Monocyte Chemoattractant Proteins Mediate Myocardial Microvascular Dysfunction in Swine Renovascular Hypertension

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Abstract

Background—Monocyte chemoattractant proteins (MCPs) play an important role in mediating inflammatory processes. Hypertension (HTN) is associated with inflammation as well as impaired cardiac microcirculatory function and structure, but the contribution of MCPs to these alterations remained unclear. This study tested the hypothesis that MCPs regulate cardiac microvascular function and structure in an experimental HTN.

Methods and Results—Pigs (n=6/group) were studied after 10 weeks of normal, renovascular HTN, or renovascular HTN+ bindarit (MCPs inhibitor, 50 mg/kg/day PO). Left ventricular (LV) function, myocardial microvascular permeability, and fractional vascular volume were assessed by fast computed tomography before and after adenosine infusion (400 μg/kg/min). Myocardial fibrosis, inflammation, and microvascular remodeling were determined *ex-vivo*. Hypertension was not altered by bindarit, but LV hypertrophy and diastolic function were improved. In response to adenosine, myocardial microvascular permeability increased in HTN (from 0.0083±0.0009 to 0.0103±0.0011 AU, p=0.038 *vs.* baseline) and fractional vascular volume decreased, while both remained unchanged in normal and HTN+bindarit pigs. HTN upregulated endothelin-1 expression, myocardial inflammation and microvascular wall thickening, which were inhibited by bindarit.

Conclusions—MCPs partly mediate myocardial inflammation, fibrosis, vascular remodeling, and impaired vascular integrity induced by hypertension. Inhibition of MCPs could potentially be a therapeutic target in hypertensive cardiomyopathy.

Keywords

MCPs; inflammation; hypertension; microvascular permeability; remodeling

Hypertension (HTN) is a leading cause of congestive heart failure in the United States and impairs left ventricular (LV) function, myocardial perfusion, and microvascular function.[1, 2] Myocardial microvascular dysfunction is an important modulator of coronary resistance and

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myocardial blood flow,[3] and often associated with changes in microvascular architecture like rarefaction or thickening.[4]

Important attributes of microvascular function include microvascular permeability (MP) and fractional vascular volume (FVV), key indices of microvascular structural integrity. MP, the rate of leakage of plasma components to the extravascular tissue, reflects endothelial barrier function.[5, 6] FVV, the volume of myocardium occupied by microvessels, represents the number and tone of functional microvessels and myocardial perfusion. [7, 8] MP and FVV are difficult to assess *in-vivo*, but can be accurately and non-invasively assessed by fast CT.[2, 7]

The mechanism by which HTN leads to cardiomyopathy may involve inflammation. Perivascular inflammation of intramyocardial arteries is an early response to pressure overload, including induction of monocyte chemoattractant proteins (MCPs) and macrophage infiltration, in particular MCP-1.[9, 10] The expression of MCP-1 and its receptor (CCR2) in HTN is regulated by mechanical strain and by release of reactive oxygen species. Furthermore, MCPs mediate vascular inflammation and remodeling by facilitating the secretion of vasoconstrictors like endothelin (ET)-1,[11] cytokines and chemokines, and may contribute to vascular endothelial dysfunction.[12,13]

However, the role of MCPs in HTN-induced alterations of myocardial microvascular structure and function are poorly understood. The current study was designed to test the hypothesis that MCPs contribute to the impairment of the myocardial microcirculation, and their blockade would improve myocardial microvascular structure and function in swine renovascular HTN.

Methods

Animal procedures were approved by the Institutional Animal Care and Use Committee. Female domestic pigs (initially weighing 25-35kg) were randomized into three groups: normal (n=6), renovascular HTN (n=6), and HTN pigs supplemented with bindarit (Angelini Research Center - ACRAF, Italy, 50 mg/kg/day P.O., n=6). Bindarit (2-Methyl-2-[[1-(phenylmethyl)-1H-indazol-3yl]methoxy]propanoic acid) is a specific inhibitor of MCPs 1, 2, and 3 synthesis,[14, 15] and at this dose inhibits MCPs' synthesis and inflammation.[16] Renovascular HTN was elicited by induction of renal artery stenosis, which increases arterial pressure within 7-10 days.[17, 18] Mean arterial pressure (MAP) was measured by a PhysioTel® telemetry system (Data Sciences) implanted at baseline in the left femoral artery.

