DISTINGUISHED SCIENTIST AWARD LECTURE

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Mechanisms of remodelling of small arteries, antihypertensive therapy and the immune system in hypertension

Abstract

This review summarizes my lecture for the 2015 Distinguished Scientist Award from the Canadian Society of Clinical Investigation, and is based mainly on studies in my laboratory on the mechanisms of remodelling of small arteries in experimental animal and human hypertension and on treatments that lower blood pressure and improve structure and function of resistance vessels. Small resistance arteries undergo either inward eutrophic or hypertrophic remodelling, which raises blood pressure and impairs tissue perfusion. These vascular changes are corrected by some antihypertensive drugs, which may lead to improved outcomes. Vasoconstriction, growth, oxidative stress and inflammation are some of the mechanisms, within the vascular wall, that can be beneficially affected by antihypertensive agents. These antihypertensive-sensitive mechanisms are reviewed in this review, together with the inflammatory and immune mechanisms that may participate in hypertension and associated cardiovascular injury. Molecular studies, based on this research, will hopefully identify novel diagnostic and therapeutic targets, which will improve our ability to prevent and treat hypertension and cardiovascular disease.

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We review here our work on remodelling of the vasculature in experimental animal and human hypertension, its mechanisms and what we have learned from the effects of treatment. Our work concentrated on small arteries, where resistance to blood flow occurs and leads to increased peripheral resistance - the hallmark of arterial hypertension. In hypertension, large arteries stiffen and develop outward hypertrophic remodelling as we age [1-3], whereas the remodeling of small arteries is classically associated with an increase in media thickness. These two types of remodelling have been described as inward eutrophic or inward hypertrophic remodelling [4]. In the first type of remodelling, found in essential (primary) hypertension in humans and in spontaneously hypertensive rats (SHR), the lumen is reduced and the media to lumen ratio is similar to that of normotensive individuals [5-7]. Remodelling is usually eutrophic when the renin-angiotensin-aldosterone system (RAAS) is mildly or inappropriately activated relative to blood pressure levels as in primary hypertension or in SHR. In hypertrophic remodelling of small arteries, which occurs in secondary hypertension such as in renovascular hypertension, primary aldosteronism or pheochromocytoma [8], in hypertension that is associated with diabetes [9,10] and in acromegaly [11], and in salt-dependent and mineralocorticoid hypertension, as well as in malignant hypertension in rodents [12-15], all conditions in which the endothelin system is activated, there is true vascular hypertrophy, meaning that the media cross-sectional area is enlarged and remodelling is indeed hypertrophic. Vascular smooth muscle cells (VSMC) from small arteries of hypertensive rats are hyperplastic, whereas aortic VSMC are hypertrophic [16,17]; however, volume and number of VSMC from small arteries in essential hypertension are similar to those of normotensive subjects [18]. The mechanisms of inward growth are unclear but may be associated with peripheral apoptosis, or they could result from vasoconstriction of arteries that are embedded in an expanded extracellular matrix [19], as there is deposition of collagen and fibronectin with an increased collagen to elastin ratio in small arteries from hypertensive humans and rodents [18,20]. These depositions may be induced by endothelin [21], angiotensin II and aldosterone [22]. Tissue transglutaminases, via interactions of extracellular matrix fibrillar components with attachment sites on VSMC

[23,24], or matrix metalloproteinases (MMP) such as MMP2 [25] may play an important role. We have previously suggested that rearrangement of cells and fibrillar components, due to altered interaction of these components through integrins, may result in collagen fibers recruited at higher distending vessel pressures leading transiently to decreased stiffness [26]; however, with increased pulsatility impacting on small arteries, these eventually become stiffer as do larger conduit arteries [27].

Our studies demonstrated that small artery remodeling may be the first manifestation of target organ damage in hypertension [26]. All stage I hypertensive subjects present small artery remodeling, whereas endothelial dysfunction is found in 60% and left ventricular hypertrophy in 45%. The presence of small artery remodeling, in fact, has prognostic significance, since those hypertensive patients with the highest media to lumen ratio had the highest incidence of cardiovascular events [28].

