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Effects of Antihypertensive Drugs on Endothelial Dysfunction

Clinical Implications

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Abstract

Essential hypertension is associated with endothelial dysfunction, which is caused mainly by the production of oxygen-free radicals that can destroy nitric oxide (NO), and impair its beneficial and protective effects on the vessel wall. In prospective studies, endothelial dysfunction is associated with increased incidence of cardiovascular events. Antihypertensive drugs show contrasting effects in terms of improvement or restoration of endothelial function.

Little evidence is available with β-blockers. Whereas treatment with atenolol has a negative effect in peripheral subcutaneous and muscle microcirculation, insufficient evidence is available to establish whether new compounds such as nebivolol, which activates the L-Arginine–NO pathway, and carvedilol, which has strong antioxidant activity, can improve endothelial function in patients with hypertension.

Calcium channel antagonists, particularly the dihydropyridines, can reverse impaired endothelium-dependent vasodilation in different vascular districts, including the subcutaneous, epicardial, renal and forearm circulation. However, conflicting results are found in the brachial artery. In the forearm circulation, nifedipine and lacidipine can improve endothelial dysfunction by restoring NO availability through a mechanism probably related to an antioxidant effect.

ACE inhibitors, on the other hand, seem to improve endothelial function in subcutaneous, epicardial, brachial and renal circulation, whereas they are ineffective in potentiating the blunted response to acetylcholine in the forearm of patients with essential hypertension. They can also selectively improve endothelium-dependent vasodilation to bradykinin, an effect not mediated by restoring NO availability but probably related to hyperpolarisation.

Recent evidence suggests angiotensin II AT_1-receptor antagonists can restore endothelium-dependent vasodilation to acetylcholine in subcutaneous microcirculation but not in that of the forearm muscle. Evidence concerning the effect of these drugs on the brachial artery in patients with atherosclerosis is positive. However, treatment with an AT_1-receptor antagonist can improve basal NO release and decrease the vasoconstrictor effect of endogenous endothelin-1.

In conclusion, despite the considerable evidence that impaired endothelium-dependent vasodilation can be improved by appropriate antihypertensive treatment, no clinical data exist demonstrating that the reversal of endothelial dysfunction is associated with a reduction in cardiovascular events. In the near future, large scale clinical trials are required to demonstrate that treatment of endothelial dysfunction can lead to better prognosis in patients with essential hypertension.

1. Endothelium and Cardiovascular Disease

Probably the most significant advance in cardiovascular medicine over the last two decades has been the identification of endothelial cells as a vasoactive organ. After the pioneering report by the 1998 Nobel Prize winner Robert Furchgott, an impressive array of evidence has made it possible to state today that the endothelium plays a primary autocrine/paracrine regulatory role by secreting substances that control both vascular tone and structure. Moreover, accumulating evidence has indicated that the dysfunctioning endothelium, which is characteristic of essential hypertension and most of the cardiovascular risk factors, is a major promoter for atherothrombosis and, consequently, cardiovascular events. As a logical consequence, endothelial dysfunction is now considered an important target for cardiovascular treatment. This review focuses on available evidence concerning the effect of antihypertensive treatment on endothelial function in humans and its possible clinical relevance.

1.1 Influence of Endothelium-Derived Relaxing and Contracting Factors on Vascular Tone and Structure

The endothelium produces several relaxing factors including nitric oxide (NO), prostacyclin and a not yet identified hyperpolarising relaxing factor

(EDHF).[1] The best characterised, and probably the most important, relaxing factor is NO,[2,3] which is derived from transformation of the amino acid L-arginine into citrulline[4] by the activity of NO synthase (NOS),[5] a constitutive enzyme present in endothelial cells. NO is produced and released either basally or under the influence of agonists, such as acetylcholine, bradykinin, substance P, serotonin and others, acting on specific endothelial receptors, and by mechanical forces, such as shear stress.[1] Endothelial cells can also induce relaxation by causing hyperpolarisation.[6] However, at the present time, arguments for the existence of EDHF in humans are plausible only on the basis that endothelium-dependent relaxation cannot be abolished by NOS antagonists, thus ruling out NO as being responsible for this activity.[6]

Endothelial cells can also produce endothelium-derived contracting factors (EDCF). The principal EDCF is endothelin-1 (ET-1). Endothelins are three 21 amino acid isopeptides (ET-1, ET-2 and ET-3), which are characterised by sustained and potent vasoconstrictor action.[7,8] ET-1, generated by the vascular endothelium, is probably the major isoform in the cardiovascular system. Human ET-1 derives from the 212 amino acid preproET-1, which is enzymatically cleaved to form the 38 amino acid precursor big ET-1, further processed to form the active peptide by the activity of endothelin-converting enzyme (ECE).[9] ET-1 acts through specific receptors named ET_A and ET_B.[10] ET_A receptors are represented only on smooth muscle cells, and have the function of promoting growth and mediating contractions.[11] In contrast, ET_B receptors are located on both endothelial and smooth muscle cells, with different effects. Smooth muscle cell ET_B receptors evoke contractions,[11,12] whereas endothelial ET_B receptors induce relaxation by production of endothelium-derived relaxing factors, including NO[13] and prostacyclin.[14]

The overall biological effect of ET-1 on vascular tone and structure derives from the balance between the direct effect via ET_A and ET_B receptors on smooth muscle cells and NO- or prostacyclin-induced activation mediated by endothelial ET_B receptors.[15] This balance could represent one crucial mechanism explaining why ET-1, which is a physiological substance, can shift to a pathological role in cardiovascular disease.

1.2 Pathological Role of Endothelial Dysfunction in Cardiovascular Disease

In particular conditions including aging, menopause and pathological conditions such as hypertension, diabetes mellitus, atherosclerosis, vasospasm and reperfusion injury, activation of endothelial cells can lead to the production and release of contracting factors including cyclo-oxygenase-derived EDCF, mainly represented by prostanoids [thromboxane A_2 and prostaglandin (PG) H_2][16] and oxygen-free radicals,[17] which counteract the relaxing activity of NO. Oxygen-free radicals can also impair endothelial function by causing NO breakdown.[18] It is of relevance that the concept of endothelial dysfunction is predominantly related to the parallel activation of NO and EDCF pathways. Even in the presence of preserved NO production, EDCF can impair NO availability or biological effects. Thus, in several experimental conditions, EDCF pathway blockade can lead to a complete restoration of the L-arginine-NO pathway.

2. Endothelial Dysfunction in Essential Hypertension

Endothelial dysfunction is now recognised as a characteristic of patients with essential hypertension.[19-27] By definition, endothelial dysfunction is a functional and reversible alteration of endothelial cells resulting from an impairment in NO availability.[22,23,26,27] Thus, endothelial dysfunction must be distinguished from endothelial damage, which is represented by the anatomical disruption of the endothelium.