After 10 weeks, pigs were anesthetized (ketamine 15.7 mg/kg/h and xylazine 2.3 mg/kg/h in saline), intubated, and ventilated. A pigtail catheter in the right atrium served for contrast media injection, and a side-arm for adenosine infusion. Blood samples were collected for measurement of plasma renin activity (PRA), aldosterone, and ET-1 by enzyme immunoassay. [19] Fast CT studies were then performed to assess cardiac function and structure *in-vivo*, MP and FVV (before and after adenosine), LV filling rate, and LV muscle mass (LVMM). A few days later the pigs were euthanized using pentobarbital (100mg/kg), and hearts harvested for *in-vitro* studies. LV myocardial segments were fresh-frozen or preserved in formalin, and another segment prepared for micro-CT studies. Microvascular architecture was assessed by evaluation of microvascular density and wall thickness, inflammation by the expression of MCP-1, its receptor CCR2, cyclooxygenase (COX)-1, COX-2, IL-6, and macrophage infiltration, and cardiac remodeling by myocardial expression of the angiotensin II receptor type I (AT1R), ET-1, interstitial fibrosis, and myocyte hypertrophy. Vascular integrity was also evaluated by Rho-kinase (ROCK) activity (tested by its downstream product phospho-myosin phosphatase) and tight junction protein expression. Since HTN and inflammation can increase oxidative stress, dihydroethidium (DHE) staining for superoxide production and the expression of the p47 and p67 subunits of NAD(P)H oxidase were assessed[20]. To evaluate

direct effects of MCP-1, cell culture studies were performed using human cardiac fibroblasts (HCF) incubated with MCP-1 (For detail, see the online-only Data Supplement).

In-vivo CT Studies

Pigs were scanned by either electron beam (C-150, Imatron, South San Francisco, CA) or 64-slice multidetector (Sᴏᴍᴀᴛᴏᴍ Sensation-64, Siemens, Forchheim, Germany) fast CT, which provide very similar assessments of MP and FVV[7], and images analyzed with ANALYZE® (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN) (online Data-Supplement).

In-vitro Studies (For detail, see the online-only Data Supplement)

Micro-CT was used to evaluate myocardial microvascular density.[21, 22] Myocardial remodeling was evaluated by myocyte cross-sectional areas, and fibrosis with H&E and trichrome staining. Microvascular remodeling was assessed by microvascular wall thickness to lumen ratio using anti-human α-smooth muscle actin (SMA) (DakoCytomation) staining. Immunohistochemistry assessed indices of inflammation and MP, with primary antibodies against MCP-1, macrophage CD163, zonula occludens-1 (ZO-1), and phospho-myosin phosphatase targeting subunit (Thr [696]-pMYPT1). The tight junction protein ZO-1 regulates endothelial barrier function and overexpresses in response to strain,[23] while phospho-MYPT1 reflects ROCK activity, which also regulates endothelial barrier function by inactivation of Myosin Phosphatase.[24] Western Blotting was used for detecting MCP-1, CCR2, IL-6, COX1, COX-2, AT1R, ET-1, endothelial nitric oxide synthase (eNOS), p47 and p67, and ZO-1. GAPDH was used as loading control.

Statistical Analysis

Results are presented as mean±SEM. One-way ANOVA with the Bonferroni correction evaluated differences among the groups followed by an unpaired t-test, and paired Student t tests detected changes within groups; $p<0.05$ was considered significant.

Results

Cardiac and Microvascular Function

After a 10-week observation MAP was similarly increased in HTN and HTN+bindarit compared to normal (Table 1, ANOVA p=0.01), indicating that bindarit did not influence blood pressure. PRA was not significantly different among the groups, while aldosterone levels were elevated in HTN (ANOVA p=0.03) and unaltered by bindarit (p=0.17 *vs.* HTN+bindarit), suggesting no significant effect on the systemic renin-angiotensin- aldosterone system. In response to adenosine, MAP significantly decreased in all groups (p<0.05, Table 1), while heart rate remained unchanged. The degree of MAP response was greater in HTN+bindarit compared to normal (p=0.001), but not different from HTN (p=0.68, Table 1). LVMM increased in HTN (p=0.0001 *vs.* normal), suggesting LV hypertrophy, which was significantly limited (p=0.01 *vs.* HTN) but not abolished by bindarit. There were no significant differences in stroke volume, ejection fraction, and cardiac output among the groups (p>0.05 for all), suggesting sustained cardiac systolic function. E/A ratio significantly decreased in HTN compared to normal (p=0.0006, Figure 1A), suggesting diastolic dysfunction. Importantly, E/A was increased in HTN+bindarit compared to HTN (Figure 1A, p=0.02), indicating that MCPs inhibition protected cardiac diastolic function, and was not significantly lower than normal (p=0.06).

Patlak-derived (Figure 1B) myocardial MP and FVV in the LV anterior wall were similar among the 3 groups at baseline (ANOVA p=0.60 and p=0.14, Figure 1C-D). In response to adenosine, MP significantly increased in HTN (from 0.0083±0.0009 to 0.0103±0.0011 AU,

p=0.038 *vs.* baseline) and achieved levels significantly higher than normal (0.0064±0.0011 AU, p=0.016 *vs.* HTN, Figure 1C). In contrast, in normal and HTN+bindarit pigs, MP remained unaltered during adenosine. The degree of change in MP in response to adenosine was also significantly greater in HTN compared to normal (+27±10 *vs.* -20±5%, p=0.003). Furthermore, adenosine induced a significant decrease in FVV in HTN, which bindarit attenuated (Figure 1D). Hence, HTN-induced microvascular dysfunction was blunted by MCPs inhibition.

