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D-4F Does Not Mitigate Key Parameters in Rat Model of Severe Pulmonary Hypertension

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Summary

We employed a newly developed rat model of severe pulmonary hypertension (PH) to study if treatment with apolipoprotein (apo)-mimetic peptide, D-4F, mitigates PH and related pathology. Sprague-Dawley rats underwent surgery to partially ligate their left pulmonary artery, after 3-days they were injected with monocrotaline (MCT) and then randomly selected to receive a daily injection of D-4F or saline. After 21-days of treatment the animals were examined with respect to isolated lung hemodynamic measurements and X-ray microfocal angiography. There was no significant difference in right-to-left heart weight, change in body weight, pulmonary vascular resistance (PVR), or pulmonary micro-vascular density between D-4F and saline treated rats.

Introduction

Pulmonary hypertension results in remodeling of large and small pulmonary vessels, a disturbance in the delicate balance of ventilation/perfusion, and increased load on the right heart. In late stages of the disease, severe PH is accompanied by plexiform and neointimal lesions which significantly increase pulmonary vascular resistance[1, 2]. A well accepted rat model of PH involves a single subcutaneous injection of MCT, however, this model does not result in neointimal lesions. Therefore, we have developed a rat model of severe PH that combines partial ligation of the left pulmonary artery with MCT injection resulting in the formation of neointimal lesions which more closely mimics severe PH in human[3]. The model is inherently useful for

investigating possible anti-remodeling treatments aimed at reducing cellular proliferation and, in turn, lowering pulmonary vascular resistance.

Apolipoprotein (apo) A-I and apoA-I–mimetic peptides hold great promise for treatment of atherosclerosis[4, 5]. D-4F has been shown to significantly reduce rapidly evolving atherosclerotic lesions, oxidation and inflammation; and, there is evidence that it has the ability to improve endothelial function[6-8]. For these reasons, D-4F was thought to be a good candidate for treatment of vascular remodeling in this proliferative model, in which histology also confirmed marked inflammatory infiltrates[3].

Materials and Methods

All the studies were done under approval of the Zablocki VA Medical Center IACUC review board and in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals. Fifteen (15) male Sprague-Dawley rats (280-350 gm) underwent a recovery surgery in which the rat is anesthetized, intubated, then through thoracotomy the left pulmonary artery is isolated, looped with 4-0 suture and reduced such that the outer vessel diameter was 0.61mm. The animals were allowed to recover for 3-days, then injected with MCT (60 mg/kg, sub-Q), and randomly selected to receive an injection of either D-4F [1 mg/kg/day, i.p.] (N=8), or an equal volume of saline (control C, N=7). Twenty-one (21) days after injections, the animals were anesthetized, a cardiac blood sample drawn, the main pulmonary artery and trachea cannulated, and the heart and lungs excised. The right ventricle was dissected from the heart and both segments weighed separately as a measure of right ventricular hypertrophy. The isolated intact lung was prepared as previously described[3, 9]. Briefly, the lung was ventilated, rinse of blood and perfused with a physiologic saline solution. Then the airway pressure held constant at 6 mmHg, the pulmonary vein pressure (P_{PV}) kept ambient, and pulmonary arterial (P_{PA}) pressure measured over a range of flow rates (Q, 0-40 ml/min). Finally, the perfusate was replaced with a vascular X-ray contrast medium and microfocal angiography performed. All statistical comparisons were performed using a t-test (Pd"0.02) SigmaStat 3.0 (SPSS, Chicago, IL).

