TREATMENT WITH RESOLVIN D2 ATTENUATES CARDIOVASCULAR DAMAGE IN ANGIOTENSIN II-INDUCED HYPERTENSION

Díaz del Campo L[1], García-Redondo AB[2,3,4*], Duro-Sánchez S[1], Zaragoza C[4,5], Palmas F[6], Peraza DA[7], de Benito-Bueno A[7], Socuéllamos PG[7], Rodrigues-Diez R[1,3,4], Valenzuela C[4,7], Dalli J[6], Salaices M[1,3,4], Briones AM[1,3,4*]

[1]*Department of Pharmacology and Therapeutics, Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain*

[2]*Department of Physiology, Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain*

[3]*Hospital La Paz Institute for Health Research (IdiPAZ), Madrid, Spain*

[4]*CIBER Cardiovascular, Madrid, Spain*

[5]*Hospital Ramón y Cajal Research Unit (IRYCIS), Cardiology Department, Universidad Francisco de Vitoria, Madrid, Spain.*

[6]*Lipid Mediator Unit, John Vane Science Centre, London, UK*

[7]*Instituto de Investigaciones Biomédicas Alberto Sols (CSIC-UAM), Madrid, Spain*

*Equal contribution

Correspondence to: Ana M. Briones: ana.briones@uam.es. Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma de Madrid. C/Arzobispo Morcillo 4, 28029-Madrid, Spain. Telephone: +34914975399.

Word count:

ABSTRACT

Background:

Cardiovascular damage in hypertension is greatly influenced by low-grade chronic inflammation. Resolution of inflammation is orchestrated by specialized pro-resolving mediators (SPMs) and this seems to be impaired in different cardiovascular diseases. Among SPMs, resolvins (Rv) seem to have beneficial effects in some cardiovascular pathologies, but little is known about their effect on cardiovascular damage in hypertension.

Methods:

Aorta, small mesenteric arteries, heart and peritoneal macrophages were taken from C57BL/6J mice, infused or not with Angiotensin II (AngII 1.44mg/kg/day, 14 days) in presence or absence of RvD2 (100ng/mice, every second day) starting one day before or seven days after AngII infusion, for preventive or therapeutic administration, respectively.

Results:

Aorta or heart from AngII-infused mice showed altered expression of enzymes or receptors involved in SPMs synthesis or signalling, and a different metabololipidomics SPMs profile. Preventive treatment with RvD2 partially avoided AngII-induced hypertension and cardiovascular dysfunction and remodelling, likely through improved availability of vasoprotective factors, decreased cardiovascular fibrosis, reduced leukocyte infiltration and a shift towards a proresolutive macrophages phenotype. Moreover, when administered in hypertensive animals, RvD2 did not reverse hypertension but partially improved cardiovascular function and structure, decreased fibrosis and avoided proinflammatory macrophages phenotype.

Conclusions: There might be a disbalance between proinflammatory and resolution mediators in hypertension. RvD2 treatment protects cardiovascular function and structure when administered before and after the development of hypertension by modulating vascular factors, fibrosis and inflammation. Resolution of inflammation might be a novel therapeutic strategy for the treatment of cardiovascular damage associated to hypertension.

Non-standard Abbreviations and Acronyms

EC endothelial cells

NO nitric oxide

SPMs specialized proresolving mediators

PUFAs polyunsaturated fatty acids

VCAM-1 vascular cell adhesion molecule-1

INTRODUCTION

Hypertension is the leading risk factor globally for attributable deaths ([1]GBD). Hypertension is associated with alterations in vascular reactivity, which results in reduced endothelium-dependent vasodilator responses and increased contractile responses, and vascular remodelling and stiffness, due, at least in part, to an increased extracellular matrix deposition (Schiffrin 2012;Brandes 2014, Touyz et al., 2018). Beyond vascular functional and structural alterations, hypertension is characterized by cardiac hypertrophy, fibrosis, and dysfunction (Tomek 2017).

Studies conducted over the last years suggest that innate and adaptative immune systems, with elevated levels of local and circulating proinflammatory cytokines, play an important role to the development of hypertension and the cardiovascular alterations observed in this pathology (Norlander et al., 2018, Caillon et al., 2019; Drummond et al., 2019). Many preclinical studies have reported anti-hypertensive effects and cardiovascular protection of antinflammatory and immune interventions in hypertension, particularly when treatments were given at the onset of the disease, or in global knockout animals (Murray 2021). However, therapies targeting immune mechanisms are not currently considered therapeutic options for blood pressure lowering and cardiovascular disease protection (Murray 2021). Therefore, the development of antinflammatory therapies that can reprogram the immune response to accelerate the termination of inflammation with a lower burden of side effects such as immunosuppression or limited tissue repair, has become an active and attractive area of research for a number of different pathologies including inflammatory, vascular and metabolic diseases (Dalli and Serhan 2019; Kim 2020; Spite 2014).

Resolution of acute inflammation and the consequent recovery of tissue homeostasis was initially described as a passive process, explained by a decay of inflammatory signals (Buckley 2013). However, resolution of inflammation has been redefined as an active and highly orchestrated process that involves different specialized lipid mediators (SPMs) and cell types (Serhan 2015). These endogenous autacoids derive from ω-3 and ω-6 polyunsaturated fatty acids (PUFAs) and include a growing number of members grouped into four families: lipoxins, protectins, maresins and resolvins (Serhan 2014; Conte 2018; Chiang 2017; Dalli 2015). Depending on their ω-3 PUFA precursor, eicosapentanoic acid or docosahexanoic acid, resolvins are divided into E-series resolvins (RvE1-3), and D-series resolvins (RvD1-6), respectively (Serhan 2014). SPMs produce their effects through different G-protein-coupled receptors expressed in different tissues. For example, the receptor for RvD2, named GPR18, is expressed not only in immune cells (Chiang 2015) but also in endothelial cells (Zhang 2016), vascular smooth muscle cells (VSMC) (Ulu, 2019), as well as in cardiomyocytes (Matouk 2017). Resolvins were first detected in murine exudates of acute inflammation models (Serhan 2002; Serhan 2000) and their general effects are to reduce polymorphonuclear neutrophil infiltration and activation, and promote phagocytosis and clearance by macrophages (Spite 2010;Serhan 2014).