Microvascular Density

There was no difference among the groups in overall transmural density of microvessels (diameters 20-500μm) (ANOVA p=0.90, Figure 1F). However, subepicardial and subendocardial spatial density of larger microvessels (200-500 μm) was selectively decreased in HTN (all p=0.002 vs. normal, Figure 1G), but preserved by MCPs inhibition (Figure 1G).

Inflammation and Remodeling

In HTN, myocardial MCP-1 expression significantly increased mainly in the intramyocardial arterial wall, but was inhibited by bindarit (p=0.0004, Figure 2), as was perivascular macrophage accumulation (p<0.0001, Figure 2), while IL-6 expression in HTN remained elevated (Figure 2). COX-1 and COX-2 expression was similar among the groups. Myocardial fibrosis in HTN (ANOVA p=0.003) was significantly ameliorated (p=0.04 HTN *vs.* HTN +bindarit), but not abolished, by bindarit (Figure 3A, D). Myocyte cross-sectional area increased in HTN (p=0.02 *vs.* normal) and HTN+bindarit (p=0.02 *vs.* normal) (Figure 3B, E) but was not different between them (p=0.46 *vs.* HTN), indicating no significant effect of bindarit on myocyte hypertrophy. Contrarily, microvascular media-to-lumen ratio increased from 0.08±0.01 in normal to 0.15±0.01 in HTN (p=0.006) and decreased in HTN+bindarit (to 0.10±0.02, p<0.05 *vs.* HTN, Figure 3C, F), suggesting inhibition of microvascular wall thickening.

The endothelial and perivascular expression of pMYPT1 increased in both HTN and HTN +bindarit (p=0.02 and p=0.01 vs. normal, respectively), suggesting that bindarit may not influence the ROCK pathway (online supplement, Figure 1SA, D). Endothelial ZO-1 expression was also upregulated in HTN and HTN+bindarit (online supplement, Figure 1SB, E, p=0.04 and p=0.03 vs. normal, respectively), but significantly decreased in bindarit-treated compared to untreated HTN pigs (p=0.04, online supplement, Figure 1SC, F).

In addition, HTN increased both systemic and myocardial ET-1 level, which was preserved by bindarit (Table 1, Figure 3). AT1R expression was also elevated in HTN[16], but unaffected by bindarit (p=0.24, Figure 3). On the other hand, myocardial eNOS expression was slightly but significantly decreased in both HTN and HTN+bindarit (p=0.04 and p=0.02, respectively). Superoxide production by DHE was elevated in HTN (p=0.02 *vs.* normal) and unaltered by bindarit (p=0.7 *vs.* HTN, online supplement, Figure 2S). NAD(P)H oxidase p67phox expression was increased in both HTN (p=0.01) and HTN+bindarit (p=0.04), while p47phox remained unchanged. Therefore, bindarit did not ameliorate NAD(P)H oxidase-dependent oxidative stress (online supplement, Figure 2S).

Cell culture studies have shown that MCP-1 dose-dependently and significantly increased collagen-I and collagen-III production and TIMP-1 expression in HCF (Online Supplement, Figure 3S).

Discussion

The current study shows that specific inhibition of MCPs in hypertension ameliorated the increase in microvascular permeability and decrease in fractional vascular volume in response

to increased cardiac demand, suggesting preserved microvascular integrity and endothelial barrier function. These were accompanied by attenuated microvascular remodeling and myocardial fibrosis, and by improved cardiac diastolic function. These results suggest that the myocardial and microvascular changes induced by hypertension are partly mediated by MCPs and functionally consequential.

HTN is often associated with activation of the renin-angiotensin system, ET-1, oxidative stress, and decreased nitric oxide (NO) bioavailability.[25, 26] HTN is additionally characterized by inflammation and overexpression of MCPs, predominantly MCP-1[27], a critical mediator of macrophage accumulation.[26] Activated macrophages, in turn, produce many cytokines, chemokines, growth-factors, and proteases to promote inflammation and facilitate cell proliferation, extracellular-matrix turnover, and angiogenesis. Our previous studies demonstrated that renovascular HTN increased both cardiac and renal MCP-1 protein expression and oxidative stress.[17, 25, 28] Furthermore, HTN altered myocardial microvascular function and impaired myocardial perfusion responses to challenge.[2, 28, 29] In this study we assessed several inflammatory pathways potentially activated in HTN. COX-1 and COX-2 expression remained unchanged, arguing against their involvement in myocardial alterations in our model. IL-6 increased in HTN but remained elevated in HTN+bindarit, while MCP-1 expression decreased in HTN+bindarit in association with improvement of many aspects of microvascular function and structure, suggesting that it might contribute to these alterations.