Results

There was no significant difference in key structure/function parameters of the PH model between the D-4F treated and the control rats. No significant change was detected in the right ventricular hypertrophy, as measured by the ratio of the weight of the right ventricle compared to the left ventricle plus septum, see Figure 1. There was no significant difference in the gain in body weight over the 24-day experiment, see Figure 2. Treatment with D-4F did not result in a significant decrease in pulmonary vascular resistance, $(P_{PA} - P_{PV})/Q_{100}$, where Q_{100} indicates flow rate was normalized to body weight at 100 ml/min/kg, see Figure 3. Microfocal X-ray angiography did not detect any difference in the pulmonary microvascular density, which is significantly reduced

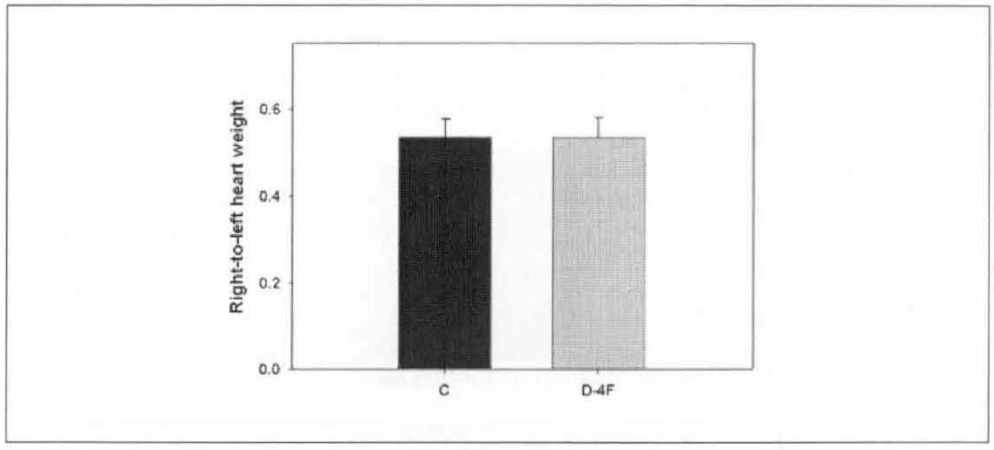

Figure 1. Ratio of the weight of the right ventricle compared to the left ventricle plus septum, as a measure of right ventricular hypertrophy.

in MCT models of PH[3], as shown in Figure 4. Interestingly, 2 of the rats that were receiving D-4F treatment died approximately 17 days into the experiment (none of the control rats died) and D-4F treated rats appeared to have more labored breathing and their lungs appeared worse compared to non-treated PH rats on gross assessment.

Conclusion

Treatment with D-4F was not shown to mitigate measures of structural or

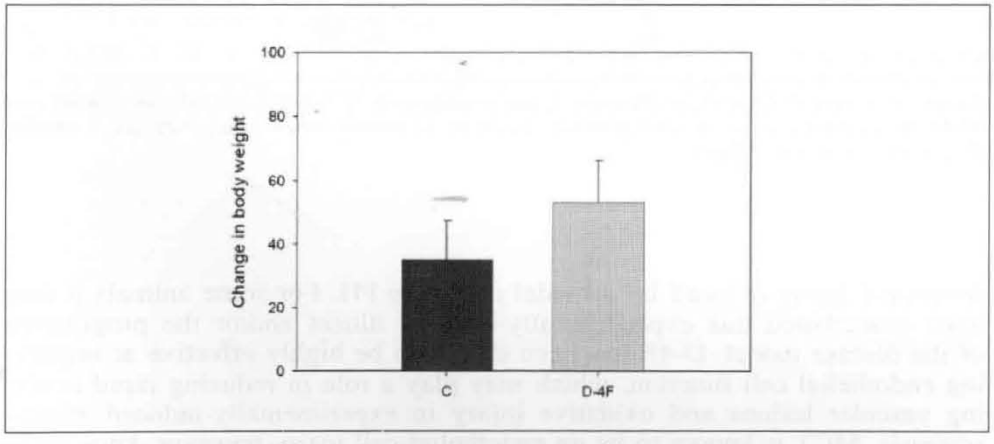

Figure 2. Gain in body weight over the 24-days of experiment.

