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Endothelial dysfunction in hypertension: The role of arginase

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1. ABSTRACT

Essential hypertension is the leading risk factor for mortality worldwide, accountable for 13% of deaths globally. Despite numerous therapies available uncontrolled hypertension is still very prevalent today and a large subset are shown to have treatment resistant hypertension. Several cardiovascular diseases including hypertension result in endothelial dysfunction and inflammation. Once thought of as a passive barrier between blood flow and tissue the endothelium is now considered a main hub for maintaining vascular tone, structure and haemostasis. Several pathways occur in the endothelium that can result in dysfunction and altered vascular stasis. Such pathways include the impairment of the vasodilator nitric oxide (NO), increases in pro-inflammatory pathways such as ROS (reactive oxygen species) production and also recent reports suggest that the enzyme arginase, associated with the L-arginine-urea cycle, may be an important factor that is increased in hypertension. These pathways may offer alternative mechanisms to treat the complications associated with hypertension rather than the conventional therapies that aim to lower blood pressure.

2. INTRODUCTION

Vascular endothelial cells play a key role in the initiation, development and progression of many cardiovascular diseases particularly hypertension. As the major risk factor for mortality worldwide much research has gone into the pathways, causes and possible pharmacotherapy's involved in hypertension. Despite growing therapies to reduce blood pressure uncontrolled hypertension is still very prevalent today. Mechanical, functional and structural changes in the vasculature such as turbulent blood flow, fluid shear stress, and vascular remodelling results in endothelial dysfunction, which further increases these changes and are also commonly associated with increases in blood pressure. Inflammatory mechanisms are also gaining interest in the context of hypertension and hypertension related vascular complications however the exact pathways involved are not yet fully understood. This review discusses current findings on the pathophysiological pathways of endothelial dysfunction and inflammation and their contribution to hypertension induced cardiovascular complications.

3. HYPERTENSION

In the 2009 WHO Global Health Risks Report hypertension is identified as the leading risk factor for mortality worldwide (1). Accountable for 13% of deaths globally it is also listed in the top five causes of disability-adjusted life years. Hypertension is defined as persistently elevated systolic blood pressure (SBP) over 140 mmHg and diastolic blood pressure (DBP) over 90 mmHg. The prevalence of elevated blood pressure is estimated to be ~23% of the adult population and by 2025 this global burden is predicted to increase to 29% (2). With age and obesity identified as two major risk factors for hypertension, it will continue to be an even greater risk as longevity and weight gain of populations increase. Other risk factors include genetic, environmental, central nervous system, cardiac, renal, gastrointestinal and endocrine factors. With its strong association with inheritability, genome-wide association studies have been conducted to assess a genetic background with raised blood pressure. Several single-nucleotide polymorphisms and various genes have found to be associated with SBP (Chr 10, 11, 12), DBP (Chr 10, 12, 15) as well as hypertension (Chr 12) (3). Co-morbidities such as diabetes mellitus, dyslipidemia, coronary heart disease, and hypercholesterolemia all correlate with increases in blood pressure. Other factors such as stress and increased sympathetic nervous system activity stimulates the cardiovascular and renal systems to increase heart rate, cardiac output, insulin resistance, platelet activation, sodium retention, and augment vascular reactivity and vascular function leading to elevations in blood pressure and the progression of atherosclerosis.

Essential or idiopathic hypertension, the most common type, involves increased peripheral resistance to blood flow particularly in small resistance arteries. In these arteries vascular remodelling can occur and changes in structural, functional and mechanical mechanisms in these vessels leads to reduced lumen diameter and increased intimal thickening. This hypertrophic phenotype coupled with altered myogenic tone results in cardiovascular complications and damaging effects to target organs. Therefore it is not surprising that hypertension is the single biggest risk factor for incidence, development and progression of coronary heart disease, stroke, chronic heart failure and chronic kidney disease (4, 5). Furthermore with each 10 increments in blood pressure the risk for developing a cardiovascular event increases (6). Several lifestyle and drug treatments provide excellent therapy in the management of hypertension. Lifestyle recommendations include physical exercise, healthy body weight, and reduced alcohol consumption. Following the DASH study (Dietary Approaches to Stop Hypertension) (7) dietary recommendations involve reduced sodium intake and increased grains, vegetables, fruits and nuts, which have found to lower blood pressure. In obese patients undertaking the DASH diet, blood pressure was lower compared to those taking potassium, magnesium and fibre supplements (8). First-line pharmaceutical treatments include thiazide diuretics, beta-blockers, ACE (angiotensin converting enzyme) inhibitors, long-acting calcium channel blockers, or angiotensin receptor blockers (ARB) (9). Ideally, these drugs are used on their own but when optimal blood pressure levels are not reached these agents are used concomitantly. While no one class of antihypertensive treatment appears superior in reducing the risk of cardiovascular disease (10) some agents are more suitable in certain cardiovascular complications. For example, ACE inhibitors and ARB are recommended for chronic renal disease to control hypertension (11). Despite many effective antihypertensive agents and the recent developments of vaccines (particularly targeting the rennin-angiotensin aldosterone system) (12) uncontrolled blood pressure and its severe effects remains an ongoing issue today. Furthermore, while some antihypertensive treatments are effective in lowering blood pressure they may not be sufficient in helping target the vascular dysfunction or remodelling (13). Therefore targeting another aspect in the progression of hypertension may help to reduce cardiovascular morbidity particularly for those difficult to treat with current antihypertensive treatments.

