CXCR4 Antagonism Attenuates the Cardiorenal Consequences of Mineralocorticoid Excess

Po-Yin Chu, PhD; Amanda Zatta, PhD; Helen Kiriakis, PhD; Jaye Chin-Dusting, PhD; Xiao-Jun Du, MD, PhD; Tanneale Marshall, BS; David M. Kaye, MD, PhD

Background—Extensive evidence implicates aldosterone excess in the development and progression of cardiovascular disease states including hypertension, metabolic syndrome, cardiac hypertrophy, heart failure, and cardiorenal fibrosis. Recent studies show that activation of inflammatory cascade may play a specific role in the sequelae of mineralocorticoid activation, although the linking mechanism remains unclear. We tested the possibility that secondary stimulation of the stromal-derived factor 1/CXC chemokine receptor 4 (SDF-1/CXCR4) pathway plays a contributory role.

Methods and Results—We investigated the effect of the highly selective CXCR4 antagonist AMD3465 (6 mg/kg per day for 6 weeks through minipump) in doxycorticosterone acetate (DOCA)-treated, uninephrectomized mice. CXCR4 antagonism significantly attenuated the induction of cardiac fibrosis, renal fibrosis, hypertension, and left ventricular hypertrophy by DOCA. Mineralocorticoid excess also stimulated the accumulation of T-lymphocytes in the heart and kidney and this was significantly blunted by CXCR4 inhibition.

Conclusions—Taken together, these data strongly implicate the SDF-1/CXCR4 axis in the pathogenesis of mineralocorticoid excess induced hypertension, inflammation, and cardiorenal fibrosis. This insight provides a new potential therapeutic approach for the treatment of specific aspects of mineralocorticoid mediated cardiovascular disease. (Circ Heart Fail. 2011;4:651-658.)

Key Words: mineralocorticoid ■ hypertension ■ cardiac fibrosis ■ inflammation ■ cytokines ■ CXCR4

A bundant evidence clearly implicates components of the rennin-angiotensin-alderosterone axis in the pathogenesis of many major cardiovascular disorders including heart failure, metabolic syndrome, and hypertension. In particular, evidence for the contribution of elements of this system are highlighted by numerous studies that document increased plasma levels of relevant hormones, supported by large-scale trials of angiotensin-converting enzyme inhibitors, angiotensin II receptor, and aldosterone antagonists in the various clinical paradigms.1–4

Clinical Perspective on p 658

Despite the long-standing recognition that neurohormonal activation accompanies the initiation and progression of hypertension and its sequelae, emerging data are further defining the responsible mechanisms. In particular, recent studies on the role of angiotensin II and aldosterone in the pathogenesis of hypertension have shown that both agents initiate a program of vascular and tissue inflammation.5 More specifically, the accumulation of T-cells in the vasculature has been suggested to play a critical role in the development of hypertension. The mechanism responsible for vascular lymphocyte recruitment remains controversial. Together with inflammation, myocardial and renal fibrosis are also major features of experimental models of mineralocorticoid and angiotensin II excess,6,7 and, in the clinical context, cardiorenal fibrosis is clearly relevant in the context of hypertensive heart disease and heart failure.

The development of fibrosis in mineralocorticoid excess models has been shown in several studies to be largely independent of blood pressure, although this remains controversial.8–11 Although strong evidence links the development of cardiac fibrosis with the presence of inflammation,12 the means by which this results in fibrosis is less certain. One potential explanation is that the inflammatory process encompasses an increase in profibrotic cytokines such as TGF-β,13 which stimulates the activation and proliferation of resident fibroblasts. Alternatively, it is possible that that inflammatory chemokines released by infiltrating T-cells promote the recruitment of profibrotic cells to the heart. Consistent with this concept, we recently demonstrated that recruitment of bone marrow–derived...
fibrocytes contributes significantly to cardiac fibrosis in an experimental model of heart failure. In conjunction, we showed that this process was associated with an increase in local levels of stromal-derived factor 1 (SDF-1), which could be driven by angiotensin II. SDF-1 is a chemotactic protein that is known to play a critical role in the trafficking of various bone marrow derived cells including lymphocytes, fibrocytoid cells, and hematopoietic stem cells through interaction with its cognate receptor, CXCR4.

