A Muscadine Grape Extract Prevents Hypertension-Induced Diastolic Dysfunction and Aortic Damage in Association with Anti-fibrotic and Anti-oxidant Mechanisms

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Introduction

Polyphenols, complex molecules composed of various combinations of benzene rings and hydroxy groups, are main components in fruits and vegetables that account in part for their health benefits. More than 8000 polyphenols were identified in plants and are broadly classified into one of four groups: phenolic acids, flavonoids, stilbenes and lignans. Muscadine grapes are abundant in the southeastern United States and are a rich dietary source of bioactive polyphenols, including ellagic acid, gallic acid, anthocyanins, and flavan-3-ols, with the highest phenolic content in the skin and seeds. We recently completed a Phase 1 clinical trial showing that a proprietary muscadine grape extract (MGE) is safe and well-tolerated by cancer patients. Ongoing Phase 2 clinical trials are investigating the effect of MGE on patients with breast and prostate cancer. However, high blood pressure (BP) is a prevalent comorbidity. Hypertension affects 46% of American adults and can lead to pathological cardiac hypertrophy, systolic and diastolic abnormalities, and eventually heart failure.

The goals of this project are to determine:

- whether hypertensive rats can be safely treated with MGE;
- if MGE reduces high BP or hypertension-associated damage to the heart or vasculature;
- the molecular mechanisms for the MGE-mediated improvement in end organ damage to the heart and blood vessels.

Methods

***Animal model*:** Sprague-Dawley rats (male, 8 weeks of age, n = 8) were treated for 4 weeks with regular drinking water (Control), MGE (0.2 mg/mL total phenolics in their drinking water), angiotensin II (Ang II, 24 µg/kg/h via implanted osmotic mini-pump) or MGE and Ang II. Administration of MGE was initiated 1 week prior to Ang II treatment. The MGE was purchased from Piedmont Research & Development Corp.

***Blood pressure (BP)*:** Indirect BP was recorded weekly via tail-cuff plethysmography.

Echocardiography: Echocardiography was performed using the VisualSonics Vevo 2100 High-Resolution Imaging System in rats sedated with isoflurane. Scans to quantify cardiac parameters were taken in M mode parasternal short axis view, Tissue Doppler in 4 chamber view at the mitral annulus, or pulse wave Doppler in 4 chamber view.

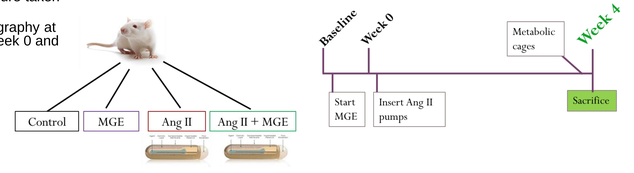
mRNA quantification: mRNA concentration was determined by reverse transcriptase-polymerase chain reaction.

Immunohistochemistry (IHC): Five micron sections of paraffin-embedded hearts or aortas were stained with Picrosirius Red, Masson's trichome, or specific antibodies. Four representative regions were acquired with a Perkin Elmer Mantra microscope at 40X.

***Statistics*:** Data are presented as mean ± SEM and analyzed by one way ANOVA, using Tukey's multiple comparisons tests for post test analysis.

Study Design

- Blood pressure taken weekly
- Echocardiography at baseline, week 0 and week 4



MGE does not affect BP but prevents Ang II-mediated diastolic dysfunction.

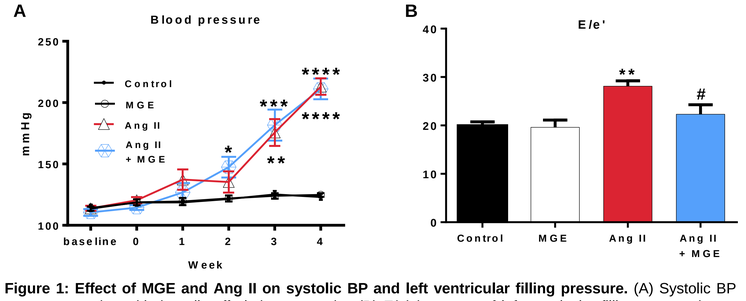


Figure 1: Effect of MGE and Ang II on systolic BP and left ventricular filling pressure. (A) Systolic BP was measured weekly by tail cuff plethysmography. (B) E/e' (measure of left ventricular filling pressure) was measured by echocardiography at week 4. (n=8; *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 compared with control, # p<0.05 compared to Ang II alone.)

