Title

Nitazoxanide activates BMP9-ALK1-SMAD signaling cascade and improves HHT vascular pathology.

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Objective

HHT is caused by loss-of-function mutations in BMP9-ALK1-SMAD signaling in endothelial cells (ECs) and leads to arteriovenous malformations (AVMs). Using a drug repurposing approach, we sought to identify SMAD1/5/8 activators in ECs capable of ameliorating HHT pathology. Here, we identified nitazoxanide and investigated its therapeutic potential.

Methods

Cell lines and human ECs were treated with nitazoxanide. SMAD1/5/8 activation, ID1 expression and mTOR signaling were assessed by Western blot, immunofluorescence, and flow cytometry. Mechanistic studies included pharmacological inhibition and siRNA-mediated silencing. In vivo efficacy was evaluated in BMP9/10-immunoblocked neonatal mice by analyzing retinal vascular abnormalities and pathway modulation.

Results

Nitazoxanide induced a robust, dose-dependent increase in SMAD1/5/8 phosphorylation and ID1 expression, with maximal effects at 1 μM, without affecting cell viability. This effect was ALK1-dependent, as pharmacological inhibition or genetic silencing of ALK1 abolished pathway activation, whereas ALK2 silencing had no effect. Under pro-angiogenic conditions, nitazoxanide sustained SMAD1/5/8 activation and inhibited mTOR signaling, as indicated by reduced S6 phosphorylation. In vivo, nitazoxanide significantly reduced AVM number and size, venous dilation, and hypervascularization in the retina. These effects were associated with restoration of SMAD1/5/8 signaling in the lung and normalization of mTOR activity in the liver. Importantly, nitazoxanide restored SMAD signaling in BOECs derived from HHT patients carrying ALK1 or SMAD4 mutations.

Conclusion

Nitazoxanide prevents HHT vascular pathology in vivo by activating SMAD1/5/8 signaling and inhibiting mTOR. These findings support its potential as a therapeutic strategy for HHT.