Chloroquine in Pulmonary Arterial Hypertension
A New Role for an Old Drug?

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Study Hypothesis
Pulmonary arterial hypertension (PAH) is a complex condition resulting from excess proliferation and impaired apoptosis of pulmonary artery smooth muscle cells (PASMCs), as well as vasoconstriction, inflammation, endothelial abnormalities, dysfunctional autophagy, and alterations in PASMC metabolism. Bone morphogenetic protein receptor II (BMPR-II) is the most common genetic abnormality in hereditary PAH and is also significantly reduced in both nonhereditary PAH and experimental PAH. The treatments currently available for PAH are primarily vasodilators, which have been shown to improve functional capacity and survival. However, despite these agents, PAH remains a fatal disease without curative therapies. In this study, Long et al examined the role of autophagy and BMPR-II degradation in experimental and human PAH. Previous work from this group identified that the decreased levels of BMPR-II in experimental models of PAH are due in part to degradation of BMPR-II by the lysosome. Therefore, they also studied the role of chloroquine, a long-established antimalarial drug and known inhibitor of autophagy, in experimental PAH.

How Was the Hypothesis Tested?
Long et al studied both human PASMCs and a well-established rat model of PAH to test the relationship among autophagy, lysosomal degradation of BMPR-II, PASMC proliferation/apoptosis, and the development of PAH. The authors used the monocrotaline rat model of PAH to study the use of chloroquine in both preventing and regressing PAH. After treatment with chloroquine, the animals were assessed with invasive hemodynamics, vascular staining for PASMC proliferation and apoptosis, as well as immunohistochemistry staining for lung p62 and LC3B-II expression, both markers for activation of autophagy. The effects of chloroquine on right ventricular function and size were assessed using Millar pressure-volume microtip catheter and right ventricular weight postmortem. Quantification of proliferation was performed through Ki67 staining, and apoptosis was quantified by TUNEL staining.

Principal Findings
Chloroquine treatment for 3 weeks in monocrotaline rats prevented the development of pulmonary hypertension while also increasing the cardiac output and contractility in these animals, thereby significantly decreasing pulmonary vascular resistance. Chloroquine similarly inhibited the muscularization of small pulmonary arteries in monocrotaline rats and reversed the medial thickening of larger arteries. Furthermore, when administered to monocrotaline rats with established PAH, chloroquine regressed pulmonary vascular disease and lowered right-sided pressures. There was less right ventricular hypertrophy observed in monocrotaline rats treated with chloroquine, and the chloroquine-treated monocrotaline rats weighed ≈10% less than their vehicle-treated counterparts.

Immunoblotting of lung samples was performed to measure the expression of the autophagy markers LC3B-II and p62. Lung p62 levels were reduced in monocrotaline rats, consistent with activation of autophagy. Chloroquine prevented this decrease in p62 and in the regression study partially restored the p62 expression in medial PASMCs. LC3B-II was increased in the small pulmonary arteries in monocrotaline rats, again consistent with increased autophagy, although chloroquine treatment did not have an effect on LC3B-II levels. However, this is to be expected because as a lysosomal inhibitor, chloroquine would actually favor the accumulation of LC3B-II.

Chloroquine treatment was associated with a decrease in monocrotaline-induced PASMC proliferation in vivo and was found to increase the apoptosis of PASMCs,
thereby addressing and treating a key imbalance in the PAH phenotype. Knockdown of ATG5 with small-interfering ATG5 in PASMCs inhibited autophagy by decreasing the expression of LC3B-II. This direct inhibition of autophagy also inhibited proliferation of PASMCs.

BMPR-II protein expression was reduced in the lungs of monocrotaline rats, and treatment with chloroquine prevented the reduction in BMPR-II expression. To determine whether the effect of chloroquine on BMPR-II expression was directly related to the effect of chloroquine on autophagy, the authors studied mouse embryonic fibroblasts deficient in ATG5. These ATG5-deficient mouse embryonic fibroblasts showed increased p62 and BMPR-II expression, supportive of the hypothesis that increased autophagy contributes to the PAH phenotype and downregulation of BMPR-II seen in nonheritable PAH. Alternative inhibition of lysosomal function in PASMCs with either chloroquine or concanamycin A (another lysosomal inhibitor) was also found to increase BMPR-II expression, further evidence of the relationship between lysosomal dysfunction and PAH.

**Implications**

Long et al show that activation of the autophagy pathways and loss of BMPR-II protein in the pulmonary hypertensive lung are prevented by chloroquine. Chloroquine therapy, through this inhibition of autophagy, decreases proliferation and increases apoptosis of PASMCs in pulmonary hypertensive arteries. In an animal model of PAH, chloroquine and another antimalarial medication, hydroxychloroquine, can prevent the development of pulmonary hypertension and decrease the progression of pulmonary hypertension in established disease.

This study has potentially direct impact on patient care. Despite existing therapies, PAH continues to carry a significant mortality, and the therapeutic agents currently available are expensive, with many requiring continuous infusion or frequent laboratory testing. The availability of the current vasodilators used in PAH is also limited by their cost, and they remain unavailable in many parts of the world. Therefore, this study provides insights into the potential therapeutic impact that chloroquine or hydroxychloroquine could have on PAH. These are old medications, well established as antimalarial agents and fiscally affordable. Their use has already been extended to inflammatory rheumatologic conditions, and in this setting, they have proven to be well tolerated when taken chronically and provide a welcome alternative as part of a steroid-sparing treatment algorithm.

Previously, it has been shown that BMPR-II is degraded in lysosomes, and activation of the lysosomal autophagy pathway is a feature of the arteries in PAH. This accounts for the benefit exhibited by chloroquine in an animal model of PAH, namely inhibition of lysosomal activation, prevention of BMPR-II downregulation, and ultimately inhibition of proliferation and increased apoptosis of PASMCs. This study, therefore, presents the opportunity for a whole new axis of therapeutics in PAH.

The study is limited by the reliance on the monocrotaline model of PAH. PAH remains a complex, multifactorial disease, which is reflected by the heterogeneous animal models of the disease. The monocrotaline rat is the longest established model but is criticized because of the marked inflammation present and myocardial involvement of the model. Other models, such as the chronic hypoxia model or the chronic hypoxia+Sugen 5416 model, are also limited, the former being predominantly driven by vasoconstriction and the latter being less proliferative than other models. However, all these models likely reflect one of the parts of PAH, rather than any one model capturing the human PAH phenotype in its entirety. It would be important to reproduce these findings in other animal models of PAH.

Although hydroxychloroquine or chloroquine may not become the single therapeutic agent for PAH, by identifying the role played by lysosomal degradation in PAH and demonstrating the potential impact that these agents can have on BMPR-II expression and pulmonary vascular disease, Long et al have significantly advanced our understanding of this fatal disease.

**Acknowledgment**

The author is a member of the Membership Committee of the American Heart Association Functional Genomics and Translational Biology Council.

**Disclosures**

None.

**Key Words:** autophagy ▪ hypertension, pulmonary ▪ therapy
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doi: 10.1161/CIRCGENETICS.113.000214
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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