Immune and inflammatory cell infiltration, particularly of perivascular fat, accompanied by up-regulation of inflammatory mediators, such as vascular cellular adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, nuclear factor (NF) kappaB, monocyte chemoattractant factor (MCP)-1, and plasminogen activator inhibitor (PAI)-1, in response to enhanced oxidative stress are recognized participants in the remodelling process [27].

Dysfunction of endothelium is often but not always found in vessels of hypertensive subjects. This dysfunction is probably a secondary phenomenon not involved in initiation of BP elevation but rather one that may contribute to progression of atherosclerosis and cardiovascular events in association with risk factors clustering with hypertension, such as dyslipidemia, obesity, metabolic syndrome, diabetes and smoking. Endothelial dysfunction may be evaluated by measuring different parameters, such as reduced endothelium-dependent vasodilation [29] or expression of endothelin-1 or inflammatory or thrombogenic agents that are produced by the endothelium [30]. A dysfunctional endothelium is characterized by an inflammatory phenotype with increased endothelial cell proliferation, altered morphology, production of C-reactive protein and other inflammatory and thrombogenic mediators including monocyte chemotactic protein (MCP)-1 and PAI-1, upregulated adhesion molecules,

enhanced thrombogenicity and adhesiveness of circulating cells and anoikis [30].

Anti-hypertensive therapy and small artery structure and function

We demonstrated that angiotensin-converting enzyme inhibitors (ACEIs) [31,32], angiotensin receptor blockers (ARBs) [33,34] and calcium channel blockers (CCBs) [35,36] improved the structure of small arteries dissected from gluteal subcutaneous biopsies in essential hypertensive patients in contrast to the beta-blocker atenolol, which did not [31-36]. Changes in these subcutaneous small arteries reflect what happens in the coronary circulation, as shown by indirect measurements [37-39] and as demonstrated in hypertensive rodents [40]. Renin-angiotensin (RAS) blockers also improved the structure of the small arteries of type 2 diabetic patients [41,42], and induced an upregulation of AT₂ receptors [43], which should improve vasodilatation, as has been demonstrated in rodents [44]. Even though it did not lower BP, a selective AT₂ receptor agonist, compound 21, reduced aortic or pericoronary collagen, small artery stiffness and aortic superoxide generation and improved endothelial dysfunction in stroke-prone SHR, especially if associated with an ARB [45]. As well, and despite the fact that the BP of hypertensive patients was normalized by the selective mineralocorticoid receptor antagonist (MRA), eplerenone, neither remodelling nor endothelial function of the small arteries improved [46]; however, stiffness of small arteries was reduced secondary to a decrease in collagen deposition and the collagen-to-elastin ratio in the media of the vascular wall.

We measured the effects of ACEI on endothelial function by evaluation of acetylcholine-induced relaxation *in vitro* (only one manifestation of endothelial function). Two-year treatment with an ACEI slightly improved the abnormal endothelium-dependent relaxation of small arteries in patients [47]. Treatment with ARBs corrected endothelial dysfunction [33,34], as did treatment with CCBs [36]. Beta blockade with atenolol did not improve endothelial function in several studies [33,34,36,47]. ARBs normalized endothelial function in early type 2 diabetes [48]; however, in advanced type 2 diabetes with hypertension, an ARB added to prior antihypertensive therapy, including ACEIs and CCBs, did not have beneficial effects on the endothelium despite the fact that structure was partially corrected [42]. Endothelial dysfunction may be more difficult to correct than the structure of the blood vessel even if BP is lowered. How different NADPH oxidases (nox1, nox2 and nox4, nox5) and their subunits in both layers of the vascu-

lar wall participate in these different effects of antihypertensive drugs remains to be established [49].

Signaling pathways for small artery remodelling

Inhibition of the RAAS contributes to improvement of vascular structure and function by blocking growth-promoting, pro-oxidant and pro-inflammatory actions of angiotensin (Ang) II [50]. Vasodilatation [51] may also play a role [52], by counteracting the small artery remodelling that results from vasoconstriction [19]. Vasodilators include ACEIs, ARBs or CCBs, and these all improve the remodelling of the vasculature. Atenolol, which induces peripheral vasoconstriction [53], has repeatedly failed to improve small artery remodelling despite its BP-lowering action [33,34,36,47].