4. RESISTANT HYPERTENSION

Resistant hypertension (RH), also termed ‘refractory hypertension’ or ‘treatment-resistant hypertension’, describes a subset of hypertensive patients that despite the use of 3 or more antihypertensive treatments usually including a diuretic, remain persistently above their goal blood pressure. RH also encompasses those patients that have controlled blood pressure but require 4 or more pharmacological treatments. However, RH should not be confused with uncontrolled elevated blood pressure, which is commonly misdiagnosed as RH and thus termed ‘pseudo-resistant hypertension’. Uncontrolled hypertension is a broad term for all hypertensive patients who cannot maintain their high blood pressure, this can be due to therapeutic inertia, poor compliance to the treatments, inappropriate or inadequate treatments prescribed, undiagnosed hypertension, or the white coat effect, which is the differences seen between clinical and home blood pressure measurements. Of these, poor adherence from the patient and therapeutic inertia that involves poor management from the physician appear to be the most common problems of uncontrolled hypertension. However, RH generally stems from those that are older, have increased adiposity, are diabetic (14) and have a history of uncontrolled elevated blood pressure.

To date the prevalence of treatment resistance hypertension has not yet been defined. Recent large antihypertensive trials with criteria including dose titration and monitored adherence provide the best estimation for determining prevalence (Table 1). The ALLHAT trial (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial) (15) with 33,000 participants and a 5 year follow-up demonstrated a third of participants still presented with uncontrolled blood pressure even with on average 2 antihypertensive medications. The proportion with elevated blood pressure despite 3 or more antihypertensive treatments was 27%. They show that DBP was a lot easier to control with ~92% demonstrating opitmal levels at the 5 year follow-up. The LIFE trial (Losartan Intervention For Endpoint reduction) (16) with

Table 1. Uncontrolled blood pressure results from recent clinical trials

Trial	Mean follow up	No. of participants	No. of anti-hypertensive drugs administered	Participants with uncontrolled BP at end of trial	Participants on greater than 3 anti-hypertensive drugs at end of trial	Reference
ALLHAT (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial)	5 years	33,357	1 or more	34.4 %	27.3 %	Cushman *et al.* (15)
LIFE (Losartan Intervention for Endpoint Reduction in Hypetension)	4.8 years	9,193	1 or more	53.5 %	24 %	Dahlof *et al.* (16)
INVEST (International Verapamil-Trandolapril Study)	2.7 years	22,576	2 or more	28.8 %	51.6 %	Pepine *et al.* (17)
CONVINCE (Controlled Onset Verapamil Investigation of Cardiovascular Endpoints)	3 years	16,602	1 or more	34.3 %	39.6 %	Black *et al.* (18)
ACCOMPLISH (Avoiding Cardiovascular Events Through Combination Therapy in Patients Living with Systolic Hypertension)	3 years	11,506	2 or more	26.1 %	--	Jamerson *et al.* (19)
HYVET (Hypertension in the Very Elderly Trial)	1.8 years	3,845	1-2	52 %	--	Beckett *et al.* (135)

just over 9000 participants demonstrated much higher incidence of uncontrolled blood pressure with those taking Losartan (an angiotensin-II receptor type 1 antagonist) where only 49% and 89% showed optimal SBP and DBP, respectively. Similarly of patients taking atenolol (beta blocker) only 46% and 89% demonstrated optimal levels. In a trial with 22,000+ participants specifically looking at hypertensive coronary artery disease, the INVEST trial (International Verapamil-Trandolapril Study) (17) compared a thiazide diuretic with a beta blocker to a calcium channel blocker with an ACE inhibitor. At 12 and 24 months half the participants in each group were still on 3 or more antihypertensive drugs and neither group was superior in lowering blood pressure. Similar results can found in the CONVINCE trial (Controlled Onset Verapamil Investigation of Cardiovascular End Points) (18) with 33% uncontrolled at 3 year follow-up and 18% with 3 or more antihypertensive agents; and more recently the ACCOMPLISH trial (Avoiding Cardiovascular Events Through Combination Therapy in Patients Living With Systolic Hypertension) (19), while demonstrating that by 6 months combination therapy is effective and safe, 20% still remained above goal.

Patients with RH are found to have a higher risk of target organ damage (20), increased left ventricular hypertrophy, increased carotid intima-media thickening, and a greater propensity of plaque development (21). Furthermore, aortic stiffening, sleep apnoea, and chronic kidney disease are all found to contribute to the risk of becoming resistant to treatment. Studies also suggest that primary aldosteronism may be a common cause of RH (20, 22, 23) with one study demonstrating 20% of RH patients presented with increased aldosterone (24). Generally, overproduction of aldosterone leads to sodium and water reabsorption and potassium excretion causing increased blood volume and hypertension. However hyperaldosteronism also results in leukocyte infiltration (25), increased expression of pro-inflammatory cytokines (26) and the production of reactive oxygen species leading to vascular fibrosis and remodelling.

