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Review Article

Genetically Modified Mouse Models Used for Studying the Role of the AT_2 Receptor in Cardiac Hypertrophy and Heart Failure

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The actions of Angiotensin II have been implicated in many cardiovascular conditions. It is widely accepted that the cardiovascular effects of Angiotensin II are mediated by different subtypes of receptors: AT_1 and AT_2. These membrane-bound receptors share a part of their nucleic acid but seem to have different distribution and pathophysiological actions. AT_1 mediates most of the Angiotensin II actions since it is ubiquitously expressed in the cardiovascular system of the normal adult. Moreover AT_2 is highly expressed in the developing fetus but its expression in the cardiovascular system is low and declines after birth. However the expression of AT_2 appears to be modulated by pathological states such as hypertension, myocardial infarction or any pathology associated to tissue remodeling or inflammation. The specific role of this receptor is still unclear and different studies involving *in vivo and in vitro* experiments have shown conflicting data. It is essential to clarify the role of the AT_2 receptor in the different pathological states as it is a potential site for an effective therapeutic regimen that targets the Angiotensin II system. We will review the different genetically modified mouse models used to study the AT_2 receptor and its association with cardiac hypertrophy and heart failure.

1. Introduction

Angiotensin II (Ang II) plays a pivotal role in the regulation of the cardiovascular system [1, 2]. It is known that the effects of Ang II are mediated by several subtypes of Ang II receptors; these receptor subtypes differ from each other on their pharmacological and biochemical properties. Up until now, the subtypes that have been identified are the Type 1 (AT_1) and the Type 2 (AT_2) present in humans, and AT_{1A} and AT_{1B} present in rodents [2]. Both of these receptors, AT_1 and AT_2, belong to the seven-transmembrane domain superfamily of receptors, and they share 34% of their nucleic acid sequence. The AT_1 receptor is widely distributed in adult tissues (blood vessels, kidney, adrenal gland, heart liver, and brain). The AT_2 receptor is ubiquitously expressed in fetal tissues but the expression of the AT_2 receptor declines rapidly after birth with very low abundance in ventricular myocytes and vascular endothelium and absence in fibroblasts [3, 4]. Most of the distinct effects of Ang II on vasomotor tone, contractility, and myocardial growth are mediated by AT_1 receptors [5, 6] but various studies from the past two decades have demonstrated involvement of the AT_2 receptor in some important actions of Ang II in the heart. However, the specific role of the AT_2 receptor still remains unclear [5–7] as there have been contradictory results in the different studies performed.

Accumulating evidence suggests that the AT_2 receptor may act as an AT_1 receptor antagonist, as its activation has been associated with opposite cellular functions of AT1, such as antigrowth, antihypertrophic, and proapoptotic effects [8, 9]. If these receptors exert opposing actions in the heart, their ratio of expression under different cardiac pathologies may determine myocardial function and structure. Experiments using hypertrophic hearts have demonstrated that the cardiac expression of AT_1 and AT_2 receptors changes during the process of cardiac hypertrophy allowing the heart to respond differently to Ang II. Studies have shown that the pathological hypertrophy and failure of human hearts

are associated with a decrease of AT_1 and an increase of AT_2 receptor expression, that is, an increase of AT_2-to-AT_1 ratio [10, 11]. In patients treated with AT_1 antagonists, circulating Ang II levels are increased and may preferentially bind to AT_2 receptors inducing several effects that still remain controversial.

In order to develop a safer and more effective therapeutic regimen by targeting the Ang II system, it becomes essential to clarify the role of the AT_2 receptor in the development of cardiac hypertrophy and failure. With the objective of clarifying the specific actions of the AT_2 receptor in cardiac hypertrophy and heart failure, different approaches have been developed including *in vivo* and *in vitro* experiments. However, results from these studies have been inconsistent. This paper will review the different genetically modified mouse models used to study the AT_2 receptor and its association with cardiac hypertrophy and heart failure.

2. Angiotensin II Type 2 Receptor Mouse Models

Mice had been extensively used as a model for cardiovascular research; not only due to their short gestation period but also because there is significant preservation of the molecular pathways that control cardiovascular development and function between mice and humans [12]. Different approaches to genetic modification in the mouse such as gene deletion or overexpression have been described [13]. These animal models have become invaluable tools to study cardiovascular genetics, developmental biology, and physiology in normal or pathologic hearts [12, 13].