Recent evidence suggests that lack or fail in resolution of inflammation might be an underlying mechanism involved in different vascular diseases (Kim 2020; Conte 2018). Moreover, decreased levels of LXA4, RvD1 and RvE1 have been described in hypertensive patients compared to normotensive controls (Yücel and Özdemir 2021). Interestingly, RvD1, RvD2 and RvE1 display direct protective actions in VSMC, modulating their cell phenotype (Miyahara 2013; Ho 2010) and contractility (Jannaway 2018), and resolvins such as RvD2 acts on endothelial

cells (EC) by stimulating NO and prostacyclin (PGI2) production, which limit excessive leukocyte infiltration and reduce pro-inflammatory cytokines (Spite 2009). More importantly, treatment with some SPMs such as RvD2 prevents atheroprogression, reduces neointima formation, inhibits aortic abdominal aneurysm formation and enhances post-ischemic recovery in kidneys, brain or skeletal muscle (Conte 2018). However, whether hypertension is associated with altered local SPMs and whether RvD2 might protect of the cardiovascular damage associated to hypertension, is unknown.

We hypothesized that cardiovascular damage associated to hypertension may be explained, at least in part, by an insufficient or inefficient resolution of inflammation. Then, treatment with SPMs such as RvD2, that increases availability of protective vasoactive factors, might result in an effective therapy in hypertension, possibly by modulation of inflammatory pathways and/or direct effects in cardiovascular cells. Our data demonstrate, for the first time, that preventive and intervention treatments with RvD2 repair the cardiovascular damage associated to hypertension by mechanisms that include increased NO and PGI2 availability, decreased fibrosis and modulation of inflammatory response in cardiovascular tissues and macrophages.

MATERIALS AND METHODS

The authors declare that all supporting data are available within the article (and its online supplementary files).

RESULTS

AngII increases the cardiovascular expression of LOXs and GPR18 and decreases specialized proresolvin mediators.

The generation and accumulation of RvD2, has been well characterized in exudate models; however, its biosynthesis in the vessels and heart is less known. AngII increased mRNA levels of enzymes involved in RvD2 synthesis *Alox5* and, *Alox15* and *Gpr18* in aorta (Figure 1A) and heart (Figure 1B). Moreover, GPR18 protein was more evident in the three layers of the vascular wall (Figure 1C) and in cardiomyocytes and cardiac infiltrating cells (Figure 1D), after AngII infusion.

We then determined cardiac lipid mediator profiling using liquid chromatography tandem mass spectrometry. Identification and quantitation of cardiac lipid mediators from the 4 major essential fatty acid metabolomes was conducted in accordance with established criteria that include matching retention times and at least 6 diagnostic ions in the MS-MS spectrum (reference?). In these cardiac samples, we identified mediators from the lipoxygenase and cyclooxygenase-derived bioactive metabolomes that include the arachidonic acid, EPA, DHA and n-3 DPA–derived resolvins, maresins, and lipoxins (Figure 1E).

Comentado [a1]: Jesmond, this is copied from one of your papers (Souza et al., Circ Res 2020). Please revise

We then next employed partial least squares discriminant analysis, which generates a regression model based on concentrations of lipid mediators that are differently expressed between the groups (reference?), to gain insights into specific mediators that were modified by AngII infusion. Assessment of the score plots demonstrated a shift in cardiac lipid mediator profiles with AngII treatment (Figure 1E). Assessment of the variable importance in projection scores, which identify the contribution of each mediator in the observed separation between groups, demonstrated a downregulation of 17R-RvD1, RvD5 and RvD6, RvE3, LxA4 and LxB4, and 22-COOH-MaR1 (add names of all reduced?). Conversely, AngII increased n3-derived DPA RvD1 and PD2, MaR1 and the prothrombotic TxB2 (Figure 1E). Together these results demonstrate that AngII leads to a

Comentado [a2]: Again copied from your paper. Please revise

decrease in cardiac lipid mediator concentrations with marked? decreases in AA, EPA and some DHA-derived SPMs.

RvD2 administration partially prevents AngII-induced hypertension and vascular dysfunction by increasing NO and PGI_2 availability.

We next questioned whether the overexpression of GPR18 in heart and aorta by AngII might enhance the effects of RvD2 in the animal model.

Flow cytometry analysis of the content of circulating immune cells revealed an increase in Cd11b cells, monocyte ($Ly6C^+$) and neutrophils ($Ly6G^+$) after AngII infusion, without changes in circulating total $Cd45^+$ cells (data not shown). These effects were completely prevented by RvD2 treatment (Figure S1).

Treatment with RvD2 before AngII infusion also partially prevented elevation of systolic blood pressure, from day 7 and until the end of the treatment (Figure 2A). Moreover, AngII infusion impaired endothelium-dependent vasorelaxation to acetylcholine in aorta and SMA, and treatment with RvD2 prevented endothelial dysfunction only in SMA (Figure 2B, 2C). No changes in vasodilator responses to the exogenous NO donor (DEA-NO) were observed between all experimental groups (Figure 2B, 2C), indicating no differences in VSMC sensitivity to NO. AngII increased vascular contractile responses to phenylephrine in aorta (Figure 2B) but not in SMA, and this aortic hypercontractility was prevented by RvD2 treatment (Figure 2C).

Then, we incubated arteries from control and AngII-infused mice with RvD2 *ex vivo* in the organ bath (10 nM, 30 min). As shown in Figure S2, RvD2 normalized contractile responses and improved vascular relaxation in aorta and/or SMA from AngII-infused mice.

Next, we analysed the possible mechanisms underlying beneficial effects of RvD2 in vascular function. The selective prostacyclin receptor antagonist RO1138452 induced spontaneous vasoconstrictor responses in aortic segments from AngII-infused mice, and this effect was greatly enhanced in RvD2-pretreated animals (Figure S3A). The NOS inhibitor, L-NAME, increased vasoconstrictor responses to phenylephrine less in aorta from AngII-infused than control mice, and RvD2 treatment restored the modulation of vasocontractile responses by NO (Figure S3B).

As observed in Figure S3C, the contribution of the EDHF pathway to the SMA vasodilation was similar between the three experimental groups. However, the contribution of the tandem NO plus COX derivates was smaller in AngII-infused animals and was partially restored with the RvD2 pretreatment (Figure S3D). Altogether these data suggest that a higher NO and/or PGI2 availability after RvD2 treatment would be responsible for the protective effects of RvD2 treatment against AngII-induced vascular dysfunction.