Blockade (by ARBs) or lack of activation of AT₁ receptors, because of reduction of circulating concentrations of Ang II under treatment with ACEIs, will abrogate intracellular signaling. Accordingly, the concentration of intracellular calcium will be reduced and activation of calcium-dependent kinases, such as Pyk-2, a member of the focal adhesion kinase family, will decrease, resulting in decreased activation of NADPH oxidase, decreased production of reactive oxygen species (ROS), abrogation of PDGF and EGF receptor transactivation and activity of the MAP kinases, including ERK1/2, p38MAPK, MAPKAP kinase 2 (MK2) [15,54] and c-Jun N-terminal kinase (JNK) as well as JAK/STAT [15,50,55]. Nuclear translocation of proto-oncogenes, such as c-fos, c-jun and c-myc, and other transcription factors is blunted, resulting in decreased VSMC growth and remodelling. The inflammatory response stimulated by Ang II is diminished, with reduced activation of NF- κ B and adhesion molecule expression. Immune cell infiltration of adventitia and perivascular fat is decreased. Reduction in the stimulation of TGF- β results in decreased fibrosis, oxidative stress and inflammation, all actions that contribute to regress small artery remodelling. Aldosterone may induce effects similar to those of Ang II. In mouse VSMC, which have AT_{1a} and AT_{1b} receptors, stimulation of ERK1/2 and JNK by aldosterone requires a functional AT_{1a}R, whereas NF- κ B phosphorylation requires both a functional AT_{1a}R and AT_{1b}R [56]. On the other hand, a functional mineralocorticoid receptor (MR) is needed for Ang II stimulation of NF- κ B phosphorylation, and AT_{1a}R, AT_{1b}R and MR are necessary for nuclear translocation of the NF- κ B in response to aldosterone or Ang II. AT_{1a}R is necessary for aldosterone-induced c-fos gene transcription. The latter has been confirmed *in vivo*, as *agtr1a*^{-/-} failed to develop aldosterone-induced hypertrophic remodelling and endothelial dysfunction of mesenteric small arteries [57], resembling previous findings where the ARB,

losartan, blocked the *in vivo* cardiac pro-fibrotic effects of aldosterone [58].

We have also studied the role of ET-1 in experimental and human hypertension. Salt-sensitive hypertensive models, such as deoxycorticosterone acetate (DOCA)-salt rats and DOCA-salt hypertension in SHR, have enhanced ET-1 expression in the endothelium [59,60], associated with hypertrophic remodelling and endothelial dysfunction that was corrected by ET antagonists [14]. Thus, hypertrophic remodelling is a signature of the effects of ET in the hypertensive process [12,13]. Aldosterone infusion increased ET-1 expression and induced hypertrophic vascular remodelling with ECM deposition of collagen types I and III and fibronectin [21] by modulating the promoter of the ET-1 gene [61]. Most models exhibiting an ET-1-mediated component are either salt-sensitive or have severe elevation of BP. Indeed, patients with stage-2 hypertension have increased expression of preproET-1 mRNA in endothelium of gluteal subcutaneous small arteries [62]. In a mouse that overexpressed human preproET-1 in the endothelium (eET-1 mouse), although BP was not elevated resistance arteries exhibited hypertrophic remodelling and endothelial dysfunction [63], with enhanced NADPH oxidase activity and ROS generation, and up-regulated of inflammatory mediators [64]. On a high-salt diet, eET-1 mice presented greater increase in BP compared to wild-type mice [65], associated with enhanced oxidative stress and endothelial dysfunction. Use of an ET_A antagonist improved the effects of the high-salt diet, whereas an ET_B antagonist worsened them. DNA microarrays of blood vessels from eET-1 had increased expression of lipid metabolism genes [66]. Four of these could be validated by qPCR (*cyp51*, *dgat2*, *scd1* and *elovl6*), supporting a role of ET-1 in atherosclerosis. We then crossed the eET-1 mice with apoE^{-/-} mice and fed all the mice a high-fat diet. Atherosclerotic plaques increased in the eET-1/ apoE^{-/-} crossed mice to a great extent than in apoE^{-/-}. There was also a rise in BP. Thus, we concluded that endothelial ET-1 expression accelerated progression of atherosclerosis and could link atherosclerosis and hypertension. We have now generated an inducible mouse model, using Cre-Lox technology, that overexpresses human ET-1 in the endothelium [67]. This mouse has elevated BP, which is lowered by ET_A receptor blockers. In chronic experiments, these mice exhibit stiffer arteries, albeit without structural or functional remodelling (S. Coelho, P. Paradis, E.L. Schiffrin, unpublished observations, 2015); however, in the long-term experiments, renal blood flow is compromised, which can contribute to the persistent elevation of BP.