MGE abrogates Ang II-induced cardiac fibrosis by modulating TGFβ/Smad/CTGF.

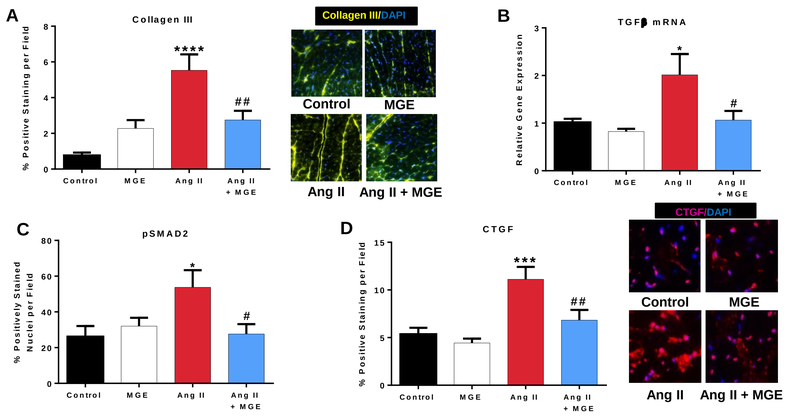


Figure 2: Effect of MGE and Ang II on cardiac fibrosis. (A) Collagen III, (C) pSmad2, and (D) CTGF were measured by IHC and (B) TGFβ mRNA was determined by RT-PCR. n=8; *p<0.05, ***p<0.001, and ****p<0.0001 compared to control; # p<0.05, ## p<0.01 compared to Ang II alone.

MGE prevents Ang II-induced oxidative stress.

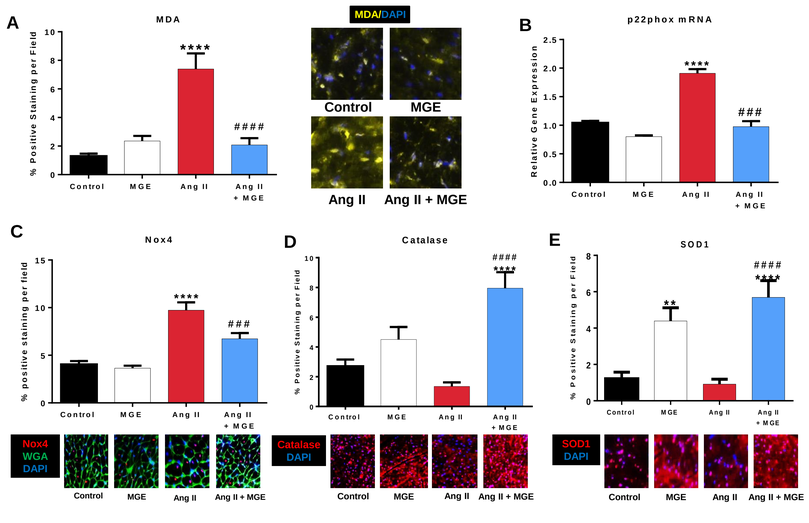
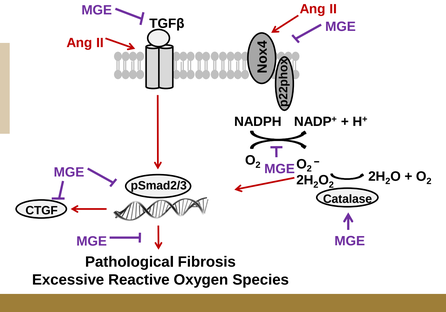


Figure 3: Effect of MGE and Ang II on oxidative stress. (A) Malondialdehyde (MDA), a lipid peroxidation marker, (C) NADPH oxidase 4 (Nox4), a ROS-producing enzyme, (D) catalase and (E) SOD1, endogenous antioxidants, were measured in left ventricular sections. (B) p22phox mRNA was quantified by RT-PCR. n=8; *p<0.05, **p<0.01, ****p<0.0001 compared to control; ### p<0.001, #### p<0.0001 compared to Ang II alone.