In the present study, we tested the hypothesis that the hypertensive and profibrotic actions of mineralocorticoid excess may be, in part, dependent on the actions of SDF-1 upregulation and that these features could be attenuated by a selective CXCR4 antagonist.

Methods

Animal Model and CXCR4 Antagonist Delivery

Male C57BL/6 mice age 6 weeks underwent a left unilateral nephrectomy. In conjunction, the mice were implanted with a 60-day, slow-release pellet in the right flank, containing either 150 mg of deoxycorticosterone acetate (DOCA, Innovative Research of America, Sarasota, FL) or a placebo containing the inert carrier. Animals were also randomly allocated to receive the selective CXCR4 antagonist AMD3465 (6 mg/kg per day, kindly provided by Drs Geoff Akita and Ralph Kelly, Genzyme Corp, Cambridge, MA) or vehicle (0.1N NaHCO3), delivered by a mini-osmotic pump (Alzet, Model 2004). Thus, the study included the following treatment groups: group 1 (control: placebo pellet, vehicle mini-osmotic pump, n=5), group 2 (CXCR4 antagonist alone: placebo pellet, AMD3465 mini-osmotic pump, n=6), group 3 (DOCA alone: DOCA pellet, vehicle mini-osmotic pump, n=6), and group 4 (DOCA+CXCR4 antagonist: DOCA pellet and AMD3465 mini-osmotic pump group, n=6). All animals received a standard diet and were provided with isotonic saline ad libitum (0.9% NaCl). Animals were followed by weekly measurements of weight and monitored for a period of 6 weeks. All experimental protocols were approved by the Alfred Medical Research and Education Precinct Animal Experimentation Ethics Committee under the guidelines of the National Medical and Health Research Council of Australia which are consistent with the US National Institutes of Health guidelines.

Functional Measurements

On the day before study completion, echocardiographic imaging of the left ventricle was performed under isoflurane anesthesia, using a PHILIPS IE33 ultrasound machine (Royal Philips Electronics, Amsterdam, The Netherlands) with a 15-MHz linear transducer. Off-line image analysis was performed in a blinded fashion. Before termination, arterial blood pressure was measured, using a 1.4F microtip transducer catheter (Millar) inserted through the carotid artery.

Morphometry and Histological Analysis

Animals were killed at the end of the experiments by deep anesthesia followed by the rapid removal of the heart, lungs, and kidney. The heart, kidney, and lung tissues were weighed, and tissue samples were either fixed in 10% formalin in PBS for sectioning or snap-frozen in liquid nitrogen for molecular biology. Four-micron, paraffin sections were stained with Masson trichrome to evaluate the distribution and localization of collagen. The extent of perivascular and interstitial collagen was quantified in each of ten random fields per section, using ImagePro Plus software (Addept Electronic Solutions Pty Ltd, Moorabin, Australia) coupled to an Olympus BH2 microscope at a magnification of ×400. The extent of fibrosis was expressed as the percentage of the total section area occupied by fibrosis. In addition, the perivascula and interstitial collagen volume fraction were also quantified separately. In this analysis, collagen immediately surrounding an intramyocardial coronary artery was considered as perivascular in location. Morphometric analysis was performed blinded as to each experimental group.