2.1 Mechanisms Responsible

Available evidence concurs in indicating that endothelial dysfunction associated with essential hypertension is characterised by impaired NO availability.[28] In patients with essential hyperten-

sion, N^G-monomethyl-L-arginine (L-NMMA) infusion causes a lesser degree in vascular tone[29] and does not significantly blunt the response to agonists such as acetylcholine or bradykinin as compared with healthy controls.[22,23,30] Taken together, these results indicate the presence of impaired basal and stimulated NO release in arteries of patients with essential hypertension. The presence of impaired basal NO release is further confirmed by the evidence of reduced plasma nitrite and nitrate levels, oxidative end-products of NO, in patients with essential hypertension.[31]

Reduced response to endothelial agonists in patients with essential hypertension does not seem to be related to a selective defect at the membrane receptor or signal transduction pathways since it is observed with agonists (acetylcholine, bradykinin, substance P)[19,20,30,32] acting on different receptors or different intracellular transduction pathways.

2.2 Role of Oxidative Stress in Determining Endothelial Dysfunction

Enquiry into the mechanisms responsible for impaired NO availability raises several different possibilities. One of most relevant mechanisms is oxidative stress production, which causes NO breakdown.[28] These reactive oxygen species, mainly superoxide anions, combine and destroy NO-producing peroxynitrates,[1] which have several negative effects on vascular function and structure. The role of oxidative stress is supported by the evidence that ascorbic acid, an oxygen-free radical scavenger, can increase the response to acetylcholine in the peripheral circulation and in the coronary epicardial artery of patients with essential hypertension.[27,33,34] Oxygen-free radicals can be generated by different enzymatic and nonenzymatic sources, including xanthine oxidase, cyclooxygenase, NOS–induced superoxide production caused by cofactor tetrahydrobiopterin (BH4) depletion and NAD(P)H-dependent oxidases.[1]

In patients with hypertension, a positive role has been demonstrated for cyclo-oxygenase activity[26,27] and excluded for xanthine oxidase,[35] while the relevance of BH4 depletion and nicotinamide adenine dinucleotide (phosphate) [NAD(P)H]-dependent oxidases has not yet been fully investigated. However, it is evident that different pathological pathways can lead to reduced NO availability and, as a consequence, endothelial dysfunction. It is worth noting that in presence of reduced NO availability, alternative pathways, including hyperpolarisation, account for endothelium-dependent vasodilation.[30]

Another attractive possibility is the production of the L-arginine analogue N^G N^G-dimethyl-L-arginine (ADMA), an endogenous competitive inhibitor of NOS, which therefore reduces NO production.[36] Recently, plasma ADMA levels were found to be positively correlated with mean arterial pressure and other cardiovascular risk factors.[37]

2.3 Interaction between Nitric Oxide and Vasoconstrictor Substances

An interaction between the NO system and endothelial vasoconstrictor substances, mainly ET-1 and angiotensin II, can participate in the pathogenesis of endothelial dysfunction. Although plasma ET-1 levels do not seem to be increased in patients with essential hypertension,[15,38] it is worth noting that the vasoconstrictor activity of the peptide has been found to be increased in parallel with diminished NO availability.[39] It is conceivable that the endothelial ET_B-induced inhibitory effect of NO on ET-1 production[40] and activity[41] is impaired in patients with essential hypertension because of the presence of decreased NO availability. Thus, the altered equilibrium between the two systems can lead to an increase in the vasoconstrictor, and possibly the proliferative, activity of ET-1.[15,38]

Angiotensin II also has different effects on the NO system. This peptide, while causing NO breakdown via AT_1 receptors and the consequent activation of NAD(P)H-dependent oxidases,[42,43] can promote NO synthesis in endothelial cells via AT_2 receptor stimulation.[44] It is possible, depending on the predominance of the activity of the two receptor subtypes or NO availability, that angiotensin II can deeply influence endothelial function or dysfunction.

In summary, given the different pathological pathways potentially leading to endothelial dysfunction, it is plausible that a variety of antihypertensive agents could act positively on these alterations, at least in certain vascular beds or with certain stimuli.

3. Clinical Relevance of Endothelial Dysfunction in Hypertension

3.1 Promotion of Atherosclerotic Lesions by Impaired Endothelium-Dependent Vasodilation

The clinical relevance of the presence of endothelial dysfunction in hypertension is attributable to the fact that NO and EDCF not only exert an opposite effect on vascular tone but also, respectively, inhibit and activate those mechanisms, such as platelet aggregation,[45] vascular smooth muscle-cell proliferation[46] and migration,[47] monocyte adhesion[48] and adhesion molecule expression;[49] all of which exert an important role in the genesis of thrombosis and atherosclerotic plaque. Endothelial dysfunction is thus a mechanism promoting atherosclerosis and thrombosis, or altering vasomotricity, and thereby contributing to cardiovascular events. This concept is reinforced by the evidence that endothelial dysfunction is not specific to essential hypertension. Rather, it is a common alteration of the major cardiovascular risk factors, including ageing,[24,25,50] menopause,[51] hypercholesterolaemia,[52,53] smoking,[54] diabetes mellitus,[55,56] and hyperhomocysteinaemia.[57,58] It is conceivable that such an alteration may not be a mechanism participating in the determination of high blood pressure values. It is more likely to be a common pathogenetic mechanism determining cardiovascular events in patients with cardiovascular risk factors.

3.2 Association of Endothelial Dysfunction with Markers of Vascular Damage and Cardiovascular Events

Evidence is mounting that the presence of endothelial dysfunction is associated with markers of vascular damage and with cardiovascular events. In patients with essential hypertension impaired forearm response to acetylcholine is correlated with intima-media thickening of carotid arteries, an index of atherosclerosis.[59] Moreover, in epicardial coronary arteries of normotensive individuals the response to acetylcholine shows an inverse correlation with intramural plaque as detected by intravascular ultrasound.[60] Finally, in epicardial coronary arteries of patients with cardiac transplantation, endothelial dysfunction is a predictor of the subsequent development of arteriolosclerosis.[61]

It is worth noting that the presence of endothelial dysfunction has been associated with the occurrence of cardiovascular events in longitudinal studies. Suwaidi et al.[62] have evaluated the outcome of patients with mild coronary artery disease on the basis of endothelial function. Only patients with severe endothelial dysfunction (assessed as coronary microcirculatory response to acetylcholine) had cardiovascular events in a mean follow-up period of 28 months (range 11 to 52 months). Similar results were obtained by Schächinger et al.,[63] who demonstrated that cardiovascular events (in a median follow-up period of 7.7 years) had a significantly greater association with coronary endothelial dysfunction, assessed as response to intracoronary acetylcholine, sympathetic activation by cold pressure test and flow-mediated dilation induced by distal infusion of papaverine. Furthermore, the presence of endothelial dysfunction in the peripheral large arteries (flow-mediated dilation in the brachial artery) has also been associated with increased coronary events.[64]

Although these studies may be biased by a small study population, concordant evidence is accumulating which demonstrates that endothelial dysfunction acts as a pathogenetic mechanism causing cardiovascular disease.