RvD2 treatment prevents AngII-induced vascular remodeling

AngII increased aortic wall thickness and type I collagen (*Col1a2*) deposition and this was prevented by RvD2 (Figure 2D).

SMAs from AngII-infused mice presented smaller lumen diameter and increased wall thickness, indicating inward remodelling. The changes in wall thickness, but not in lumen diameter, were partially restored by RvD2 treatment (Figure S4A). In-depth analysis of vascular wall structure by confocal microscopy showed that AngII did not change adventitia thickness, but increased media thickness, and this was prevented by RvD2 (Figures S4B). AngII did not modify adventitial or SMC number but increased VSMC nuclei area; RvD2 treatment decreased

VSMC number, but it did not alter nuclei size (Figure S4C, S4D). AngII also augmented vascular stiffness, as shown by the leftward shift of the stress-strain relationship and the increase in β-value and this was unaffected by RvD2 (Figure S4E).

RvD2 treatment *in vivo* or incubation *ex vivo* did not modify blood pressure, vascular contractile or relaxant responses, or vascular structure in any vascular bed from control mice (data not shown) suggesting that an inflammatory stimulus, such as AngII, is needed to observe RvD2 beneficial effects.

RvD2 protects against cardiac dysfunction, hypertrophy and fibrosis induced by AngII

AngII infusion decreased ejection fraction and increased cardiac hypertrophy, cardiomyocytes size, and cardiac fibrosis measured by Masson staining and augmented mRNA expression of hypertrophic and profibrotic factors *Nppb* (B natriuretic peptide) and *Tgfb* (transforming growth factor b) (Figure 3). All these effects were prevented by RvD2 treatment (Figure 3).

RvD2 treatment prevents AngII-induced leukocyte infiltration and changes the proinflammatory profile of peritoneal macrophages

AngII infusion increased the aortic infiltration of lymphocytes and macrophages, as shown by the increased expression of the lymphocyte T antigen Cd3 and the macrophage marker *Runx1*, as well as the intercellular adhesion molecule 1 (*Icam1*), effects that were prevented by RvD2 (Figure 4A).

In the heart, AngII tended to increase Cd3 expression and augmented *Runx1* and the chemokine monocyte chemoattractant protein-1 (*Mcp1)* mRNA (Figure 4B). As in aorta, RvD2 limited AngII-induced macrophage and monocyte infiltration

(Figure 4B). Moreover, , the analysis of principal components showed a clear difference in the metabololipidomic profile of hearts from AngII-infused mice treated with RvD2 (Figure 4C). JESMOND, WE ARE NOT SURE WHAT TO HIGHLIGHT FROM THE VIP ANALYSIS HERE.

We then used peritoneal macrophages as a model to assess changes in proinflammatory profile with potential impact in vascular function (Olivencia 2021). Peritoneal macrophages from AngII-infused mice exhibited altered electrophysiological properties (i.e augmented KV currents, use-dependent effects and a greater degree of inactivation together with a faster time constant of inactivation (Figures 4D, 4E and S5), as previously reported for classical activation or (M1) macrophages (Carmen add one reference if needed). AngII also increased the expression of *Kcna3* and proinflammatory cytokines such as *Il1ß*, *Il6* and *Cox2* (Figure 4F) and reduced the expression of the antioxidant enzyme, hemooxygenase 1 (Ho-1). Importantly, RvD2 prevented the electrophysiological alterations, downregulated the expression of all M1 proinflammatory markers induced by AngII and increased the expression of M2 anti-inflammatory markers *Ho-1* and *Cd163* (Figure 4F).

RvD2 protects cardiovascular function and structure when administered seven days after AngII infusion

We then questioned whether RvD2 might also confer cardiovascular protection when administered in mice with elevated blood pressure.

Intervention treatment with RvD2 did not reverse the AngII-induced circulating Cd11b positive cells or neutrophils (Ly6G+) but decreased the number of monocytes (Ly6C+) (Figure S1).

RvD2 did not reverse AngII-induced hypertension (Figure 5A). Moreover, RvD2 did not prevented AngII-induced hypercontractility (data not shown) or endothelial dysfunction in aorta (Figure 5B). However, RvD2 partially improved endothelial function in SMA (Figure 5B). Moreover, RvD2 treatment decreased AngII-induced vascular hypertrophy in both aorta (Figure 5C) and SMA (Figure S6A). The beneficial effects of RvD2 treatment in SMA remodeling were due to a decreased media thickness and VSMC number without modifying adventitia thickness, adventitial cells number or vascular stiffness (Figures S6B-S6E). Intervention treatment with RvD2 also normalized cardiac function (Figures 5D) and reversed AngII-induced cardiomyocyte hypertrophy and cardiac fibrosis (Figures 5E, F).

Regarding immune cells infiltration in cardiovascular tissues, therapeutic treatment with RvD2 produced moderate effects with only *Icam1* and *Runx1* significantly decreased in aorta and heart respectively (Figures 6A,6B). Interestingly, RvD2 treatment shifted peritoneal macrophages phenotype to a more anti-inflammatory profile as shown by normalized electrophysiological properties (Figure 6C,6D,S5) and downregulation of proinflammatory genes *Kcna3*, *Il1b*, *Il6*, and *Cox2* without significant modification of M2 markers (Figure 6E).

DISCUSION

Regulation of the inflammatory response by SPMs has encouraged investigation into their therapeutic and diagnostic use in cardiovascular pathologies. Our study demonstrates, for the first time, that experimental hypertension might be associated with low cardiovascular SPMs that may enable progression of chronic cardiovascular inflammation. Systemic delivery of RvD2 prevented and attenuated cardiovascular alterations induced by hypertension by mechanisms

that include augmented availability of protective vasoactive factors, decrease in cardiovascular remodeling and fibrosis, prevention of immune cells infiltration and shift towards proresolutive macrophages phenotype.