5. ENDOTHELIAL STRUCTURE AND FUNCTION

Endothelial cells (EC) line the vasculature to form the endothelium, which acts as a semi-permeable monolayer between the lumen and the vessel wall. Their structure and integrity are essential in maintaining vascular tone and haemostasis. Prenatally these cells originate from same precursor as haematopoietic cells, the hemangioblast ($CD34^+$), which can differentiate into endothelial precursor cells ($VEGFR3^+$) to become vascular endothelial cells ($VEGF\text{-}R3^+$, podoplanin$^-$, $PAL\text{-}E^+$). Recent studies have shown that vasculogenesis also occurs postnatally with endothelial progenitor cells found to mobilize from the bone marrow (27) and other sites in the body including the peripheral blood, liver (28), and adipose tissue (29) to help repair and regenerate vessel walls in adults.

Despite the total mass of the endothelium only weighing between 100 – 500 g the amount of surface area exposed to blood flow is thought to be highly active and up to 350 m^2 (30). Through its paracrine, endocrine and autocrine functions the endothelium helps to regulate various cardiovascular processes. Blood wall exchanges occur through the abundant ion channels (K^+, Ca^{2+}, Na^+, Cl^-), G-proteins, caveolae and tyrosine kinase receptors in the plasma membrane lipid bilayer of the endothelium. Such exchanges include the release of vasomotor factors such nitric oxide (NO) or prostacyclin (PGI_2) that inhibit platelet aggregation and cause relaxation as well as release of endothelium-derived hyperpolarizing factor (EDHF) that leads to activation of outward K^+ currents causing vascular smooth muscle cell hyperpolarization (31). These responses result from stimuli such as thrombin, bradykinin, ADP or changes in blood flow or pressure. Conversely, vasoconstriction factors such as thromboxane A_2, endothelin-1, angiotensin-II (AngII), prostaglandins,

reactive oxygen species, free radicals, pro-coagulant, pro-thrombotic factors and pro-inflammatory mediators are stimulated during disease states leading to impaired endothelium-derived vasodilatation or endothelial dysfunction (ED).

Under basal conditions fluid shear stress and pulsatile stretch results in continuous release of compensatory vasoactive substances. The magnitude of endothelial shear flow in vessels is dependent on the velocity of blood flow, direction, obstructions along the vessel as well as the location of flow in the vascular tree. Under physiological conditions flow of blood along straight vessels, also known as undisturbed laminar flow, results in high shear stress (HSS) values (15 – 70 dyn/cm^2) and several cardioprotective properties. Indeed, cultured endothelial cells from different human vessels demonstrated reduced inflammation following exposure to high shear stress compared to static conditions (32). Physiological shear stress conditions have also shown to lead to anti-inflammatory effects with reduced TNF-alpha induced adhesion molecule expression (33). However conflicting reports exist, where one study has shown that using MRI technology, one patient was found to develop plaque ulceration at the location with the highest shear stress (34). This suggests HSS may not be protective in areas of vulnerable plaques. However, it is well established that sites in the vascular tree most vulnerable to atherosclerotic plaques include the inner curve of vessels as well as at bifurcations and branches. At these sites disturbed laminar flow is shown to be the most prominent (35, 36) and presents in two forms: unidirectional which results in low shear stress (LSS) values (<12 dyne/cm^2) or bidirectional that leads to oscillatory shear stress (OSS) or turbulent flow. LSS and OSS has been found to be associated with decreased NO bioavailability, upregulation of LDL, degradation of the ECM, apoptosis, promotion of oxidative stress, inflammation as well as vascular and plaque remodelling (37).

6. ENDOTHELIAL DYSFUNCTION IN HYPERTENSION

6.1. Characteristics and measurements

Under physiological conditions damage to the vasculature leads to several haemostatic processes signalled by the endothelium to reduce blood flow. These include vasoconstriction, formation of a haemostatic plug, initiation of the coagulation cascade, repair of the damaged site via endothelial progenitor cells, local endothelial cells and smooth muscle cells and finally fibrinolysis. However, under pathological conditions such as hypertension, atherosclerosis, diabetes, and coronary heart disease endothelial dysfunction occurs and the endothelium is unable to help regulate these processes. In 1980 the seminal paper, Furchgott and Zawadzki (38) demonstrated that damage to the integrity of the endothelium led to impaired relaxation when stimulated with acetylcholine (ACh) compared to vessels where the endothelium was preserved. They further demonstrated that the vasorelaxation observed was mediated by the release of an endothelium derived relaxing factor (EDRF), later identified by Ignarro and colleagues as NO (39). Endothelial dysfunction is generally defined as impaired endothelium-dependent vasodilatation to specific stimuli and characterised by an imbalance between vasoconstriction and vasodilatation factors, predominantly NO. However, there is growing literature to support that endothelial dysfunction also involves pro-inflammatory states, which will be discussed later. It is recognised that ED is the initial and reversible step in the pathological process of cardiovascular diseases such as hypertension and diabetes mellitus but is also implicated to be essential in the progression of many infections and autoimmune diseases due to its angiogenic properties the pathogenesis of certain cancers (40).