Immunohistochemistry was performed on paraformaldehydefixed frozen sections using a biotin-avidin-peroxidase technique and visualized with diaminobenzidine. Serial cryostat sections (6 μm) were cut, air-dried onto Superfrost plus microscope slides (Thermo Fisher Scientific Inc, Erembodegem, Belgium), and fixed in acetone at −20°C for 20 minutes. Sections were preincubated with PBS containing 3% hydrogen peroxide to inhibit endogenous peroxidase activity. The primary antibodies, including rabbit–anti-mouse SDF-1α (1:100, Fitzgerald Industries International, Concord, MA), rat–anti-mouse CD4 antibody (1:10, clone RM4–5, BD Biosciences, CA), and rat–anti-mouse CD68 antibody (1:100, clone FA-11, AbD Serotec, Oxford, UK), were added and incubated overnight at 4°C for identification of SDF-1, CD4+ T-lymphocytes, and CD68+ macrophages. After washing 3 times in PBS, sections were incubated with the appropriate biotinylated secondary antibody (30 minutes, Vector, Burlingame, CA) followed by incubation with an avidin-biotin-peroxidase complex (Vectastain ABC kit, Vector, Burlingame, CA), with the subsequent antibody detection using diaminobenzidine (Vector). Histological quantification of CD4+ T-lymphocyte and CD68+ macrophage abundance was carried at ×400 magnification in 10 separate sections per animal.

Molecular Biology

Real-time PCR was performed to determine collagen types I and III and SDF-1α mRNA expression in the heart and kidney. Total RNA was isolated using the TRIzol (Invitrogen) purification system and reverse transcribed using TaqMan reagents (Applied Biosystems). Real-time PCR (ABI Prism 7300 sequence detection system, Applied Biosystems) was conducted using a 50-ng template. The primers sequences for real-time PCR included collagen I (fwd 5'-GGAGATGATGGGGAAGCTG-3', (rev) 5'-TTTCAGGGTCAT-ACAGCAACTCCCA-GGAATGGAGCAAGACAGTCTTTG-3', (rev) 5'-TGCG-AATCTCATGTAGGGTAGTCTCA-3'; SDF-1 (fwd 5'-AAG-CACCCATGCTCATTTC-3', (rev) 5'-TTTCCAGGCTCAT-GAGACAGTCT-3' and GAPDH (fwd 5'-ACGAAACTCCCAA-CTTCTCC-3', rev 5'-CTTCTTTGCTCATTGTC-3'). Expression values were determined by calculating the ΔΔCt value for each reaction, and data were expressed as fold difference compared with control.

Vascular Pharmacology

To assess the potential actions of AMD3465 on vascular tone, aortic rings were prepared from C57BL/6 mice as described previously. In brief, aortic ring segments (2 mm in length) were mounted into an isometric myograph (Myograph Model 610 mol/L, JP Trading, Copenhagen, Denmark). After a 30-minute equilibration period, each vessel was subjected to a passive length-tension stretch. This procedure enabled each vessel to be normalized to an internal circumference equivalent to 90% the transmural pressure of 100 mm Hg. To investigate the vasodilator potential of AMD3465, full concentration-response curves (10 nmol/L to 10 μmol/L) were constructed using vessels preconstricted with phenylephrine.

Statistical Analyses

The procedures were carried out using the Statistical Package for the Social Sciences (SPSS) software (SPSS 17.0 for Windows, SPSS, Chicago, IL). Values are presented as mean±SEM. Comparisons between 3 or more groups were performed with ANOVA, with post hoc testing performed using the Bonferroni correction. Where applicable, between-group comparisons were performed, using Student t test. A probability value of <0.05 was considered to be significant.
Results

CXCR4 Antagonism Attenuates Fibrosis and Hypertension in Mineralocorticoid Excess

Administration of the mineralocorticoid DOCA, was associated with a significant increase in systolic blood pressure (DOCA versus vehicle: 133±15 versus 90±5 mm Hg, \( P=0.004 \)) together with a 47% increase in heart weight and a 116% increase in kidney weight (both \( P<0.001 \), Table 1). Surprisingly, in DOCA mice, coadministration of a CXCR4 antagonist, AMD3465, caused a marked reduction in blood pressure (98±3 mm Hg, \( P=0.02 \) versus DOCA, Figure 1), whereas AMD3465 alone was without any effect on blood pressure. Consistent with its lack of direct antihypertensive effect, AMD3465 was also completely without effect on vascular tone as examined in isolated aortic rings (data not shown). AMD3465 treatment significantly reduced heart weight in DOCA mice (\( P<0.05 \)), returning to near control levels (Table 1). In mice treated with the CXCR4 antagonist alone, there was no significant change in body weight, heart weight, or kidney weight.

