Paper

The angiotensin type 1 receptor antagonist valsartan attenuates pathological ventricular hypertrophy induced by hyperhomocysteinemia in rats

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Abstract

Introduction. Clinical and experimental studies have reported the role of homocysteine in ventricular hypertrophy. Activation of the renin-angiotensin system mediated by angiotensin II type 1 (AT_1) receptor has also been suggested to contribute to the pathogenesis of ventricular hypertrophy. There are also reports suggesting the affect of angiotensin II (Ang II) on cardiac hypertrophy is mediated by hyperhomocysteinemia. However, there is limited information on the mechanisms of the possible relationship between homocysteine and Ang II in ventricular hypertrophy. In this study we tested the hypothesis that hyperhomocysteinemia induced ventricular hypertrophy and remodelling may be mediated through activation of Ang II AT_1-receptors in rats.

Methods. This study was conducted on control non-treated rats (n=13), methionine-treated rats (1.5 mg/kg/day, n=18) and methionine plus oral AT_1 antagonist (valsartan, 30 mg/kg/day, n=13) treated rats for 56 days. Systolic blood pressure (SBP) was determined in rats at baseline, 28 and 56 days. Echocardiography was also performed in all rats after eight weeks, and blood samples were obtained for determination of plasma tHcy. Rats were then sacrificed for histopathological and biochemical assessment of cardiac structure.

Results. The SBP in the methionine-treated rats was significantly higher compared with controls and significantly lower compared with the methionine-valsartan group at 28 and 56 days ($p<0.001$). In addition, left ventricular wall thickness (LVWT) in the methionine-valsartan group (4.36±0.11 mm) was significantly lower compared with the methionine group (5.0±0.23 mm, p=0.03). Furthermore, cardiac collagen to total protein ratio was significantly lower in the methionine-valsartan group (2.19±0.11%) compared with the methionine group (2.64±0.08%, p=0.026). Fractional shortening (FS) was not significantly different between groups.

Conclusion. Results from this study suggest that hyperhomocysteinemia-induced hypertension and ventricular hypertrophy in rats are mediated, at least partly; by Ang II activation of AT_1-receptors.

Introduction

Hyperhomocysteinemia is identified as an independent risk factor for cardiovascular diseases such as arteriosclerosis, and coronary artery and cerebrovascular disorders.[1,2] Emerging evidence for the role of homocysteine in ventricular hypertrophy is supported by clinical and experimental research. Clinical studies have indicated a positive association between total plasma homocysteine (tHcy) and left ventricular mass after adjustment for other factors.[3,4] Experimentally, moderate hyperhomocysteinemia for 10 weeks in rats directly caused ventricular hypertrophy characterised by myocyte hypertrophy, increased perivascular and interstitial collagen and coronary arterial wall thickening, in the absence of significant blood pressure changes.[5] In addition, a two-fold increase in serum homocysteine caused a significant increase in left ventricular systolic and diastolic dimensions and decreased posterior wall thickness after only two weeks in rats.[6]

Although the pathogenesis of ventricular hypertrophy is complex and poorly understood, activation of the renin-angiotensin system (RAS) appears to play an important role.[7] Angiotensin II type-1 (AT_1) receptor activation is involved in vascular and cardiac hypertrophy, cardiac remodelling, endothelial dysfunction, neointima formation and processes leading to atherothrombosis.[8] Valsartan, a selective AT_1-receptor antagonist, has been used to prevent ventricular hypertrophy.

Animal studies on the association between hyperhomocysteinemia and the RAS in the pathogenesis of ventricular hypertrophy suggest that vasomotor responsive-ness of isolated vascular smooth muscle cells (VSMC)[9] or carotid rings[10] to angiotensin II (Ang II) is enhanced in the presence of high serum tHcy levels. Furthermore, it is reported that Ang II enhanced the stiffness and collagen deposition of small arteries in mice with mild hyperhomo-cysteinemia.[11] However, the role of Ang II and hyperhomocysteinemia in cardiac hypertrophy is still unclear. Therefore, in this study we tested the

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hypothesis that pathological changes induced by hyperhomocysteinemia may be mediated through the activation of AT_1-receptors in rats.

