Cardiovascular hypertrophy in diabetic spontaneously hypertensive rats: optimizing blockade of the renin–angiotensin system

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A B S T R A C T

The aim of the present study was to compare the antihypertrophic effects of blockade of the renin–angiotensin system (RAS), vasopeptidase inhibition and calcium channel antagonism on cardiac and vascular hypertrophy in diabetic spontaneously hypertensive rats (SHR). SHR with streptozotocin-induced diabetes were treated with one of the following therapies for 32 weeks: the angiotensin-converting enzyme (ACE) inhibitor captopril (100 mg/kg); the angiotensin AT₁ receptor antagonist valsartan (30 mg/kg); a combination of captopril with valsartan; the vasopeptidase inhibitor mixanpril (100 mg/kg); or the calcium channel antagonist amlodipine (6 mg/kg). Systolic blood pressure and cardiac and mesenteric artery hypertrophy were assessed. Mean systolic blood pressure in diabetic SHR (200 ± 5 mmHg) was reduced by captopril (162 ± 5 mmHg), valsartan (173 ± 5 mmHg), mixanpril (176 ± 2 mmHg) and amlodipine (159 ± 4 mmHg), and was further reduced by the combination of captopril with valsartan (131 ± 5 mmHg). Captopril, valsartan and mixanpril reduced heart and left ventricle weights by approx. 10%. The combination of captopril and valsartan further reduced heart weight (−24%) and left ventricular weight (−29%). Amlodipine did not affect cardiac hypertrophy. Only mixanpril and the combination of captopril and valsartan significantly reduced mesenteric weight. The mesenteric wall/lumen ratio was reduced by all drugs, and to a greater extent by the combination of captopril and valsartan. We conclude that optimizing the blockade of vasoconstrictive pathways such as the RAS, particularly with the combination of ACE inhibition and AT₁ receptor antagonism, is associated with antitrophic effects in the context of diabetes and hypertension. In contrast, calcium channel blockade, despite similar effects on blood pressure, confers less antitrophic effects in the diabetic heart and blood vessels.

INTRODUCTION

Diabetes and hypertension are major risk factors for cardiovascular diseases. About three-quarters of adults with diabetes will die from cardiovascular diseases. The prevalence of hypertension is increased in the diabetic population, and hypertension in combination with diabetes further increases the risk of cardiovascular mortality. Both diabetes and hypertension are associated with cardiac and vascular hypertrophy, which are considered to be important pathological processes leading to the subsequent development of cardiovascular diseases [1,2].

Key words: angiotensin-converting enzyme, angiotensin receptor, calcium channel antagonist, cardiovascular hypertrophy, diabetes, vasopeptidase inhibitor, hypertension.

Abbreviations: ACE, angiotensin-converting enzyme; HbA1c, glycated haemoglobin; NEP, neutral endopeptidase; RAS, renin–angiotensin system; SHR, spontaneously hypertensive rats; VPI, vasopeptidase inhibitor.

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Currently, the most important therapeutic approach to preventing diabetic cardiac and vascular complications, in addition to strict glycaemic control, is rigorous blood pressure control [3]. Angiotensin-converting enzyme (ACE) inhibitors, because of their protective effects on the cardiovascular system and the kidney, are regarded as the first-line antihypertensive therapy for patients with diabetes [4–6]. Increasing evidence suggests that angiotensin AT1 receptor antagonists offer similar protection as ACE inhibitors [7,8], and they are regarded as alternative drugs for patients suffering from ACE-inhibitor-related adverse effects, specifically persistent cough. However, blockade of the renin–angiotensin system (RAS) with either an ACE inhibitor or an AT1 antagonist only retards the development of the diabetic complications. The pathological process leading to cardiovascular disease continues, albeit at a lower rate. Therefore new strategies to prevent and treat these complications are urgently needed.

It has been postulated that the combination of an ACE inhibitor with an AT1 receptor antagonist confers greater organ protection, specifically in the kidney, compared with either drug alone [9]. We have shown recently that in diabetic spontaneously hypertensive rats (SHR) [10], in sub-totally nephrectomized rats [11] and in diabetic Ren2 rats [12] this combination results in additive lowering of blood pressure and renal protection. However, the cardiac and vascular effects of such a combination in diabetes remain largely unknown.