As demonstrated in online-only Data Supplement Figure 1, histological analysis of heart tissue showed that mice treated with DOCA for 6 weeks were characterized by extensive fibrosis in both the perivascular and interstitial spaces of the left ventricle as compared with sham control. This effect was significantly attenuated in mice receiving AMD3465. By quantitative analysis (Figure 2A), DOCA-treated mice exhibited an increase in the perivascular collagen volume fraction from 5.8±0.9% to 36.1±0.6% (\( P<0.001 \)) and in the interstitial collagen volume fraction from 0.4±0.1% to 7.6±0.5% (\( P<0.001 \)). AMD3465 coadministration attenuated the development of both perivascular fibrosis (to 15.7±1.3%, \( P<0.001 \)) and interstitial fibrosis (to 1.2±0.6%, \( P<0.001 \)) compared with DOCA animals. Together with the development of cardiac fibrosis, the administration of DOCA also caused a renal fibrosis, and this was also significantly attenuated by the addition of the CXCR4 antagonist (online-only Data Supplement Figure 2). Quantitative analysis of the kidney (Figure 2B) demonstrated that DOCA-treated mice exhibited an increase in the collagen volume fraction from 0.4±0.04% to 16.7±0.6% (\( P<0.001 \)), and cotreatment with AMD3465 significantly attenuated the development of both fibrosis (to 5.3±0.1%, \( P<0.001 \)), whereas AMD alone was without effect.

In addition to the histological evaluation of the fibrotic response to mineralocorticoid excess, we determined the tissue expression collagen I and III mRNA in the heart and kidney by RT-PCR. In the heart, there was a significant increase in collagen I and III mRNA levels (\( P<0.001 \) and \( P=0.049 \), respectively), and in the kidney there was also an increase in collagen I and III mRNA (\( P=0.004 \) and \( P=0.029 \), respectively), as shown in Figure 3A and 3B. Consistent with the histological data, the presence of AMD3465 significantly attenuated the DOCA-mediated upregulation of collagen I and collagen III gene expression in both the heart and kidney (Figure 3A and B). Treatment with the CXCR4 antagonist alone was without effect.

Table 1. Body Weight and Organ Weights in Control Mice and Mice Treated With CXCR4 ANT, DOCA, or CXCR4 ANT Plus DOCA (n=5 to 6)

<table>
<thead>
<tr>
<th></th>
<th>Sham Control</th>
<th>CXCR4 ANT</th>
<th>DOCA</th>
<th>DOCA+CXCR4 ANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body, g</td>
<td>31.04±1.3</td>
<td>29.12±0.2</td>
<td>27.74±0.96</td>
<td>28.42±1.0</td>
</tr>
<tr>
<td>Heart, mg</td>
<td>134±6*</td>
<td>120±2*</td>
<td>197±14</td>
<td>148±7*</td>
</tr>
<tr>
<td>Kidney, mg</td>
<td>296±17*†</td>
<td>271±5*†</td>
<td>640±39</td>
<td>511±56</td>
</tr>
</tbody>
</table>

CXCR4 indicates CXC chemokine receptor 4; ANT, antagonist; and DOCA, dexoycorticosterone acetate.

Values are mean±SEM. *\( P<0.01 \) versus DOCA; †\( P<0.01 \) versus DOCA+CXCR4 ANT.

Figure 1. Bar graphs showing blood pressure (BP) in control and treated mice. *\( P<0.05 \), control versus dexoycorticosterone acetate (DOCA); †\( P<0.05 \), DOCA+CXC chemokine receptor 4 (CXCR4) antagonist (ANT) versus DOCA.

