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Effects of antihypertensive drugs on conduit artery endothelial function

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Abstract

Essential hypertension is associated with endothelial dysfunction, which is caused by decreased nitric oxide (NO) availability, resulting in an impairment of 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 in peripheral conduit arteries. Little evidence is available with diuretics. Treatment with the ß-adrenoceptor antagonists atenolol and nebivolol is negative. Insufficient evidence is available to establish whether new compounds such as carvedilol, which has strong antioxidant activity, can improve endothelial function in hypertensive patients. Calcium antagonists that can reverse impaired endothelium-dependent vasodilation in different vascular districts, including epicardial and forearm microcirculation, show conflicting results in the brachial artery of essential hypertensive patients. ACE-inhibitors, on the other hand, seem to improve endothelial function in epicardial and peripheral conduit arteries, whereas they are ineffective in the peripheral microcirculation. However, they selectively improve endothelium-dependent vasodilation to bradykinin, an effect probably related to hyperpolarization. Finally, evidence concerning the effect of angiotensin II receptor antagonists on the brachial artery in patients with hypertension and atherosclerosis is inconclusive.

In conclusion, despite the considerable evidence that impaired endothelium-dependent vasodilation can be improved by appropriate antihypertensive treatment, no clinical data are available to demonstrate that reversal of endothelial dysfunction is associated with a reduction in cardiovascular events. To acquire further knowledge on this issue, large scale clinical trials will be required to demonstrate that treatment of endothelial dysfunction can lead to better prognosis in essential hypertensive patients.

Keywords: Arteries, Endothelium, Flow mediated dilation, Hypertension, Treatment.

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Introduction

The most significant advance in cardiovascular medicine over the last two decades has probably been the identification of endothelial cells as a vasoactive organ. After the pioneering report by the 1998 Nobel Prize-winner Robert Furchgott[1], 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 the dysfunctioning endothelium, which is characteristic of essential hypertension and most cardiovascular risk factors, as a major promoter for athero-thrombosis and, consequently, cardiovascular events. A logical consequence of this growing body of knowledge is that endothelial dysfunction is now considered an important target for cardiovascular treatment. The present review will focus on available data concerning the effect of antihypertensive treatment on conduit artery endothelial function in humans.

Endothelium-derived relaxing and contracting factors

The endothelium produces several relaxing factors including nitric oxide (NO), prostacyclin and a not yet identified hyperpolarizing relaxing factor (EDHF)[2]. The best characterized, and probably the most important, relaxing factor is NO[3], which is derived from transformation of the amino acid L-Arginine into citrulline by the activity of NO synthase, a constitutive enzyme present in endothelial cells[4]. 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[2]. Endothelial cells can also induce relaxation by causing hyperpolarization[5]. 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 NO synthase antagonists, thus ruling out NO as responsible for this activity[5].

Endothelial cells can also produce endothelium-derived contracting factors (EDCFs). The principal EDCF is endothelin-1 (ET-1), which acts through specific receptors named ETA and ETB[6] and induces a sustained and potent vasoconstrictor action[7]. ETA receptors are represented only on smooth muscle cells and have the function of promoting growth and mediating contractions[6]. In contrast, ETB receptors are located on both endothelial and smooth muscle cells, with opposite effects. Smooth muscle cell ETB receptors evoke contractions[6,8], whereas endothelial ETB receptors induce relaxation by production of endothelium-derived relaxing factors, including nitric oxide (NO)[9].

In particular conditions such as aging or menopause, and in certain pathological conditions, for example hypertension, diabetes mellitus, atherosclerosis, vasospasm and reperfusion injury, activation of endothelial cells can lead to production and release of contracting factors including cyclo-oxygenase-derived endothelium-dependent contracting factors. These are mainly represented by prostanoids (thromboxane A_2 and prostaglandin H_2)[10] and oxygen free radicals[11], which counteract the relaxing activity of NO. Oxygen free radicals can also impair endothelial function by causing NO breakdown[12]. It is of relevance that the concept of endothelial dysfunction is predominantly related to parallel activation of the NO and EDCF pathways. Even in the presence of preserved NO production, EDCFs can impair NO availability or biological effects. Thus in several experimental conditions, EDCF pathway blockade can lead to complete restoration of the L-arginine-NO pathway.

Definition and assessment of endothelial dysfunction

Endothelial dysfunction is defined as a functional and reversible alteration of endothelial cells resulting from an impairment in NO availability. This alteration leads to a deep derangement of endothelial equilibrium, resulting in the functional prevalence of EDCFs and consequent activation of pathways that bring about functional and structural vascular alterations. This in turn promotes the development of atherosclerosis. Note, in this context, that the presence of endothelial dysfunction has been associated with the occurrence of cardiovascular events in longitudinal studies[13]. Endothelial dysfunction is thus an important alteration, which must however be distinguished from endothelial damage. The latter is represented by the anatomical disruption of the endothelium. This differentiation is crucial since endothelial dysfunction is in general an early alteration which is potentially reversible by appropriate treatment. In contrast, endothelial damage is a more serious event since endothelial cell regeneration is far more difficult to achieve. Moreover regenerated cells very often present an irreversible dysfunction.