Vasopeptidase inhibitors (VPIs) are a new class of drugs comprising single molecules that simultaneously inhibit both ACE and neutral endopeptidase (NEP). NEP is an enzyme that plays an important role in the degradation of natriuretic peptides and bradykinin [13]. Thus treatment with VPIs, such as mixanpril (S21402), is associated with a decrease in the concentration of the vasoconstrictor angiotensin II and accumulation of the aforementioned vasodilators. Thus VPIs have been suggested to offer increased antihypertensive efficacy and cardiovascular protection compared with ACE inhibition alone [14–16]. The long-term cardiac and vascular effects of VPIs in the context of diabetes and hypertension have not been studied previously.

Calcium channel antagonists are widely used antihypertensive agents. In diabetes-related vascular hypertrophy in the absence of hypertension, we have reported that these agents confer similar vascular protection as observed with the RAS blockers [17]. However, the effects of calcium channel antagonists on cardiac and vascular hypertrophy in the presence of hypertension and diabetes, particularly in the long term, have not been studied.

Studying the combination of the two major cardiovascular risk factors, hypertension and diabetes, has been explored experimentally by inducing diabetes in SHR [18,19]. We used this model to assess the cardiac and vascular effects of RAS blockade, a VPI and a calcium channel antagonist; the latter achieves similar antihypertensive efficacy without influencing the RAS significantly.

METHODS

Male SHR (230–270 g), housed at the Biological Research Laboratory of the Austin and Repatriation Medical Centre, were used in this study. The research protocol was approved by the Animal Welfare Committee of the Austin and Repatriation Medical Centre. Diabetes was induced by tail-vein injection of streptozotocin (Boehringer-Mannheim, Mannheim, Germany) at a dose of 45 mg/kg in citrate buffer after an overnight fast. Long-acting insulin (Ultralente; Novo Industries A/S, Copenhagen, Denmark) at a dose of 4 units/day was injected subcutaneously to prevent ketouria and promote weight gain. The animals had free access to water and standard rat chow (Clark King & Co., Melbourne, Australia).

The rats were allocated randomly to one of seven groups and treated for 32 weeks. These groups were as follows: group 1, control SHR (n = 17); group 2, diabetic SHR without drug treatment (n = 14); group 3, diabetic SHR treated with the ACE inhibitor captopril (Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ, U.S.A.) at a dose of 100 mg/kg in drinking water (n = 9); group 4, diabetic SHR treated with valsartan (Novartis Pharma AG, Basel, Switzerland) at a dose of 30 mg/kg by gavage (n = 11); group 5, diabetic SHR treated with captopril (100 mg/kg in drinking water) plus valsartan (30 mg/kg by gavage) (n = 7); group 6, diabetic SHR treated with mixanpril (IRIS Pty Ltd, Courbevoie, France) at a dose of 100 mg/kg in drinking water (n = 9); group 7, diabetic SHR treated with amlodipine (Pfizer, New York, NY, U.S.A.) at a dose of 6 mg/kg by gavage (n = 12). The doses of these compounds were based on our own previously published studies, which indicated that the doses used inhibited the appropriate enzymes or receptors in target organs [11,14,20]. Systolic blood pressure was measured by tail-cuff plethysmography in conscious preheated rats every 4 weeks. Samples for plasma renin activity and glycated haemoglobin (HbA1c) were collected from the tail veins of conscious rats before the animals were killed at week 32. Plasma renin activity was measured by RIA [21]. HbA1c was measured by HPLC (Bio-Rad, Richmond, CA, U.S.A.) [22].

At the end of the experiment, the animals were anaesthetized by intravenous injection of pentobarbitone sodium (Boehringer Ingelheim, Artarmon, NSW, Australia) at a dose of 60 mg/kg. The mesenteric arteries were perfused in vivo at arterial pressure via an intra-aortic cannula with saline and then with 2.5% glutaraldehyde.
RESULTS

Metabolic parameters and systolic blood pressure

Diabetes was associated with reduced weight gain and increased HbA1c levels compared with non-diabetic animals (Table 1). These parameters were not influenced by any drug treatment.