Figure 2. Bar graphs: A, Myocardial perivascular (black bars) and interstitial collagen (gray bars) volume fraction; B, renal collagen volume fraction. *\( P<0.001 \) versus control. †\( P<0.001 \) versus dexoycorticosterone acetate (DOCA). ANT indicates antagonist; CXCR4, CXC chemokine receptor 4.
Renal and Cardiac Inflammation in DOCA Hypertension

To establish the role of inflammation in the hemodynamic and fibrotic response to mineralocorticoid excess and its amelioration by CXCR4 antagonism, we performed an immunohistochemical assessment of the extent of tissue infiltration by T-lymphocytes and monocytes/macrophages. As illustrated in online-only Data Supplement Figure 3, DOCA administration induced a robust accumulation of lymphocytes in the myocardium and kidney, and this was substantially reduced by AMD3465 (Figure 4, \(P < 0.001\)). By contrast, there was only a modest number of infiltrating macrophages in the heart and kidney, which did not significantly differ among the treatment groups (Figure 4).

Cardiac Function

As shown in Table 2, the addition of DOCA was associated with a significant increase in the thickness of the interventricular septum and left ventricular posterior wall. Over the course of the 6-week study, there was no change in left ventricular chamber size or fractional shortening. AMD3645 alone was without effect on any echocardiographic index of left ventricular size or function. The addition of the CXCR4 antagonist in DOCA-treated mice was accompanied by a profound reduction toward near normal values for ventricular wall thickness. At a molecular level, BNP mRNA expression in the left ventricle was increased 3.7-fold \((P = 0.005)\) in DOCA-treated mice compared with control mice, and this was not altered in DOCA mice treated with AMD3465 (3.7±1.2-fold compared with control).

Mineralocorticoid Excess Upregulates SDF-1

Given that mineralocorticoid exposure induced a strong fibrotic and inflammatory response in the heart, we assessed the expression of myocardial SDF-1 expression by immunohistochemistry. As illustrated in Figure 5A, DOCA administration significantly upregulated SDF-1 mRNA in the myocardium, and this was decreased by AMD3465. In support of these findings, SDF-1 immunoreactivity was

Figure 3. Bar graphs: Myocardial (A) and renal (B) mRNA expression levels for collagen I and collagen III. *\(P < 0.05\), **\(P < 0.01\) versus control; †\(P < 0.05\), ††\(P < 0.01\) versus deoxycorticosterone acetate (DOCA). ANT indicates antagonist; CXCR4, CXC chemokine receptor 4.

Table 2. Serial Echocardiographic Examination Performed in Control Mice and Mice Treated With CXCR4 ANT, DOCA, or CXCR4 ANT Plus DOCA (n = 5 to 6)

<table>
<thead>
<tr>
<th></th>
<th>Sham Control</th>
<th>CXCR4 ANT</th>
<th>DOCA</th>
<th>DOCA+CXCR4 ANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVSd, mm</td>
<td>0.76±0.04†</td>
<td>0.69±0.05†</td>
<td>1.27±0.05</td>
<td>0.86±0.03†</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>3.96±0.14</td>
<td>3.98±0.06</td>
<td>3.60±0.24</td>
<td>4.08±0.10</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>0.96±0.08†</td>
<td>0.76±0.04†</td>
<td>1.28±0.06</td>
<td>0.95±0.02†</td>
</tr>
<tr>
<td>IVSs, mm</td>
<td>1.19±0.08†</td>
<td>1.07±0.05†</td>
<td>1.69±0.11</td>
<td>1.30±0.04*</td>
</tr>
<tr>
<td>LVIDs, mm</td>
<td>2.80±0.10</td>
<td>2.85±0.02</td>
<td>2.60±0.28</td>
<td>2.87±0.14</td>
</tr>
<tr>
<td>LVPWs, mm</td>
<td>1.18±0.03*</td>
<td>1.01±0.08*</td>
<td>1.50±0.08</td>
<td>1.12±0.07†</td>
</tr>
<tr>
<td>FS, %</td>
<td>29.38±0.72</td>
<td>28.4±1.09</td>
<td>28.44±3.7</td>
<td>29.89±2.4</td>
</tr>
</tbody>
</table>

CXCR4 indicates CXC chemokine receptor 4; ANT, antagonist; and DOCA, deoxycorticosterone acetate; IVSd, interventricular septum (diastole); LVIDd, left ventricular internal diameter (diastole); LVPWd, left ventricular posterior wall (diastole); IVSs, interventricular septum (systole); LVIDs, left ventricular internal diameter (systole); LVPWs, left ventricular posterior wall (diastole); FS, fractional shortening.