3.3 Possible Implications for Antihypertensive Therapy

This line of reasoning suggests that in patients with essential hypertension impaired endothelium-

dependent vasodilation, although not involved in the pathogenesis of increased blood pressure values, could act as a promoter of the atherosclerotic lesions that are one of the most serious complications of essential hypertension. Such an hypothesis raises the issue that reversing impaired endothelium-dependent vasodilation could constitute an important goal for antihypertensive therapy.

Awareness that mere blood pressure normalisation is not sufficient to normalise response to acetylcholine[65] or methacholine[66] in patients with essential hypertension is of crucial importance. It implies that antihypertensive drugs must be endowed with the ability to restore endothelial function, a specific property that goes far beyond blood pressure reduction.

Antihypertensive drugs must therefore be reconsidered in terms of specific effect on endothelial function. Experimental studies indicate that the majority of available agents have the potential to improve endothelium-dependent relaxation.[67,68] Drugs can act by different mechanisms including activation of NOS, a scavenger activity on oxidative stress or by decreasing the production of oxygen-free radicals (table I). However, when the same agents have been tested in a clinical setting, positive animal evidence has not always been confirmed.

This review examines available evidence documenting the effect of antihypertensive treatment on endothelial function in patients with essential hypertension.

Before presenting the results of clinical studies in detail, several specific issues must be addressed in order to correctly interpret these results. Firstly, when discussing the effect of treatment in patients with essential hypertension, the results must be considered in relation to the specific pathology. There is an unjustified tendency to transfer positive results obtained in populations with different pathologies (for instance atherosclerosis) to patients with essential hypertension. We see in section 4 that some drugs are effective in patients with atherosclerosis but not in those with essential hypertension.

Table I. Possible mechanisms responsible for the beneficial effect of antihypertensive drugs on endothelial dysfunction

Drugs activating L-arginine-NO pathway
Nebivolol
AT_1-receptor antagonists
Drugs with antioxidant effects
'Scavenger' of oxygen-free radicals
Carvedilol
Doxazosin (6- and 7-hydroxy-metabolites)
Improvement of cellular redox state
Dihydropyridine calcium channel antagonists
Phenylalkylamine calcium channel antagonists
Reduction of oxygen-free radical production
ACE inhibitors
AT_1-receptor antagonists

ACE = Angiotensin converting enzyme; **AT_1** = angiotensin II B1; **NO** = nitric oxide.

A second problem concerns duration of treatment. Very often results obtained after short-term drug administration are not confirmed by studies performed to evaluate long-term treatment. Since essential hypertension is a chronic disease, results obtained after single drug administration must be confirmed by a more appropriate experimental design requiring prolonged drug administration.

Thirdly, endothelial function is an autocrine-paracrine mechanism. Results must be applied to the specific vascular district tested in the study. Again, results in the peripheral microcirculation cannot be extrapolated to large peripheral arteries or coronary micro- and macrocirculation. Available evidence indicates a low, although statistically significant, correlation (an r-value of 0.36) between endothelium-dependent vasodilation tested in different vascular beds in the same individuals.[69] It may not be warranted to start with results obtained in peripheral circulation and extrapolate conclusions on the presumption that they will be applicable to more important vascular districts such as the coronary arteries.

A final issue, and probably the most important, regards the widespread concept that treatment-induced augmented response to an endothelial agonist is an index of increased NO production. This argument is highly misleading. As previously discussed in section 2, in essential hypertension, re-

duced endothelium-dependent vasodilation is characterised by impaired NO availability while compensatory mechanisms (hyperpolarisation?) sustain the residual endothelial responsiveness.[30] Thus, when no experimental demonstration is given (for instance by the use of the selective NO-synthase inhibitor L-NMMA), in several circumstances the mere increase in agonist-induced vasodilation cannot be extrapolated as an increase in NO availability. This is a crucial point since at the present time no information exists in humans to show whether augmented hyperpolarisation or decreased activity of contracting factors would lead to the same beneficial effect as restoration of NO availability.

4. Antihypertensive Drugs and Endothelial Function

4.1 β-Blockers

Studies specifically designed to test the effect of treatment with classical β-adrenoceptor antagonists (β-blockers) on endothelium-dependent vasodilation are not available. However, the selective β_1-blocker atenolol has frequently been employed as a control treatment in studies designed to assess the effectiveness of different compounds, including calcium channel antagonists or angiotensin converting enzyme (ACE) inhibitors. In subcutaneous microcirculation (resistance-size small arteries dissected from a gluteal subcutaneous biopsy studied on a wire myograph), Schiffrin et al.[70] showed in several studies that treatment with atenolol for 1 year did not improve the response to acetylcholine. Moreover treatment with atenolol for 3 years did not improve the impaired endothelium-dependent vasodilation to both acetylcholine and bradykinin in the forearm vasculature of patients with essential hypertension.[71]

However, a different effectiveness has been proposed for novel agents of the same class. Experimental evidence suggests that high concentrations of nebivolol, a selective β_1-blocker with vasodilating properties, cause endothelium-dependent relaxations.[72] In healthy volunteers, nebivolol infused into the brachial artery at a supratherapeutical concentration caused a modest increase in forearm blood flow (FBF). This effect was significantly inhibited by L-NMMA and restored when L-arginine was administered simultaneously with the NOS inhibitor, indicating that acute intravascular nebivolol administration evokes slight NO-dependent vasodilation.[73]

Conflicting results are available in patients with essential hypertension. Although Dawes et al.[74] demonstrated that intrabrachial nebivolol administration increased FBF, an effect sensitive to L-NMMA, in our studies the compound failed to induce vasodilation. Only in the presence of ascorbic acid, an antioxidant, did we observe a restoration of the nebivolol vasodilating effect, which was sensitive to L-NMMA administration (results not yet published). This discrepancy may be explained by different selection of the hypertensive study population. However, is unlikely that the modest vasodilating effect of high and nontherapeutic nebivolol concentrations could be responsible for the pronounced decrease in peripheral vascular resistance induced by the drug during oral administration. Moreover, the intra-arterial administration of nebivolol is not an adequate model to assess its clinical mechanism of action. This drug, when administered orally, is almost totally metabolised and no evidence is available on the vascular effects of these active metabolites. Further studies are needed to explore the real mechanism involved in nebivolol-induced vasodilation with therapeutic and oral administration.