Table 1 Metabolic parameters and plasma renin activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Body weight (g)</th>
<th>HbA1c (%)</th>
<th>PRA (nmol of angiotensin I·h⁻¹·l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17</td>
<td>431 ± 7</td>
<td>4.6 ± 0.3</td>
<td>5.8 × 1/1.2</td>
</tr>
<tr>
<td>Diabetes</td>
<td>14</td>
<td>356 ± 7*</td>
<td>9.4 ± 0.6*</td>
<td>9.6 × 1/1.2</td>
</tr>
<tr>
<td>Diabetes + captopril</td>
<td>9</td>
<td>369 ± 10*</td>
<td>8.0 ± 0.6*</td>
<td>45.3 × 1/1.2*</td>
</tr>
<tr>
<td>Diabetes + valsartan</td>
<td>11</td>
<td>340 ± 8*</td>
<td>9.0 ± 0.7*</td>
<td>24.4 × 1/1.5*</td>
</tr>
<tr>
<td>Diabetes + captopril + valsartan</td>
<td>7</td>
<td>354 ± 14*</td>
<td>8.0 ± 0.3*</td>
<td>87.4 × 1/1.4*</td>
</tr>
<tr>
<td>Diabetes + mixanpril</td>
<td>14</td>
<td>376 ± 10*</td>
<td>9.5 ± 0.4*</td>
<td>10.1 × 1/1.1</td>
</tr>
<tr>
<td>Diabetes + amlodipine</td>
<td>12</td>
<td>361 ± 10*</td>
<td>8.3 ± 0.4*</td>
<td>ND</td>
</tr>
</tbody>
</table>

Data are shown at week 32 as means ± S.E.M., except for plasma renin activity (PRA), which is shown as the geometric mean ×/−1 tolerance factor. Significance of differences: *P < 0.05 compared with control; †P < 0.05 compared with untreated diabetes. ND, not done.

Plasma renin activity

Plasma renin activity was slightly elevated in diabetic SHR compared with control SHR (P = 0.05; Table 1). Captopril and valsartan increased plasma renin activity by 4- and 5-fold respectively compared with untreated diabetic SHR. The combination of captopril and valsartan resulted in a further increase in plasma renin activity when compared with the monotherapy groups. Mixanpril therapy did not affect plasma renin activity.
Diabetes reduced by the combination of captopril plus valsartan and by mixanpril. Amlodipine did not influence these cardiac parameters. The right ventricle weight/body weight ratio was increased in diabetic SHR compared with untreated diabetic SHR. The combination of captopril plus valsartan reduced these parameters to a greater extent than observed when either drug was given as monotherapy. Amlodipine did not influence these cardiac parameters. The right ventricle weight/body weight ratio was increased in diabetic SHR compared with non-diabetic SHR. The right ventricle weight/body weight ratio was not influenced by any treatment when compared with the untreated diabetic SHR.

**Cardiac hypertrophy**

Total heart weight, left ventricle weight and right ventricle weight in relation to body weight are shown in Table 2. The heart weight/body weight ratio and left ventricle weight/body weight ratio were similar in diabetic SHR and non-diabetic SHR. Captopril, valsartan and mixanpril reduced the heart weight/body weight ratio and the left ventricle weight/body weight ratio compared with untreated diabetic SHR. The combination of captopril plus valsartan reduced these parameters to a greater extent than observed when either drug was given as monotherapy. Amlodipine did not influence these cardiac parameters. The right ventricle weight/body weight ratio was increased in diabetic SHR compared with non-diabetic SHR. The right ventricle weight/body weight ratio was not influenced by any treatment when compared with the untreated diabetic SHR.

**Mesenteric hypertrophy**

The mesenteric artery weight/body weight and medial wall/lumen ratios are presented in Table 3. Compared with non-diabetic SHR, diabetic SHR had an increased mesenteric artery weight/body weight ratio, which was reduced by the combination of captopril plus valsartan and by mixanpril.

There was a modest increase in the medial wall/lumen ratio of approx. 10% in diabetic SHR. The medial wall/lumen ratio was reduced by captopril, by valsartan, by the combination of captopril plus valsartan and by mixanpril. Amlodipine also reduced the medial wall/lumen ratio, albeit to a lesser extent than other treatments.

**Correlation of systolic blood pressure with cardiovascular parameters**

In diabetic animals, mean systolic blood pressure was correlated with the heart weight/body weight, left ventricle weight/body weight and media/lumen ratios (Figure 2). No correlation of systolic blood pressure was found with the right ventricle weight/body weight or mesenteric artery weight/body weight ratios (results not shown). Amlodipine therapy, despite having similar antihypertensive efficacy to the three other monotherapies, had less of an antitrophic effect on cardiac and left ventricular weights or on the mesenteric media/lumen ratio.