Values are mean±SEM.*\(P < 0.05\) versus DOCA; †\(P < 0.01\) versus DOCA.
readily detected in DOCA-treated animals, and this was abrogated by AMD3465 (Figure 5B). SDF-1 was not detectable in the presence of AMD3465 alone (data not shown). By contrast, altered renal SDF-1 mRNA expression was not detectable in mice at the 6-week time point.

**Discussion**

The present study provides evidence that the CXCR4/SDF-1 axis is involved in the pathogenesis of the adverse cardiovascular actions of mineralocorticoid excess. Specifically, we demonstrated that AMD3465, a selective CXCR4 antagonist, markedly attenuated both cardiac and renal fibrosis, together with a reduction in cardiac hypertrophy and blood pressure in unilaterally nephrectomized mice treated with DOCA.

Recent evidence has accumulated to suggest that inflammation may be a critical factor in the pathogenesis of fibrosis and hypertension in the setting of mineralocorticoid excess.\(^21,22\) This concept has arisen from studies demonstrating the activation of proinflammatory signaling pathways, together with data showing that various immunomodulatory can attenuate the development of mineralocorticoid-induced hypertension.\(^5,23–25\) Despite these observations, the mechanism by which immune activation occurs in mineralocorticoid excess and how it causes hypertension remains unclear. Recently, considerable emphasis has been directed toward the role of T-lymphocytes, which accumulate in the kidney and vessels in the setting of various forms of hypertension.\(^5\)

Consistent with previous studies, we showed that DOCA administration was accompanied by a robust accumulation of T-lymphocytes, in both the kidney and heart. By contrast, we did not observe any accumulation of macrophages, in comparison to the modest recruitment of macrophages reported elsewhere.\(^26\) The mechanism responsible for the selective accumulation of T-cells in the heart, kidney and vessels has not been clearly established, and in the current study we hypothesized that the recruitment of lymphocytes could be mediated through the local expression of chemokines such as SDF-1. This hypothesis was based on recent data from our group showing that SDF-1 expression is increased in heart failure and that in cell culture models angiotensin II increases SDF-1 secretion.\(^14\)

In further support of the putative role of SDF-1 in the setting of mineralocorticoid excess, it has also been shown that aldosterone increases SDF-1 mRNA levels.\(^27\) In keeping with this hypothesis, we showed that the selective CXCR4 antagonist AMD3465 markedly attenuated the development of hypertension and T-lymphocyte accumulation in the kidney and heart. The selectivity of AMD3465 for the CXCR4 receptor specifically has been previously well established.\(^19\) AMD3465 in the absence of DOCA did not alter blood pressure and had no effect on vascular tone, making it unlikely that the effects observed were mediated by a direct action on blood vessels.

It has also been suggested that activation of other potentially important cytokines may be of relevance in the causation of mineralocorticoid-mediated and angiotensin II–mediated hypertension, including the CCR5 ligand RANTES as well as other inflammatory mediators, including TNF-\(\alpha\) and ICAM1.\(^5,28–30\) In the present study, we did not specifically measure the effect of prolonged CXCR4 antagonism on peripheral blood T-lymphocyte numbers. Previous studies have demonstrated that prolonged exposure to the CXCR4 antagonist AMD3100 does not cause any immunodepletion,\(^31\) therefore suggesting that our observations are likely not explained by a simple reduction in T-cell numbers.