Assessing ED can be based on a variety of biomarkers, cellular markers and gross vasoreactivity techniques. Serum concentration of ICAM, VCAM, E-selectin, P-selectin as well as von Willebrand Factor and microalbuminuria have been used as biomarkers due to their expression on vascular endothelial cells during dysfunction and their consequent release into the bloodstream. Despite the current controversy in determining specific surface markers for endothelial progenitor cells, typically CD34+/KDR+/CD133+, a vast amount of literature demonstrate that these cells are found to inversely correlate with endothelial dysfunction in patients with coronary artery disease, diabetes and other CVD risk factors and co-morbidities. Increases in mature circulating endothelial cells, which are products of endothelial wall turnover and apoptosis during endothelial damage are another cellular marker implicated in cardiovascular diseases. Emerging evidence also suggests increased endothelial microparticles, which are continually shed blebbings of endothelial cells into the bloodstream, are elevated in CVD (41). Non-invasive tests of endothelial function include flow-mediated dilatation (FMD) of the brachial artery, which measures change in diameter of the brachial artery via an ultrasound, laser Doppler examination, pulse wave analysis, and pulse amplitude tonometry. While more invasive techniques include venous occlusion plethysmography that is used to assess change in forearm blood flow and arterial stiffness via infusion of various vasorelaxants. Cardiac catherization is the most invasive and expensive and assesses changes in epicardial diameter and blood flow.

Despite the various techniques available it is still unclear whether endothelial dysfunction is a cause or a consequence of hypertension. In hypertensive animal models, rats fed on a fructose-rich diet were found to have impaired endothelial-mediated vasodilatation 10 days before the rats were shown to have increased blood pressure (42), a similar result is also seen in eNOS knockout mice. However, many other models demonstrate chronic hypertension can lead to damaging effects on the endothelium (43, 44). Clinical studies also show confounding results where a study conducted by Rossi and colleagues (45) using 952 normotensive post-menopausal women demonstrate that each decrease in flow-mediated dilation predicted an increased risk in the development of hypertension during a 3.5 year follow-up even when a adjusted for multiple factors This suggests impaired

endothelial-mediated vasodilatation precedes future development of hypertension in this cohort. In a recent report from the MESA (Multi Ethnic Study of Atherosclerosis) looking at FMD and hypertension in 3500 participants they demonstrate a different finding over a 4.8 year follow-up (46). While at baseline reduced FMD correlated with increased prevalence in blood pressure but when adjusted for various factors such as age, sex, ethnicity, BMI, cholesterol levels and other metabolic factors the association was not found. Therefore they suggest that endothelial dysfunction is a consequence of hypertension not a cause.

6.2. Impaired NO

The most widely studied biological mechanism of endothelial dysfunction in hypertension is the decreased bioavailability of nitric oxide (NO). NO is not only a potent vasodilator and essential in regulating vascular tone and blood pressure but it also contributes to the regulation of haemostasis, platelet and leukocyte adhesion as well as vascular smooth muscle cell proliferation. NO is a very small lipid soluble molecule with a half-life of just a few seconds before it is converted into nitrates and nitrites that are ultimately excreted. In the various systems NO can act in many ways such as a neurotransmitter (nervous system), a vasodilator (cardiovascular system) and an inhibitor of viral replication (immune system).

Nitric oxide synthase (NOS), a family of P450 mono-oxygenase-like enzymes, catalyses the production of NO and exists in three distinct isoforms; NOS-1, NOS-2, NOS-3. They differ not only in their genetic origin (47, 48) but also their location. NOS-1 or neuronal NOS (nNOS) is found in the central and peripheral nervous system but also skeletal muscle, pancreas and endometrium and has role in neurotransmission and glomerular interactions (49). NOS-2 or inducible NOS (iNOS) is found in activated macrophages, heart, liver, smooth muscle and the endothelium and has a role in inflammation (50, 51). Finally NOS-3 or endothelial NOS (eNOS) is found predominantly in the endothelium but also in the brain and epithelial cells. eNOS is involved in vascular relaxation, regulating platelet adhesion/aggregation and angiogenesis (52, 53). The process of NO synthesis involves firstly the oxidation of arginine to N^G-hydroxy-$_L$-arginine (NHA) using NADPH (nicotinamide adenine dinucleotide phosphate) and O_2 catalyzed by the nitric oxide synthases (NOS), a family of P450 mono-oxygenase-like enzymes (54). The second step involves the production of NO when NHA is converted to $_L$-citrulline via NOS. Actions of NOS are accelerated by the cofactors flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and tetrahydrobioptern (BH_4). In the endothelium eNOS is localized in the plasma membrane and more specifically in highly rich lipid invaginations or caveloae (55) where it is bound in an 'inactive' state to the coat protein caveolin-1 (Cav-1). Besides NO production, caveolae, caveolins and cavins (regulators of caveolins) are involved in the regulation of various signaling cascades involved in vascular remodeling, intracellular calcium (Ca^{2+}), and microvascular permeability. Activation of eNOS also involves heat shock protein 90, calmodulin binding, phosphorylation of Ser1179 and dephosphorylation of Thr497 domains and subcellular localization. Under normal shear flow there is an influx of Ca^{2+} causing calmodulin to bind to eNOS and causing its subcellular localization to either the cytosol or the Golgi (56) or possibly to the mitochondria (57) which ultimately results in activation. Once NO is produced it can then stimulate soluble guanylate cyclase (sGC) in vascular smooth muscle cells (VSMC) to increase cyclic GMP (cGMP) that leads to relaxation and reduced Ca^{2+}. Impaired NO bioavailability commonly seen in various cardiovascular diseases including hypertension can be due to either impaired production or increased degradation of NO. Given the already established pathway of NO synthesis, impaired production may be a result of reduced eNOS activity, substrate and cofactor availability and the localization of eNOS or the presence of endogenous inhibitors.