Compared to *in vitro* cardiomyocyte culture and pharmacological intervention, genetically modified mouse models have provided a novel and powerful method to study the physiological function of the AT_2 receptor. First, this technique allows us to study the function of the gene of interest in a physiological setting; second, it reduces the off-target effects of pharmacological inhibitors. It was hoped that genetic deletion or overexpression of the receptor would provide a much clear picture of AT_2 in cardiac hypertrophy and failure. Currently, there are two AT_2 overexpression and two AT_2 knockout mouse models that have been generated [14–17]. Unexpectedly, the results from these mouse models are contradictory and have raised more questions in the field.

2.1. Transgenic Mice with Cardiomyocyte-Specific Overexpression of AT_2. Two transgenic (TG) mouse models with cardiomyocyte-specific AT_2 overexpression have been generated [14, 15]. In the first model, the AT_2 receptor was overexpressed in both atria and ventricles, using the α-myosin heavy chain promoter in C57BL/6 mice [15]. Studies using this transgenic mouse model showed that the TG mice did not present any abnormality in myocardial development or phenotype when compared to nontransgenic (NTG) mice [15, 18]. Under baseline condition, the heart weight (HW) to body weight (BW) ratio was similar between TG and NTG mice. However, TG mice showed a higher end-diastolic wall thickness [18, 19]. Heart rate was similar between TG and NTG, while ejection fraction (EF%) was higher in TG mice [18]. Aortic stenosis (AS) in adult mice or with chronic Ang II infusion significantly increased HW/BW in mice compared to control mice; but HW/BW was not different between TG and NTG mice [19]. Ang II infusion in mice reduced HR in TG mice, but did not increase apoptosis [18]. Myocardial infarction (MI) increased left ventricular mass index (LVMI) in mice, with no difference between TG and NTG mice. LV wall thickness and EF%, however, maintained higher in TG versus NTG mice after MI [18] (Table 1).

Our laboratory has generated a mouse model with ventricular myocyte-specific overexpression of the AT_2 receptor using α-myosin heavy chain 2v (MLC 2v) promoter in FVB/n mice [14]. We generated four lines of mice with different copy number of the AT_2 gene [14]. This allowed us to study the dose-response of AT_2 overexpression. We have studied two lines of AT_2 transgenic mice with relatively high (AT_2^{high}TG) and low (AT_2^{low}TG) expression of AT_2. We found that under baseline condition, the left ventricular to body weight ratio (LV/BW) was increased in AT_2^{high}TG mice; this was accompanied by a decrease of LV wall thickness, an increase of cardiomyocyte area and length, and an increase of interstitial spaces and the deposition of fibrillar collagen in AT_2^{high}TG mice [14]. LV systolic function, as assessed by echocardiography and hemodynamic measurements, was significantly depressed in AT_2^{high}TG [14]. The contractile function of cardiomyocytes isolated from AT_2^{high}TG mice was significantly decreased under baseline and in response to Ang II [20]. These results suggest that excessive AT_2 overexpression can induce pathological cardiac remodeling and failure. Mice with low AT_2 overexpression (AT_2^{low}TG), however, did not demonstrate a significant change in cardiac morphology and function at baseline [14]. We further tested whether AT_2 overexpression would modify cardiac remodeling in aortic stenosis- (AS-) induced hypertrophy using AT_2^{low}TG mice [21]. Our results showed that 70 days after AS, LV/BW and LV wall thickness were increased in AS mice, with no difference between AT_2^{low}TGAS and NTGAS mice. However, LV myocyte diameter was smaller and the percentage of LV collagen was lower in AT_2^{low}TGAS versus NTGAS mice. LV systolic pressure and peak dP/dt± were lower in AT_2^{low}TGAS versus NTGAS mice, with no decrease in wall thickness. LV end diastolic pressure was lower in AT_2^{low}TGAS versus NTGAS mice [21]. These results suggest that lower level AT_2 overexpression did not accelerate cardiac hypertrophy and failure in AS mice; it is likely that the diastolic compliance was improved in AT_2^{low}TGAS mice (Table 1).

