The Effects of The Pharmaceutical BH4 Supplement Sapropterin Dihydrochloride (Kuvan®) on Vascular Function & Structure in A Mouse Model of Marfan Syndrome

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INTRODUCTION

Marfan Syndrome (MFS) is an autosomal dominant disorder caused by mutations in the fibrillin-1 (FBN-1) gene, leading to systemic connective tissue abnormalities, with aortic aneurysm and rupture as primary causes of mortality [1]. Our recent findings in a transgenic MFS model have shown that MFS aortic aneurysm is associated with pathological changes in other vasculatures such as carotid and posterior cerebral arteries in the mouse model of MFS [2]. In the context of aortic root aneurysm, mutations in the *FBN-1* gene disrupt the sequestration of the latent transforming growth factor beta (TGF-β) complex within the extracellular matrix (ECM), leading to aberrant and excessive activation of the downstream mitogen activated protein kinase ERK1/2 signaling pathway and overexpression of metalloproteinases (e.g., MMP2, MMP9), which serves a role in aortic wall elastin degradation and endothelial dysfunction [3, 4]. It is well established that MFS aneurysm is associated with endothelial dysfunction, highlighted by a significant reduction in nitric oxide (NO) production and bioavailability [5]. Current pharmacological management primarily involves the use of angiotensin II (Ang-II) type 1 receptor (ATR1) blockers (ARBs) such as losartan, which is believed to slow down aortic root dilation by not only reducing the hemodynamic stress on the aortic wall, but also, by inhibiting deleterious ATR1-mediated downstream activation of MMP2/MMP9 [6]. It is also suggested that losartan's efficacy might be linked to a direct impact on NO production [6,7]. The importance and critical contribution of endothelial dysfunction during the progression of aortic aneurysm has prompted us to explore approaches that could potentially improve endothelial function and NO bioavailability within the aortic wall. Tetrahydrobiopterin (BH4), is considered as a critical cofactor for eNOS activity, enhancing NO production, therefore, providing a potential therapeutic target to improve endothelial function and attenuate MFS-associated vascular remodeling [8,9]. In this study, we ***hypothesize that BH4 supplementation with sapropterin dihydrochloride (Kuvan®) will reduce aortic root aneurysm (aortic root diameter growth) and wall stiffness (pulse wave velocity), while normalizing carotid artery wall thickness and distensibility, and posterior cerebral artery blood flow in a transgenic mouse model of MFS.***

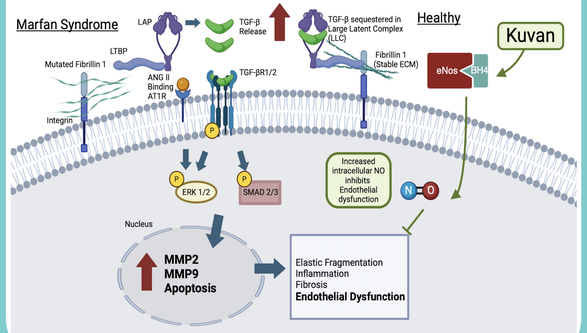


Figure 1. Pathophysiology of Marfan Syndrome.

EXPERIMENTAL DESIGN

Control Mouse (*FBN1+/+*) — **MFS Mouse (*FBN1-/+*)**

Week 4 — **+/- Kuvan® (0.1g/Kg)** — N=12 — N=12

Week 24 — **Ultrasound Imaging**

- **Aortic root – Sinus of Valsalva**
- **Aortic PWV**
- **Carotid artery wall thickness and distensibility**
- **Carotid artery PWV**
- **Posterior cerebral artery peak blood flow**