Since the works of Huang and colleagues (58) and Shesely and colleagues (59) demonstrated a hypertensive phenotype in eNOS knockout models studies have been utilizing these models extensively and have demonstrated am essential role of eNOS in the vasculature. In animal models, supplementation with the eNOS substrate L-arginine leads to enhanced NO synthesis in diabetic (60) and pulmonary hypertensive rats (61, 62) as well as reduced atherosclerotic lesions in rabbits (63) and cerebral infarcts in various experimental models of stroke (64). More importantly, clinical studies have also shown supplementation increases NO synthesis and enhances vascular reactivity. Indeed, hypertensive patients have paradoxically high levels of L-arginine in their plasma (65) yet display impaired L-arginine transport in platelets, red blood cells (66) and endothelial cells and are therefore unable to adequately produce optimum NO. Consequently, L-arginine activity may be rate-limiting for NO production and this is seen even in normotensive patients with a family history of hypertension (67). Increased transport can be seen in various studies to improve vascular function where De Meirelles and collegues (68) demonstrated hypertensive patients undergoing 12 weeks of aerobic exercise had significantly improved L-arginine transport as well as NOS activity as well as reductions in fibrinogen and C-reactive protein.

Against this backdrop, it is thus of little surprise that L-arginine supplementation has been reported to improve endothelial function. Certainly, supplementation of L-arginine in humans has been delivered via several modes, including intra-arterially, intravenously and oral supplementation and in high risk patients it has been shown to increase NO production and decrease leukocyte adhesion, platelet aggregation and hyperplasia of the intimal layer (69). Despite this, owing to the diverse role of L-arginine, its supplementation is likely to result in increased metabolism via pathways other than NO synthesis, such as those that increase ornithine, polyamines, creatine, proline and spermine (70). It is thus important to note that although L-arginine therapy can produce its effects via NO dependent mechanisms, effects independent of NO may also play a functional role. L-arginine is also a potent hormone secretagogue. It has long been used for the

assessment of growth hormone release by the pituitary gland (71) and L-arginine administration results in an approximate twofold increase in plasma growth hormone, insulin and glucagons release (72), (73). L-arginine also releases prolactin (74) and insulin either directly or indirectly (by the release of other endothelium dependent agents such as muscarinic agonists) (73). The mechanisms regulating the endocrine secretagogue effect on various hormones in response to L-arginine remains largely unknown. However, the secretagogue effect is of important consideration when evaluating the role of L-arginine on the vasculature, since many of these resultant hormones have been independently reported to act on vascular smooth muscle cells. Apart from its complex metabolism and potent secretagogue effects, dietary L-arginine supplementation is also less than ideal since it is an amino acid that undergoes considerable first pass metabolism. Ingestion of L-arginine may not have large physiological effects due to its low bioavailability, reported to be as low as 21% through to 67% (75), (76).

Another cause of L-arginine reductions may involve the eNOS inhibitor ADMA (asymmetric dimethylarginine) an endogenous analogues of L-arginine, which is not only increased essential hypertension but also inversely correlated with forearm blood flow (65). The enzyme arginase (discussed later) may also be another determinant of reduced cytosolic L-arginine and impaired NO production.

Reduced L-arginine, increased ADMA or BH_4 deficiency can also lead to uncoupling or dysfunction of eNOS and other NOS isoforms. eNOS contains two dimers with an N-terminal oxygenase domain that binds BH_4, L-arginine, iron, and calmodulin ions as well as a C-terminal reductase domain that binds FAD, FMN and NADPH. In the pathological setting, BH_4 is oxidised to BH_2 causing altered electron flow from FMN and FAD to L-arginine and the uncoupling of eNOS dimers. This results in free radical production particularly superoxide from O_2 in replace of NO. Studies show in hypertension there is a downregulation of BH_4 and in a animal nephrectomy model BH_4 supplementation normalised SBP levels (77). Other mechanisms causing uncoupling of eNOS include impaired Akt kinase phosphorylation of the serine (Ser^{1179}) domain (78), (79) or increased phosphorylation of the theronine (Thr^{497}) domain via protein kinase C (80), (81). In pulmonary hypertension it is suggested that the tight binding of Cav-1 and eNOS may play a major role leading to reduced angiogenesis and increased cell proliferation. While the binding of Cav-1 is generally shown to suppress eNOS activity, Cav-$1^{-/-}$ mice in response to vascular shear stress demonstrate reduced blood flow but no change in lumen diameter and an increase in vessel wall thickness and cellular proliferation compared to controls (82). This study then showed that reconstituting Cav-1 into the vessels the effects were then ablated, suggesting in these knockout mice eNOS is unable to localize in the caveolae causing impaired response to shear stress. Therefore Cav-1 is found to not only regulate eNOS activation but also is essential in its localization. While eNOS is found to produce more NO when bound to the membrane compared to cytosolic eNOS it appears that the location is more important (52). Sanchez *et al* (56) suggest that eNOS translocation from the caveolae to the Golgi via acetylcholine may correspond to a vasodilation pathway whereas they show with platelet-activating factor (PAF), which is an inflammatory marker and vasoconstrictor causes eNOS to locate to the cytosol. Therefore cytosolic relocation of eNOS, and particularly mitochondrial bound eNOS (57) may relate to an inflammatory response. Indeed, arginase II is known to compete with eNOS for L-arginine and is found to be not only bound to the mitochondria but is also shown to regulate eNOS activity (83).