Hypertension induces myocyte hypertrophy, interstitial fibrosis, and consequent cardiac stiffness, which are implicated in increased LVMM and impaired diastolic function (e.g. decreased E/A). In this study MCPs synthesis inhibition improved cardiac diastolic function, likely by ameliorating cardiac stiffness and increasing compliance. Interestingly, our HCF study suggests that MCP-1 can directly increase myocardial matrix production and fibrosis, thus its action is not necessarily mediated only by monocyte recruitment. Bindarit decreased interstitial fibrosis, but not myocyte hypertrophy, likely because blood pressure remained elevated, resulting in incomplete improvement of LVMM. Our observations are underscored by previous studies in rodents, in which MCP-1 was inhibited with anti-MCP-1 neutralizing antibody or indirectly reduced using ROCK inhibitor, antioxidants and inactivation of CCR-2.[30-33]

Importantly, bindarit is able to modulate the levels and attenuate over-production of MCPs in response to inflammation, without entirely blocking their physiological activity or inadvertently increasing MCPs expression, as observed with anti-MCP-1 antibody.[34] In line with previous studies showing that bindarit dose-dependently inhibits MCP-1/CCL-2 production and is quite selective among chemokines[14, 35], we found that in our model it did not decrease the upregulated IL-6 expression.

Interestingly, we observed that HTN increased MP and decreased FVV in response to adenosine, suggesting vascular dysfunction possibly mediated by inflammation. Hypertension initially evokes coronary endothelial dysfunction and abnormal myocardial perfusion regulation, and subsequently vascular remodeling and dysfunction.[4] Endothelial barrier function is important for maintaining vascular integrity and regulation of vascular tone. MCP-1 directly increases blood-brain barrier permeability in inflammation by regulating the expression and rearranging endothelial tight junction proteins[36, 37] that maintain endothelial barrier integrity. We found increased expression of the endothelial tight junction component ZO-1, a membrane protein that links the actin cytoskeleton and tight junctions.[38] Its endothelial overexpression in HTN may imply tight junction reassembly, a compensatory effect that may reflect disruption of endothelial barrier integrity.[39] Notably, subtle changes in barrier function may be disclosed only during challenges like increased cardiac demand.[40] ROCK also mediates some of the effects of MCP-1 on brain MP[37], but does not seem to be a major modulator of

myocardial MP in our model, because bindarit improved MP without changing MYPT (and hence ROCK activity). Nevertheless, other tight-junction proteins might contribute to loss of endothelial integrity in HTN.

Myocardial FVV decreases during cardiac challenge in HTN also indicate microvascular dysfunction, possibly consequent to microvascular wall remodeling and architectural changes[4]. Indeed, microvascular rarefaction is commonly observed in HTN. We observed a selective decrease in density of larger microvessels (200-500 μm).[25] The unchanged total myocardial microvascular density in HTN pigs may reflect an earlier increase in the number of small microvessels needed to support developing LV hypertrophy.[2, 4, 25, 28] Prevention of microvascular rarefaction by bindarit in HTN is likely through decreased myocardial and perivascular fibrosis.[41] In addition, we observed in HTN increased media-to-lumen ratio, which possibly precedes luminal narrowing.[25] The improvement achieved by MCPs inhibition implicates them in microvascular remodeling in HTN. Overall, MCPs inhibition decreased inflammation-induced vascular remodeling, rarefaction, and dysfunction, and blunted the reactive decrease of FVV in response to adenosine.

In addition to microvascular remodeling, myocardial vascular volume (FVV) could be affected by changes in vascular tone. Adenosine normally induces vasodilatation that involves both endothelial-dependent and –independent mechanisms.[2] During endothelial dysfunction, a decrease in NO and increased abundance of vasoconstrictors (e.g. ET-1) may lead to paradoxical vasoconstriction.[2, 29] By upregulating ET-1[11] MCPs might contribute to endothelial dysfunction in HTN. This notion is underscored by our observation that MCPs blockade decreased systemic and myocardial ET-1 levels and ameliorated the decrease in FVV in response to cardiac demand. Notably, since FVV responses improved without altering eNOS expression or oxidative stress, a decrease in NO availability was unlikely a critical factor. Adenosine infusion may amplify a decrease in FVV by disclosing functional impairments, a failure in capillary recruitment, or microvascular rarefaction in HTN.[42]

Systemic PRA increases shortly after induction of experimental renovascular HTN,[17, 18] but in the chronic phase returns to basal levels[43] and may sustain HTN by activating secondary mechanisms. However, the effects of bindarit in our model were not mediated by inhibition of the renin-angiotensin-aldosterone system, which remained unchanged (PRA, myocardial AT-1R, or circulating aldosterone levels). Because MCP inhibition did not significantly blunt oxidative stress or the renin-angiotensin system, systemic hypertension remained unabated, and the beneficial effects of bindarit on cardiac function were likely derived from direct attenuation of cardiac remodeling (Figure 4). Further studies are needed to dissect the potential interaction of MCPs with the renin-angiotensin system.

Our study was limited by the use of a relatively small number of young pigs. We also used in all groups two fast-CT scanners, which provide comparable assessments of MP and FVV.[7] It is possible that some of the tissue studies sampled some unbound circulating proteins, but exanguinated myocardium contains little blood volume, and the preparation procedure was the same for all the groups. Therefore, the fraction of retained unbound cytokines might be negligible and comparable among the groups, and result in minimal interference with our result interpretation. Bindarit inhibits MCPs 1, 2, and 3, but MCP-1 is the key member of the MCP family that has been most commonly and directly linked to cardiovascular disease and hypertension, so that the effects of the drug are likely attributable mainly to blockade of MCP-1. Nevertheless, we cannot exclude the possibility that inhibition of MCPs 2 and 3 contributed to these benefits. Additionally, the dose of bindarit may incompletely inhibit MCPs expression and consequently introduce some variability.