Local vascular synthesis of SPMs has been described in different vascular settings although the specific origins have yet to be fully elucidated, and likely include both direct synthesis and cell-cell interactions with exchange of intermediates (Kim; Conte 2018; Sansbury and Spite 2016; Chatterjee et al., 2017). It has been proposed that in different types of vascular injury including atherosclerosis, abdominal aortic aneurysms (AAA), and restenosis, there might be defective resolution (Conte 2018; Merched 2008) or an imbalance between proinflammatory and proresolving signals (Fredman 2016). Moreover, at the clinical level lower circulating levels different SPMs such as RvDs have been found in patients with peripheral arterial disease (Ho *et al.,* 2010), coronary artery disease (Elajami *et al.,* 2016), hypertension (Yücel and Özdemir 2021) or presenting an acutely symptomatic carotid plaque rupture event (Bazan *et al.,* 2017). SPMs synthesis depends on LOX enzymes expression and location. Thus, 5-LOX in the perinuclear region participates in the biosynthesis of proinflammatory LTB4 and LTC4 (Radmark et al., 2015). However, 5-LOX translocation from nucleus to cytoplasm is associated with SPM biosynthesis in vascular cells (Chatterjee *et al.,* 2017). 15LOX is mainly involved in SPMs synthesis such as RvDs, protectins and lipoxins (Serhan and Levy, 2015). Our data suggest that proresolving lipid mediators might be being temporarily synthesized throughout the course of chronic inflammation in hypertension. Thus, we found that both Alox5, but not LTC4S (data not shown), and Alox15 and RvD2 receptor Gpr18 genes, are increased by AngII infusion in aorta and heart. Moreover, we found a clearly different profile of cardiac SPMs with several

mediators specifically decreased after AngII infusion, suggesting that the cardiovascular inflammatory milieu during hypertension might be partially determined by defective SPMs. Whether this is due to alterations in LOXs activity, protein expression, or subcellular location, need to be further examined.

Previous studies have demonstrated a protective role of RvD2 in the development of atherosclerosis, aneurysms, and restenosis (Viola *et al.,* 2016; Pope *et al.,* 2016). However, few studies have demonstrated modulation of cardiovascular functional responses by SPMs. For example, RvE1, RvD1 and RvD2 inhibited vasoconstriction induced by U46619, a thromboxane A2 agonist, in rat thoracic aorta and human pulmonary arteries (Jannaway 2018). We found that RvD2 treatment prevented AngII-induced hypercontractility in aorta and endothelial dysfunction in resistance arteries, by mechanisms that likely include augmented NO and PGI2 availability. In agreement, RvD2-induced activation of NO/NOS pathway and PGI2 production has been demonstrated in human or murine endothelial cells (Miyahara *et al.,* 2013, Spite *et al.,* 2009; Zuo *et al.,* 2018). Moreover, RvD2 treatment produced a significant reduction of AngII-induced vascular remodeling in large and small arteries, and prevented cardiac dysfunction by mechanisms that likely include decreased collagen deposition, lower number of VSMC and attenuated cardiomyocyte hypertrophy. Our data add novel evidence of the protective effects of RvD2 in vascular damage reported for neointima hyperplasia (Miyahara *et al.,* 2013; Akagi *et al.,* 2015), atherosclerosis (Viola *et al.*, 2016), AAA (Pope et al., 2016) and hindlimb ischemia (Zhang *et al.,* 2016), and extend beneficial effects of this specific mediator to hypertensive heart pathology. More importantly, not only RvD2 was able to prevent AngII-induced cardiovascular damage, but also when it was administered in fully hypertensive mice, RvD2 was able to protect the cardiovascular system by increasing vascular

relaxation, decreasing pathological vascular remodeling, and improving cardiac function, structure and fibrosis. RvD2 treatment also partially prevented AngII-induced hypertension, but it was not able to revert the elevated blood pressure levels, probably due to the short length of RvD2 administration in this specific regression model. Together, our results suggest that SPMs such as RvD2 might be a new therapeutic strategy to protect the cardiovascular system from hypertension.

While most direct effects of RvDs have been studied on leukocytes, especially on macrophages, resolvins also act on VSMC and endothelial cells where they down-regulate cell adhesion molecules therefore limiting leukocyte:vascular cells interaction and infiltration (Viola *et al.,* 2016; Pope *et al.,* 2016; Norling *et al.,* 2012; Spite *et al,.* 2009). For example, RvD1, D2 or protectin D1 reduced the expression of vascular cell adhesion molecule 1 (VCAM-1) or ICAM-1 in retinal or aortic endothelium or in VSMC (Tian 2009; Miyahara 2013; Merched 2008). Also, previous evidence showed that total leukocyte infiltration is reduced by local delivery of RvD2 (Miyahara 2013; Akagi 2015). We observed that preventive RvD2 treatment reduced the AngII-induced upregulation of *Icam-1* and *Mcp1* genes in aorta and heart respectively, and this was accompanied by decreased lymphocytes and/or macrophage infiltration. Moreover, preventive RvD2 treatment normalized the elevated numbers of circulating leukocytes (both monocytes and neutrophils) induced by AngII. Interestingly, when administered in hypertensive mice, RvD2 did not normalize circulating inflammatory cells, and minor effects were observed at local level despite of clear cardiovascular benefits, suggesting that the observed cardiovascular phenotype induced by RvD2 does not primarily rely on a systemic but rather a local, lesion-inherent effect likely in cardiovascular and infiltrated immune cells. In this sense, RvD2 incubation *ex*

vivo also improved vascular function in aorta and resistance arteries from AngII-infused mice, and earlier studies demonstrated that RvD2 reduced VSMC proliferation and migration *in vitro* (Miyahara et al., 2013) and *in vivo* (Akagi et al., 2015).

It is well accepted that different SPMs, including RvD2, shift macrophage phenotype towards a resolutive M2 profile that is associated with tissue repair (Conte 2018; Dalli and Serhan 2017), and this was found in the context of AAA (Pope *et al.,* 2016), atherosclerosis (Viola *et al.,* 2016), neointima hyperplasia (Akagi *et al.,* 2015), or aortic valve disease (Artiach *et al.,* 2020). Macrophage functions are dependent on Kv currents which are determined by heterotetrameric Kv1.3/Kv1.5 channels (Moreno *et al.,* 2013; Vicente *et al.,* 2006; Villalonga *et al.*, 2010), with increased Kv current due to augmented Kv1.3/Kv1.5 ratio and/or formation of Kv1.3 homotetramers, in classical activated M1 macrophages (refs Carmen, Moreno *et al.,* 2013; Villalonga *et al.,* 2010). We have recently described that AngII increases the expression of *Kv1.3* channels in mice aorta and peritoneal macrophages where it also upregulates Kv current and M1-like cytokines (Olivencia et al., 2021). Here, we found that both preventive and therapeutic treatment with RvD2 clearly restored electrophysiological properties and decreased the expression of *Kv1.3* and several M1 cytokines while upregulating M2 markers in peritoneal macrophages, pointing to immune cells as a major target for RvD2. In agreement, RvD2 addition to activated peritoneal macrophages reduced IL-1b secretion (Lopategui *et al.,* 2018), and human macrophages incubated with RvD2 displayed augmented Cd163 expression (Chiang et al., 2015).