Production of reactive oxygen species (ROS) are another set of molecules that may result from and cause eNOS uncoupling, decreased NO production and increased NO degradation. While essential for cell metabolism and signaling when there is an imbalance in the production of oxidants or ROS to antioxidants in blood vessels this leads to a pro-oxidant state and the pathogenesis of oxidative stress causing endothelial dysfunction, increased contractility, vascular smooth muscle cell growth and apoptosis, monocyte migration, lipid peroxidation, inflammation and increased deposition of extracellular matrix proteins. Furthermore, ROS has been shown to activate signal transduction pathways and induce gene expression and growth factors (84, 85). As reviewed extensively endothelial dysfunction in hypertension and many cardiovascular diseases is due in part to an increase in ROS production (43), (86)-(87). ROS are produced from the mitochondria and subcellular sources such as the mitochondrial electron transport chain, NADPH oxidase, xanthine oxidase, cytochrome P450, cyclooxygenase, lipoxygenase, and uncoupled eNOS. Initial formation begins with the reduction in one electron of molecular oxygen causing formation of superoxide anions ($\cdot O_2^-$). Superoxide can then go on to produce hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2) via superoxide dismutase (SOD), and peroxynitrite ($ONOO^-$) from scavenging NO.

Experimental models of hypertension including DOCA-salt rats (88), spontaneous hypertensive rats (89), L-NAME hypertensive rats (90), and hypertriglyceridaemic rats (91) all demonstrate increases in ROS production. In some models, increases in ROS is found to precede hypertension suggesting that production of ROS may contribute to the initiation and the progression of hypertension (92). Furthermore, treatment with antioxidants improves vascular function and structure, prevents target-organ damage, and reduces blood pressure in animal models of hypertension (92, 93). However, clinical studies have been less conclusive. Indeed, ROS levels are enhanced in hypertensive patients with reports of increased levels of H_2O_2 (94) and upregulation of vascular NADPH (95). However most clinical trials demonstrate no beneficial effects on blood pressure (96). Of the many toxic affects excess ROS has on the vasculature, inflammation appears to be the greatest and is involved in the initiation and development of atherosclerotic plaques commonly associated with hypertension and various cardiovascular diseases.

6.3. Endothelial inflammation

Recent reports suggest that hypertension induced endothelial dysfunction involves low-grade inflammation

that can progress into hypertension induced atherosclerosis and several cardiovascular complications. This comes as no surprise as these conditions are commonly correlated and both have similar risk factors (age, obesity, diet, diabetes, smoking) and result in vascular remodeling and dysfunction. Indeed, inflammatory markers that are reportedly upregulated in hypertensive patients include tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP) (97), interleukin (IL)-6, IL-1b and angiotensin II (AngII) (98-100). Many of these inflammatory markers are involved in the initial step in atherosclerotic plaque development, the adhesion cascade. Some animal studies have shown a relationship exists between high intraluminal pressure and plaque development. Indeed, in ApoE knockout mice with induced hypertension via either eNOS ablation (101), renal artery clamping (102), or aortic constriction (103) increases in atherosclerosis and expression of adhesion molecules were seen including intracellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin. This effect is also seen in clinical studies where essential hypertensive patients demonstrate increases in ICAM-1, VCAM-1 and E-selectin in their serum following a cold pressor test (104). Furthermore, studies show that when an aortic stenosis is administered to rabbits, hypertension causes monocyte adhesion to be increased only in the proximal region to the stenosis (105) which is where the highest oscillatory shear stress occurs. While it is clear that high intraluminal pressure results in leukocyte adhesion and atherosclerotic development, the exact mechanisms and pathways are still unclear. Several studies have implicated various factors including ROS production, NF-kB activation, endothelin-1 (ET-1), and the rennin-angiotensin-aldosterone system (RAAS).

ROS production as previously stated is important in the progression of inflammation and atherosclerotic plaques. They are involved in the gene expression of many adhesion molecules such as ICAM-1, VCAM-1, E-selectin and monocyte chemoattractant protein-1 (MCP-1) (106) and in disease states such as hypertension play a role in regulating the inflammatory response to shear stress. Ang II a well-regarded mediator of the RAAS and well characterized in hypertension and vascular dysfunction is shown to stimulate leukocyte adhesion through angiotensin type 1 (AT_1) and 2 (AT_2) receptors. However, it is also shown Ang II acts on ROS production and proinflammatory transcription factors. For example, rats perfused with Ang II and an NADPH oxidase inhibitor present with reduced ICAM-1 expression and macrophage infiltration compared to those perfused with Ang II alone and this was shown to be independent of blood pressure changes (107). Suggesting that Ang II induced inflammation is mediated via NADPH oxidase and ROS production.