In summary, we observed that hypertension induces myocardial inflammation, fibrosis, and vascular remodeling, and impairs vascular integrity. These effects are partly mediated by the endogenous inflammatory mediator MCPs, and its inhibition attenuated LV hypertrophy and improved cardiac diastolic function (Figure 4). Inhibition of MCPs could therefore be a potential therapeutic target for the downstream end-organ damage induced by hypertension, such as hypertensive cardiomyopathy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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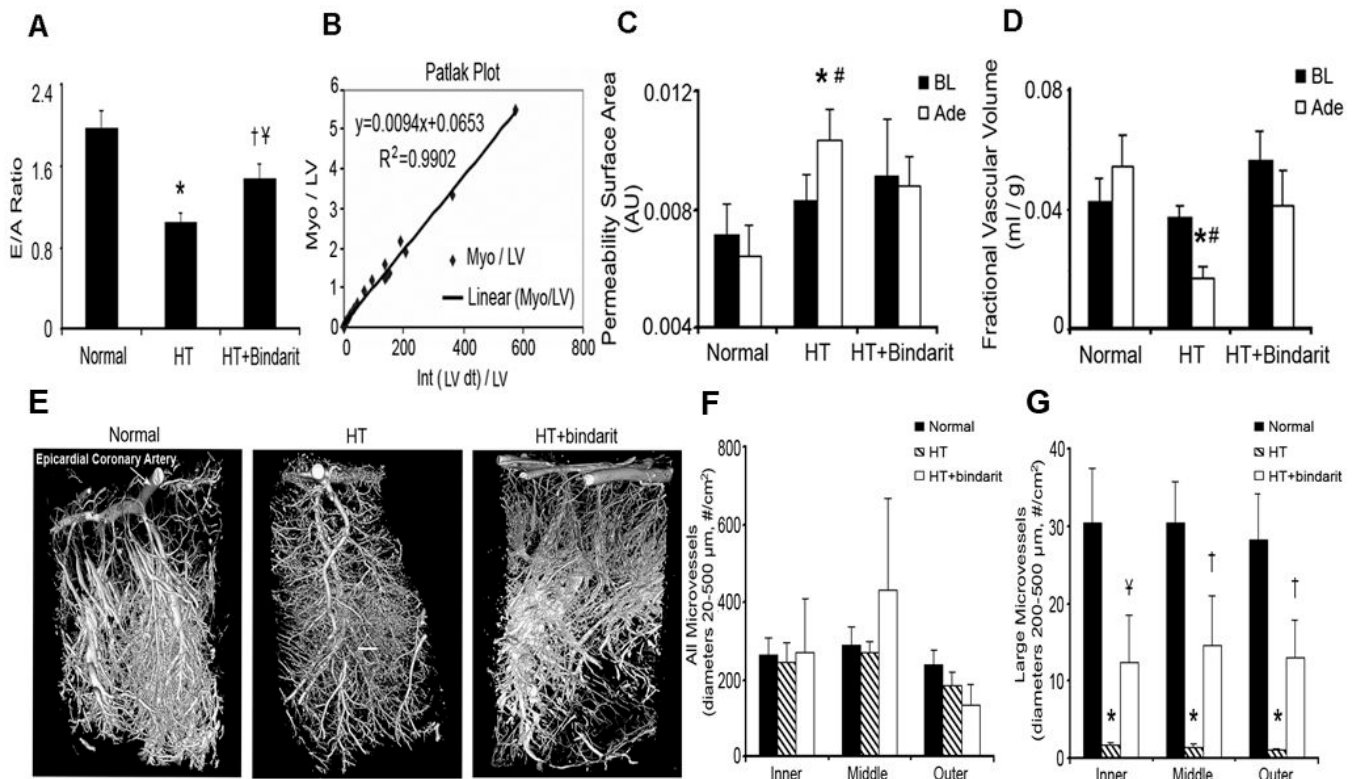
Figure 1.
Cardiac diastolic function evaluated by the ratio of left ventricular early (E) to late (A) filling rates, myocardial microvascular permeability (MP) and fractional vascular volume (FVV) assessed by the Patlak graphical analysis method using fast CT, and microvascular density assessed by micro-CT. A. Cardiac diastolic function evaluated by E/A in normal, hypertension (HTN), and HTN+bindarit pigs (n=6 each). E/A ratio significantly decreased in HTN compared to normal and increased in HTN+bindarit compared to HTN. B. Representative Patlak plot from which MP (slope) and FVV (y-axis intercept) are calculated. C, D. MP and FVV at baseline (BL, black bars) and during adenosine (Ade, white bars) infusion. Basal anterior wall myocardial MP and FVV were