Methods

Animal groups

Male Fisher rats were divided into three groups: control non-treated (n=13), methionine-treated (n=18) and methionine plus valsartan-treated (n=13) groups. Animals were housed three per cage in a temperature-controlled room (23°C) with a 12:12-hour dark-light cycle and free access to food and water. The Institutional Animal Care and Use Committee at the College of Medicine and Medical Sciences, Arabian Gulf University approved all experimental procedures in this study.

Induction of hyperhomocysteinemia and administration of the AT_1 antagonist

Hyperhomocysteinemia was induced by methionine (Sigma Chemical Co.) that was delivered in drinking water as we previously described.[12] The concentration of methionine in water was adjusted to deliver 1.5 g/kg/day per rat based on average water intake. Methionine is metabolized to produce homocysteine via S-adenosyl methionine and S-adenosyl homocysteine. In addition, methionine and its intermediate products can inhibit homocysteine methyltransferase and thereby block the metabolism of homocysteine, further leading to increased tHcy levels.[13] Valsartan (Diovan, Novartis Pharmaceuticals, Basel, Switzerland) was administered orally in drinking water at a concentration to deliver 30 mg/kg/day per rat.

Measurement of arterial pressure in conscious rats

Systolic pressures were measured by the tail cuff technique using an automated sphygmomanometer (Technical & Scientific Equipment, Kronberg, Germany). After proper conditioning for one week, rats were placed in individual restrainers during pressure measurements. Three pressure readings were recorded and the data were displayed and averaged on a computer program.

Echocardiography

After eight weeks, echocardiography was done in the three groups under anaesthesia with a ketamine/xylazine mixture (60 mg/5 mg/kg/rat) administered intraperitoneally as previously described.[6] The ventral thorax was shaved and covered with an ultrasonic transmission gel and measurements were carried out using HP 4500 Sono echocardiogram system using a 12 MHz transducer. Measurements were utilised to image the left ventricle (LV) in the short-axis view. Interventricular septum thickness (IVS), posterior LV wall thickness (PW), and LV end-diastolic dimension (LVEDD) were measured at the end of diastole and LV end-systolic dimension (LVESD) was measured at the end of systole using visual frame analysis. LV wall thickness (LVWT) was calculated as IVS+PW and LV fractional shortening (FS) was calculated as (LVEDD-LVESD)/LVEDD. All measurements are expressed as ± standard error of the mean (SEM) of three determinations and performed by a cardiologist who was blinded with regard to the treatments.

Histomorphometric analysis

Histomorphometric analysis was carried out according to the method previously described.[5] Briefly, horizontal sections of ventricular myocardium (midway between apex and atrioventricular ring) were fixed in 10% neutral buffered formalin and embedded in paraffin. Serial sections (5 µm) including both left and right ventricles were stained with *picrosirius red* for fibrillar collagen. Coronary arteriolar remodelling and both perivascular and interstitial collagen volume fraction were then estimated. Images were taken through an Olympus BX 41 microscope with a digital camera (DP 12) and were then analyzed using NIH Image software provided by National Institute of Health (http://rsb.info.nih.gov/nih-image/). Quantitative assessment of collagen was expressed as the percentage of red area in each screen. At least four areas were examined in each heart.

Spectrophotometeric assessment of cardiac collagen

This method is based on the selective binding of Sirius red with collagen and of fast green with non-collagen protein.[14] Briefly, the paraffin-embedded tissue was cut in 15 µm sections, and the deparaffinised sections were subsequently treated with a saturated solution of picric acid in distilled water containing 0.1% fast green FCF and 0.1% Sirius red F3BA. After several washes of unbound dyes with de-ionised water, the dyes were eluted with NaOH-methanol and absorbencies were measured in a spectrophotometer at 540 nm and 605 nm (corresponding to the maximum absorbencies of Sirius red and fast green, respectively). An estimate of collagen content, expressed as a percentage of total protein, was obtained using a previously described formula.[15]

Statistical analysis

All data in this study were analyzed using SPSS version 13.0 and presented as means±SEM. One-way analysis of variance (ANOVA) and *post hoc* multiple comparison (Tukey HSD) were employed to determine the significant differences in SBP, echocardiogram and histopathological parameters between and within three groups of

Table 1
Echocardiographic measurements in control (n=13), methionine (n=18) and methionine plus valsartan-treated (n=13), rats after 56 days.