**DISCUSSION**

The present study has evaluated a range of pharmacological strategies aimed at preventing cardiac and vascular hypertrophy in the potentially clinically im-

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**Table 2 Cardiac weights**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Heart weight/body weight (g/kg)</th>
<th>Left ventricle weight/body weight (g/kg)</th>
<th>Right ventricle weight/body weight (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17</td>
<td>3.88 (3.77–3.98)</td>
<td>2.98 (2.90–3.05)</td>
<td>0.62 (0.60–0.65)</td>
</tr>
<tr>
<td>Diabes</td>
<td>14</td>
<td>3.99 (3.83–4.13)</td>
<td>2.93 (2.87–3.04)</td>
<td>0.72 (0.66–0.77)*</td>
</tr>
<tr>
<td>Diabes + captopril</td>
<td>9</td>
<td>3.69 (3.44–3.82)†</td>
<td>2.62 (2.59–2.76)†</td>
<td>0.70 (0.65–0.76)</td>
</tr>
<tr>
<td>Diabes + valsartan</td>
<td>11</td>
<td>3.71 (3.46–4.03)†</td>
<td>2.67 (2.59–2.88)†</td>
<td>0.72 (0.67–0.80)</td>
</tr>
<tr>
<td>Diabes + captopril + valsartan</td>
<td>7</td>
<td>3.05 (2.52–3.58)§</td>
<td>2.08 (1.63–2.54)§</td>
<td>0.71 (0.64–0.78)</td>
</tr>
<tr>
<td>Diabes + mixanpril</td>
<td>14</td>
<td>3.60 (3.50–3.76)†</td>
<td>2.61 (2.46–2.81)†</td>
<td>0.66 (0.57–0.73)</td>
</tr>
<tr>
<td>Diabes + amlodipine</td>
<td>12</td>
<td>3.90 (3.63–4.17)§</td>
<td>2.80 (2.61–2.99)§</td>
<td>0.73 (0.65–0.81)</td>
</tr>
</tbody>
</table>

**Table 3 Mesenteric vascular weight**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mesenteric weight/body weight (g/kg)</th>
<th>Mesenteric wall/lumen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17</td>
<td>0.34 (0.28–0.40)</td>
<td>0.30 (0.28–0.34)</td>
</tr>
<tr>
<td>Diabes</td>
<td>14</td>
<td>0.51 (0.44–0.60)*</td>
<td>0.34 (0.29–0.38)</td>
</tr>
<tr>
<td>Diabes + captopril</td>
<td>9</td>
<td>0.47 (0.26–0.62)*</td>
<td>0.22 (0.17–0.27)†</td>
</tr>
<tr>
<td>Diabes + valsartan</td>
<td>11</td>
<td>0.52 (0.40–0.61)*</td>
<td>0.27 (0.21–0.34)†</td>
</tr>
<tr>
<td>Diabes + captopril + valsartan</td>
<td>7</td>
<td>0.30 (0.18–0.42)§</td>
<td>0.20 (0.13–0.26)§</td>
</tr>
<tr>
<td>Diabes + mixanpril</td>
<td>14</td>
<td>0.37 (0.29–0.51)†</td>
<td>0.26 (0.20–0.31)†</td>
</tr>
<tr>
<td>Diabes + amlodipine</td>
<td>12</td>
<td>0.52 (0.45–0.59)*</td>
<td>0.27 (0.20–0.34)†</td>
</tr>
</tbody>
</table>
Figure 2  Linear correlation of systolic blood pressure with cardiovascular parameters in diabetic SHR

D, diabetic; Cap, captopril; Val, valsartan; Mix, mixanpril; Aml, amlodipine.

important setting of long-term diabetes in combination with hypertension. The major findings of the present study are: (1) drugs blocking the RAS were effective in preventing cardiovascular hypertrophy in the context of diabetes associated with systemic hypertension; (2) calcium channel antagonism with amlodipine had only minimal effects on cardiovascular hypertrophy despite similar blood pressure reduction; (3) the combination of ACE inhibition with AT$_1$ receptor antagonism offered a clear advantage over either drug alone in preventing cardiovascular hypertrophy in this model.

The RAS, via its effector molecule angiotensin II, is regarded as playing a key role in the pathogenesis of diabetic cardiovascular complications [3,4,6]. Angiotensin II has both pressor and trophic effects. Blockade of the RAS with either an ACE inhibitor or an AT$_1$ receptor antagonist has been shown to reduce cardiovascular hypertrophy in non-diabetic SHR [25]. The present study demonstrated similar blood pressure reduction and antihypertrophic effects on the cardiovascular system in SHR with long-term diabetes. This is in agreement with a large number of clinical and experimental studies that have demonstrated cardiovascular protection by RAS blockers [6].