The mechanism by which the activation and recruitment of inflammatory cells leads to hypertension has also been investigated. Previous studies have strongly implicated heightened oxidative stress, arising from various sources including the activation NADPH oxidase as a key mediator in the pathogenesis of hypertension.\(^32\) Guzik et al\(^5\) showed that angiotensin II stimulates the production of superoxide by T-lymphocytes, providing a potential link between T-cell accumulation, activation, and the pathogenesis of hypertension. Although both renal and vascular inflammation is reported in experimental models of hypertension, it has been proposed that the level of oxidative stress in the kidney particularly correlates most closely with the development of hypertension.\(^32\) By contrast, the specific role of nonrenal vascular inflammation is less clear. For example, although the heme oxygenase-2 knockout mouse is characterized by selective vascular inflammation and heightened oxidative stress, blood pressure is not elevated.\(^33,34\)

The DOCA-salt model of hypertension has been widely used to investigate the pathogenesis and reversibility of left ventricular hypertrophy and myocardial fibrosis,\(^35,36\) and in the present study we showed that CXCR4 antagonism significantly abrogated the effect of DOCA on left ventricular mass and myocardial fibrosis. Our findings in this regard are limited by the absence of a control
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antihypertensive group; however, the use of a control antihypertensive agent is confounded by their own secondary neurohormonal sequelae. Although the model is accompanied by significant hypertension, previous studies have suggested that the development of cardiac hypertrophy and fibrosis in the DOCA model is at least partly independent of the extent of hypertension. In this context, although the data relating to the antifibrotic effects of CXCR4 antagonism in the heart and kidney could be explained by a blood pressure–independent mechanism, we are not able to exclude the possibility that these actions were partially or entirely dependent on blood pressure lowering. Interestingly, we found that left ventricular BNP mRNA expression was unaffected by CXCR4 administration in the DOCA mice. Given that myocardial BNP gene expression is determined partially by wall stretch, it is possible that the sustained increase in BNP mRNA was the result of intravascular volume expansion. In support of this, we did not observe any effect of AMD3465 on the levels of BNP mRNA in primary cultures of mouse neonatal mouse cardiomyocytes (data not shown). This hypothesis is in accord with previous data showing that the DOCA mediated in myocardial ANP expression was probably mediated by changes in intravascular volume.

In the present study, we showed that CXCR4 antagonism abrogated the induction of renal fibrosis by DOCA. Renal fibrosis is a fundamental feature of chronic kidney disease and the presence of renal fibrosis per se has also been suggested to contribute to the ongoing loss of renal function through a range of mechanisms, including hypoxia. Previous studies using the DOCA model of mineralocorticoid excess have shown that several blood pressure–independent interventions, including statins and the tetrapeptide N-acetyl-Ser-Asp-Lys-Pro, can reduce DOCA-induced fibrosis. These studies also confirmed that the reduction in DOCA-induced fibrosis was accompanied by an improvement in creatinine clearance, sodium excretion, and urinary albumin. As a corollary, we may also predict that CXCR4 antagonism would afford a similar renal protective action, although we did not measure creatinine clearance, albumin excretion, or sodium excretion in our study.

In the current study, we did not specifically demonstrate the mechanism by which SDF-1 expression leads to the development of fibrosis. Previously we showed that SDF-1 stimulates both the homing and proliferation of cardiac fibroblasts, and in this study we demonstrated the induction of myocardial SDF-1 expression in response to mineralocorticoid excess. This raises the possibility that SDF-1 could be directly responsible for the development of fibrosis, through stimulation of fibroblasts and/or recruitment of fibrocytes. In support of this possibility, CXCR4 antagonism has been shown to reduce scar formation after myocardial infarction. Interestingly we also observed a reduction in the expression of SDF-1 mRNA and protein in CXCR4 antagonist–treated animals. One potential explanation is that the decrease in lymphocyte accumulation in AMD3465-treated mice reduced the tissue levels of cytokines, which can upregulate SDF-1. By contrast, there was no change in the expression of SDF1 mRNA in the kidney at 6 weeks. This finding is possibly explained by previous observations in other experimental paradigms that the effect of CXCR4 antagonists on SDF-1 expression appears to be variable over time. Beyond the recruitment of fibrocytes, it is also possible that the mobilization of inflammatory cells by SDF-1 could subsequently lead to fibrotic response through the release of other profibrotic cytokines. Although T-lymphocyte accumulation and the presence of fibrosis were observed together and both were attenuated by CXCR4 antagonism, it is not possible to directly implicate the accumulation of T-cells in the pathogenesis of fibrosis. For example, in the mdx mouse, T-cell depletion does not alter the development of skeletal muscle fibrosis.