Proposed Actions of MGE in the Myocardium



MGE inhibits Ang II-mediated aortic stiffness.

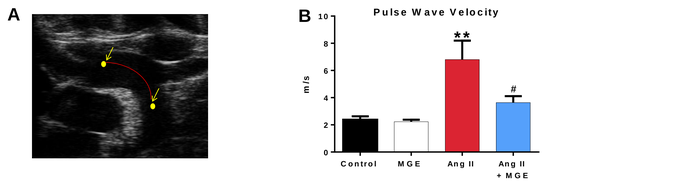


Figure 4: Effect of MGE on Ang II-induced aortic stiffness. (A) Representative image of aorta and calculation of pulse wave velocity, quantified in (B). n=4-6; **p<0.01 compared with control, # p<0.05 compared to Ang II alone.

Ang II-induced fibrosis and oxidative stress are prevented by MGE.

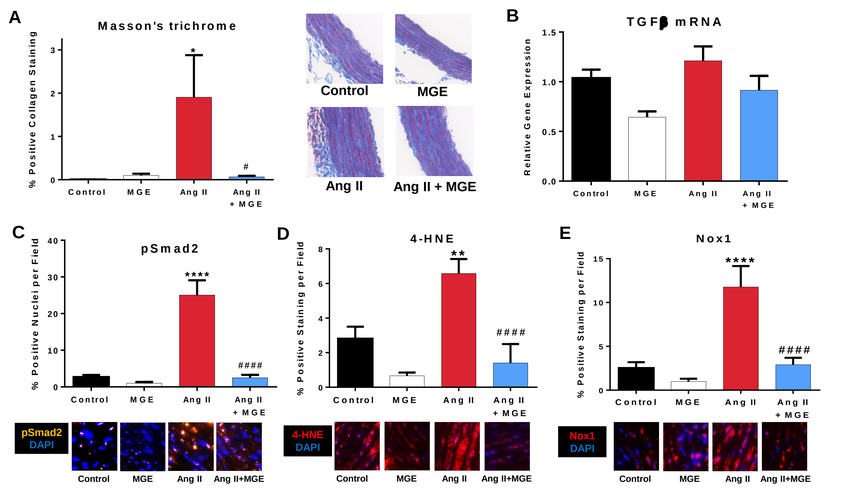


Figure 5: MGE reduces Ang II-mediated aortic fibrosis. (A) Aortas were stained with Masson's trichrome to identify total collagen. (B) TGFβ mRNA was quantified by RT-PCR. Aortas were stained with pSmad2 (C), 4-HNE (D), or Nox 1 (E) antibodies and positive staining per field was quantified in the media. n=8; *p<0.05, **p<0.01, ****p<0.0001 compared with control; # p<0.05, #### p<0.0001 compared to Ang II alone.

MGE inhibits Ang II-induced upregulation of inflammatory markers.

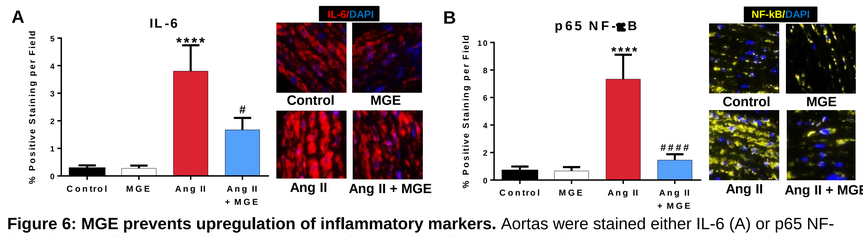


Figure 6: MGE prevents upregulation of inflammatory markers. Aortas were stained either IL-6 (A) or p65 NF-κB (B) antibodies and positive staining per field was quantified in the media. n=8; ****p<0.0001 compared with control; # p<0.05, #### p<0.0001 compared to Ang II alone.

Summary and Clinical Significance

- MGE reduces both cardiac and aortic fibrosis in association with either an attenuation of the TGFβ-dependent or –independent Smad pathway.
- MGE reduces markers of oxidative stress by upregulating antioxidant enzymes and inhibiting increase in Nox enzymes.

MGE may prevent subsequent cardiac damage and improve cardiac and aortic function in hypertensive patients without an effect on blood pressure.

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