These two transgenic mouse models are different in several aspects: (1) the strain of mice (C57BL/6 versus FVB/n), (2) the AT_2 overexpression site (atria + ventricles versus ventricles), and (3) the overexpression level of AT_2. The site of AT_2 overexpression may be the cause of HR changes in mice using α-MHC promoter [15], which was not observed in mice using MLC2v promoter [14]. Despite the difference of the models, the results from these studies demonstrate that the expression level of AT_2 is a key determinant of outcome. Excessive AT_2 overexpression can lead to cardiac failure, while lower AT_2 overexpression may improve cardiac performance under stress.

2.2. AT_2 Knockout Mouse Models. Two AT_2 knockout (KO) mouse models were generated at the same time by two independent research groups [16, 17]. Both models were generated by targeted disruption of the AT_2 gene on the X chromosome [16, 17]. The results from these two models, however, are different. The first model was generated in C57BL/6 mice [16]. Under baseline condition, blood pressure (BP) was higher in KO mice. LVW/BW, wall thickness and LV mass (LVM) were lower in KO versus wild-type (WT) mice [16, 22, 23]. Cardiac function did not change in KO versus WT [22]. The most striking result from this model is that AT_2 knockout prevented the cardiac hypertrophic response to both aortic stenosis (AS) and chronic Ang II infusion in mice [22, 23]. Cardiac function was either similar between KO and WT (AS) or improved in KO versus WT (Ang II infusion) mice [22, 23]. These results suggest that AT_2 is essential for the development of cardiac hypertrophy and dysfunction (Table 1).

The second AT_2 KO mouse model was generated in FVB/n mice [17]. Unlike the first model, under baseline condition, BP was not different between KO and WT and no changes in cardiac morphology were observed [24]. Aortic stenosis resulted in a similar increase of HW/BW in KO versus WT mice, while the perivascular fibrosis was higher in KO mice [24]. Acute myocardial infarction resulted in higher mortality rate, higher LVW/BW, lung/BW, ratio and decreased EF% in KO versus WT mice [25] (Table 1). These results suggest that AT_2 has antihypertrophic remodeling effects and may be important for maintaining cardiac function under certain stress.

The contradictory results from these two models may be caused by the different mouse strains and the different disease models used. However, they also suggest that traditional gene deletion approach in mice may lead to the activation of compensatory mechanisms and ultimately different phenotypes.

3. Limitations of the Existing Mouse Models

The existing AT_2 mouse models have several limitations: (1) the models cannot recapitulate the AT_2 receptor expression patterns during pathological hypertrophy and failure. Studies have shown that AT_2 receptor expression is high in the fetus [3], significantly decreased in adult hearts, and increased again in diseased hearts in humans [7]. While AT_2 receptors are chronically overexpressed or disrupted in these models; (2) the expression level of AT_2 receptors in transgenic mouse hearts may not represent the increase of AT_2 receptors in a diseased heart. Studies in transgenic mice clearly showed a dose-relationship between AT_2 overexpression and cardiac remodeling and function. Further experiments by using mice with cardiac AT_2 receptors expression similar to that in diseased hearts are needed; (3) the cell type of AT_2 receptor overexpression may be not accurate. Studies have shown that fibroblasts are the major cell type that expresses AT_2 receptors in diseased human hearts [26]. In current mouse models the AT_2 receptor is overexpressed in cardiomyocytes; (4) chronically manipulation of AT_2 receptors expression may activate compensatory mechanisms, which may lead to phenotypes that are not related to AT_2 receptors.

3.1. In Vitro Studies. In order to understand better the role of AT_2 receptors in the heart and in cardiac pathology, it is important to review what *in vitro* studies have shown and how they differ from the mouse models mentioned above. Studies using cultured rat neonatal cardiomyocytes, fibroblasts, and coronary endothelial cells have shown that the stimulation of the AT_2 receptor inhibits cell growth and proliferation and opposes the effects of the AT1 receptor [27, 28]. Nakajima C. et al. used AT_2 receptor expression vectors to evaluate the growth of cultured aortic vascular smooth muscle cells (VSMC) with overexpression of these receptors versus controls. In this study, VSMCs with transfection of the AT_2 receptor presented a decrease of 70% in neointimal area when compared to controls, suggesting that the AT_2 receptors have an inhibitory effect of neointimal growth. Moreover, this effect was blocked with PD123319, an AT_2 receptor antagonist [29].

