Targeting the MetAP2 Pathway in Pulmonary Arterial Hypertension

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Background and Hypothesis

Pulmonary hypertension (PH) is characterized by elevations in pulmonary arterial pressures leading to hypoxemia and right ventricular (RV) dysfunction. PH complicates and worsens the prognoses of many pulmonary and systemic diseases. Current therapies for PH enhance survival modestly1-3 but do not significantly reverse the accumulation of smooth muscle cells (SMCs) and endothelial cells (ECs). Therapies that reverse the proliferation of these cells would be predicted to impact positively both quality-of-life and survival in PH.

Methionine aminopeptidase-2 (MetAP2) is a metalloprotease that cleaves the initiator methionine off polypeptides, a process necessary for many downstream, post-translational modifications of certain proteins. Fumagillin is a fungal metabolite4 that irreversibly inactivates the enzymatic activity of MetAP2,5,6 an enzyme which has been implicated in the regulation of cell proliferation.6,7 Fumagillin and its analogues potently inhibit the proliferation of SMCs8,9 and ECs.9

We propose that inhibition of MetAP2 by fumagillin will attenuate the proliferation of SMCs and ECs and reverse pulmonary artery remodeling in animal models of PH.

Results

Figure 1. Fumagillin Prevents PH and RV Hypertrophy in MCT-Injured Rats

(A) Early, but not late, treatment with fumagillin inhibits PH in MCT-injured rats. (B) Early AND late treatment with fumagillin prevents RV hypertrophy in MCT injury (*P<0.05).

Figure 2. Treatment of MCT-Injured Rats with Fumagillin Leads to Improved RV Function

Treatment of rats with fumagillin early after injury leads to significant improvement in (A) right ventricular ejection fraction, (B) right ventricular minimum dP/dt, (C) right ventricular end-diastolic pressure, and (D) the ratio of right ventricular to pulmonary artery elastance, Ees/Ea. Note stepwise improvement in these hemodynamic parameters as fumagillin treatment is delivered late vs. early (*P<0.05, early v. VEH).

Figure 3. Late Treatment with Fumagillin Inhibits RV Cardiomyocyte Hypertrophy

(A) Uninjured + vehicle, (B) uninjured + fumagillin, (C) MCT + vehicle, week 2, (D) MCT + fumagillin early, and (E) MCT + fumagillin late (bar=20 μm). The area of cardiomyocytes cut in cross section was measured (F). MCT injury led to a significant increase in cardiomyocyte cross-sectional area, which was attenuated by treatment with fumagillin (*P<0.05, one-way ANOVA, followed by Bonferroni’s post-hoc test).

Figure 4. Treatment with Fumagillin Decreases the Number of Apoptotic Cells in Rat Hearts

At five weeks after MCT injury, animals were killed and the free wall of the right ventricles were prepared for TUNEL staining: (A) Uninjured + vehicle, (B) uninjured + fumagillin, (C) MCT + vehicle, week 2, (D) MCT + fumagillin early, and (E) MCT + fumagillin late (magnification x400, bar=20 μm). The number of TUNEL+ cells in the right ventricles were increased by MCT injury but attenuated by fumagillin treatment (F) (*P<0.05, one-way ANOVA, followed by Bonferroni’s post-hoc test, n=3-5 animals per group).

Figure 5. Fumagillin Protects Rats from PA Thickening in MCT-Injured Rats

H&E staining of lungs from (A) uninjured, vehicle-treated, (B) uninjured, fumagillin-treated, (C) MCT-injured, vehicle treated, or (D) MCT-injured, early fumagillin treated. Arrows point to thickened pulmonary arteries (bar=50μm). Inset images are x400 magnification and show IH for α- SMA (brown) and vWF (gray). PA thickness was measured by Movat staining (E) All vessels <250 μm, (F) vessels <50 μm, (G) vessels <30 μm. (*P<0.05, n=4-6 animals).

Conclusions

Early treatment with fumagillin prevented MCT-induced PH, but the protective mechanism of fumagillin in MCT injury is far more complex than a simple effect on smooth muscle cell proliferation.

Animals treated early with fumagillin exhibited decreased vessel thickness. We uncovered an anti-inflammatory effect of fumagillin following MCT injury, as we found fewer CD45+ leukocytes in the lungs of MCT-injured, fumagillin-treated animals compared to controls. Perhaps our most surprising observation was that late treatment of MCT-injured animals with fumagillin disconnected RV remodeling from PH.

The MCT-injured, late fumagillin-treated animals exhibited both decreased RV mass and decreased RV cardiac myocyte cross-sectional area compared to the MCT-injured vehicle controls. No clear difference was detected between the numbers of capillaries in MCT-injured animals treated with fumagillin late or the vehicle, suggesting that the effect of fumagillin in the heart was not a result of altered neoangiogenesis.

We suggest that the effect of fumagillin on the mycardium is at least partially protective. MCT-injured rats treated with fumagillin beginning 14d after injury exhibited decreased weight loss compared to the vehicle controls. Our data support a growing body of literature suggesting that RV failure in PH is not solely explained by RV pressure overload.10,11 Our future studies will focus on understanding the molecular mechanisms underlying the effect of fumagillin on the myocardium.

References