Vascular inflammation in hypertension and diabetes: molecular mechanisms and therapeutic interventions

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ABSTRACT
More than 80% of patients with Type 2 diabetes mellitus develop hypertension, and approx. 20% of patients with hypertension develop diabetes. This combination of cardiovascular risk factors will account for a large proportion of cardiovascular morbidity and mortality. Lowering elevated blood pressure in diabetic hypertensive individuals decreases cardiovascular events. In patients with hypertension and diabetes, the pathophysiology of cardiovascular disease is multifactorial, but recent evidence points toward the presence of an important component dependent on a low-grade inflammatory process. Angiotensin II may be to a large degree responsible for triggering vascular inflammation by inducing oxidative stress, resulting in up-regulation of pro-inflammatory transcription factors such as NF-κB (nuclear factor κB). These, in turn, regulate the generation of inflammatory mediators that lead to endothelial dysfunction and vascular injury. Inflammatory markers (e.g. C-reactive protein, chemokines and adhesion molecules) are increased in patients with hypertension and metabolic disorders, and predict the development of cardiovascular disease. Lifestyle modification and pharmacological approaches (such as drugs that target the renin–angiotensin system) may reduce blood pressure and inflammation in patients with hypertension and metabolic disorders, which will reduce cardiovascular risk, development of diabetes and cardiovascular morbidity and mortality.

INTRODUCTION
The frequency of diabetes mellitus is increasing rapidly worldwide even in developing countries [1,2]. Hypertension often co-exists with diabetes, such that 60% of patients with diabetes are hypertensive, and up to 20% of subjects with hypertension are diabetic [3]. Patients with diabetes and hypertension are at high risk, and require effective BP (blood pressure)-lowering. Indeed, diabetes mellitus doubles the risk of cardiovascular events in patients with hypertension [4–6]. In patients with Type 2 diabetes and in hypertension, the pathophysiology of cardiovascular disease is multifactorial, and endothelial dysfunction, vascular inflammation and increased urine albumin excretion develop with time and are independently associated with mortality [7]. The endothelium is the organ that bridges several cardiovascular risk factors (e.g. hypertension, dyslipidaemia, smoking, diabetes and congestive heart failure) and may be at the initiation of the development of vascular...
inflammation and atherosclerosis [8–10]. Endothelial dysfunction is characterized by impaired vasomotor response (reduced vasodilation and increased endothelium-dependent contraction), cell proliferation, platelet adhesion/aggregation, vascular permeability and leucocyte–endothelial interactions that participate in vascular inflammation. Endothelial dysfunction is often associated with microalbuminuria [11], and the latter has been considered by some to be a manifestation of impaired endothelial function. Microalbuminuria is perhaps the most important indicator of the initiation of systemic vascular injury and associated target organ damage.

Hypercholesterolaemia, hypertension and insulin resistance contribute to endothelial dysfunction and inflammation in the vascular wall, as well as to increased lipoprotein oxidation, smooth muscle cell proliferation, extracellular matrix deposition, cell adhesion and thrombus formation, all processes involved in the development of atherosclerosis and ischaemic heart disease [10,12,13]. Endothelial dysfunction promotes vascular inflammation by inducing the production of vasoconstrictor agents, adhesion molecules and growth factors [10,13]. Thus inflammation is a central mechanism contributing to the progression of cardiovascular disease, and may be involved in the triggering of myocardial ischaemia [13,14].

