Cardiac Remodeling Attenuation by Angiotensin-(1-7) During Angiotensin II-Induced Hypertension

Juline Machado

ABSTRACT

Heart failure (HF), a type of cardiovascular disease, is characterized by cardiac remodeling and loss of hemodynamic function. Cardiac remodeling is characterized by an increase in interstitial fibrosis (mediated by cardiac fibroblasts), and myocyte hypertrophy, which results in decreased cardiac function. It is abundantly clear that the renin-angiotensin system (RAS) is involved in numerous aspects of HF. Therapeutic inhibition of the RAS has been shown to provide some defense against cardiac remodeling. ACE2, a newly discovered enzyme of the RAS pathway, is responsible for converting Angiotensin II (Ang II) to Ang (1-7). We have found that the over expression of ACE2 prevents fibrosis and hypertrophy induced by chronic infusion of Ang II. Since Ang (1-7) is a product of the ACE2 enzyme, we hypothesized that chronic infusion of Ang (1-7) would also prevent cardiac fibrosis and hypertrophy. Infusion of Ang II in Sprague-Dawley rats resulted in increases in blood pressures, cardiac myocyte diameter and interstitial fibrosis of the heart, the latter being the hallmarks of cardiac remodeling. Co-infusing Ang (1-7), inhibited the interstitial fibrosis and the increase in myocyte diameter, however Ang (1-7) did not alter the increase in blood pressure induced by Ang II infusion. Ang(1-7) has been reported to work through a variety of mechanisms that may result in this apparent cardiac protective action. It has been hypothesized that a potential mechanism by which Ang-(1-7) may mediate this action is through alteration of the number of the classical angiotensin receptors. We evaluated this hypothesis and our results demonstrated that Ang (1-7) did not alter either AT1 or AT2 receptor subtypes. It has also been hypothesized that Ang-(1-7) may mediate its cardioprotective effects by stimulating its own reported receptor, the Mas receptor. We therefore utilized a compound, [D-Ala]-Ang-(1-7) (A779), an antagonist to Ang (1-7) receptor, to evaluate this possible mechanism for the anti-remodeling effects of Ang (1-7). Our results suggest that Ang (1-7) may work through its own specific receptor, to produce anti-remodeling effects in cardiac tissue without altering classical cardiac angiotensin receptor populations. Our results also demonstrated that the anti-remodeling effect of Ang-(1-7) is independent on blood pressure, further suggesting that the effects of Ang (1-7) are mediated directly on the cardiac tissue.

INTRODUCTION

As a common and detrimental ailment in the US, heart failure results from chronic cardiac remodeling and subsequent hemodynamic failure. Cardiac remodeling causes structural changes of the heart including...
fibrosis, thickening of the coronary arteries as well as myocyte hypertrophy. Both myocyte hypertrophy and interstitial fibrosis lead to decreased functioning of the heart and can result from chronic hypertension.

The renin angiotensin-system (RAS) plays a major role in cardiac remodeling and heart failure. Though the RAS has been formally viewed as a single end-product pathway, it is now known that Ang I is converted to multiple products that play key roles in tissue functioning (Santos 2004). Ang II has both remodeling effects on the heart that are mediated through its AT1R (angiotensin II type 1 receptor) and possible anti-remodeling effects mediated by activation of the AT2R (angiotensin II type 2 receptor). Angiotensin II works through the AT1R to increase fibroblast concentration and expression as well as increase myocyte hypertrophy, which can lead to cardiac remodeling and eventually heart failure. Although the concentrations of AT2R are normally less than that of AT1R, during heart failure the number of AT2R increase and have been hypothesized to do so in an attempt to reduce various aspects of cardiac remodeling.

Angiotensin II is converted to Angiotensin (1-7) by a recently discovered enzyme called ACE2. Ang (1-7) has been shown to have anti-remodeling effects by attenuating Ang II actions, which result in increasing myocyte hypertrophy and interstitial fibrosis (Tallant et al. 2005 and Grobe et al. 2006). Grobe et al. (2006) and Santos et al. (2003), have previously observed that co-infusion of Ang II and Ang (1-7) in another model of hypertension decreased cardiac hypertrophy and fibrosis; however, we are unaware of the mechanism(s) by which Ang (1-7) produces these effects. There are several proposed mechanisms by which Ang (1-7) may decrease cardiac remodeling. Some of these hypothesized mechanisms include alteration of AT1R and AT2R numbers, antagonizing the action of AT1R, antagonistic of other aspects of the RAS and/or binding to the MAS receptor. Mas receptors are G-coupled proteins that are involved in both Ang (1-7) and Ang II activities. Kostenis et al. (2005) have suggested that Mas receptors act as antagonists to the AT1 receptors, making it unavailable for Ang II to bind. [D-Ala²]-ANG-(1–7) (A779) is an antagonist to the Mas receptor, which can counteract the anti-remodeling effects of Ang (1-7).