Comentado [a3]: Carmen would it be possible to remove one or even two references?

It is important to mention that RvD2 treatment also modulated the SPMs profile at the cardiac level likely due to the ability of these mediators to function through positive loops (Sugimoto *et al.,* 2016; Haworth *et al.,* 2008). A complex and unresolved issue is which and how each specific SPMs modulated by RvD2 might have affected cardiovascular outcomes. Interestingly, administration of RvD2 increased RvD5n3-DPA which has been shown to reduce platelet-leukocytes aggregates, vascular TXB_2 concentrations and aortic lesions in $ApoE^{-/-}$ mice (Colas et al., 2018). We propose that RvD2 administration has initiated different signaling cascades that have led to reprogramed cardiovascular and immune cells leading to protective phenotypes.

Several aspects of our study need further consideration. We performed SPMs analysis in heart but not in vascular tissues. However, expression of Lox genes and GPR18 were modified by AngII in a similar direction, and we found beneficial effects of RvD2 both in the heart and in the vasculature. It may be also argued that the beneficial effects of RvD2 at the cardiovascular level could be due to the partial decrease in hypertensive responses induced by this compound. However, as discussed earlier, when administered in fully hypertensive mice RvD2 did not decrease blood pressure but elicited cardiovascular protection and macrophages modulation. Also, there were some differences in the effects of RvD2 in the different vascular beds, with beneficial effects of RvD2 administration in endothelial function mostly in resistance arteries and improved vascular contractility in aorta. Interestingly, *ex vivo* incubation with RvD2 improved aortic contraction and relaxation which could be explained by the different RvD2 concentrations achieved in *in vivo* an *in vitro* studies. Finally, peritoneal macrophages might not recapitulate features of cardiac or vascular resident macrophages and future studies are warranted to confirm our findings in these

specific cell types. However, we previously demonstrated that conditioned media from AngII-stimulated peritoneal macrophages can induce endothelial dysfunction likely due to their M1-like phenotype (Olivencia et al., 2021). In addition, other studies have demonstrated the ability of SPMs to modulate macrophage phenotype affecting vascular function. For example, gene deletion of ChemR23, favours a proinflammatory phenotype of peritoneal macrophages that increase VSMC proliferation (Artiach *et al.,* 2018), and increase oxLDL uptake and decreased phagocytosis in atherosclerosis (Laguna-Fernández *et al.,* 2018).

In summary, our results suggest that there seems to be a disbalance in the overall production of specialized proresolving mediators by AngII. RvD2 treatment facilitates resolution phenotype in cardiovascular tissues and immune cells and promotes protection of cardiovascular function and structure when administered before and after the development of hypertension. Thus, we propose that resolution of inflammation might be a novel effective therapy against the target organ damage associated to hypertension.

Perspectives

Novelty and relevance

What Is New?

What Is Relevant?

Clinical/Pathophysiological Implications?

Acknowledgements

This work was supported by the Ministerio de Ciencia e Innovación and Fondo Europeo de Desarrollo Regional (FEDER)/FSE (SAF2016-80305P; PID2020-116498RB-I00), Comunidad de Madrid (CM) (B2017/BMD-3676 AORTASANA) FEDER-a way to build Europe. RRD was supported by a Juan de la Cierva contract (IJCI-2017-31399). Please are your funding.

REFERENCES

1. Akagi D, Chen M, Toy R, Chatterjee A, Conte MS. Systemic delivery of proresolving lipid mediators resolvin D2 and maresin 1 attenuates intimal hyperplasia in mice. *FASEB J*. 2015;29(6):2504-2513.
2. Artiach G, Carracedo M, Plunde O, Wheelock CE, Thul S, Sjövall P, Franco-Cereceda A, Laguna-Fernandez A, Arnardottir H, Bäck M. Omega-3 Polyunsaturated Fatty Acids Decrease Aortic Valve Disease Through the Resolvin E1 and ChemR23 Axis. *Circulation*. 2020;142(8):776-789.
3. Bazan HA, Lu Y, Jun B, Fang Z, Woods TC, Hong S. Circulating inflammation-resolving lipid mediators RvD1 and DHA are decreased in patients with acutely symptomatic carotid disease. *Prostaglandins Leukot Essent Fatty Acids*. 2017;125:43-47.
4. Brandes RP. Endothelial dysfunction and hypertension. *Hypertension*. 2014;64: 924–928.
5. Buckley CD, Gilroy DW, Serhan CN, Stockinger B, Tak PP. The resolution of inflammation. *Nat Rev Immunol*. 2013;13(1):59-66.
6. Caillon A, Paradis P, Schiffrin EL. Role of immune cells in hypertension. Br J *Pharmacol*. 2019;176(12):1818-1828.
7. Chatterjee A, Komshian S, Sansbury BE, Wu B, Mottola G, Chen M, Spite M, Conte MS. Biosynthesis of proresolving lipid mediators by vascular cells and tissues. *FASEB J*. 2017;31(8):3393-3402.
8. Chiang N, Dalli J, Colas RA, Serhan CN. Identification of resolvin D2 receptor mediating resolution of infections and organ protection. *J Exp Med*. 2015;212(8):1203-1217.

9. Chiang N, Serhan CN. Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. *Mol Aspects Med*. 2017;58:114-129.
10. Colas RA, Souza PR, Walker ME, Burton M, Zasłona Z, Curtis AM, Marques RM, Dalli J. Impaired Production and Diurnal Regulation of Vascular RvDn-3 DPA Increase Systemic Inflammation and Cardiovascular Disease. *Circ Res.* 2018;122(6):855-863.
11. Conte MS, Desai TA, Wu B, Schaller M, Werlin E. Pro-resolving lipid mediators in vascular disease. *J Clin Invest*. 2018;128(9):3727-3735.
12. Dalli J, Chiang N, Serhan CN. Elucidation of novel 13-series resolvins that increase with atorvastatin and clear infections. *Nat Med*. 2015;21(9):1071-1075.
13. Dalli J, Serhan CN. Identification and structure elucidation of the pro-resolving mediators provides novel leads for resolution pharmacology. *Br J Pharmacol*. 2019;176(8):1024-1037.
14. Drummond GR, Vinh A, Guzik TJ, Sobey CG. Immune mechanisms of hypertension. *Nat Rev Immunol*. 2019;19(8):517-532.
15. Elajami TK, Colas RA, Dalli J, Chiang N, Serhan CN, Welty FK. Specialized proresolving lipid mediators in patients with coronary artery disease and their potential for clot remodeling. *FASEB J*. 2016;30(8):2792-2801.
16. Ellis A, Pannirselvam M, Anderson TJ, Triggle CR. Catalase has negligible inhibitory effects on endothelium-dependent relaxations in mouse isolated aorta and small mesenteric artery. *Br J Pharmacol.* 2003;140:1193–1200.