similar among the groups. Infusion of adenosine induced in HTN a significant increase in MP and decrease in FVV compared to baseline, which were both blunted by bindarit. E. Representative micro-CT images of myocardial microvessels. F, G. Quantitation of microvascular density. There was no difference among the groups in overal microvascular transmural density (from 20 to 500μm in diameter). However, the spatial density of larger microvessels (200-500 μm) was selectively decreased in HTN compared to normal in both the subepicardium and subendocardium, but was significantly preserved by bindarit. * p<0.05 *vs.* normal, # p<0.05 *vs.* baseline (BL), † p<0.05 *vs.* HTN and ¥ p=0.06 *vs.* normal.
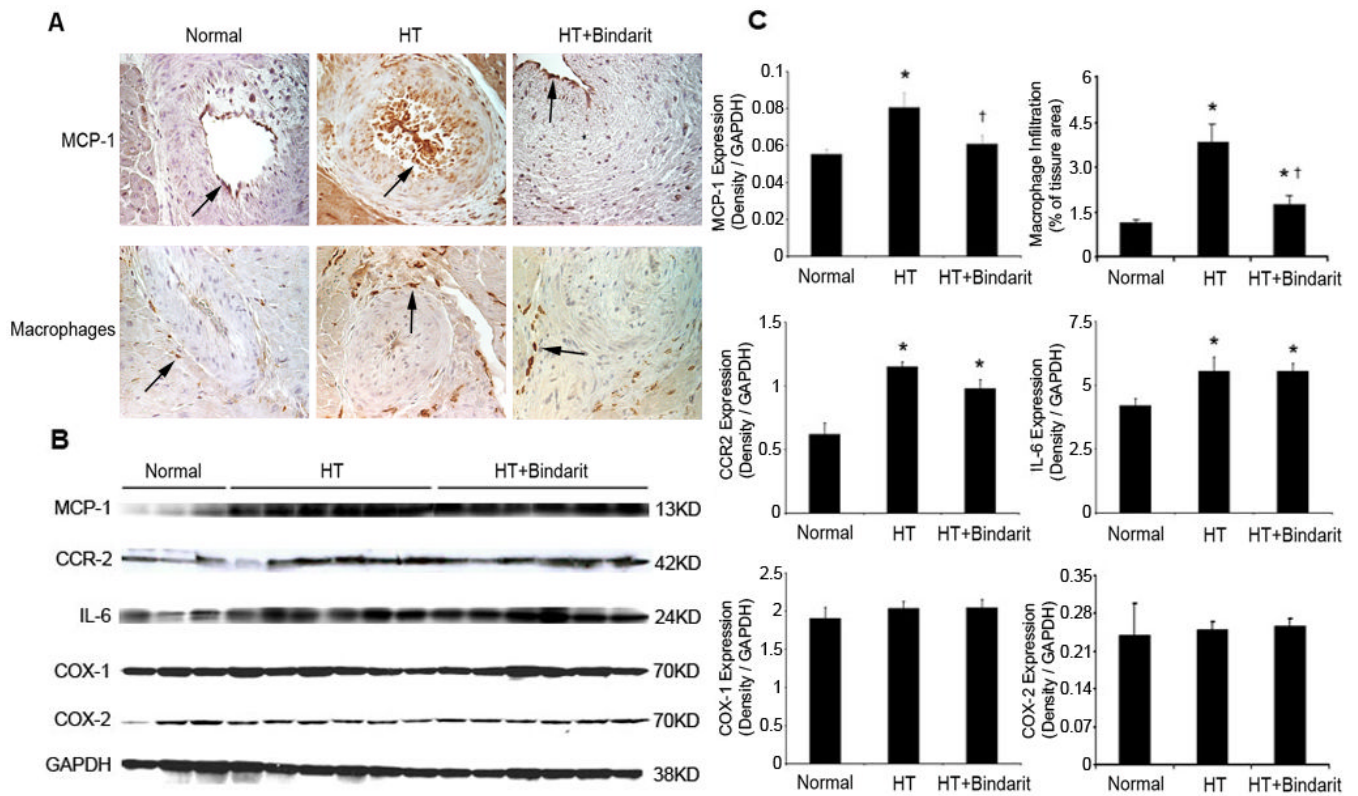
Figure 2.
The expression of myocardial MCP-1, its receptor CCR2, cyclooxygenase (COX)-1, COX-2, IL-6, and macrophage infiltration in normal, hypertension (HTN), and HTN+bindarit pigs detected by immunohistochemistry. A. Representative images (magnification ×40) of MCP-1 and macrophage staining (brown, arrows), showing increase in HTN and decrease by bindarit. B. Western blots, showing increased expression of MCP-1, CCR-2 and IL-6 in both HTN and HTN+bindarit. COX-1 and COX-2 expression was similar among the groups. C. Quantitation and densitometry normalized by GAPDH. * p<0.05 *vs.* normal and † p<0.05 *vs.* HTN.
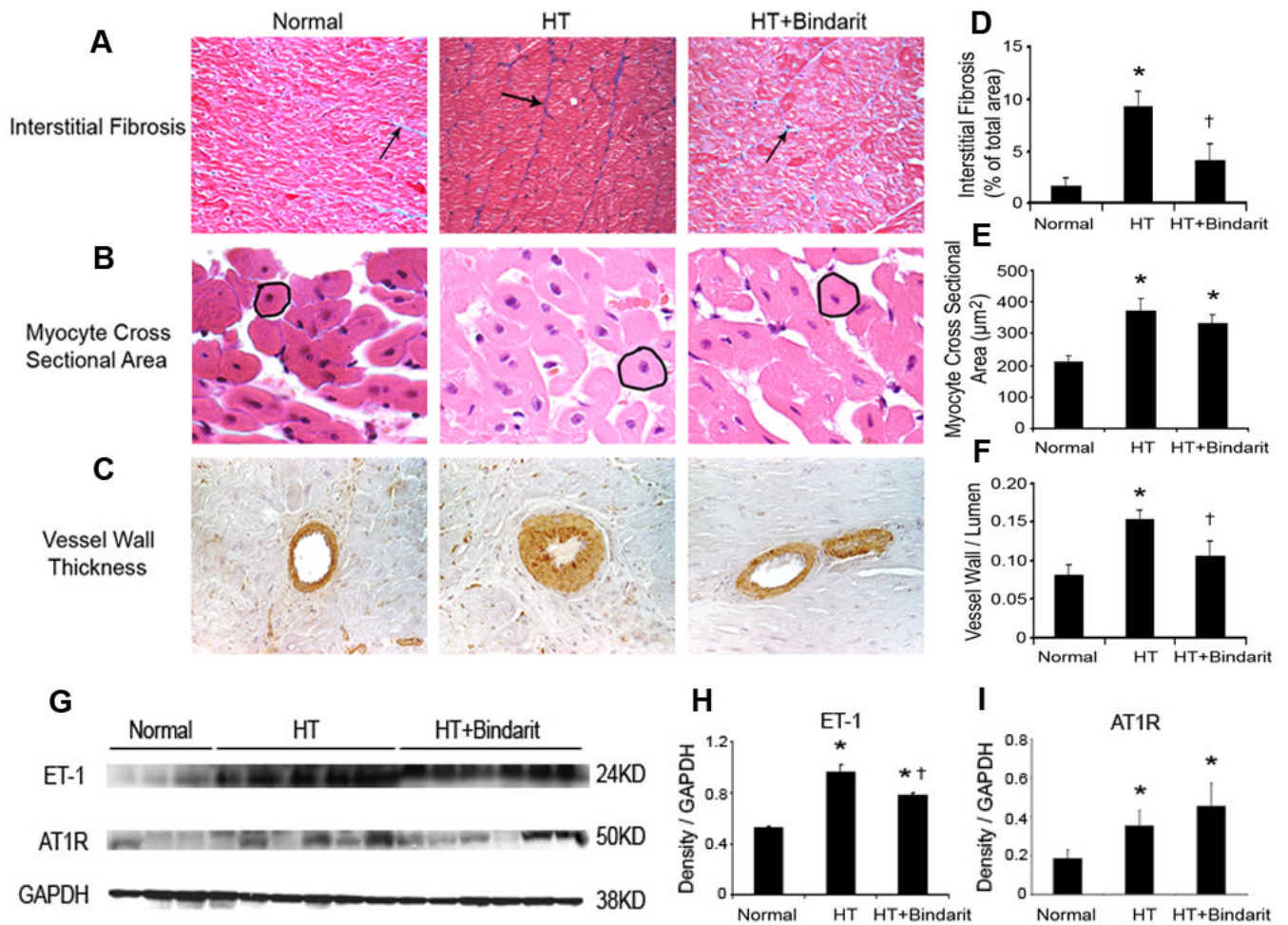