Patients with cardiovascular disease present with increased expression and plasma concentration of inflammatory markers and mediators [14,15], which include CRP (C-reactive protein) and adhesion molecules, such as selectins (P-selectin, E-selectin and L-selectin), ICAM-1 (intercellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1). Moreover, increased plasma levels of the primary inflammatory cytokine TNF-α (tumour necrosis factor-α), and the secondary inflammatory cytokine IL (interleukin)-6, as well as ICAM-1, VCAM-1, E-selectin, vWF (von Willebrand factor) and CRP, have been demonstrated in patients with hypertension [16]. High levels of inflammatory mediators, particularly IL-6, ICAM-1 and CRP, may be independent risk factors for the development of hypertension [17,18]. They may also be associated with increased risk of diabetes [19] and cardiovascular disease. Inflammation measured by these markers, mainly CRP, may be included in the definition of the metabolic syndrome, a constellation of abnormalities (including abdominal obesity, high blood glucose/impaired glucose tolerance, dyslipidaemia and high BP) that increase the risk of overt diabetes mellitus and cardiovascular events [20]. In the UKPDS (United Kingdom Prospective Diabetes Study), the incidence of complications of diabetes was strongly associated with elevated BP [6], and tight BP control in hypertensive patients with Type 2 diabetes decreased the risk of macrovascular disease, stroke and deaths related to diabetes [22]. Hence lowering BP as well as therapeutic approaches to control vascular inflammation, particularly in patients with glucose intolerance or diabetes, may provide significant clinical benefits.

In this review, we focus on the pathophysiological role of low-grade inflammation in the vasculature of patients with hypertension and diabetes, as well as the role of inflammatory markers in cardiovascular disease, in view of potential therapeutic interventions to reduce cardiovascular risk.

**RAAS (RENIN–ANGIOTENSIN–ALDOSTERONE SYSTEM) AND VASCULAR INFLAMMATION**

Activation of RAAS plays a key role in the development and pathophysiology of cardiovascular disease by several mechanisms [9]. Ang II (angiotensin II) is one of the final products and the main known mediator of the renin–angiotensin system. Ang II induces vascular injury through several mechanisms, including vasoconstriction, cell growth, oxidative stress production and inflammation. Ang II modulates vascular inflammation by inducing cytokine release [23] and pro-inflammatory transcription factors such as NF-κB (nuclear factor κB) [24]. NF-κB, in turn, regulates adhesion molecule (VCAM-1 and ICAM-1) and cytokine expression in several cell types [9,25]. These molecules induce and maintain inflammation within the vascular wall, stimulate deposition of extracellular matrix and promote hypertrophy and/or hyperplasia of VSMCs (vascular smooth muscle cells) [26]. Ang II also stimulates the production of PAI-1 (plasminogen-activator inhibitor-1) [27], which contributes to the prothrombotic state as well as to atherosclerotic plaque rupture. Moreover, Ang II is involved in atherosclerotic lesion progression and plaque instability by stimulating the activation of MMPs (matrix metalloproteinases), which can digest the fibrous cap and thereby participate in the triggering of plaque rupture [28].

ROS (reactive oxygen species) act as signalling molecules, modulating vascular tone and structural changes in the circulation [29], and participate in the development and progression of atherosclerosis [30]. ROS [mainly O_2•− (superoxide) and H_2O_2] activate multiple signalling molecules including MAPKs [mitogen-activated protein kinases; (p38MAPK, JNK (c-Jun N-terminal kinase), ERK (extracellular-signal-regulated kinase)-1/2 and ERK-5], non-receptor tyrosine kinases [Src, JAK-2 (Janus kinase-2), STAT (signal transducer and activator of transcription), p21Ras, Pyk-2 and Akt], receptor tyrosine kinases [EGFR (epidermal growth factor receptor), IGFR (insulin-like growth factor receptor) and PDGFR (platelet-derived growth factor receptor)], protein tyrosine phosphatases and redox-sensitive transcription factors [NF-κB, AP-1 and HIF-1 (hypoxia-inducible factor-1)] (Figure 1) [26,29]. The major source of vascular ROS is NADPH oxidase, which is expressed in endothelial cells, VSMCs, fibroblasts and monocytes/macrophages.
Ang II, ET (endothelin)-1 and inflammatory mediators can modulate basal NADPH oxidase-induced \( \text{O}_2^{•−} \) production by the expression of NADPH oxidase subunits [33]. Ang II-mediated activation of NADPH oxidase [34] involves cSrc, PKC (protein kinase C), PLA2 (phospholipase A2) and PLD (phospholipase D) pathways, as well as increased synthesis of most of its subunits [29,35]. In hypertension, these processes may be enhanced [33], contributing to increased activation of NADPH oxidase and consequent oxidative stress in the vascular wall [9].