17. Fredman G, Hellmann J, Proto JD, Kuriakose G, Colas RA, Dorweiler B, Connolly ES, Solomon R, Jones DM, Heyer EJ et al. An imbalance between specialized pro-resolving lipid mediators and pro-inflammatory leukotrienes promotes instability of atherosclerotic plaques. *Nat Commun*. 2016;7:12859.
18. GBD 2019 Risk Factors Collaborators. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet.* 2015;396(10258):1223-1249.
19. Halade GV, Kain V, Serhan CN. Immune responsive resolvin D1 programs myocardial infarction-induced cardiorenal syndrome in heart failure. *FASEB J*. 2018;32(7):3717-3729.
20. Haworth O, Cernadas M, Yang R, Serhan CN, Levy BD. Resolvin E1 regulates interleukin 23, interferon-gamma and lipoxin A4 to promote the resolution of allergic airway inflammation. *Nat Immunol.* 2008; *9*:873–879.
21. Ho KJ, Spite M, Owens CD, Lancero H, Kroemer AH, Pande R, Creager MA, Serhan CN, Conte MS. Aspirin-triggered lipoxin and resolvin E1 modulate vascular smooth muscle phenotype and correlate with peripheral atherosclerosis. *Am J Pathol*. 2010;177(4):2116-2123.
22. Jannaway M, Torrens C, Warner JA, Sampson AP. Resolvin E1, resolvin D1 and resolvin D2 inhibit constriction of rat thoracic aorta and human pulmonary artery induced by the thromboxane mimetic U46619. *Br J Pharmacol*. 2018;175(7):1100-1108.
23. Kain V, Ingle KA, Colas RA, Dalli J, Prabhu SD, Serhan CN, Joshi M, Halade GV. Resolvin D1 activates the inflammation resolving response at splenic and ventricular site following myocardial infarction leading to improved ventricular function. *J Mol Cell Cardiol*. 2015;84:24-35.

24. Kim AS, Conte MS. Specialized pro-resolving lipid mediators in cardiovascular disease, diagnosis, and therapy. *Adv Drug Deliv Rev*. 2020;159:170-179.

25. Laguna-Fernandez A, Checa A, Carracedo M, Artiach G, Petri MH, Baumgartner R, Forteza MJ, Jiang X, Andonova T, Walker ME, et al. ERV1/ChemR23 Signaling Protects Against Atherosclerosis by Modifying Oxidized Low-Density Lipoprotein Uptake and Phagocytosis in Macrophages. Circulation. 2018;138(16):1693-1705.

26. Leung SW, Vanhoutte PM. Endothelium-dependent hyperpolarization: age, gender and blood pressure, do they matter? *Acta Physiol*. 2017;219:108–123.

27. Liu G, Gong Y, Zhang R, Piao L, Li X, Liu Q, Yan S, Shen Y, Guo S, Zhu M et al. Resolvin E1 attenuates injury-induced vascular neointimal formation by inhibition of inflammatory responses and vascular smooth muscle cell migration. *FASEB J*. 2018;32(10):5413-5425.

28. Lopategi A, Flores-Costa R, Rius B, López-Vicario C, Alcaraz-Quiles J, Titos E, Clària J. Frontline Science: Specialized proresolving lipid mediators inhibit the priming and activation of the macrophage NLRP3 inflammasome. *J Leukoc Biol*. 2019;105(1):25-36.

29. Matouk AI, Taye A, El-Moselhy MA, Heeba GH, Abdel-Rahman AA. The Effect of Chronic Activation of the Novel Endocannabinoid Receptor GPR18 on Myocardial Function and Blood Pressure in Conscious Rats. *J Cardiovasc Pharmacol*. 2017;69(1):23-33.

30. Merched AJ, Ko K, Gotlinger KH, Serhan CN, Chan L. Atherosclerosis: evidence for impairment of resolution of vascular inflammation governed by specific lipid mediators. *FASEB J*. 2008;22(10):3595-3606.

31. Merched AJ, Ko K, Gotlinger KH, Serhan CN, Chan L. Atherosclerosis: evidence for impairment of resolution of vascular inflammation governed by specific lipid mediators. *FASEB J*. 2008;22(10):3595-3606.

32. Miyahara T, Runge S, Chatterjee A, et al. Miyahara T, Runge S, Chatterjee A, Chen M, Mottola G, Fitzgerald JM, Serhan CN, Conte MS. D-series resolvin attenuates vascular smooth muscle cell activation and neointimal hyperplasia following vascular injury. *FASEB J*. 2013;27(6):2220-2232.

33. Moreno C, Prieto P, Macías Á, Pimentel-Santillana M, de la Cruz A, Través PG, Boscá L, Valenzuela C. Modulation of voltage-dependent and inward rectifier potassium channels by 15-epi-lipoxin-A4 in activated murine macrophages: implications in innate immunity. *J Immunol*. 2013;191(12):6136-6146.

34. Murray EC, Nosalski R, MacRitchie N, Tomaszewski M, Maffia P, Harrison DG, Guzik TJ. Therapeutic targeting of inflammation in hypertension: from novel mechanisms to translational perspective. *Cardiovasc Res*. 2021;117(13):2589-2609.

35. Norlander AE, Madhur MS, Harrison DG. The immunology of hypertension. *J Exp Med*. 2018;215(1):21-33.

36. Norling LV, Dalli J, Flower RJ, Serhan CN, Perretti M. Resolvin D1 limits polymorphonuclear leukocyte recruitment to inflammatory loci: receptor-dependent actions. *Arterioscler Thromb Vasc Biol*. 2012;32(8):1970-1978.

