MS Title: Loss of Cavin1 and Expression of p-Caveolin-1 in Pulmonary Hypertension: Possible Role in Neointima Formation. #42832

Response to the Reviewers:

The authors would like to thank the Reviewers for their valuable comments.

Reviewer #1:

This is a very well designed study using a rat model aiming to elucidate whether loss of cavin-1 and p-cav-1 occur in PH that might explain the mechanism of neointima formation. The authors analyzed the expression of cav-1, p-cav-1 and cav-1-related proteins cavin-1, cav-2, vascular endothelial cadherin (VECad), and pERK1/2 in rats treated with MCT, hypobaric hypoxia and a group treated with MCT and exposed to hypoxia. This study also included lung histology and haemodynamic data in rats. It is a difficult study, well performed with clear results and messages which to my opinion deserves to be published in the World Journal of Cardiology in the form it has been submitted

Thank you for your kind remarks.

Reviewer #2:

The authors evaluate the effects of the pulmonary hypertension induced by chronic hypoxia (4 weeks), endothelial cell disruption by monoctrotaline and both on vascular wall remodelling and activation of proliferative and anti-apoptotic pathways in endothelial and smooth muscle cells. Previously published studies by the same group, in particular Exp Biol Med (Maywood) 2012;237(8):956-65, evaluated similar parameters and factors in the monoctrotaline-induced pulmonary hypertension in rats. In the present work, the addition of the hypoxia and monoctrotaline+hypoxia groups of rats reveals the mechanism of the hypoxia-induced pulmonary hypertension and underscores the role of endothelial damage in triggering the vascular wall remodelling. My only major concern is related to the similarity of the study protocol to the previously published studies, focusing on the caveolin and cavin expression in the endothelial and smooth muscle cells.

In our previous study (Exp Biol Med 2012; 237:956-965), using the monocrotaline (MCT) model of PH, we had shown the progressive loss of endothelial caveolin-1 (cav-1) accompanied by other EC membrane proteins, and reciprocal activation of pro-proliferative and anti-apoptotic pathways, increasing expression and activity of MMP2. At 4 weeks ≈85% of arteries exhibited loss of endothelial cav-1 and ≈29% of the arteries exhibited vWF loss. vWF loss was observed only in the arteries exhibiting extensive endothelial cav-1 loss. The enhanced expression of caveolin-1 was observed in smooth muscle cells (SMC) of ≈23% arteries; only in the arteries with extensive endothelial damage/loss. We did not observe any neointima formation. In order to accelerate the disease process, we exposed the MCT-treated rats to hypoxia. At 4 weeks these rats develop higher pulmonary artery pressure and increased right ventricular hypertrophy.

Importantly, small arteries exhibit neointima and occlusion. In the present study we examined cav-1, p-cav-1 and cav-1 related proteins cavin-1, VE-cadherin and ERK1/2. Loss of cavin-1 strongly suggests loss of caveolae, which will move cav-1 to non-caveolar site where it is thought to lose its normal function. Our results suggest that the dual role of cav-1 may be critical in the progression of the disease. It is significant that hypoxia-induced PH does not disrupt endothelial cells, nor does it affect endothelial cav-1 levels and nor is there enhanced expression of cav-1 in SMC.

The authors use monoctrotaline (MCT) in rats to evoke the endothelial cell disruption in the animal model of pulmonary hypertension. Is there any correspondence to the pathogenesis of PH in human?

We have shown the sequence of events in the progression of PH in the MCT and MCT + Hypoxia model as follows: 1. progressive loss of endothelial cav-1 and disruption of endothelial cells; 2. Extensive disruption/loss of endothelial cav-1 is followed by enhanced expression of cav-1 in smooth muscle cells; 3. neointima formation and obstruction of small arteries. We have observed the same sequence of events in human cases: IPAH and HPAH [12], PAH associated with congenital heart defect [10], PAH associated with drug toxicity [11]. The integrity of endothelial cells appears to be a key factor underlying the progression toward irreversibility.

Minor concerns regard the form of the manuscript. There are several references and discussion points in the Result section. In my opinion, they should be moved to Discussion. Also, the manuscript seems fragmented and there are several errors of punctuation; both may result from numerous revisions with deletions/additions of the content. The authors should check again these aspects of the manuscript before resubmitting.

The references and discussion points have been moved from the Result Section to the Discussion Section. We have tried to correct the typographical errors.

Reviewer #3

This is an interesting study. However, the work in this paper is needed to be improved. 1. The design is good and writing well. Maybe the language should improve. 2. The format of references should be corrected.

Thank you for the comment. We have edited the paper and corrected the typographical errors. We have changed the format of references.