Increased ROS levels in hypertension impair endothelium-dependent vascular relaxation by reducing NO (nitric oxide) bioavailability [34] and increasing vascular
contractile responses. Ang II-stimulated NADPH oxidase induces ICAM-1 expression, macrophage infiltration and vascular hypertrophy, independently of BP elevation [36]. Macrophages infiltrating the adventitia or the media of blood vessels may mediate oxidative stress generated by NADPH oxidase, also in response to elevated BP [36]. We recently reported [37] that osteoporotic mice deficient in mCSF (murine colony-stimulating factor) and accordingly monocytes/macrophages in the vascular wall present less oxidative stress and less induction of inflammatory molecule up-regulation in the vasculature by Ang II, and develop less endothelial dysfunction and vascular remodelling, suggesting a central role for macrophages and pro-inflammatory mediators in Ang II-induced vascular injury.

Ang II induces aldosterone synthesis through stimulation of AT1 (Ang II type 1) receptors in the adrenal cortex. Aldosterone increases tissue ACE (angiotensin-converting enzyme) activity [38] and up-regulates angiotensin receptors [39], which suggests the existence of a vicious cycle that may potentiate the effect of the RAAS. The activation of mineralocorticoid receptors may contribute to cardiovascular dysfunction, inflammation, fibrosis and vascular damage. Several animal models have confirmed that aldosterone and other mineralocorticoids can cause injury of the vasculature of the brain, heart and kidneys by inducing ROS formation and endothelial dysfunction [40]. Mineralocorticoid antagonist attenuates this damage by mechanisms that appear to be independent of changes in BP, involving direct pro-inflammatory and pro-fibrotic effects that may involve activation of the ET system [41–44]. Mineralocorticoid receptor blockade also improved endothelial function and reduced oxidative stress in Ang II-infused rats [45], suggesting that aldosterone induces actions usually attributed to direct effects of Ang II. Moreover, aldosterone may induce endothelial dysfunction and inflammation through activation of COX-2 (cyclo-oxygenase-2) in normotensive and hypertensive rats [46].

Ang II-induced inflammation via NF-κB and AP-1 activation involves, in part, ET receptors [47]. ROS are potent stimulators of ET-1 synthesis by endothelial cells and VSMCs [48]. ET-1 activates NADPH oxidase, as well as other sources of ROS, including xanthine oxidase and mitochondria, to produce increased oxidant stress in VSMCs and blood vessels [49–52]. ET-1-induced oxidative stress elicits inflammatory responses and contributes to the vascular remodelling and endothelial dysfunction found in hypertensive models that exhibit an ET-mediated component [53]. ETA receptor antagonism decreases oxidative stress, normalizes hypertrophic remodelling, decreases collagen and fibronectin deposition, and reduces ICAM-1 levels in the vasculature of aldosterone-infused rats [44]. When human preproET-1 was transgenically overexpressed in the endothelium, mice exhibited endothelial dysfunction and increased activity of NADPH oxidase, leading to enhanced oxidative stress and vascular inflammation [51].

Considering the pro-inflammatory effects of Ang II and aldosterone, agents that interfere with the components of RAAS, such as ACE inhibitors, ARBs (angiotensin receptor blockers) and mineralocorticoid receptor antagonists (spironolactone or the more selective eplerenone), represent logical therapeutic tools to reduce vascular inflammation and cardiovascular risk, as suggested in large clinical trials in patients with hypertension and diabetes. However, BP reduction by itself may also influence inflammation, since calcium channel blockers not only decreased BP in patients with hypertension, but also reduced plasma concentrations of ICAM-1, E-selectin and vWF [54].