37. Olivares-Silva F, De Gregorio N, Espitia-Corredor J, Espinoza C, Vivar R, Silva D, Osorio JM, Lavandero S, Peiró C, Sánchez-Ferrer C, Díaz-Araya G. Resolvin-D1 attenuation of angiotensin II-induced cardiac inflammation in mice is associated with prevention of cardiac remodeling and hypertension. *Biochim Biophys Acta Mol Basis Dis*. 2021;1867(12):166241.

38. Olivencia MA, Martínez-Casales M, Peraza DA, García-Redondo AB, Mondéjar-Parreño G, Hernanz R, Salaices M, Cogolludo A, Pennington MW et al. K_V 1.3 channels are novel determinants of macrophage-dependent endothelial dysfunction in angiotensin II-induced hypertension in mice. *Br J Pharmacol*. 2021;178(8):1836-1854.

39. Pope NH, Salmon M, Davis JP, Chatterjee A, Su G, Conte MS, Ailawadi G, Upchurch GR Jr. D-series resolvins inhibit murine abdominal aortic aneurysm formation and increase M2 macrophage polarization. *FASEB J*. 2016;30(12):4192-4201.

40. Rådmark O, Werz O, Steinhilber D, Samuelsson B. 5-Lipoxygenase, a key enzyme for leukotriene biosynthesis in health and disease. *Biochim Biophys Acta.* 2015;1851(4):331-9.

41. Sansbury BE, Spite M. Resolution of Acute Inflammation and the Role of Resolvins in Immunity, Thrombosis, and Vascular Biology. *Circ Res*. 2016;119(1):113-130.

42. Schiffrin EL. Vascular remodeling in hypertension: Mechanisms and treatment. *Hypertension.* 2012; 59(2):367–374.

43. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol*. 2008;8(5):349-361.

44. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *J Exp Med*. 2000;192(8):1197-1204.

45. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med*. 2002;196(8):1025-1037.

46. Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. *J Clin Invest.* 2018;128(7):2657-2669.

47. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. *Nature*. 2014;510(7503):92-101.

48. Spite M, Clària J, Serhan CN. Resolvins, specialized proresolving lipid mediators, and their potential roles in metabolic diseases. *Cell Metab*. 2014;19(1):21-36.

49. Spite M, Norling LV, Summers L, Yang R, Cooper D, Petasis NA, Flower RJ, Perretti M, Serhan CN. Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. *Nature*. 2009;461(7268):1287-1291.

50. Spite M, Serhan CN. Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. *Circ Res*. 2010;107(10):1170-1184.

51. Sugimoto MA, Sousa LP, Pinho V, Perretti M, Teixeira MM. Resolution of Inflammation: What Controls Its Onset? *Front Immunol.* 2016; *7*:160.

52. Tian H, Lu Y, Sherwood AM, Hongqian D, Hong S. Resolvins E1 and D1 in choroid-retinal endothelial cells and leukocytes: biosynthesis and mechanisms of anti-inflammatory actions. *Invest Ophthalmol Vis Sci*. 2009;50(8):3613-3620.

53. Tomek J, Bub G. Hypertension-induced remodelling: on the interactions of cardiac risk factors. *J Physiol*. 2017;595(12):4027-4036.

54. Touyz RM, Alves-Lopes R, Rios FJ, Camargo LL, Anagnostopoulou A, Arner A, Montezano AC. Vascular smooth muscle contraction in hypertension. *Cardiovasc Res.* 2018;114(4):529-539.

55. Ulu A, Sahoo PK, Yuil-Valdes AG, Mukherjee M, Van Ormer M, Muthuraj PG, Thompson M, Anderson Berry A, Hanson CK, Natarajan SK, Nordgren TM. Omega-3 Fatty Acid-Derived Resolvin D2 Regulates Human Placental Vascular Smooth Muscle and Extravillous Trophoblast Activities. Int J Mol Sci. 2019 Sep 7;20(18):4402.

56. Vicente R, Escalada A, Villalonga N, Texidó L, Roura-Ferrer M, Martín-Satué M, López-Iglesias C, Soler C, Solsona C, Tamkun MM, Felipe A. Association of Kv1.5 and Kv1.3 contributes to the major voltage-dependent K+ channel in macrophages. *J Biol Chem*. 2006;281(49):37675-37685.

57. Villalonga N, David M, Bielanska J, Vicente R, Comes N, Valenzuela C, Felipe A. Immunomodulation of voltage-dependent K+ channels in macrophages: molecular and biophysical consequences. *J Gen Physiol*. 2010;135(2):135-147.

58. Viola JR, Lemnitzer P, Jansen Y, Csaba G, Winter C, Neideck C, Silvestre-Roig C, Dittmar G, Döring Y, Drechsler M et al. Resolving Lipid Mediators

Maresin 1 and Resolvin D2 Prevent Atheroprogression in Mice. *Circ Res*. 2016;119(9):1030-1038.

59. Wu J, Montaniel KR, Saleh MA, Xiao L, Chen W, Owens GK, Humphrey JD, Majesky MW, Paik DT, Hatzopoulos AK, Madhur MS, Harrison DG. The origin of matrix-producing cells that contribute to aortic fibrosis in hypertension. *Hypertension.* 2016;67:461–468.

60. Yücel H, Özdemir AT. Low LXA4, RvD1 and RvE1 levels may be an indicator of the development of hypertension. Prostaglandins Leukot Essent Fatty Acids. 2021 Nov;174:102365. doi: 10.1016/j.plefa.2021.102365. Epub 2021 Oct 29. PMID: 34740030.

61. Zhang MJ, Sansbury BE, Hellmann J, Baker JF, Guo L, Parmer CM, Prenner JC, Conklin DJ, Bhatnagar A, Creager MA, Spite M. Resolvin D2 Enhances Postischemic Revascularization While Resolving Inflammation. *Circulation*. 2016;134(9):666-680.

62. Zhang MJ, Sansbury BE, Hellmann J, Baker JF, Guo L, Parmer CM, Prenner JC, Conklin DJ, Bhatnagar A, Creager MA, Spite M. Resolvin D2 Enhances Postischemic Revascularization While Resolving Inflammation. *Circulation*. 2016;134(9):666-680.

63. Zuo G, Zhang D, Mu R, Shen H, Li X, Wang Z, Li H, Chen G. Resolvin D2 protects against cerebral ischemia/reperfusion injury in rats. *Mol Brain.* 2018;11(1):9.