The proinflammatory transcription factors NF-kB aand enodthelin-1 (ET-1) also correlate with increases in Ang II resulting in increases in proinflammatory genes, cytokines and signaling pathways (108). RAAS independent inflammatory pathways have also been implicated where increases in shear flow and pressure has shown to increase NF-kB binding to DNA in cells (109) and upregulate expression in vessels (110). Furthermore, when pressurized vessels were incubated with an NF-kB inhibitor this dramatically reduced the number of adhered monocytes to the endothelium and the expression of adhesion molecule (111). This study also showed that addition of Ang II to vessels at various pressures was not sufficient to increased adhesion. This suggests that upregulation of the adhesion cascade may result from more mechanical factors such as intraluminal pressure and stretch independent of Ang II. Indeed, this effect is also seen in studies with rats undergoing aortic constriction in which they demonstrate that increased blood pressure and not RAAS upregulated the adhesion molecules ICAM-1 and P-selectin (112). This is also seen in terms of ROS production where NADPH oxidase deficient mice demonstrated lower blood pressure compared to controls and this was not increased with Ang II was administered (113). Therefore these results suggest antoher pathway may be involved in endothelial inflammation in hypertension.

6.4. Role of arginase

The enzyme arginase may be one factor involved in an alternative pathway involved in endothelial dysfunction and inflammation in hypertension (Figure 1). Arginase is a fundamental manganese metalloenzyme in the hepatic urea cycle that hydrolyses L-arginine to urea and L-ornithine. Arginase exists in two distinct isoforms (I and II) where they differ in intracellular, gene and tissue expression, gene transcription and transduction regulators as well as metabolism. While both enzymes are found throughout the body arginase I or hepatic arginase is a cytosolic enzyme found abundantly in the liver but also in red blood cells whereas arginase II or extra-hepatic arginase is a mitochondrial enzyme expressed more widely and is seen in the kidney, brain, gastrointestinal tract, prostate and the vasculature. It has been shown vascular endothelial cells and smooth muscle cells express both isoforms, but it appears that the distribution is vessel and species dependent (114-116). In particular arginase II appears to be the predominant isoform in human endothelial cells (115). Arginase isoforms share ~59% homology, with arginase I composed of 322 amino acide residues, 11.5 kbp long and 8 exons on chromosome 6q23, where arginase II appears to have 344 amino acid residues and 8 exons but is on chromosome 14q24.1-q24.3. Products of arginine hydrolysis involve increased urea production and L-ornithine leading to increases in polyamines, proline, and glutamate (involved in cell growth and proliferation) as well as a reduction in NO.

Initially the role of arginase in the body was mainly thought of as disposing excess nitrogen via amino acid and nucleotide metabolism. Recently studies suggest an important role for arginase in the vasculature. In the endothelium arginase is now regarded to help regulate NO levels by competing with eNOS for L-arginine. Arginase is also thought to modulate eNOS (117) and this is most likely to occur when eNOS is translocated from the caveolae to the cytosol and perhaps even the mitochondria under proinflammatory states. As such increased arginase activity/expression has been implicated in many vascular