A drug that potentially could restore endothelial function in patients with essential hypertension is carvedilol, a selective β_1-blocker with additional α_1-adrenoceptor antagonist properties and, importantly, an elevated antioxidant effect. Preliminary observations indicate that carvedilol can increase hyperaemic flow-mediated dilation, an endothelium-dependent stimulus, of the brachial artery of patients with essential hypertension.[75]

4.2 Calcium Channel Antagonists

4.2.1 Effect on Endothelium-Dependent Vasodilation in Humans

Experimental data indicate that calcium channel antagonists increase endothelium-dependent relaxations in different vessels from a number of animal models.[68,76,77] A positive effect of this class of drugs (mainly of the dihydropyridine type) on endothelial function in different vascular beds has also been documented in patients with essential hypertension. Thus, treatment with nifedipine GITS (gastrointestinal therapeutic system) for 1 year, but not with the β-blocker atenolol, improved relaxation to acetylcholine in gluteal subcutaneous resistance-size small arteries of patients with essential hypertension.[70]

This beneficial effect of calcium channel antagonists on endothelial function was confirmed in the coronary vascular bed, in a study evaluating the acute effect of intracoronary infusion of two different calcium channel antagonists, the dihydropyridine nicardipine and the benzothiazepine-like diltiazem, on endothelium-dependent dynamic exercise-induced vasomotion in normal and stenotic epicardial vessels both of normotensive individuals and patients with essential hypertension.[78] In normal segments of epicardial coronary arteries of normotensive individuals, dynamic exercise caused coronary vasodilation. In nonstenotic vessels of patients with hypertension dynamic exercise failed to induce vasodilation, suggesting the presence of endothelial dysfunction. Moreover, exercise caused a paradoxical vasocostriction in the stenotic coronary epicardial arteries of normotensive individuals, which was even more severe in patients with essential hypertension indicating well-documented endothelial dysfunction. Furthermore, with neither of the two calcium channel antagonists did acute intracoronary administration modify endothelium-dependent vasomotion in nonstenotic vessels of normotensive individuals (vessels characterised by preserved endothelium-dependent vasodilation). On the other hand, it did reverse endothelial dysfunction in nonstenotic segments from patients with essential hypertension, and in the stenotic segments from both normotensive individuals and patients with hypertension. It is worth noting that in this study calcium channel antagonists also increased the vasodilating response to nitroglycerin. Whether the compounds, under acute intracoronary administration (an experimental model fairly remote from the usual clinical conditions), directly interacted with NO (released either by endothelial cells or nitroglycerin) or whether it is a case of nonspecifically increased coronary vasomotricity, still remains unclear.

However, the positive results achieved by calcium channel antagonists in the coronary circulation are confirmed by the Evaluation of Nifedipine and Cerivastatin sodium on Recovery of Endothelial Function (ENCORE) study (personal communication, TF Lüscher). This study assessed the effect of nifedipine GITS, the HMG-CoA reductase inhibitor cerivastatin, the combination of nifedipine and cerivastatin, and placebo treatment on the epicardial coronary arteries of patients with atherosclerotic coronary disease. The findings showed that only the calcium channel antagonist treatment resulted in a significantly greater improvement of response to acetylcholine compared with the placebo group. But it is also worth noting that the cerivastatin and nifedipine plus cerivastatin groups showed a small positive effect similar to that exerted by placebo, indicating that at least part of the drug effects were dependent on the clinical characteristics (in particular concomitant treatment) of the different study groups.

Another study on the peripheral macrocirculation demonstrates that nifedipine, but not the diuretic hydrochlorothiazide, can improve flow-mediated dilation in the brachial artery of patients with essential hypertension (n = 10).[79] This positive study is at variance with preliminary results from our laboratory indicating that in two different populations (n = 35 and n = 36, respectively) of patients with essential hypertension, 6-months treatment with two different dihydropyridine calcium channel antagonists, namely nifedipine and amlodipine, did not improve flow-mediated brachial artery dilation (unpublished observations). A

possible explanation for these contrasting results could reside in the marked difference in sample size between the two studies. This is a crucial issue, especially considering the low reproducibility of the determination of flow-mediated dilation. On the other hand, a negative result with amlodipine treatment is also reported in the Brachial Artery Normalization of Forearm Function (BANFF) study.[80] Although the latter study was not conducted in patients with essential hypertension, but rather in patients with coronary disease, amlodipine 5 mg/day for 2 months failed to increase flow-mediated dilation.[80]

Several positive studies are available in the forearm microcirculation of patients with essential hypertension. A first study[81] demonstrated that treatment with amlodipine for 2 months increased the vasoconstrictor effect of intrabrachial infusion of L-NMMA, a selective antagonist for NOS. Since the degree of vasoconstriction with this agent is directly related to the basal production of NO, this finding seems to indicate that amlodipine can increase basal NO release.[81] Such an effect may not be drug or class specific since the impaired tonic NO release observed in patients with essential hypertension is a consequence of elevated blood pressure values. It is therefore possible that mere blood pressure reduction could be sufficient to restore a normal basal NO release.

The only study describing a negative effect of calcium channel antagonist treatment on endothelial function is by Higashi et al.,[82] who used maximal vasodilation after forearm ischaemia as an endothelium-dependent stimulus. This study does not assess the effect of single compounds, but of different compounds of the same class, demonstrating that only ACE inhibitor-based treatment, and not calcium channel antagonists, β-blockers or diuretics, can successfully increase post-ischaemic vasodilation. This effect seems to be specific since it is sensitive to L-NMMA and not observed on the relaxing response to sublingual nitroglycerin. The major limitations of the Higashi study are attributable to the fact that forearm post-ischaemic vasodilation is not universally considered an endothelium-dependent stimulus[83] and that the majority of the total study population (250 of 296 patients) consisted of previously treated patients.

Assessment of the effect of calcium channel antagonists on agonist-induced endothelium-dependent vasodilation showed that treatment with lacidipine 6 mg/day for 2 and 8 months significantly increased the vasodilation to acetylcholine or bradykinin.[84] These results are reinforced by the finding that treatment with nifedipine GITS 60 mg/day for 6 months increased the vasodilating effect of acetylcholine (unpublished observations). In these same patients nifedipine blunted the vasoconstrictor effect of phenylephrine and ET-1. Finally, isradipine treatment can also increase the response to acetylcholine in the forearm microcirculation of patients with essential hypertension.[85]

4.2.2 Possible Mechanisms of Effect on Endothelial Dysfunction

The following question now arises: does the mere increase in vasodilation to acetylcholine truly represent a restoration of NO availability? The potential mechanism by which calcium channel antagonists could exert their beneficial activity on endothelial dysfunction is very unlikely to be a calcium-dependent mechanism since endothelial cells do not express voltage-operated calcium channels.[86] Experimental evidence suggests that calcium channel antagonists exert an antioxidant effect and therefore could protect endothelial cells against free-radical injury.[87,88] If this is the case, they would offer protection against the main mechanism that leads to an impairment in NO availability and consequently to endothelial dysfunction in hypertension.