FIGURE LEGENDS

Fig 1. Angiotensin II (AngII) regulates the expression of lipoxygenases (LOX) and RvD2 receptor. Lipoxygenase 5 (*Alox5*), 15 (*Alox15*) and *Gpr18* gene expression in aorta (A) and heart (B). Representative immunohistochemical images showing GPR18 expression in aorta (C) and heart (D). (E) 2-dimentional score plot (left) and variable importance in projection (VIP) scores (right) of murine heart tissues of control and AngII-infused mice. Areas in the score plot denote 95% confidence regions. *p<0.05 *vs* Control. Data represent mean ± SEM. n=10 to 20.

Fig 2. Resolvin D2 partially prevents hypertension, hypercontractility, endothelial dysfunction and vascular remodeling induced by AngII. (A) Systolic blood pressure of control, AngII-infused mice and AngII-infused mice treated with RvD2 for 14 days. (B-C) Concentration-response curves to acetylcholine (Ach), diethylamine nonoate (DEA-NO), and phenylephrine (Phe) in aorta (B) and small mesenteric arteries (SMA) (C) from the three experimental groups. (D) Media thickness quantification and representative images of hematoxylin-eosin (HE) staining (10x magnification) of paraffin-embedded aortic sections. (E) Col1a2 gene expression levels in aorta. *p<0.05 vs Control, #p<0.05 vs AngII. Data represent mean ± SEM. n=7 to 23.

Fig 3. RvD2 prevents cardiac hypertrophy, fibrosis and dysfunction induced by AngII. (A) Cardiac function measured by echocardiography, (B) cardiac hypertrophy measured as left ventricle weight (LVW)/tibial length (TL) ratio, (C) representative images of Wheat Germ Agglutinin staining of cardiomyocytes (63x magnification) and quantification of cardiomyocyte size, (D) representative images of Masson's trichrome staining of left ventricle (4x magnification) and

measurement of cardiac fibrosis, and (E) gene expression of cardiac hypertrophy (*Nppb*) and profibrotic (*TgfB*) markers, of control, AngII-infused mice and AngII-infused mice treated with RvD2 for 14 days . *p<0.05 vs control, #p<0.05 vs AngII. Data represent mean ± SEM. n=6 to 15.

Fig 4. RvD2 prevents AngII-induced immune infiltration and changes in metabololipidomics profile and normalizes macrophages phenotype. (A-B) gene expression levels of immune cells markers in aorta (A) and heart (B) from control, AngII-infused mice and AngII-infused mice treated with RvD2 for 14 days. (C) 2-dimentional score plot (left) and VIP scores (right) of murine heart tissues of AngII-infused mice with or without RvD2 treatment. Areas in the score plot denote 95% confidence regions. D) Representative current recordings obtained after applying the pulse protocol shown in the upper part of the figure in peritoneal macrophages obtained from control (n=22 macrophages, n=5 mice), AngII-infused (n=23 macrophages, n=8 mice), and AngII+preRvD2-infused (n=27 macrophages, n=6 mice). The right panel shows the I-V relationships in each experimental condition. E) Current records showing the cumulative KV inactivation measured after applying trains of 15 pulses of 250 ms from -80 to +50 mV at 2 Hz in macrophages obtained from control (n=22 macrophages, n=7 mice), AngII-infused (n=21 macrophages, n=11 mice), and AngII+preRvD2-infused (n=16 macrophages, n=6 mice). Right panel shows the peak current amplitude at each pulse normalized to the peak current amplitude of the first pulse. Data were fitted to a monoexponential function. Results are presented as means ± SEM. *P<0.05 vs control, and #P<0.05 AngII+preRvD2 vs AngII. Significance was analyzed by a one-way ANOVA followed by a Tukey's post hoc test. (F) gene expression levels of M1 and M2 markers in peritoneal macrophages

from the three experimental groups. *p<0.05 vs control, #p<0.05 vs AngII. Data represent mean ± SEM. n=7 to 13.

Fig 5. Intervention RvD2 partially reversed AngII-induced alterations in vascular and cardiac function and structure. (A) Systolic blood pressure, (B) concentration-response curves to Ach in aorta and SMA, (C) media thickness quantification and representative images of HE staining (10x magnification) of paraffin-embedded aortic sections, (D) cardiac function measured by echocardiography, (E) quantification of cardiomyocyte size and cardiac fibrosis and representative images of wheat germ agglutinin staining of cardiomyocytes (63x magnification) and Masson's trichrome staining of left ventricle (4x magnification), and (F) and gene expression of cardiac hypertrophy (*nppb*) and profibrotic markers (*tgfB*) in heart, from control, AngII-infused mice and AngII-infused mice treated with RvD2 starting 7 days after initiation of AngII infusion. Because experiments were run simultaneously, data from control and AngII-infuse mice are the same as Figures 2 and 3. *p<0.05 vs Control, #p<0.05 vs AngII. Data represent mean ± SEM. n=7 to 23.

Fig 6. Intervention RvD2 partially decreases AngII-induced immune infiltration and normalizes macrophages phenotype. Gene expression levels of immune cells markers in aorta (A) and heart (B) from control, AngII-infused mice and AngII-infused mice treated with RvD2 starting 7 days after initiation of AngII infusion. C) Representative current recordings obtained after applying the pulse protocol shown in the upper part of the figure in peritoneal macrophages obtained from control (n=22 macrophages, n=7 mice), AngII-infused (n=23 macrophages, n=10 mice), and AngII+RvD2-infused (n=36 macrophages, n=6 mice). The right panel shows the I-V relationships in each experimental condition.

D) Current records showing the cumulative KV inactivation measured after applying trains of 15 pulses of 250 ms from -80 to +50 mV at 2 Hz in macrophages obtained from control (n=19 macrophages, n=7 mice), AngII-infused (n=21 macrophages, n=11 mice), and AngII+RvD2-infused (n=36 macrophages, n=6 mice). Right panel shows the peak current amplitude at each pulse normalized to the peak current amplitude of the first pulse. Data were fitted to a monoexponential function. Results are presented as means ± SEM. *P<0.05 vs control, and #P<0.05 AngII+RvD2 vs AngII. Significance was analyzed by a one-way ANOVA followed by a Tukey's post hoc test. (E) gene expression levels of M1 and M2 markers in peritoneal macrophages from the three experimental groups. Because experiments were run simultaneously, data from control and AngII-infuse mice are the same as Figure 4. *p<0.05 vs control, #p<0.05 vs AngII. Data represent mean ± SEM. n=7 to 18.