In the clinical setting, it is increasingly apparent that cardiac fibrosis contributes substantially to the phenotype of a range of cardiovascular disease states. Notably, recent research on the syndrome of heart failure with a preserved ejection fraction has highlighted the important contribution of cardiac fibrosis, which leads to increased myocardial stiffness. Given the central role of cardiac fibrosis in the pathogenesis of heart failure with a preserved ejection fraction, several large-scale clinical trials of angiotensin-converting enzyme inhibitors and angiotensin II antagonists have recently been conducted in patients with heart failure. In the majority of these studies, there has been no clear evidence of benefit, providing a clear impetus for ongoing evaluation of antifibrotic strategies.

In conclusion, these data demonstrate that the CXCR4/SDF-1 signaling pathway plays a central role in the pathogenesis of experimental hypertension induced by DOCA and of the accompanying cardiac and renal fibrosis. Furthermore, our study also points toward a coocordinated process in which the induction of an inflammatory cascade is dependent, at least in part, on SDF-1. Taken together, our study results suggest that further investigation of the potential clinical utility of CXCR4 antagonists in the context of hypertensive heart and kidney disease is warranted.

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Disclosures
None.

References


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Elevated levels of neurohormones are fundamental to the pathogenesis of many cardiovascular disease states, including hypertension and heart failure. This notion is strongly supported by abundant clinical trial data, including studies demonstrating the beneficial actions of angiotensin II and aldosterone antagonists in both of these paradigms. Beyond their classic actions on systemic and renal vascular tone, oxidative stress, and salt and water balance, recent evidence has accumulated to suggest that activation of an inflammatory cascade may contribute significantly to the induction of hypertension by both angiotensin II and aldosterone. Within such a model, the cardiac and renal fibrosis that classically accompany mineralocorticoid excess may arise from the sequelae of inflammation. In the present study, we postulate that induction of cardiorenal fibrosis and inflammation by mineralocorticoid excess might be consequent on the stimulation of the chemokine stromal-derived factor-1 (SDF-1). Our study demonstrated that the chronic administration of an antagonist of CXCR4 (the receptor for SDF-1) was able to significantly attenuate the adverse effects of prolonged exposure to mineralocorticoid excess in an experimental mouse model. In particular, CXCR4 antagonism prevented the development of hypertension, reduced cardiac and renal fibrosis, and markedly reduced the accumulation of inflammatory cells within the heart and kidney. Taken together, these data highlight the importance of the SDF-1:CXCR4 pathway in the cardiovascular sequelae of mineralocorticoid excess and provide support for the potential clinical application of CXCR4 antagonists in relevant cardiovascular disorders.
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SUPPLEMENTARY FIGURE LEGENDS

Figure 1. Masson’s trichrome stained sections of left ventricle. Control (A), CXCR4 ANT (B), DOCA (C) and DOCA+CXCR4 ANT (D) mouse hearts

Figure 2. Masson’s trichrome stained sections of kidney. Control (A), CXCR4 ANT (B), DOCA (C) and DOCA+CXCR4 ANT (D) mouse hearts

Figure 3. T-cell (CD4 positive) staining in frozen sections from heart (left) and kidney (right) in Control (upper row), DOCA (middle row) and DOCA+CXCR4 ANT (lower row) treated mice.
Figure 1

CONTROL

DOCA

CXCR4 ANT

DOCA + CXCR4 ANT
Figure 2