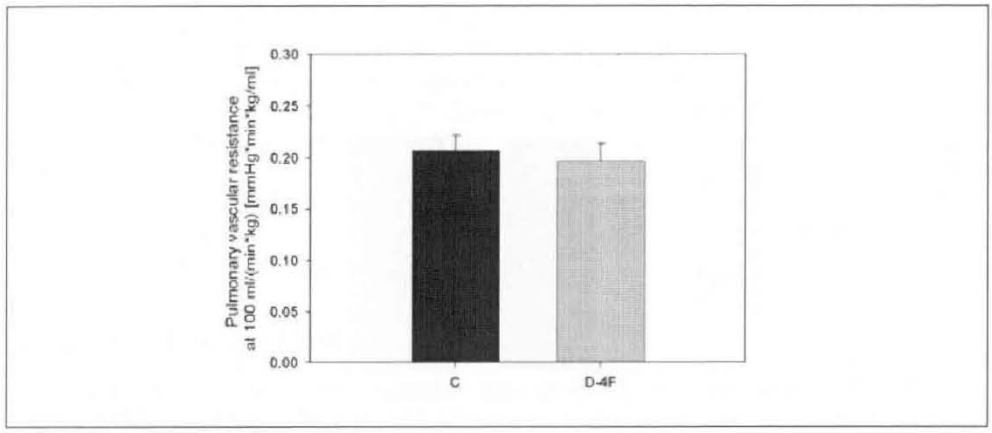

Figure 3. Pulmonary vascular resistance at a flow rate of 100 ml/min/kg.

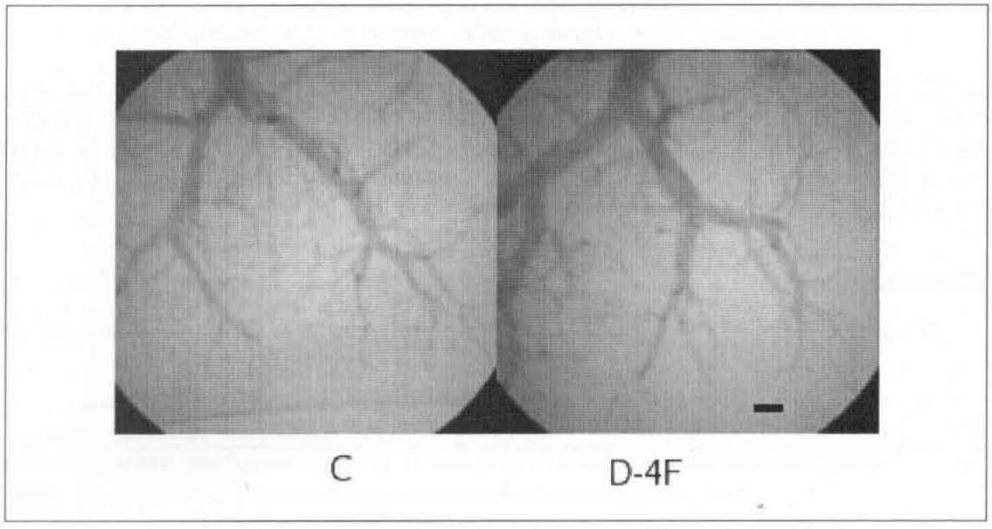

Figure 4. Example high-magnification X-ray angiograms of lower lung lobe in control and D-4F treated rats. Intravascular pressure is set to 21 mmHg and airway pressure 6 mmHg. Scale on left image 100μm.

functional injury induced by a model of severe PH. For some animals it may have exacerbated this experimentally-induced illness and/or the progression of the disease model. D-4F has been shown to be highly effective at improving endothelial cell function, which may play a role in reducing rapid evolving vascular lesions and oxidative injury in experimentally-induced atherosclerosis. MCT is known to be an endothelial cell toxin, however, knowledge

of the role and amount of oxidative stress in the MCT model is limited[10]. One possibility is that D-4F may accelerate vascular occlusive lesions in this model of severe PH by protecting endothelial cells from apoptotic cell death. D-4F may rescue endothelial cells that sloughed off of the vessel wall, allowing them to remain in circulation longer than normal and providing them greater opportunity to become trapped in the lung and subsequently complicate and accelerate vasculopathy in the microvasculature of this model of PH. Supported by Department of Veterans Affairs.

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