Obesity, metabolic syndrome and diabetes

We demonstrated that peroxisome proliferator activator receptors (PPAR) α [68] and PPAR γ agonists [69] protect rat vessels from the growth-promoting, inflammatory and oxidant effects of Ang II. We have now generated an inducible VSMC-selective PPAR γ gene deletion mouse by crossing PPAR γ floxed mice with mice that express tamoxifen-inducible Cre recombinase, under the control of a smooth muscle myosin heavy chain promoter [70]. Deletion of the PPAR γ gene in VSMC significantly exaggerated Ang II-induced vascular remodelling and endothelial dysfunction by enhancing vascular superoxide generation and inflammatory response, confirming a vascular protective role for PPAR γ . We believe that this should be taken into account for development of novel therapeutic agents for cardio-metabolic disease.

In New Zealand Obese (NZO) mice, which are a model of metabolic syndrome with a mild elevation of BP, we showed that resistance arteries have hypertrophic vascular remodelling and endothelial dysfunction [71]. The latter can be improved by the superoxide dismutase mimetic, Tempol. The vessels of these mice showed evidence of eNOS uncoupling, with increased superoxide and peroxynitrate, increased NADPH oxidase activity, enhanced TNF α expression, adhesion molecule expression, immune cell infiltration, and diminished adiponectin production in perivascular adipose tissue (PVAT), as well as adipocyte hypertrophy. PVAT from control NZB mice induced a reduction of norepinephrine vasoconstriction, but was unaffected by PVAT from NZO mice. This indicates that NZB PVAT has anti-contractile properties that are absent in NZO mice, possibly due to a deficiency in adiponectin [71,72]. With Italian colleagues, we studied severely obese individuals and showed that despite not being hypertensive, they presented with hypertrophic small artery remodelling [89], in agreement with the experimental results we published on NZO mice [73].

Immunity, the microbiome and cardiovascular injury

The prevalence of cardiovascular disease [74] and hypertension [75] is elevated in inflammatory and immune diseases. Indeed, low-grade inflammation participates as a mechanism of cardiovascular injury and BP elevation [76]. It is unclear how inflammation and immune mechanisms are activated in hypertension. Genetic susceptibility and excess salt, obesity and other environmental conditions may act on the brain to stimulate the sympathetic nervous system, inducing direct actions or acting indirectly via small rises in BP. The BP rise could generate damage-associated molecular patterns (DAMPs) recognized by pattern-recognition receptors (PRRs) that activate

innate immunity, and also neo-antigens that may activate adaptive immunity. The enhanced sympathetic activity may, by itself, influence the immune system. There is increasing evidence of interactions between the sympathetic system, the intestinal microbiome and the very abundant immune cells in the wall of the intestine, which are a source of systemic T regulatory and Th17 lymphocytes, and these lymphocytes may then migrate to secondary lymphoid organs, such as the lymph nodes or the spleen. Treg/T effector (Th1 or Th17) ratios may fluctuate with ratios of Bacteroidetes (protective)/Firmicutes (inflammatory), contributing to hypertensive or normotensive phenotypes [77]. As well, infectious agents acting through pathogen-associated molecular patterns (PAMPs), recognized by toll-like receptors (TLRs), could also contribute to activation of immune responses. Activation of dendritic cells leads to generation of isoketals that then stimulate T lymphocytes [78]. Effector T lymphocytes may be activated through other antigen presenting cells such as macrophages [79-81]. Upsetting the balance between T effector and T regulatory lymphocytes leads to an inflammatory response that contributes to cardiovascular injury. We demonstrated, in osteopetrotic mice that have a mutation in the *monocyte/macrophage colony stimulating factor* (*csf1*) gene, that Ang II infusion [79] or DOCA-salt [80] fail to induce vascular injury, endothelial dysfunction or elevate BP in homozygous *csf1^{Op/Op}* mice, whereas in heterozygous *csf1^{Op/+}* effects were intermediate, which confirms that innate immunity is involved in BP elevation and vascular remodelling. Wenzel *et al.* [81] elegantly extended these results, demonstrating that in mice with inducible expression of diphtheria toxin receptor (LysMiDTR mice), depletion of lysozyme M-positive (LysM^E) myelomonocytic cells by low-dose diphtheria toxin prevented Ang II-induced vascular dysfunction, endothelial dysfunction and BP elevation.