Figure 3.
Myocardial fibrosis (A), myocyte cross sectional area (B), and vessel wall to lumen ratio (C) quantified in trichrome, H&E, and SMA stained slides (magnification ×40) and myocardial expression of ET-1 and AT1R detected by western blotting from normal, hypertension (HTN), and HTN+bindarit pigs. Myocardial fibrosis was increased in HTN compared to normal, and significantly ameliorated, but not abolished, by bindarit (D). Myocyte cross sectional area was increased in both HTN and HTN+bindarit compared to normal, but was not different between the two groups (E), indicating that bindarit had no effect on myocyte hypertrophy. Microvascular media-to-lumen ratio increased in HTN compared to normal and decreased in HTN+bindarit compared to HTN, yet remained higher than normal (F). Myocardial tissue ET-1 level significantly increased in HTN, which was significantly preserved by bindarit; AT1R expression was elevated in HTN, but unaffected by bindarit (G, H). * $p < 0.05$ *vs.* normal and [†] $p < 0.05$ *vs.* HTN.
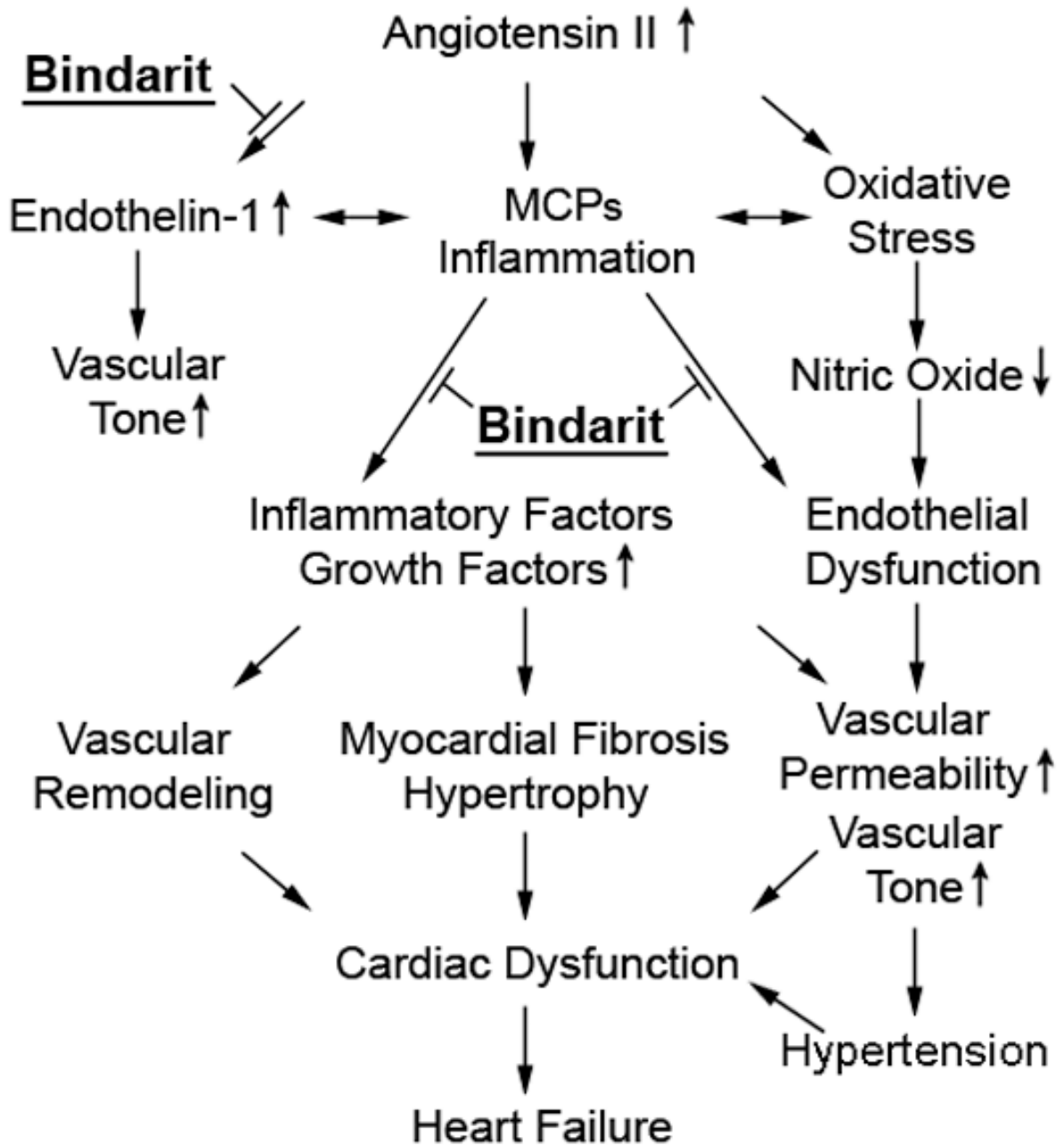
Figure 4.
A schematic outlining the pathways blocked by bindarit that are likely responsible for its effects to improve cardiac and myocardial microvascular function. However, MCP inhibition did not substantially blunt oxidative stress or the renin-angiotensin system, which drive systemic hypertension in this model.