This hypothesis is reinforced by evidence that treatment with nifedipine GITS 30 to 60 mg/day for 3 months restored NO availability and decreased oxidative stress in 12 patients with essential hypertension.[89] In the study in question, response to acetylcholine at baseline was found to be impaired in comparison with matched normotensive controls and resistant to L-NMMA inhibition. Intrabrachial ascorbic acid increased vasodilation

to acetylcholine and restored L-NMMA–mediated inhibition, confirming the role of oxidative stress in determining a reduction in NO availability in patients with essential hypertension. Nifedipine treatment not only increased vasodilation to acetylcholine but also restored the inhibiting effect of L-NMMA while preventing the potentiating effect of ascorbic acid (figure 1). Taken together, these results suggest that nifedipine increases the vasodilating effect of acetylcholine by restoring the NO pathway through an antioxidant mechanism. Further support for this argument is provided by findings in an adjunctive group of patients with essential hypertension, in whom nifedipine treatment reduced systemic oxidative stress by decreasing circulating plasma lipoperoxides and isoprostanes, and increasing plasma antioxidant capacity.[89]

Another study performed in our laboratory confirms this beneficial effect of calcium channel antagonists. In a double-blind, randomised trial we compared the effect of treatment with lacidipine and atenolol for 3 months on vasodilation to acetylcholine and bradykinin and the degree of inhibition exerted by L-NMMA, in order to assess NO availability. Despite a similar antihypertensive effect, lacidipine but not atenolol increased the response to acetylcholine and bradykinin, restoring the ability of L-NMMA to blunt the response to both endothelial agonists.[71] It is relevant that lacidipine but not atenolol was able to reduce plasma lipoperoxides, low density lipoprotein (LDL)/peroxides, and EC/ROS (endothelial cells/reactive oxygen species), and to increase LDL lag phase, confirming the antioxidant properties of this compound in particular and the class in general.[71]

As an additional argument corroborating the antioxidant effect of calcium channel antagonists, it is important to mention that nifedipine[90] and isradipine[91] treatment can improve endothelial function in normotensive patients with hypercholesterolaemia, an effect exerted without decreasing blood pressure or plasma cholesterol levels. Note that in hypercholesterolaemia, as in essential hypertension, endothelial dysfunction is characterised by an alteration in NO availability[53] caused by oxidative stress.[92]

Finally, preliminary evidence from our laboratory indicates that verapamil, a diphenylalkylamine, increases vasodilation to acetylcholine and restores the inhibiting activity exerted by L-NMMA on the endothelial agonist in the forearm circulation of patients with essential hypertension. These results appear to indicate that the beneficial effect of calcium channel antagonists on endothelial function and in particular on the restoration of NO availability is not limited to the dihydropyridine subtype, but is probably a class effect.

In summary, calcium channel antagonists exert activity on endothelial dysfunction in different vascular beds, including the coronary macrocirculation and peripheral microcirculation. However, conflicting results have been presented concerning the effect of dihydropyridine agents on peripheral large arteries, where available evidence is not concordant on the beneficial effect of these drugs. It should be remembered that calcium channel antagonists can improve endothelial function by restoring NO availability, an effect probably related to antioxidant properties.

4.3 ACE Inhibitors

4.3.1 Possible Mechanisms of Effect on Endothelial Dysfunction

ACE inhibitors have been studied for their potential to improve endothelial function. They are known to increase plasma levels of bradykinin, an endothelium-dependent vasodilator, by inhibiting degradation of the peptide. Moreover, since angiotensin II can cause endothelial dysfunction by inhibiting NO-synthase activity or by inducing oxidative stress through activation of membrane NADH-oxidase,[42] considerable interest is focused on the action of ACE inhibitors.

The potentially beneficial effect of ACE inhibitors has been confirmed in different models of experimental hypertension, including the spontaneously hypertensive rat (SHR) and in the L-NAME–induced hypertension model. However, in contrast to the striking improvements obtained in

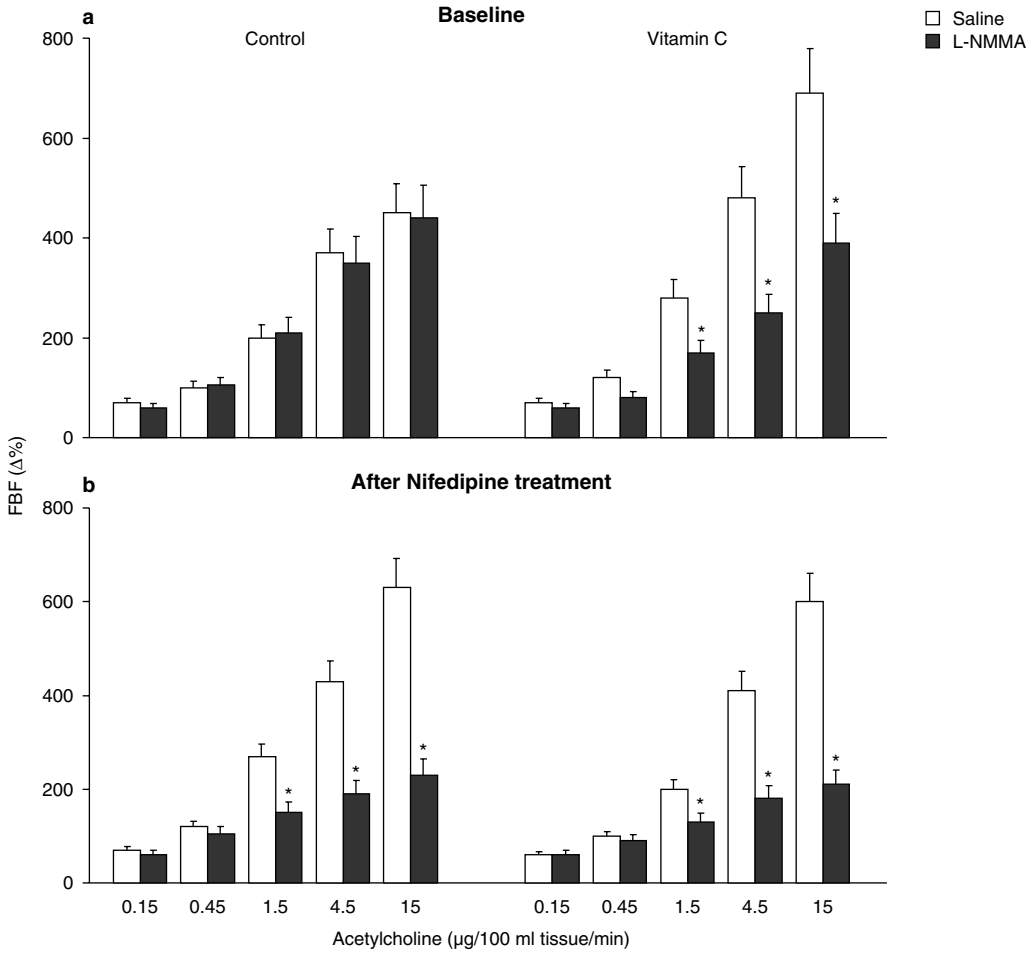

Fig. 1. Bars indicate acetylcholine-induced increase in forearm blood flow (FBF) in the absence and presence of ascorbic acid under control conditions and in the presence of N^G-monomethyl-L-arginine (L-NMMA) in patients with essential hypertension at baseline (**a**) and after 12-weeks of treatment with the dihydropyridine calcium channel antagonist nifedipine GITS 30 to 60 mg/day (**b**). At baseline the response to acetylcholine is resistant to L-NMMA. Ascorbic acid potentiated the vasodilating effect of acetylcholine and restored the inhibiting ability of L-NMMA. Taken together these results indicate the presence of oxidative stress, which reduces nitric oxide (NO) availability in patients with essential hypertension. Treatment with nifedipine increased the response to acetylcholine, restored L-NMMA–induced inhibition and prevented the beneficial effect of ascorbic acid. Thus, nifedipine increases endothelium-dependent vasodilation and restores NO availability probably by an antioxidant effect.[72] **GITS** = gastrointestinal therapeutic system; * = significant difference between infusion with and without L-NMMA ($p \leq 0.05$)

experimental models of hypertension, results in patients with essential hypertension are controversial.