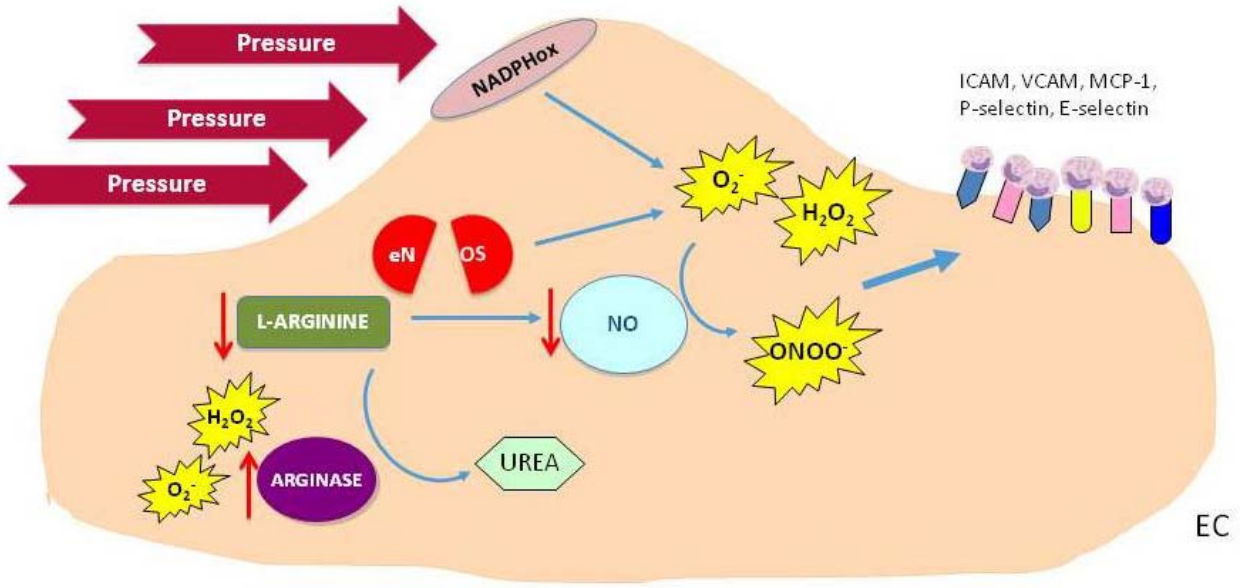

Figure 1. Our hypothesis is that high blood pressure induces an increase in reactive oxygen species (ROS) production in endothelial cells (EC) possibly via NADPH oxidase (NADPHox) which causes an increase in endothelial arginase expression and/or activity which decreases available L-arginine, this increases endothelial nitric oxide synthase (eNOS) expression and/or activity and the 'uncoupled' eNOS produces superoxide ($\cdot O_2^-$) over NO resulting in the production of hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) and pro-inflammatory factors leading to the expression of adhesion molecules.

pathologies including hypertension (118, 119), ischaemia-repurfusion (120), uremia (121), aging (117), sexual arousal (122, 123), diabetes (124, 125) and atherosclerosis (115), (126). In hypertension arginase activity and expression studies demonstrate increased arginase activity reduces NO mediated dilation in hypertensive pigs, which was then normalized using an arginase inhibitor (116). These results area also seen in other models of hypertension including Dahl rats with salt induced hypertension (127) and in bovine pulmonary arterial endothelial cells where NO production was increased and urea was decreased when both L-arginine and L-valine were used to inhibit arginase activity (128). And in a model of chronic hypertension treatment with an arginase inhibitor for 10 weeks in older aged spontaneous hypertensive rats decreased blood pressure and cardiac fibrosis and improved vascular function (129). In one small clinical study assessing attenuated reflex cutaneous vasodilatation in essential hypertension increased NO-dependent vasolation was found when arginase was inhibited and not with L-arginine supplementation (130). Interestingly, it has also been reported that arginase expression of both isoforms is increased in spontaneous hypertensive rats before overt hypertension develops and is positively correlated to systolic blood pressure in these rats (131). Therefore these results suggest that in genetic hypertension vessels have the propensity to develop endothelial dysfunction before established hypertension is even developed. This may also occur before inflammatory mechanisms take place, as increased inflammatory markers are not seen till these rats are adults (131, 132), suggesting underlying molecular/transcriptional mechanisms, possibly via NF-kB pathway.

Despite these recent findings it is still unclear the exact mechanisms/pathways that induces arginase upregulation in hypertension. Several studies have examined a possible link with ROS production that may help link hypertension induced endothelial dysfunction and inflammation. Ryoo and colleagues (126) demonstrate that in atherogenic prone ApoE$^{-/-}$ when arginase II activity was reduced via either inhibition or gene deletion NO bioavailability was increased and ROS production was reduced, resulting in improved endothelial function and reduced vascular stiffness. They also show that plaque area, thickness and foam cells were all reduced in thoracic aora treated with the arginase inhibitor BEC, S-(2-boronoethyl)-L-cysteine. The ROS/arginase link is may also be reciprocal where intraluminal H_2O_2 is found to upregulate arginase expression and impair NO-mediated dilation in porcine coronary arteries and these affects were attenuated when arginase inhibitors DFMO or nor-NOHA reduced H_2O_2 and increased vascular function. Inflammatory cytokine TNF-a has also shown to upregulate arginase in ischemia-repurfusion resulting in reduced bioavailability of L-arginine and the uncoupling of eNOS producing increased superoxide production (133). This action may be via NADPH oxidase. Where one study has demonstrated that in alveolar macrophages treated with apocynin, NADPH oxidase inhibitor, arginase is also attenuated (134).

7. SUMMARY

Endothelial dysfunction plays a significant role in the initiation and development of hypertension and its progression to cardiovascular related diseases. Impaired

NO production is a strong indicator of endothelial dysfunction and continues to be an essential component when measuring vascular impairment. Recently, reports suggest that inflammatory mechanisms in the vasculature may also be an important factor determining dysfunction in the endothelium. While it is still unclear the exact pathways involved in the progression of essential hypertension to cardiovascular diseases there is increasing interest in the role of arginase and reactive oxygen species play in the inflammatory progression of hypertension.

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Abbreviations: RH: resistant hypertension; HSS: high shear stress; LSS: low shear stress; OSS: oscillatory shear stress; NO: nitric oxide; ED: endothelial dysfunction; ROS: reactive oxygen species; $\cdot O_2^-$: superoxide; H_2O_2: hydrogen peroxide; $ONOO^-$: peroxynitrite; OH^-: hydroxyl radical; eNOS: endothelial nitric oxide synthase; Cav-1: caveolin-1; ICAM: intracellular adhesion molecule; VCAM: vascular cell adhesion molecule.

Key Words: Hypertension, Resistant Hypertension, Endothelial Dysfunction, Inflammation, Arginase, Reactive Oxygen Species, Nitric Oxide, Endothelial Nitric Oxide Synthase, Review

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