Table 1

Systemic and cardiac function and myocardial microvascular density (mean±SEM) assessed by micro-CT in normal, hypertension (HTN), and HTN+bindarit pigs.

	Normal n=6	HTN n=6	HTN+bindarit n=6
Mean arterial pressure (mmHg)	101±4	120±5[*]	124±9[*]
Change post-adenosine (%)	-6±1[#]	-14±5[#]	-17±2[*#]
Heart Rate (bpm)	75±7	67±4	74±3
Plasma Renin Activity (ng/ml/hour)	0.17±0.03	0.17±0.02	0.16±0.06
Systemic Aldosterone (pg/ml)	684±68	1194±156[*]	971±139[*]
Endothelin-1 (pg/ml)	5.6±0.2	7.9±0.8[*]	5.9±0.3[†]
Stroke Volume (ml)	54±5	46±3	47±5
Cardiac Output (Liter/min)	3.5±0.4	2.8±0.1	3.4±0.5
E/A Ratio	1.98±0.17	1.04±0.10[*]	1.47±0.15[†]
LV Muscle Mass / Body Weight (g/kg)	2.14±0.16	3.43±0.16[*]	2.79±0.16[*†]

[*] $p<0.05$ *vs.* normal;

[†] $p<0.05$ *vs.* HTN;

[#] $p<0.05$ *vs.* baseline

MAP: mean arterial pressure; LV: left ventricle