Transforming growth factor β is a protein that has been reported to elevate in disease states such as hypertension, cardiomyopathy and both Type I and II diabetes (Peterson 2005). Our lab found TGFβ important to study because of its relation to promoting fibrosis and scarring—which can lead to cardiac remodeling in the heart. Denten et al. (2001) have found that TGFβ cytokines contribute to remodeling of the heart.

The purpose of this experiment was to determine if Ang (1-7) infusion can: i) protect the heart from the remodeling effects induced by Ang II; ii) alter the AT1R, AT2R receptor number; iii) produce its cardioprotective actions through its Mas receptor and via actions on TGFβ; and iv) alter the blood pressure increase induced by Ang II.

**MATERIALS AND METHODS**
Angiotensin II (Ang II) was placed into osmotic minipumps, which were subcutaneously implanted into male Sprague-Dawley rats to induce hypertension and subsequent cardiac remodeling. Angiotensin (1-7) and A779 were also infused in the rat with subcutaneously implanted osmotic minipumps. Four groups were constructed to determine the effects of Angiotensin (1-7) on Ang II: untreated controls, Ang II alone, Ang II + Ang (1-7), Ang II + Ang (1-7) + A779. All compounds were obtained from Sigma (St. Louis, MO). The rate of infusion of each substance was 100 ng/kg/min for four weeks. Indirect, systolic blood pressures were recorded weekly, via the tail-cuff method. After four weeks the animals were sacrificed and the hearts were harvested for further analysis. Once the hearts were sectioned and stained by Picro-Sirius Red, myocyte diameter and interstitial fibrosis was measured using an ImageJ program. AT1 and AT2 receptor binding was measured by freezing the cross sections and performing autoradiography. Blood from the atria was also collected at sacrifice in order to determine the amount of transforming growth factor-β (TGFβ) in the plasma. Once the plasma was isolated via centrifugation, ELISA was used to measure levels of TGFβ. For analysis of fibrosis, a more stringent, non-parametric Kruskal-Wallis one-way ANOVA was utilized for the percent area data. A one-way ANOVA followed by a post hoc Fisher test was used to determine significance for other variables. Significance was set at P<0.05.

RESULTS

Myocyte Hypertrophy

Results for myocyte hypertrophy are summarized in Figure 1. Treatment with Ang II produces a significant induction of cardiac hypertrophy as assessed by the measurement of myocyte diameter from ventricles of the treated animals. When compared to the Ang II group, there was a statistically significant attenuation of myocyte hypertrophy in the Ang II + Ang (1-7) group. Although A779, in the Ang II + Ang (1-7) + A779 group, did not significantly (P < 0.06) reverse the positive effects of Ang (1-7) on hypertrophy, this group was also not statistically different from the control group.
Figure 1. Myocyte hypertrophy was induced by chronic infusion of angiotensin II (ANG II), and this hypertrophy was significantly attenuated by coinfusion of angiotensin-(1–7) [ANG-(1–7)]. Sham, n = 12; ANG II, n = 14; ANG II + ANG-(1–7), n = 10; ANG II + ANG-(1–7) + [D-Ala7]-ANG-(1–7) (A779), n = 6. *P < 0.05 vs. Sham; †P < 0.05 vs. ANG II (Grobe et al. 2007)

**Interstitial fibrosis**

Similar to the results found in myocyte hypertrophy, Ang (1-7) reversed the Ang II-induced interstitial cardiac fibrosis (Fig. 2). Chronic treatment with Ang (1-7) significantly reduced the interstitial fibrosis when compared to the group treated with Ang II alone. Again, although A779 did not significantly reverse the fibrotic effects of Ang II, the fibrosis observed in this group was not significantly elevated over that of the control group.
Figure 2. Interstitial fibrosis was induced in the mid myocardium by chronic infusion of ANG II, and this was attenuated by coinfusion of ANG-(1–7). A: example pictures at x100 magnification. Bars, 100 µm. B: quantified data from all animals. Sham, $n = 12$; ANG II, $n = 14$; ANG II + ANG-(1–7), $n = 10$; ANG II + ANG-(1–7) + A779, $n = 6$. *$P < 0.05$ vs. Sham; †$P < 0.05$ vs. ANG II (Grobe et al. 2007).