Adaptive immunity may also be involved in hypertension and vascular injury. Indeed, a blunted Ang II-induced BP rise, aortic and small artery remodelling and vascular oxidative stress were found in *Rag1^{-/-}* mice that are deficient in T- and B-lymphocytes [82]. Adoptive transfer of effector T cells, but not B lymphocytes from control mice, corrected these abnormal responses of *Rag1^{-/-}* mice. The potential role of Th17 lymphocytes has been highlighted by a study that showed that Ang II infused into *IL17^{-/-}* mice and induced an elevation of BP that was less sustained, with less T cell infiltration in the perivascular fat and less superoxide production in the aortic rings [83]. As well, Th17 lymphocytes are involved in the stiffening of the aorta that occurs during hypertension in the mouse [84].

Salt-sensitive hypertension may have an enhanced adaptive immune mechanism. We studied consomic rats (SSBN2) bear-

ing chromosome 2 from normotensive Brown Norway (BN) rats on a Dahl salt-sensitive genetic background. Chromosome 2 bears loci for many pro-inflammatory genes, such as IL-2, IL-6 receptor, fibroblast growth factor 2, VCAM-1 and angiotensin AT_{1b} receptor [85]. The consomic strain exhibited less vascular inflammation and remodelling [86], particularly in response to high salt intake [87], associated with up-regulation of CD4⁺CD25⁺ and CD8⁺CD25⁺ lymphocytes (Treg) numbers and their activity, expression Treg of the transcription factor responsible for Treg maturation (Foxp3), and enhanced production of the anti-inflammatory mediators IL-10 and TGF β by Treg. The vasculature of Dahl salt-sensitive rats showed up-regulation of inflammatory cytokines (IL-1 β , IL-2, IL-6, TNF α and IFN- γ), expressed low levels of Foxp3b and did not produce TGF- β and IL-10. We have also found that adoptive transfer of Treg from untreated mice into Ang II-infused mice lowered their telemetric systolic BP, reduced small artery stiffness, decreased generation of superoxide and immune cell infiltration in vascular and perivascular tissue and improved endothelial dysfunction [88]. More recently [89], we injected *Rag1^{-/-}* mice with T cells from wild-type or Scurfy mice, which lack Treg, or wild-type Treg, alone or with Scurfy T cells, and then infused them with Ang II. Ang II increased systolic BP in all groups, but increased diastolic BP only in wild-type and Scurfy groups. Ang II induced endothelial dysfunction, microvascular remodelling and stiffness, and oxidative stress in perivascular adipose tissue in mesenteric artery of wild-type T cell-injected *Rag1^{-/-}* mice, but to a much greater degree in Scurfy T cell-injected *Rag1^{-/-}* mice. Angiotensin II increased monocyte chemotactic protein-1 expression in the vascular wall and PVAT, monocyte/macrophage infiltration and pro-inflammatory polarization in PVAT and the renal cortex, and T cell infiltration in the renal cortex only in Scurfy T cell-injected *Rag1^{-/-}* mice. Wild-type Treg co-injection with either the vehicle or with Scurfy T cells prevented or reduced the effects of angiotensin II. In conclusion, Treg counteracted angiotensin II-induced microvascular injury by modulating innate and adaptive immune responses. Aldosterone induced effects similar to Ang II, except that adoptive transfer of Treg from untreated mice did not lower BP, and hypertrophic remodelling was corrected [90], indicating that the effects may be independent of hemodynamics. The effects could be mediated, in part, by the anti-inflammatory actions of IL-10.

Conclusion

The different mechanisms that contribute to the remodelling of small arteries in hypertension, metabolic disease and other cardiovascular diseases have been progressively identified. This

will, hopefully, allow for the identification of new biomarkers and therapeutic targets, leading to novel therapies to treat hypertension and other cardiovascular diseases and result in improved outcomes by reducing target organ injury.

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