**INFLAMMATORY MARKERS, CLINICAL OUTCOME AND THERAPEUTIC BENEFITS IN CARDIOVASCULAR PATIENTS**

**CRP**

Endothelial damage/dysfunction is associated with markers of inflammation in patients with hypertension and/or diabetes. hsCRP (high-sensitivity CRP) has been related to insulin resistance, systolic BP, pulse pressure and hypertension [55,56], and to markers of endothelial dysfunction (plasma levels of vWF, tissue plasminogen activator and cellular fibronectin) [57]. Elevated levels of CRP predict development of the metabolic syndrome, at least in women [58]. This association is even stronger when combined with BMI (body mass index). hsCRP and PAI-1 levels are elevated in subjects with insulin resistance, with levels that are higher than in patients with coronary artery disease [59]. Thus low-grade inflammation in the pre-diabetic state is associated with increased insulin resistance. Although subjects with insulin resistance had greater adiposity, levels of hsCRP were not influenced by BMI. However, hsCRP levels were higher among the high-BMI subgroup of subjects who did not develop diabetes during follow-up.

Numerous epidemiological studies have shown that plasma hsCRP level is a powerful predictor of ischaemic cardiovascular events in patients with stable or unstable angina, appears to correlate with softer plaques that are more prone to rupture, and may even predict cardiovascular events among apparently healthy subjects [60,61]. It may thus be useful in targeting medium-risk patients who could benefit from aggressive cardiovascular preventative therapy. Furthermore, hsCRP levels are positively associated with systolic BP, pulse pressure and incident hypertension [55]. Thus CRP and high BP in combination have additional predictive value for cardiovascular outcomes, as they contribute as independent determinants of cardiovascular risk.
CRP may be more than an inflammatory marker of increased cardiovascular risk. CRP has been demonstrated in atherosclerotic plaques and appears to be involved in foam cell formation, promotes monocyte chemotaxis and facilitates LDL (low-density lipoprotein) uptake by macrophages in vitro [62,63]. In endothelial cells, CRP facilitated the release of PAI-1 [64] and ET-1 [65], and increased the expression of cell adhesion molecules [66], reduced NO bioavailability [67] and NO-mediated dilation in the vasculature. In VSMCs, CRP induced the expression of AT1 receptors and enhanced AT1 receptor-mediated ROS formation, which reduced NO bioavailability [68] and activated stress-activated p38 MAPK and JNK [69]. However, CRP may also have anti-inflammatory actions by inhibiting neutrophil activation and adhesion [70] and blocking platelet aggregation in vitro [71].

A Mediterranean-style diet has beneficial effects on endothelial function and vascular inflammatory markers in patients with metabolic syndrome. Patients consuming a Mediterranean-style diet had a significant reduction in serum concentration of hsCRP, IL-6, IL-7 and IL-18, as well as in insulin resistance [72]. Exercise training, together with weight loss, reduced hsCRP levels significantly, although not in proportion to weight reduction [73]. CRP decrease was observed in the middle weight-reduction quartile, suggesting that there may be an optimal link between exercise and weight loss with respect to the inflammatory status [73].

In patients with high LDL-cholesterol levels, those with low hsCRP have better clinical outcomes than those with higher levels [74,75]. Fenofibrate lowered hsCRP levels in patients with hypertriglyceridaemia or combined hyperlipidaemia [76]. Statin-induced reduction of atherosclerosis progression was significantly related to greater reduction of hsCRP levels. Simvastatin or atorvastatin induced the reduction of CRP in coronary patients with hyperlipidaemia [77]. Interestingly, in patients with diabetes with hypercholesterolaemia, simvastatin combined with the ACE inhibitor ramipril significantly reduced CRP levels more than monotherapy alone [78].

CRP-induced up-regulation of the AT1 receptor was attenuated by losartan [69]; on the other hand, losartan, irbesartan nor candesartan reduced serum CRP significantly in patients with hypertension [79]. A recent study, the Val-MARC (Valsartan-Managing blood pressure Aggressively and evaluating Reductions in hsCRP) study, has suggested that valsartan, but neither hydrochlorothiazide nor both combined, reduced CRP slightly [80].