RESULTS

Impact of Kuvan® on Ascending Aorta

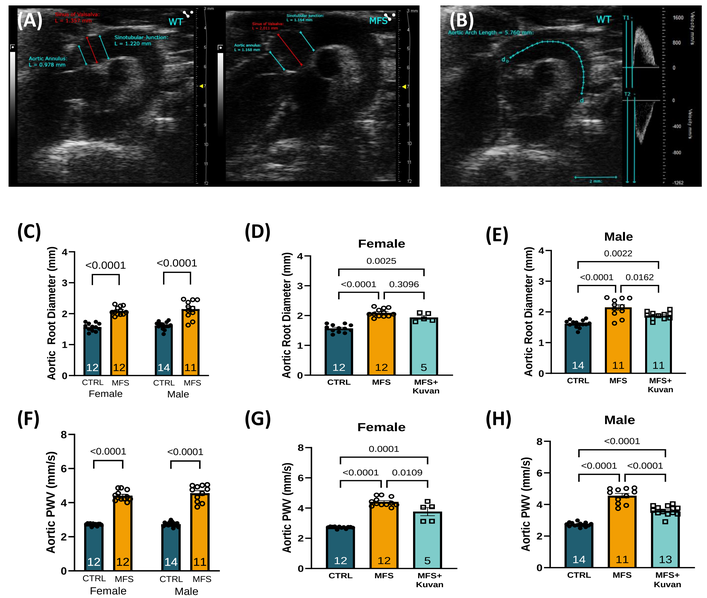


Figure 2. Measurements of aortic root diameters and wall stiffness. A) B-Mode ultrasound image of aortic root diameter in a 6-month-old CTRL and MFS mice. **B)** B-Mode image of a traced aortic arch length and PW Doppler Mode waveform of the ascending aortic arch (Time 1, T1) and descending aortic arch (Time 2, T2) in a 6-month-old female CTRL mouse. **C)** At 6 months of age, both female and male MFS mice demonstrate significant increases in aortic root diameters at the sinus of Valsalva compared to age- and sex-matched CTRL subjects. **D)** At the current power (low sample size) Kuvan® is showing little effects on aortic root growth in female MFS mice. **E)** Kuvan® significantly decreases aortic root diameters in 6-month-old male MFS mice. **F)** Female and male MFS mice exhibit increased aortic PWV (aortic wall stiffness) as compared to age- and sex-matched CTRL mice. **G)** and **H)** Kuvan® significantly decreases aortic PWV in female and male MFS. [Two-Way ANOVA followed by Tukey's Test, Mean ± SEM, $P < 0.05$]

Impact of Kuvan® on Posterior Cerebral Artery

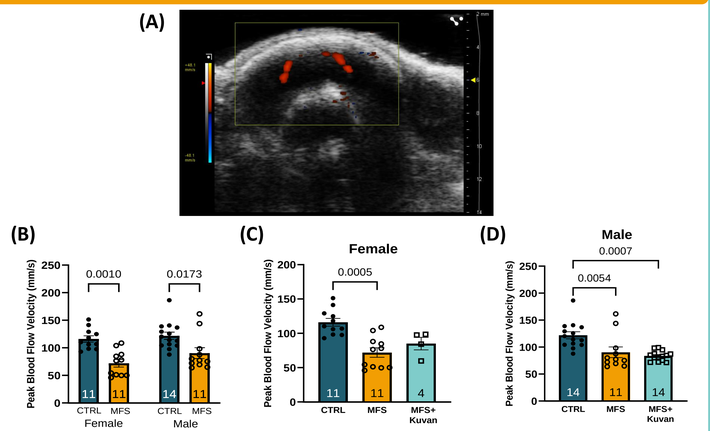


Figure 3. Measurements of the posterior cerebral artery (PCA) peak systolic velocity. A) B-Mode color Doppler view of the right and left PCA in a 6-month-old CTRL mouse. **B)** PCA peak systolic velocity was significantly decreased in male and female MFS mice compared to sex- and age-matched CTRL groups. **C)** and **D)** Kuvan® does not improve PCA blood flow in both female and male MFS mice. [Two-Way ANOVA followed by Tukey's Test, Mean ± SEM, $P < 0.05$]

