The contribution of vascular smooth muscle cells mechanics in the vascular wall stiffening

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Summary

This study highlights verteporfin's (VP) potential to mitigate arterial stiffening in cardiovascular diseases by modulating vascular smooth muscle cell (VSMC) mechanics and adhesion. VP reduces vessel stiffness by enhancing stress relaxation, increasing VSMC adhesion to type I collagen, and decreasing adhesion to fibronectin. It also alters cytoskeletal organization, implicating YAP/TAZ signaling. These findings support VP as a promising therapeutic for vascular stiffness.

Introduction

Arterial stiffening is associated with age-related cardiovascular diseases (CVDs), including hypertension, atherosclerosis, and atrial fibrillation. While prior studies on arterial stiffness have primarily focused on endothelial or extracellular matrix (ECM)-related mechanisms [1], the role of vascular smooth muscle cells (VSMCs) in this process remains poorly understood. VSMCs are highly responsive to mechanical cues such as shear stress and cyclic strain from blood flow, with mechanotransduction pathways involving integrins, stretch-sensitive ion channels, and focal adhesion complexes mediating their response. Dysregulation of these pathways in CVDs leads to abnormal VSMC behavior, contributing to vascular stiffness and maladaptive remodeling [2]. The paralogous transcriptional coactivators, yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), have been identified as critical mechanotransducers at cell-cell and cell-matrix interfaces. Our studies using a spontaneous hypertensive rat (SHR) model demonstrated a significant increase in VSMC stiffness and dynamic activity compared to wild-type (WT) controls [3]. Notably, verteporfin (VP), a potential YAP/TAZ inhibitor, effectively regulated VSMC stiffness and integrin-mediated adhesion in an ECM-dependent manner.

Methods

Rats used in this study were housed in accordance with the National Institutes of Health (NIH) guidelines as outlined in the 8th edition of the *Guide for the Care and Use of Laboratory Animals*. The animal use protocol was approved by the Laboratory Animal Use Committee of Texas Tech University. Euthanasia was performed using carbon dioxide (CO_2) asphyxiation, and the descending aorta was surgically harvested. Vessel segments (2 mm in length) were treated with 2 μM verteporfin (VP) in cell culture medium containing 10% FBS for 1 hour. Vessel contraction and relaxation were evaluated using a multi-wire myograph, while vessel stiffness was assessed with a Stable Micro Systems mechanical tester at a stretch rate of 10 mm/min. During testing, vessel rings were kept moist with a droplet of PBS.

VSMCs were enzymatically isolated from the descending aorta and cultured in DMEM F-12 medium supplemented with 10% FBS. Cells were maintained in a humidified incubator at 37°C with 5% CO_2. Once the cells reached 60-80% confluency, they were treated with 2 μM VP in complete cell culture medium containing 10% FBS. Stiffness measurements of VSMCs were conducted using atomic force microscopy (AFM) with a spherical probe (5 μm diameter) at room temperature. Scans were performed over a 40 × 40 μm cell surface area with an indentation frequency of 0.75 Hz and an approach/retraction velocity of 3 μm/sec. The parabolic Hertz equation was used to estimate VSMC stiffness.

Results and Discussion

Our results showed that VP treatment significantly enhanced stress relaxation by approximately 40% without affecting contractile stress compared to sham control. These findings suggest that VP has the potential to regulate vessel rigidity without impairing contractile function, likely through modulating VSMC mechanics rather than the mechanics of ECM proteins. Additionally, VP treatment resulted in a significant decrease in vessel E-modulus but had no significant effect on ultimate tensile stress compared to sham control.

Furthermore, our results revealed a differential effect of VP on VSMC adhesion to COL1 and FN. Specifically, VP treatment significantly increased adhesion events and forces to type I collagen (COL1), while reducing adhesion events and forces to fibronectin (FN). These findings suggest that VP enhances integrin-mediated VSMC cytoskeletal adhesion to COL1 while attenuating the adhesion axis involving the cytoskeleton, integrin, and FN. Summarized data also revealed a reduction in submembranous cytoskeletal fiber polarity following VP treatment, accompanied by a significant increase in submembranous cytoskeletal fiber density. These findings suggest the involvement of YAP signaling in cytoskeletal organization, although the precise mechanism linking YAP to cytoskeletal dynamics remains unclear.

Conclusions

VP treatment significantly reduces vessel stiffness by modulating VSMC stiffness and adhesion to distinct ECM proteins through a YAP/TAZ-dependent mechanism.

Acknowledgments

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References

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