4.3.2 Effect on Endothelium-Dependent Vasodilation in Humans

Cilazapril therapy for 2 years, but not 1 year, improved the blunted response to acetylcholine in the subcutaneous microcirculation of patients with essential hypertension.[93,94] Similar results were obtained after treatment with lisinopril for 3 years.[95]

In patients with essential hypertension, acute endovenous administration of the ACE inhibitor perindoprilat (1mg) restored normal vascular re-

sponse to endothelial stimuli (such as the cold-pressure test and flow-mediated dilation induced by papaverine) in coronary epicardial arteries without overt atherosclerosis, thus restoring normal endothelium-dependent vasodilation (figure 2).[96] This effect seems to be specific since peridomprilat did not change the vasodilating response to nitroglycerin.

In addition to this positive study, the TREND (Trial on Reversing Endothelial Dysfunction) study[97] used a double-blind, randomised, placebo-controlled design to evaluate the effect of the ACE inhibitor quinapril on coronary artery diameter response to intracoronary infusion of acetylcholine in normotensive patients with coronary artery disease. Quinapril 40 mg/day for 6 months significantly decreased epicardial coronary vasoconstriction to acetylcholine (from 14.3% at baseline to 2.3% at follow-up). The major problems with interpretation of these results are related both to the lack of a control infusion, such as an exogenous nitrate, for a possible nonspecific effect of the ACE inhibitor, and also to the study population characteristics, consisting of patients with ischaemic coronary artery disease. Caution should be exercised in extrapolating these results to patients with essential hypertension.

The beneficial effect of ACE inhibitors on large arteries seems to have been confirmed by several studies in the peripheral circulation. In our laboratory, perindopril 4 mg/day for 6 months increased flow-mediated dilation in a large population of patients with essential hypertension (personal communication, L. Ghiadoni). Our findings are in line with the previously quoted BANFF study[80] demonstrating that quinapril 20 mg/day improves flow-mediated dilation in patients with coronary artery disease. On the other hand, in the BANFF study enalapril 10 mg/day failed to exert this positive effect. However, it should be pointed out that a daily dose of enalapril at 10mg is not equipotent, in terms of 24-hour duration of action, to a daily dose of quinapril at 20mg.

In patients with coronary artery disease, ramipril 10 mg/day for 4 weeks improves flow-mediated dilation of the brachial artery.[98] In these patients the potentiating effect exerted by ramipril was blunted by L-NMMA, indicating that the treatment improves NO availability. Moreover, since ramipril administration prevents the facilitating activity of ascorbic acid on endothelium-dependent vasodilation, it is very likely that the ACE inhibitor has an antioxidant activity.

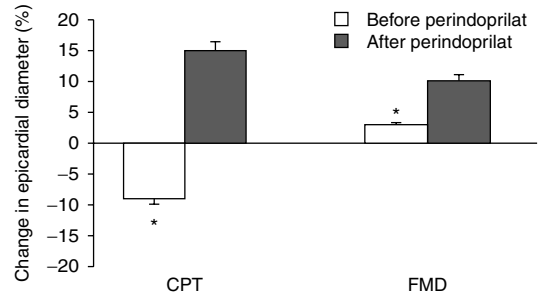

Fig. 2. Bars indicate the effect of intravenous infusion of perindoprilat on endothelial function in epicardial coronary arteries of patients with essential hypertension. It is evident that the ACE inhibitor causes the paradoxical vasoconstriction induced by the cold pressor test (CPT), an index of endothelial dysfunction, to revert to vasodilation and increases the impaired flow mediated dilation (FMD; induced by distal injection of papaverine).[71]
* = significant difference before and after perindoprilat.

The beneficial effect of ACE inhibitors on endothelial function has been also documented in the renal circulation. In this vascular district of patients with essential hypertension, ACE inhibitors can specifically improve the renal vasodilating response to systemic L-arginine and restore cyclic guanosine monophosphate excretion.[99]

Finally, in the forearm vasculature, enalapril can increase the vasoconstrictor effect exerted by L-NMMA, thus improving tonic NO release.[81] However, as mentioned earlier in section 4.2.1, this effect could be nonspecific since it is probably related to blood pressure normalisation.

Higashi et al.,[82] demonstrated that treatment with an ACE inhibitor increased post-ischaemic vasodilation in the forearm microcirculation of patients with essential hypertension. Although these authors showed that the potentiating effect exerted by the ACE inhibitor is sensitive to L-NMMA in-

fusion, it must be kept in mind that post-ischaemic vasodilation is mainly an expression of structural alteration. An extensive body of evidence now indicates that ACE inhibitors are highly potent in achieving regression of vascular structural alteration.

Impaired endothelium-dependent vasodilation in response to methacholine or actylcholine was not altered by treatment with captopril or enalapril for 2 months,[66] or cilazapril for 5 months[100], respectively, in patients with essential hypertension. Similar negative results on vasodilationin response to acetylcholine were confirmed after treatment with lisinopril for 1 year in patients with hypertension.[101] On the other hand, in the same group of patients lisinopril increased vasodilatation in response to bradykinin, an effect probably dependent on inhibition of kinin breakdown. It has been shown that the potentiating effect of lisinopril on response to bradykinin is resistant to L-NMMA, but sensitive to ouabain, an inhibitor of the Na^+/K^+ adenosine triphosphate (ATP)ase pump, which antagonises the effect of hyperpolarisation (figure 3).[30] Therefore, ACE-inhibitor-induced potentiation of the vasodilating effect of bradykinin cannot be ascribed to increased NO availability, but rather to an ouabain-sensitive pathway, possibly hyperpolarisation.

In summary, ACE inhibitors can restore endothelial function above all in large coronary and peripheral arteries, while this effect is more difficult to obtain in the peripheral microcirculation. There is a lack of clear information on whether the beneficial effect of ACE inhibitors on the large arteries is related to restoration of NO availability. It should be remembered that in the peripheral microcirculation, selective potentiation of vasodilation to bradykinin is independent of the L-arginine–NO pathway and probably related to hyperpolarisation.