Other agents may have anti-inflammatory vascular actions, such as the PPAR (peroxisome-proliferator-activated receptor-γ) agonist rosiglitazone, which reduced CRP levels in patients with Type 2 diabetes [81]. Rosiglitazone also inhibited the CRP-induced attenuation of survival, differentiation and function of endothelial progenitor mediated, in part, by reducing the expression of eNOS (endothelial NO synthase) [82].

Cytokines (TNF-α and ILs)

TNF-α is a primary inflammatory cytokine secreted by several cell types involved in vascular inflammation (endothelial cells, VSMCs and macrophages). It enhances monocyte recruitment into atherosclerotic lesions in early stage atherosclerosis [83]. Patients with increased risk of recurrent coronary events have persistently elevated plasma levels of TNF-α. Weight loss in obese patients reduced plasma levels of TNF-α [84]. In patients with hyperlipidaemia, treatment with simvastatin and the PPAR-γ agonist fenofibrate significantly reduced plasma levels of TNF-α [85]. Moreover, in patients with hypertension or obese subjects, candesartan or rosiglitazone lowered TNF-α [86,87].

IL-1 is an inflammatory cytokine involved in the early stages of the inflammatory process. Plasma levels of IL-1 are elevated in patients with coronary artery disease [88]. IL-6 is a secondary inflammatory cytokine which induces the increase of plasma concentrations of fibrinogen, PAI-1 and CRP and, in healthy men, elevated levels of IL-6 are associated with increased risk of future myocardial infarction [89]. IL-18 is an IFN-γ (interferon-γ)-inducing factor, which is one of the strongest predictors of cardiovascular death as well as of development of Type 2 diabetes [90,91]. Weight loss, a Mediterranean-style diet and exercise reduce serum concentrations of IL-6, IL-7 and IL-18 in obese subjects [72,92]. Statins induce a significant reduction in IL-1 and IL-6 levels [93]. Inhibition of RAAS with either enalapril or losartan in patients with stable angina contributed to a decrease in the release of IL-1 and IL-6 [94]. PPAR-α ligands inhibit production of IL-6 as well as prostaglandins and COX-2. These effects occur as a consequence of PPAR-α-induced inhibition of signalling by the pro-inflammatory mediator NF-κB and induction of apoptosis [95]. The action of PPAR-γ activators on inflammatory markers is a matter of controversy. Some studies have demonstrated that PPAR-γ activators inhibit the expression of TNF-α, IL-1 and IL-6 [96]. On the other hand, rosiglitazone did not reduce IL-6 levels in patients with Type 2 diabetes, and may enhance inflammatory responses in epithelial cells by potentiating TNF-α-induced production of IL-6 and/or IL-8 [97], although it reduces CRP levels [81,96]. Nevertheless, several studies have demonstrated that activation of PPAR-γ exerts anti-atherosclerotic actions [98].

Adhesion molecules, CD40 ligand and MCP-1 (monocyte chemoattractant protein-1)

Adhesion molecules, which regulate leucocyte migration into the vascular wall, have been implicated in
atherosclerosis, thrombosis and restenosis following coronary angioplasty [99]. Increased expression of ICAM-1, E-selectin and VCAM-1 have been demonstrated in the media of blood vessels in experimental models of hypertension, including SHR (spontaneously hypertensive rats) and Ang II-infused rats [37,100]. Serum concentrations of VCAM-1 and ICAM-1 are increased in subjects with systemic vascular inflammation, Type 2 diabetes and cardiovascular disease. A stepwise increase of age-adjusted soluble ICAM-1 and VCAM-1 levels was shown across tertiles for carotid intima-media thickness [101]. In the Physician’s Health Study, subjects with ICAM-1 levels in the highest quartile presented higher cardiovascular risk than patients in the lowest quartile [102]. Moreover, patients with coronary artery disease have serum concentration of adhesion molecules higher than healthy control subjects [103].