Specifically, we explored whether combining ACE inhibition with AT$_1$ receptor antagonism offers any additional benefits compared with either therapy alone. The combination resulted in improved blood pressure control as well as superior cardiac and vascular protection. This may be an additive or possibly a synergistic effect of the combination. In non-diabetic SHR, synergistic effects of ACE inhibitors and AT$_1$ receptor antagonists on blood pressure and left ventricle hypertrophy have been reported [25,26]. In clinical studies, the combination of ACE inhibitors and AT$_1$ receptor antagonists has been shown to offer increased antihypertensive efficacy and renoprotection, particularly in the setting of diabetes [9,27,28].

The effects of VPIs in cardiovascular disease are under clinical and experimental investigation. This class of drugs inhibits both ACE and NEP simultaneously. NEP inhibition results in decreased breakdown of vasoactive natriuretic peptides, which have diuretic, natriuretic and vasorelaxant effects [16]. Thus VPIs are postulated to confer superior end-organ protection when compared with ACE inhibitors in cardiovascular diseases [14–16]. The present study evaluated the effects of the VPI mixanpril in long-term diabetes. The decreases in blood pressure, left ventricular hypertrophy and vascular hypertrophy following mixanpril treatment were comparable with those with the ACE inhibitor captopril, with no clear-cut demonstration of superior vasoprotective effects with this agent. The relevance of the effects of VPIs, specifically NEP inhibition preventing the degradation of natriuretic peptides (such as atrial natriuretic peptide), in mediating the end-organ protection conferred by these agents remains controversial. Plasma atrial natriuretic peptide has been clearly demonstrated to be elevated in diabetic animals [22,29], but its role as an inhibitor of vascular and cardiac hypertrophy has not been fully clarified.

Long-term VPI (mixanpril) administration was not associated with a significant increase in plasma renin activity, normally seen with ACE inhibition. This may be
due to direct inhibition of renin synthesis in the kidneys by augmentation of local natriuretic peptide levels [30,31]. This finding is in agreement with a short-term study of mixanpril in SHR [15] and with a study of another NEP/ACE inhibitor in a rat model of heart failure [32]. In these previous studies, renal ACE activity was inhibited by a VPI, but the reactive rise in plasma renin activity was blunted.

In the present study, the calcium channel antagonist amloidipine had antihypertensive effects comparable with those of agents blocking the RAS. However, unlike drugs that interact with the RAS, only a minimal effect of this drug on cardiovascular hypertrophy was observed, suggesting a degree of dissociation between blood pressure reduction and protection from cardiovascular hypertrophy. Consistent with the findings in the present study, another group has reported that amloidipine was less effective than drugs blocking the RAS in reducing left ventricle and aortic weights in stroke-prone SHR [33]. In addition to a difference between agents that block the RAS and calcium channel blockers in influencing vascular and cardiac hypertrophy, these agents appear to differ in their degree of renal protection. For example, our group has reported previously that although the calcium antagonist lacidipine and the ACE inhibitor perindopril achieved similar blood pressure reduction, only the ACE inhibitor retarded the development of albuminuria in diabetic SHR [34].

The finding that the diabetic SHR had significantly lower blood pressure when compared with the control SHR is in accordance with our previous studies [10,18,19]. The mechanisms responsible for the lower blood pressure in diabetic SHR are unknown, but may be due to reduced growth in these animals as a result of chronic hyperglycaemia. Plasma renin activity was slightly increased in diabetic SHR in the present study, consistent with our previous report in this model [10]. This minor increase in plasma renin activity in diabetic SHR is unexplained. However, changes in plasma renin activity in diabetes may be related to renal injury, hyperglycaemia or altered activation of the RAS [35].

The concept that blockade of the RAS may confer superior end-organ protection compared with other classes of antihypertensive agents has been clearly demonstrated in clinical studies of diabetic patients with proteinuria. Indeed, in the IDNT (Irbesartan Diabetic Nephropathy Trial) study, despite similar blood pressure reduction, the AT$_1$ receptor antagonist irbesartan was associated not only with a reduction in renal events but also with a decrease in hospitalization for heart failure when compared with amloidipine treatment [8]. Of particular relevance are the recent findings of the LIFE (Losartan Intervention For Endpoint reduction in hypertension) study [36], where, particularly in diabetic cohort, the AT$_1$ receptor antagonist losartan, despite similar blood pressure reduction as with the β-blocker atenolol, was associated with less left ventricular hypertrophy, which translated to fewer cardiovascular events [36]. The findings of the present study emphasize that blood pressure reduction plays a crucial role in decreasing cardiac and vascular hypertrophy in diabetes and hypertension, but also emphasizes that the mode of reducing blood pressure is important. In particular, agents that interfere with vasoactive hormone pathways, including the RAS, appear to confer superior antitrophic effects compared with other antihypertensive regimens.

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