On the other hand, a direct prohypertrophic action of AT_2 receptors on cardiomyocytes was demonstrated by D'Amore et al. when using adenoviruses encoding AT_1 and AT_2 to coexpress these receptors in isolated cardiomyocytes [30]. Overexpression of the AT_2 receptor on cardiomyocytes using adenoviruses provoked an increase in the basal hypertrophy of these cells. This was unaffected by Ang II or AT_2 receptor ligands such as PD123319 or CGP42112A. The major outcome of this study was the lack of evidence to demonstrate that the AT_2 receptor opposes the actions of the AT_1 receptor, a widely proposed view. When the expression of the AT_2 receptor was increased, the Ang II-mediated hypertrophy through the AT_1 receptor was not inhibited; moreover, the AT_2 receptor-mediated enhanced basal hypertrophy was unchanged and it was added to that of the AT_1receptor. These findings suggest that the AT_1 and AT_2 receptor might use different pathways.

Results from *in vitro* cell culture have provided invaluable information regarding the role of the AT_2 receptor in mediating the Ang II signaling and the interaction of the AT_1 and AT_2 receptor in specific cell types. Different studies involving the AT_2 receptors showed that there is marked tissue heterogeneity, likely a reflection of the balance of AT_1/AT_2 receptor expression [31]. The various growth effects of Ang II seen in the *in vitro* studies were determined by the type of AT_2 receptor expressed in the cultured cell. For example, the AT_2 receptors are constitutively expressed in cultured endothelial cells but not in cultured vascular smooth muscle cells (VSMC); consequently, the AT_2 receptor antiproliferative effects will counteract the AT_1 receptor growth promoting effects in endothelial cells but not in vascular smooth muscle cells [27, 29]. This might explain why the results of the different *in vitro* studies are not 100% consistent and why these results differ from *in vivo* experiments. Furthermore, cell culture may not reflect the complex cross-talk among different cell types in the heart *in vivo*. In regards to the studies of the diseased heart even

Table 1: Cardiac phenotype and function in mice with AT2 overexpression (TG) or knock out (KO).

Mouse model	Strain	Baseline	Disease state	References
AT_2 TG mice (cardiomyocyte-specific, α-MHC)	C57BL/6	↔HW/BW ↑PW ↑EF%	AS: ↔ HW/BW Ang II: ↔ HW/BW, ↓HR MI: ↔LVMI, ↑PW, ↑EF%	[15, 18, 19]
AT_2 TG mice (ventricular myocyte-specific, MLC-2v)	AT_2^{high}TG	↑LVW/BW, ↓wall thickness ↑interstitial collagen ↑myocyte area and length ↓LV contractile function ↑apoptosis		[14]
	AT_2^{low}TG	↔ cardiac morphology and function	AS: ↔LVW/BW ↔wall thickness ↓myocyte diameter ↓interstitial collagen ↓LVSP ↓LVEDP	[21]
AT_2 KO mice	C57BL/6	↑BP ↑HR ↓LVW/BW ↓wall thickness ↓LVMI ↔contractile function	AS: No hypertrophy ↓interstitial collagen ↔cardiac function Ang II: No hypertrophy ↔BP ↓interstitial collagen ↑diastolic function	[16, 22, 23]
AT_2 KO mice	FVB/n	↔BP ↔ cardiac morphology	AS: ↔hypertrophy ↑perivascular fibrosis ↑coronary arterial thickening AMI: ↑LVW/BW ↑Lung/BW ↓EF%	[17, 24, 25]

HW: heart weight; LVW: left ventricular weight; BP: blood pressure; HR: heart rate; LVMI: left ventricular mass index; PW: posterior wall thickness; EF: ejection fraction; AS: aortic stenosis; Ang II: Ang II infusion; MI: myocardial infarction.

though it is well known that AT_2 receptors are upregulated in cardiac fibroblasts in the presence of cardiac pathology [26, 32, 33], it is not known whether the ratio of AT_1/AT_2 by overexpression of these receptors in cell cultures represented that in a hypertrophied/failing heart.

4. Conclusions

Transgenic mouse models with specific AT_2 overexpression or disruption have provided new information on this receptor. However, these results need to be interpreted with caution. New transgenic mouse models that conditionally overexpress or disrupt AT_2 in specific cell types in addition to cardiomyocytes in the heart may be used for more precisely studying the pathophysiological role of AT_2 receptors.

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