	IVS (mm)	PW (mm)	LVWT (mm)	FS (%)
Control	1.81±0.04	1.91±0.15	3.72±0.11	24.86±3.06
Methionine	2.49±0.09*	2.51±0.15*	5.00±0.23*	36.03±3.65
Methionine + valsartan	2.19±0.06†*	2.17±0.08†	4.36±0.11†	40.60±5.01†

Data are expressed as mean ± SEM. * = p<0.05 compared with controls, † = p<0.05 compared with methionine group. IVS = interventricular septum, PW = posterior wall of left ventricle, LVWT = left ventricular wall thickness, FS = fractional shortening.

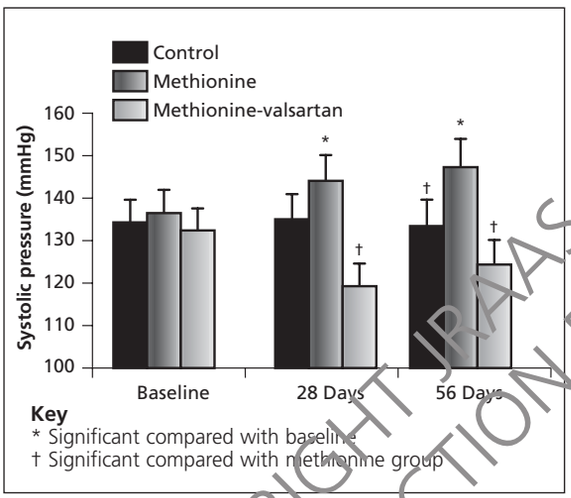

Figure 1
Systolic blood pressure in control, methionine and methionine-valsartan treated groups.

control, methionine and methionine-valstarten treated rats, respectively. A p value <0.05 was considered to be statistically significant.

Results

Serum homocysteine

Serum tHcy concentrations were significantly higher in both the methionine (21.67±5.82 µmol/L and methionine-valsartan (21.71±5.30) groups compared with the control group (8.42±1.56 µmol/L, p=0.02) after oral methionine administration at a dose of 1.5 g/kg/day for 56 days.

Systolic pressure

The SBP recorded in the three groups of rats at baseline, 28 days and 56 days is illustrated in figure 1. ANOVA showed that systolic blood pressure (SBP) was not significantly different between the three groups at baseline. However, SBP was significantly higher after 28 and 56 days in the methionine-treated group compared with baseline values (p=<0.001). In addition, in *post hoc* within group analysis, after 28 days the SBP was significantly higher in the methionine group compared with the methionine-valsartan group (p=<0.001) but not the control group (p=0.261). However after 56 days SBP was significantly higher in the methionine-treated group compared with the controls (p=0.008) and even more significantly higher than in the methionine-valsartan treated group (p=<0.001).

Table 1 shows the 2D echocardiographic parameters in the methionine and methionine-valsartan groups. Compared with controls, PW and LVWT were significantly higher in the methionine group (p<0.05 in all the variables). In contrast, IVS was marginally higher (p=0.04) in the methionine-valsartan group while other parameters were not significantly different from controls. In addition, IVS, PW and LVWT were significantly lower in the methionine-valsartan compared with the methionine group (p<0.05). No significant differences in FS were found between the three groups.

Ventricular weights

ANOVA showed that ventricular weight and ventricular/body weight ratio were significantly higher in the methionine treated group compared with controls and the methionine-valsartan treated group (p=0.04). In addition *post hoc* analysis showed that compared with the methionine group, ventricle weights and ventricle/body weight ratios were significantly lower in the methionine-valsartan group by 15.4% and 11.6%, respectively (p=0.03 in each).