4.4 Angiotensin II Receptor Antagonists

4.4.1 Possible Mechanisms of Effect on Endothelial Dysfunction

Experimental data indicate that angiotensin II exerts a negative effect on endothelial function through release of ET-1 from vascular cells,[102] production of the vasoconstrictor prostanoid PG H_2 from endothelium[103] and inhibition of NOS activity by activation of protein kinase C.[104] Moreover, both in cultured vascular smooth muscle cells and in rats, treatment with angiotensin II increased oxygen-free radical production via membrane-bound NADH/NADPH-driven oxidases, an effect associated with impaired relaxation to acetylcholine.[42,43] Additional studies

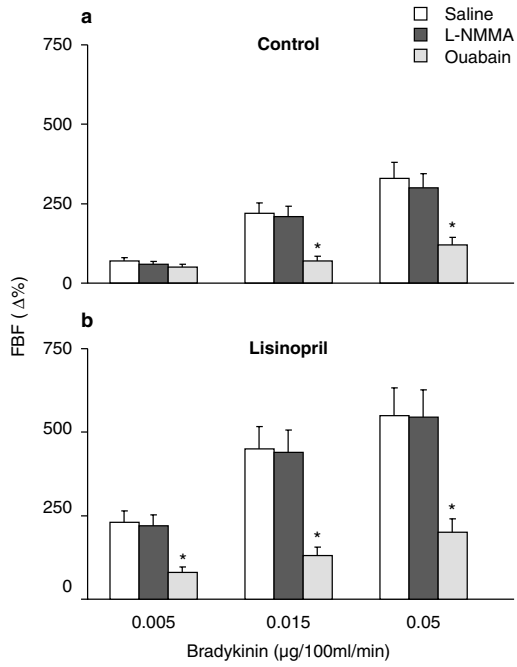

Fig. 3. Bars indicate bradykinin-induced increase in forearm blood flow (FBF) under control conditions and in the presence of N^G-monomethyl-L-arginine (L-NMMA) or ouabain in patients with essential hypertension before and after single oral administration of the ACE inhibitor lisinopril 20mg. It is evident that the response to bradykinin is sensitive to ouabain, but not to L-NMMA, indicating the presence of impaired nitric oxide (NO) availability. Lisinopril administration increases the vaodilating effect of bradykinin but the response remains resistant to L-NMMA. Thus, the ACE inhibitor potentiates the endothelium-dependent relaxation to bradykinin but it does not restore NO availability.[53] * = significant difference between infusion with and without ouabain ($p \leq 0.01$)

have shown that when losartan, an angiotensin type-1 (AT_1) receptor antagonist, was administered simultaneously with angiotensin II, vascular superoxide anion production and relaxation to acetylcholine were normalised, demonstrating a role for the AT_1 receptor in these processes.[43] It has also been found that in the presence of an AT_1 receptor antagonist, angiotensin II can bind to unblocked AT_2 receptors,[105] which may stimulate NO synthesis in endothelial cells[44] and in isolated coronary microvessels.[106] Finally, in aortic rings from SHR, prolonged antihypertensive treatment with losartan reversed endothelial dysfunction not only by enhancing NO-dependent relaxation but also by reducing cyclo-oxygenase–dependent EDCF formation.[107]

On the basis of these findings it could be hypothesised that AT_1 receptor antagonists may restore NO availability by reducing this angiotensin II-mediated negative influence on endothelium.

4.4.2 Effect on Endothelium-Dependent Vasodilation in Humans

A study exploring this hypothesis found that in patients with essential hypertension, therapy with losartan 50 to 100 mg/day for 1 year was able to restore the vasodilating effect of acetylcholine in the subcutaneous microcirculation (figure 4).[108] In contrast, in forearm microcirculation, candesartan 8 to 16 mg/day for up to 1 year did not improve the impaired response to acetylcholine or the lack of inhibition exerted by L-NMMA.[109] Despite this, candesartan produced a beneficial effect on other aspects of endothelial function. It increased the vasoconstrictor effect of L-NMMA, indicating that treatment with AT_1-receptor antagonists can increase tonic NO release (figure 5).[109]

However, the real new and interesting finding of this study is that candesartan reduced the vasodilating effect of TAK-044, a nonselective antagonist for ET-1 receptors (fig. 5).[109] The degree of vasodilation in response to this agent is an indirect index of the vasoconstrictor activity of ET-1. Previous evidence demonstrated that the vasodilating effect of TAK-044 is augmented in patients with essential hypertension compared with healthy controls,[39] suggesting an increased vasoconstrictor activity for endogenous ET-1 in essential hypertension. Therefore, this beneficial effect of candesartan on the biological activity of ET-1, possibly caused by blockade of the positive feed-back of angiotensin II on endothelin synthesis, could be relevant for preventing or reversing cardiovascular functional and structural alterations ascribable to ET-1.

No published studies are available on the effects of AT_1-receptor antagonists on large arteries in patients with essential hypertension. In our laboratory we have observed that telmisartan 40 to 80 mg/day for up to 6 months failed to improved flow-mediated dilation in patients with essential hypertension [n = 30] (unpublished data). Conflicting results are available in patients with atherosclerosis. Whereas the BANFF study[80] demonstrated that losartan did not increase flow-dependent dilation (although a nonstatistically significant positive trend was observed), Prasad et al.[110] demonstrated that oral losartan for 8 weeks improved flow-dependent dilation without effect on the response to nitroglycerin. Similar results with losartan have been obtained in the study by Horning et al.[98] In this study, losartan 100 mg/day for

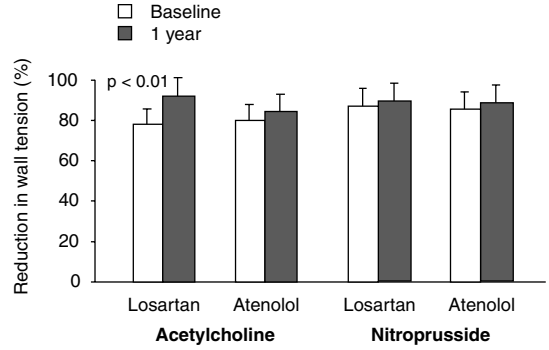

Fig. 4. Bars represent the vasodilating effect of acetylcholine and sodium nitroprusside in the subcutaneous microcirculation of patients with essential hypertension at baseline and after 1 year of treatment with the b-blocker atenolol or the AT_1-receptor antagonist losartan. Losartan, but not atenolol, increased the relaxing response to acetylcholine. Responses to sodium nitroprusside were not affected by either treatment.[89]
AT = angiotensin II.

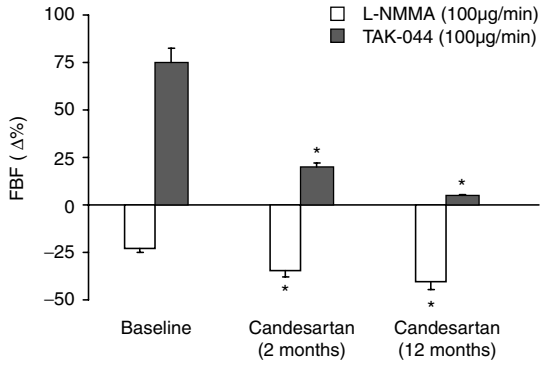

Fig. 5. Bars represent forearm blood flow (FBF) modification induced by intra-arterial N^G-monomethyl-L-arginine (L-NMMA), a selective nitric oxide (NO)-synthase inhibitor, or TAK-044, a non selective antagonist of endothelin (ET)-1 receptors, at baseline and after 2 and 12 months' treatment with the AT_1-receptor antagonist candesartan (8-16 mg/day). At baseline, L-NMMA causes vasoconstriction, an index of NO contribution to basal vascular tone, while TAK-044 induces vasodilation, an index of the vasoconstrictor influence exerted by endogenous ET-1. Candesartan increases the vasoconstriction induced by L-NMMA and decreases the vasodilation to TAK-044, indicating that this treatment can increase basal NO production and reduce endogenous ET-1 synthesis.[90] **AT** = angiotensin II; * = significant different between baseline and 2 and 12 months' treatment.