Impact of Kuvan® on Left Common Carotid Artery

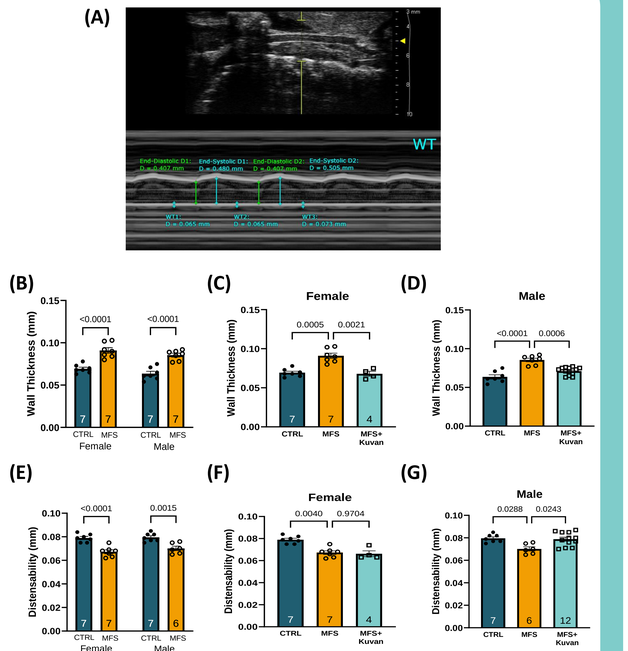


Figure 4. Measurements of the left common carotid artery wall thickness and distensibility. A) M-Mode ultrasound view of the LCCA in a 6-month-old CTRL mouse. **B)** Male and female MFS mice exhibit significant increases in the carotid artery wall thickness. **C)** and **D)** Kuvan® significantly decreases LCCA thickening in both female and male MFS mice. **E)** At 6 months of age, carotid artery wall distensibility is significantly decreased in both male and female MFS mice experience compared to CTRL mice. **F)** At the current power (low sample size) Kuvan® has little effect on carotid wall distensibility in female MFS mice. **G)** Kuvan® treatment completely normalized carotid wall distensibility in male MFS mice. [Two-Way ANOVA followed by Tukey's Test, Mean ± SEM, $P < 0.05$]

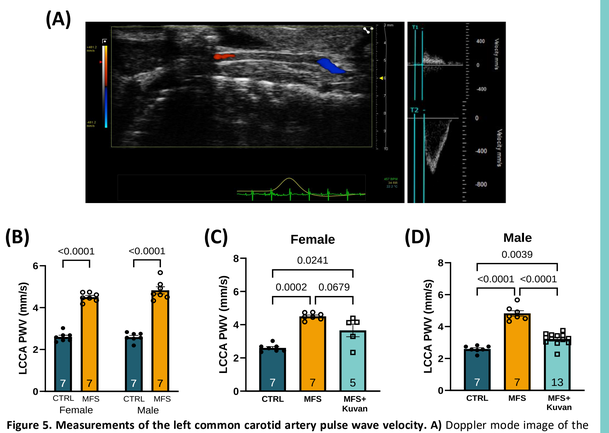
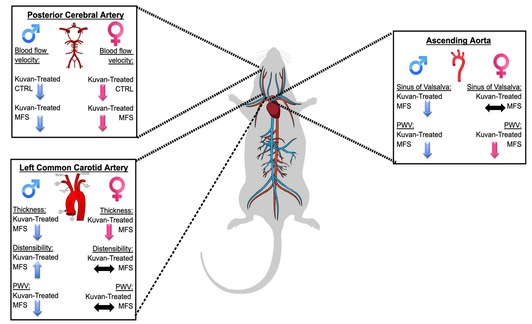


Figure 5. Measurements of the left common carotid artery pulse wave velocity. A) Doppler mode image of the left common carotid artery and PW Doppler Mode waveform of the proximal carotid artery (T1) and distal carotid artery (T2). **B)** Carotid pulse wave velocity is significantly increased in male and female MFS mice compared to sex- and age-matched CTRL groups. **C)** and **D)** Kuvan® significantly decreases PWV in both female and male MFS mice. [Two-Way ANOVA followed by Tukey's Test, Mean ± SEM, $P < 0.05$]

DISCUSSION



- Kuvan® shows promise in reducing aortic root dilation and stiffness and improving carotid artery vascular structure in MFS mice.

Limitations

- The partial or absent response in certain parameters (e.g. aortic root changes and carotid artery distensibility in females) may be attributed to the current small sample size, as the data collection is ongoing.

Therapeutic Implications

- Kuvan has the potential to serve as a therapeutic option in diseases where carotid artery vascular pathology is implicated, such as Alzheimer's and neurodegenerative diseases.
- Current studies show that hypoperfusion from the carotid artery, increases oxidative stress and disrupts clearance of toxic proteins within the brain, worsening cerebral blood flow and exacerbating cognitive impairment within these neurodegenerative diseases.

Future Direction

- The future direction our lab will be taking includes histological and biochemical analyses of the arterial wall to help identify the treatment effect on TGF-B signaling with resultant MMP expression, and collagen and elastin composition.

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