Assessment of cardiac remodelling

Picrosirius-stained histopathological sections from the left ventricles taken from control (A & B), methionine-treated (C & D) and methionine-valsartan (E & F) groups are illustrated in figure 2. In the methionine-treated group, interstitial and perivascular collagen and arteriolar wall thickness were increased compared with controls. Treatment with valsartan attenuated the increase in both interstitial and perivascular collagen. The numerical analysis of histopathological parameters

Table 2
Biometric and histopathological findings in control (n=13), methionine (n=18) and methionine valsartan-treated (n=13) rats after 56 days.

Parameter	Control	Methionine	Methionine + valsartan
Body weight (g)	255.4±13.5	258.0±11.0	246.0±12.1
Ventricle weight (mg)	336.1±29.5	463.8±31.7*	392.3±24.1*†
Ventricle wt/body wt ratio (mg/g)	1.32±0.07	1.80±0.08*	1.59±0.07*†
Perivascular collagen	1.15±0.14	2.09±0.18*	1.57±0.14*†
Interstitial collagen (%)	1.23±0.18	1.85±0.12*	1.42±0.15*†
Collagen/total protein (%)	1.54±0.16	2.64±0.08*	2.19±0.11*†
Coronary arteriole media/lumen ratio	2.15±0.22	3.21±0.39*	2.86±0.35

Data are expressed as mean±SEM. Perivascular collagen is expressed as ratio of collagen to luminal surface area.
* = p<0.05 compared with control group and † = p<0.05 compared with the methionine group.

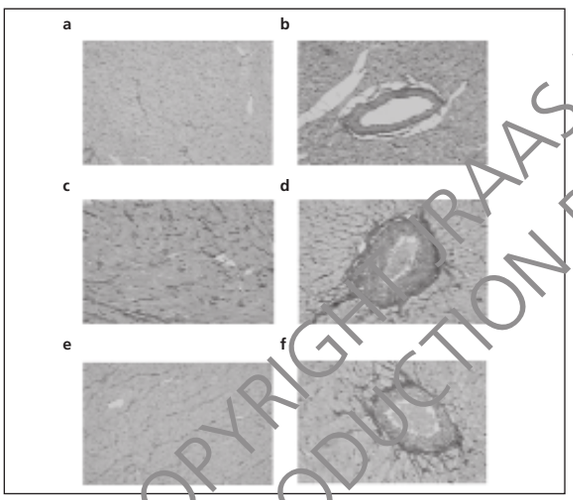

Figure 2
Picrosirius-stained sections from the left ventricle in the studied rat groups. In the methionine group (**c** & **d**), interstitial and perivascular collagen and arteriolar wall thickness were increased compared with controls (**a** & **b**). The methionine-valsartan group (**e** & **f**) demonstrates reduced interstitial and perivascular collagen compared with the methionine group. Original magnification for interstitial collagen = Γ 200 and for perivascular collagen = Γ 400.

is outlined in table 2. Perivascular and interstitial collagen were significantly lower in the methionine-valsartan compared with the methionine group (p=0.04 & 0.05, respectively). Furthermore, spectrophotometrically-assessed ventricular collagen/total protein was significantly lower in the methionine-valsartan compared with the methionine treated group (2.19±0.11% versus 2.64±0.08%, respectively, p=0.03).

Discussion

This study examined the role of angiotensin AT_1-receptors on structural and functional cardiac changes in hyperhomocysteinemic rats. Serum tHcy concentrations were increased by approximately 2.5-fold in both methionine and methionine-valsartan-treated groups after 56 days. This was associated with a significant increase in arterial pressure, ventricular hypertrophy and increased cardiac collagen deposition in methionine-treated rats. However, these pathological changes were significantly attenuated in methionine-valsartan treated rats.