4 weeks improved flow-mediated dilation of the brachial artery by restoring NO availability in patients with coronary artery disease. The degree of the beneficial effect of losartan was similar to that exerted by ramipril.

In summary, very little and discordant evidence is available concerning the effect of AT_1-receptor antagonists on endothelial function in patients with essential hypertension.

5. Is the Restoration of Endothelial Function Clinically Relevant?

Several issues must be addressed before the actual clinical relevance of reversing endothelial dysfunction in patients with essential hypertension can be appropriately evaluated. In particular, one of the most striking discoveries to emerge from the results dicussed in section 4 is that the effectiveness of pharmacological treatment may differ according to which vascular district is explored or which endothelial stimulus is applied. Treatment-induced reversal of endothelial dysfunction is clearly not a constant phenomenon. On the other hand, these different responses are probably in agreement with the concept that endothelial cells act in an autocrine/paracrine manner and can be activated through different pathways (mechanical forces, receptors).[1] Even agonist-evoked endothelial activation acts through different signal transduction pathways.[1] Therefore, the crucial problem is to establish whether clinical relevance is affected if endothelial dysfunction is assessed in large arteries or resistance arterioles or investigated with agonists (acetylcholine, bradykinin, etc), or via response to flow (flow-mediated dilation) or mixed stimuli (dynamic exercise, cold-pressor test).

The only available evidence suggests that in patients with coronary artery disease, response to acetylcholine in the coronary microcirculation and to acetylcholine, cold-pressor test and flow-mediated dilation in epicardial coronary arteries can predict cardiac events.[62,63] As regards the peripheral circulation, another study[64] in patients with ischaemic heart disease showed a slight association between brachial artery flow-mediated dilation and cardiac outcome.

Unfortunately, no evidence is available concerning the prognostic impact of endothelial dysfunction (tested in different vascular districts and with different stimuli) in patients with cardiovascular risk factors, including essential hypertension. If no information is available regarding the clinical impact of endothelial dysfunction in patients with essential hypertension it is difficult to establish whether pharmacologically-induced improvement of this alteration can represent an adjunctive beneficial effect beyond blood pressure normalisation.

What can be probably stated is that reversing endothelial dysfunction is very unlikely to contribute to blood pressure control. This possibility is in line with the evidence that the calcium channel antagonist lacidipine normalises blood pressure values and increases endothelium-dependent vasodilation in patients with essential hypertension.[71]

However, after a 2-week drug withdrawal, although patients reverted to being hypertensive, the beneficial effect of the drug on endothelial function remained. Thus, these results seem to separate the improvement of endothelium-dependent vasodilation from blood pressure control.

As for the relationship between restoration of endothelial function and better cardiovascular prognosis, one possible argument against the clinical relevance of improvement in endothelium-dependent vasodilation is that no evidence is available to demonstrate a greater beneficial effect of drug classes that are effective in reversing endothelial dysfunction (such as calcium antagonists or ACE inhibitors) compared with other drug classes in morbidity and mortality trials in patients with essential hypertension.[111] However, this observation is probably not pertinent since such trials last no longer than 5 years (usually around 3 to 4 years) and may not fully reflect the clinical situation of patients with hypertension treated over several decades. Trials have shown that in the very short term (3 to 5 years) the most important mechanism effective in reducing morbidity and mortality in patients with essential hypertension is blood pressure reduction, independently of the drug class employed.[110] There is no information available from long-term prospective studies on the effect of different drugs. If endothelial dysfunction is a promoter of atherosclerosis, it is conceivable that the potential beneficial effect obtained by prevention of this alteration could be more clearly revealed by prolonged treatment of middle-aged patients (a realistic clinical condition) rather than by short-term treatment of relatively aged, high-risk patients.

However, other possibilities must be taken into serious consideration. Despite the knowledge that endothelial cells can interact not only with vascular tone, but also with mechanisms involved in platelet aggregation, cell proliferation and migration, vascular permeability and coagulation, vasodilation is usually considered as an integrated marker for endothelial function. This extrapolation can be quite dangerous, since it cannot be ruled out that a given approach may increase vasodilation, but worsen other aspects of endothelial function. For instance, estrogen administration can increase endothelium-dependent vasodilation,[112,113] but in certain circumstances it worsens inflammation by increasing expression of endothelial adhesion molecules,[114] levels of C-reactive protein,[115] and coagulation.[115,116] Thus, the clinical effect of a given treatment is clearly very hard to predict if it derives from a balance between opposite effects on the various aspects of endothelial function. The possibility can not be excluded that an increase in endothelium-dependent vasodilation may not in itself be a valid surrogate marker for improvement of the entire complex function of the endothelium.

Therefore, before considering endothelial dysfunction as an established target for antihypertensive treatment, further large trials are necessary to investigate whether the beneficial effect of treatment in terms of cardiovascular events could be directly related to reversal of endothelial dysfunction. Without this kind of information, at the present time the impairment of endothelial function remains a mechanism of disease; no clinical demonstration indicates that pharmacological improvement of this alteration might also improve the prognosis of patients with essential hypertension.

6. Conclusions

Endothelial dysfunction occurs in essential hypertension and involves enhanced release of EDCF including prostanoids, oxygen-free radicals and ET-1 as well as decreased availability of NO caused by oxidative stress-induced breakdown. Endothelial dysfunction may be of particular clinical relevance since it can be a promoter of atherosclerotic and thrombotic damage, which is a typical complication of hypertension. Thus, although not clearly demonstrated, the suggestion that antihypertensive pharmacological treatment could reverse endothelial dysfunction might be important.

At the present time, convincing results are available that calcium channel antagonists can restore endothelium-dependent vasodilation in different vascular beds. These compounds can also restore NO availability by a mechanism possibly related to

antioxidant activity. ACE inhibitors, on the other hand, seem to improve endothelial function in subcutaneous, epicardial and renal circulation, whereas they are ineffective in potentiating the blunted response to acetylcholine in the forearm of patients with essential hypertension. In addition, they can selectively improve endothelium-dependent vasodilation to bradykinin, an effect probably mediated by a hyperpolarising factor and not by restoring NO availability. AT_1-receptor antagonists can restore endothelium-dependent vasodilation to acetylcholine in the subcutaneous microcirculation, but not in that of forearm muscle. They also display the ability to improve basal NO release and decrease the vasoconstrictor effect of endogenous ET-1.

Thus despite considerable evidence that impaired endothelium-dependent vasodilation can be improved by appropriate antihypertensive treatment, further large scale clinical trials are required to prove conclusively whether reversal of endothelial dysfunction offers a clinical advantage in patients with essential hypertension.

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