Soluble ICAM-1 and E-selectin have been positively correlated with central obesity. In obese subjects, a weight reduction programme by diet and physical exercise induced reductions of ICAM-1 and E-selectin, together with body fat, after 3 months [104]. The benefit of lifestyle modification on metabolic and inflammatory parameters was also recorded in postmenopausal women who after a 2-week high-fibre and low-fat diet together with aerobic exercise, had reduced serum glucose concentrations, improved insulin sensitivity and lowered hsCRP and ICAM-1 levels [105].

Several pharmacological approaches to improve metabolic parameters and BP induce favourable effects on adhesion molecules, as for other inflammatory markers discussed above. In patients with hypercholesterolaemia, simvastatin as well as candesartan significantly reduced ICAM-1 levels [106,107]. In subjects with hypertriglyceridaemia, the PPAR-α agonist fenofibrate induced a reduction in serum adhesion molecules [108]. In non-diabetic patients with coronary artery disease, the PPAR-γ activator rosiglitazone had a neutral effect on ICAM-1 and VCAM-1, as well as on flow-mediated dilation, although it reduced insulin resistance, hsCRP and vWF significantly [109].

CD40 ligand is a pro-inflammatory molecule involved in atherothrombotic processes. It is expressed on T-lymphocytes (CD4+) and activated platelets, and interacts with CD40 expressed on endothelial cells [110]. Plasma levels of soluble CD40 ligand are particularly elevated in patients with unstable angina [111], and represent an independent predictor of cardiac death or recurrent myocardial infarction in patients with acute coronary syndrome [112]. In patients with hypercholesterolaemia, statins reduced plasma levels of CD40 ligand [113], possibly by reducing NF-κB signalling [114]. Moreover, ARBs lowered serum levels of CD40 ligand in patients with hypertension, independently of BP reduction [115]. In diabetic ischaemic patients, PPAR-γ activators induced the same favourable effect [116]. MCP-1 is a member of the C-C chemokine family and is a potent chemotactic factor for monocytes [117]. It is produced constitutively, or after induction by oxidative stress, cytokines or growth factors, by a variety of cell types, including monocytes, smooth muscle cells and endothelial cells [118]. Increased expression of MCP-1 mRNA or protein has been observed in animals and humans with arteriosclerosis or atherosclerosis [119]. MCP-1 levels are elevated in patients with angina, and a further increase was observed in patients with unstable angina [120]. Physical exercise, mainly when combined with statin therapy, reduced MCP-1 levels in subjects with metabolic syndrome [121]. In patients with hypertension, ARBs reduced MCP-1 levels [122]. In the presence of hypercholesterolaemia in patients with hypertension, combination therapy with statins and either ARBs or ACE inhibitors was better than monotherapy in reducing MCP-1 levels [123,124]. Finally, PPAR-γ activators have beneficial effects on MCP-1, as demonstrated in experimental [125] and clinical settings [81].

CONCLUSIONS

Low-grade inflammation occurs in the vasculature in several conditions that predispose to cardiovascular disease, including hypertension and diabetes. Non-pharmacological (weight loss, exercise and Mediterranean-style diet) and pharmacological approaches (statins, ACE inhibitors and ARBs) reduce vascular inflammation in patients with diabetes and hypertension, resulting in reduced cardiovascular events in randomized clinical trials. Novel therapeutic approaches with PPAR-α and PPAR-γ activators have increasingly been demonstrated to exert cardiovascular-protective effects, independently from their metabolic actions. Indeed PPAR activators lowered BP, induced favourable effects on the heart and corrected endothelial dysfunction through antioxidant, anti-inflammatory, antiproliferative, antihypertrophic and antifibrotic effects. Although this class of drugs could become useful in the prevention of cardiovascular disease, recent studies with dual PPAR activators have cast doubts on their clinical efficacy in cardiovascular prevention compared with the original PPAR activators currently marketed. This being said, there is increasing evidence of the pathophysiological role played by inflammation in the progression of cardiovascular and metabolic disease and in the triggering of cardiovascular events, and the need to counter these mechanisms pharmacologically to improve cardiovascular outcomes.

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