascular hyperpermeability, inflammation, and tumor progression through negative regulation of eNOS activity, it was conversely shown to exert cardioprotective effects in myocardial ischemia-reperfusion
experiments by increasing NO release.\textsuperscript{17–19} In this setting, delivery of a Cav-1–derived peptide was shown to enhance endothelium-derived NO release through inhibition of protein kinase-C activity and reactive oxygen species production.\textsuperscript{19}

**Conclusions**

Short-term administration of a cell-permeable Cav-1 peptide prevents the development of pulmonary artery medial hypertrophy, PH, and RV hypertrophy in the MCT rat model. Mechanistically, administration of AP-Cav to MCT rats prevents the decreased expression of Cav-1 and Cav-2, and the hyperactivation of the STAT3 signaling cascade, and the upregulation of cyclin D1 and D3 protein levels. Taken together, our results demonstrate that reduced pulmonary Cav proteins expression is not only a marker of but also a contributor to the development of PH. Importantly, administration of a Cav-1–scaffolding domain peptide might become an alternative treatment for PH. Future studies are necessary to evaluate the long-term effect of such a cell-permeable Cav-1 peptide on both the prevention and therapy of different types of PH.

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**Disclosures**

None.

**References**


CLINICAL PERSPECTIVE

Our present results and previous reports have revealed a reduction in pulmonary caveolin (Cav) protein expression in several animal models of pulmonary hypertension. Importantly, these results appear relevant to human pulmonary arterial hypertension because a reduction in Cav-1 gene expression was previously reported in the lungs of patients with primary pulmonary hypertension. Accordingly, immunohistochemistry analysis later revealed the reduction in both Cav-1 and Cav-2 protein levels in musculature precapillary arterioles and plexiform lesions of lungs of patients with pulmonary arterial hypertension. Interestingly, the reductions in Cav protein were observed in both the endothelial cells and smooth muscle cells of the plexiform lesions. Downmodulation of the Cav protein expression in the lungs of patients with pulmonary arterial hypertension could thus represent an initiating mechanism leading to endothelial and smooth muscle cell proliferation and consequently to the formation of plexiform lesions. Therefore, reduced pulmonary Cav expression might emerge as a new marker and an important contributor to the development of human pulmonary arterial hypertension. However, future studies are warranted to determine whether Cav proteins also could be involved in the development of other types of human pulmonary hypertension. Importantly, our present results demonstrate that daily administration of a cell-permeable Cav-1 peptide can complement the decreased expression of endogenous caveolin proteins and consequently prevent the development of pulmonary hypertension in the monocrotaline rat model of pulmonary arterial hypertension. Therefore, modulation of Cav protein levels could ultimately become a new and alternative treatment for pulmonary arterial hypertension.
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