**Blood Pressure**

Weekly indirect, blood pressure recordings demonstrated that Ang II significantly increased blood pressure in all treated groups as compared to untreated sham control animals. There was no significant difference in systolic blood pressures between the Ang II, Ang II + Ang (1–7) or the Ang II + Ang (1–7) + A779 group.
Figure 3. Chronic infusion of ANG II causes significant hypertension ($P < 0.005$ vs. Sham). Coinfusion of ANG-(1–7) or ANG-(1–7) + A779 fails to reduce the blood pressure (BP) increase due to ANG II infusion ($P = 0.178$ and $P = 0.295$ vs. ANG II, respectively); $n = 6$ animals per group (Grobe et al. 2007).

**Receptor Binding**

Both AT1 and AT2 receptors were not affected by the chronic infusion of Ang II or Ang (1-7).
Figure 4. Chronic infusion of ANG II and/or ANG-(1–7) had no effect on cardiac (A) ANG II type 1 (AT1) or AT2 (B)-receptor binding; n = 6 animals per group (Grobe et al. 2007).

**TGFβ**

Transforming growth factor β, with its pro-remodeling cytokine functions, was found to decrease with co-infusion of Ang II and Ang (1-7) by 40% (non-significant) when compared to the Ang II treated group, while Ang II alone had no effect on TGFβ levels. TGFβ levels in the Ang II + Ang (1-7) group were decreased significantly by 50% when compared to the sham group.

**DISCUSSION**

We have found that Ang (1-7) significantly opposes the anti-remodeling effects of angiotensin II. Myocyte hypertrophy and interstitial fibrosis were significantly lower in animals co-infused with Ang II + Ang (1-7) suggesting that Ang (1-7) works directly on cardiac tissue to attenuate Ang II effects. Another observation was that AT1R and AT2R levels were not affected by infusion of Ang (1-7). When compared to the Ang II + Ang (1-7) and the sham groups, the Ang II + Ang (1-7) + A779 group was not significantly higher (like the Ang II group) in myocyte hypertrophy or interstitial fibrosis. This data suggests that Ang (1-7) does not work by reducing the numbers of the classical AT1 and AT2 receptors but may work through the actions at its own Mas receptor—which is sensitive to A779. Other data to support the idea that Ang (1-7) works through different receptors than the AT1R, is the fact that Ang (1-7) did not significantly decrease blood pressure. These data suggest that Ang (1-7) does not work antagonistically with Ang II for the AT 1 receptor to decrease remodeling effects, because, when AT1 receptors are inhibited, mean arterial pressure decreases (Tallant et al. 2005). Since no significant effects were observed with A779, further studies will need to be conducted to determine the role A779 plays and to verify if Ang (1-7) works through the Mas receptor.

Pro-remodeling cytokines, such as TGFβ, increased in the plasma as a result of infusion of Ang II. TGFβ, is found in every type of cell and has a large role in aggravating cardiac hypertension (Almendral et al. 2005). When released in large quantities, TGFβ increases collagen production and decreasing collagen breakdown, leading to fibrosis (Almendral et al. 2005). We found that infusion of Ang (1-7) decreases the amount of TGFβ found in the plasma by approximately 40%. Though this implies that Ang (1-7) effects might be correlated with circulating TGFβ to prevent fibrosis caused by Ang II, cytokine levels in the heart would better indicate whether Ang (1-7) works with TGFβ specifically in the cardiac system.

The following scheme may be the way in which the RAS modulates cardiac remodeling. Ang II induces aspects of remodeling via activation of the AT1R. There are conflicting literature reports as to the anti-remodeling effects of AT2R activation and there is correlative data suggesting AT2R and Ang-(1-7) forming activity (Zisman, et al. 2003). Ang (1-7) appears to reduces cardiac remodeling and from our results may do so by reducing the formation of TGFβ. The question of whether this is a direct effect of Ang-(1-7) to inhibit TGFβ synthesis or
increase its degradation or some indirect effects on modulating the AT1R stimulation of TGFβ awaits further experimentation.

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**REFERENCES**