The results from this study are consistent with animal and clinical studies indicating that homocysteine may play an important role in hypertension. Long-term administration of a high methionine diet in minipigs[16] and rats[17] resulted in hypertension and vascular pathological changes, although other studies suggest that increasing serum tHcy by two-fold, for six weeks,[5] or five-fold for 10 weeks[13] failed to cause a significant increase in arterial pressure in rats. Clinical studies also indicated a positive relationship between hyperhomocysteinemia and hypertension.[18] The findings from this study that SBP in methionine-valsartan-treated rats was significantly lower compared with methionine-treated group indicate that activation of angiotensin system is one of the mechanisms involved in homocysteine-mediated hypertension. However, other mechanisms by which homocysteine can promote hypertension include decreasing endothelial production of nitric oxide (NO), inducing accumulation of the endogenous NO synthase inhibitor asymmetric dimethylarginine, lowering NO bioactivity through induction of oxidative stress and increasing the arterial stiffness.[19] Further studies would be required to separate RAS-mediated and non-mediated effects on hyperhomocysteinemia-induced hypertension.

The finding that hyperhomocysteinemia induces an increase in LVWT and collagen deposition in

rats is consistent with previous experimental studies suggesting that hyperhomocysteinemia may be directly involved in the development of ventricular hypertrophy and cardiac remodelling.[5,6] We further demonstrated that AT_1-receptor antagonism significantly reduced the increase in LVWT and collagen content in hyperhomocysteinemic rats. This attenuation in cardiac collagen deposition with AT_1-receptor antagonism is unlikely to be mediated through altering the homocysteine metabolic pathway since both methionine and methionine-valsartan groups developed comparable levels of hyperhomocysteinemia. These results suggest that activation of Ang II, through AT_1-receptors, appears to play a role in the cardiac hypertrophy in hyperhomocysteinemic rats. However, an alternative explanation could be that the blunted blood pressure elevation observed in valsartan treated rats is responsible for the attenuation of cardiac enlargement because of haemodynamic mechanisms independent of the role of RAS in methionine induced LVH. Further studies to dissect the role of haemodynamic and non-haemodynamic factors in hyperhomocysteinemia induced ventricular hypertrophy would be required.

The interactions between homocysteine and angiotensin II in the cardiovascular system have been previously examined. Hyperhomocysteinemia enhances vascular responsiveness to Ang II in isolated aortic VSMC and carotid rings.[10] On the other hand, Ang II enhances the homocysteine-induced contraction of cardiac rings chemically denuded of endothelium[20], and also enhances the stiffness and collagen deposition of small arteries in mice with mild hyperhomocysteinemia.[7] Studies by Miller *et al.*[21] indicated that chronic hyperhomocysteinemia in rats is associated with increased collagen and transforming growth factor-β (TGF-β) in cardiac tissues along with endocardial endothelial dysfunction. TGF-β. has been shown to increase the synthesis of angiotensin-converting enzyme (ACE) in rat cardiac fibroblasts.[22] Furthermore, homocysteine may modulate the Ang II signalling linked to gene transcription.[23] The interactions between homocysteine, Ang II, AT_1-receptors, ACE and TGF-β still require further elucidation in future research.

In this study, despite the increased interstitial and perivascular collagen in hyperhomocysteinemia, FS was within normal limits. Therefore, hyperhomocysteinemia for 56 days may be associated with compensated ventricular hypertrophy either due to increasing cardiomyocyte size or compensatory neurohumoral control mechanisms. Similar observations were reported in rats with hyperhomocysteinemia for 10 weeks, despite the presence of diastolic dysfunction.[5] In addition, recent clinical studies also reported a positive association between systolic function and hyperhomocysteinemia in patients with a high risk of coronary artery disease.[24] Long-term experimental studies are required to address the changes in systolic function in models of hyperhomocysteinemia. In addition, the role of Ang II in diastolic dysfunction associated with hyperhomocysteinemia remains to be studied.

Conclusion

In conclusion, the present study demonstrates that moderate hyperhomocysteinemia causes hypertension and pathological ventricular hypertrophy. These cardiovascular changes were significantly attenuated by blocking Ang II AT_1-receptors. These data indicate that Ang II, through AT_1-receptors, plays an important role in mediating the hypertension and ventricular hypertrophy in rats with hyperhomocysteinemia.

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