Before describing the techniques employed to assess endothelial responses, attention should focus on two major issues that significantly affect the interpretation of results derived from clinical studies. First, since the endothelium is an autocrine/paracrine system, results obtained in

any given vascular bed should be limited to the district explored. Yet results are frequently extrapolated systemically, an inference which is incorrect. This means that results obtained in the peripheral micro-circulation cannot be extrapolated to large peripheral arteries or coronary micro- and macrocirculation. Second, what we call "endothelial function" or "endothelium-dependent vasodilation" is a complex event resulting from the interaction of different substances and pathways. Endothelium-dependent vasodilation is not always equivalent to NO-dependent vasodilation, since the relaxing activity of endothelial cells can be sustained by other substances including EDHFs or prostacyclin. Only by keeping in mind the complexity of endothelial responses is it possible to appropriately interpret the information derived from the available experi-mental approach.

Endothelial function is usually assessed by vascular reactivity tests[14]. In several vascular districts it is possible to activate or inhibit endothelial cells and measure the vessel changes induced by the experimental perturbation. Endothelial cells can be activated by agonists operating through specific receptors (acetylcholine, bradykinin, substance P etc.) or by increasing shear stress. In addition it is possible to block pathways involved in endothelial responses such as NO synthase activity (by L-NMMA), hyperpolarization (by ouabain), cyclooxygenase activity (by indomethacin) or oxidative stress (by antioxidants such as vitamin C or E and several drugs).

When describing the approach to evaluation of endothelium-dependent mechanisms in humans, the most important first step is to consider which vascular bed is investigated. A distinction must be made between the microcirculation and large arteries. These two vessel types are differently regulated and results obtained in microvascular districts cannot be extrapolated to large arteries. The microcirculation can be evaluated in skin, subcutaneous tissue, peripheral muscle (usually forearm) and coronary circulation. Large arteries, on the other hand, include the brachial, radial, femoral and epicardial arteries.

Skin microcirculation can be assessed by laser-doppler fluxmeter[15] while endothelial cell stimulation is obtained by drug administration via iontophoresis. Subcutaneous micro-circulation can be studied by an *ex-vivo in-vitro* technique with a myograph device after gluteal biopsy[16]. The assessment of endothelium-dependent vasodilation in the forearm microcirculation requires cannulation of the brachial artery, allowing infusion of agonists (acetylcholine, bradykinin etc.) and antagonists (L-NMMA, vitamin C etc.) at systemically ineffective rates with simultaneous evaluation of forearm blood flow changes by strain gauge venous plethysmography[17,18,19]. A similar approach is employed to evaluate coronary microcirculation. Thus compounds can be injected directly into an epicardial coronary artery during an angiographic test, coronary microvascular modifications then being measured by doppler[20].

Study of endothelial function at large artery level is performed by means of quantitative angiography for epicardial arteries[20] and vascular ultrasounds for brachial, radial and femoral arteries[21]. Endothelial cell activity can be stimulated or inhibited by specific agonists (acetylcholine, bradykinin) or antagonists (L-NMMA), by increasing shear stress or by mixed stimuli such as the dynamic exercise or cold pressor tests, which activate the endothelium by alpha-2 receptor stimulation and shear-stress increase.

The most widely used method to measure endothelial function in humans is the determination of brachial artery flow-mediated dilation (FMD). This method is of interest because it is non invasive and ap-parently simple[21]. Briefly, it is sufficient to perform distal ischemia, followed by vascular ultrasound measurement of the change in brachial artery diameter induced by post-ischemic flow increase (Figure 1). End-diastolic frames (ECG-triggered)

Stimuls: Reactive Hypertemia (blood flow increase)

Endothelium-dependent response: brachial artery diameter increase
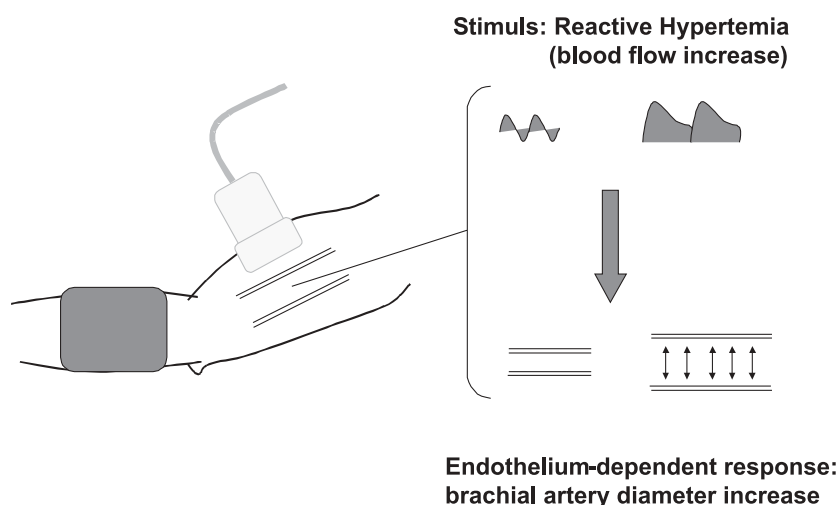
Figure 1 – Schematic representation of evaluation of flow-mediated dilation in the brachial artery. For further explanations see text.

from B-mode scan of the brachial artery are usually obtained in longitudinal section between 5 cm and 10 cm above the elbow using a 7-10 MHz linear array transducer[22]. A cuff is placed around the forearm just below the elbow. The cuff is inflated for five minutes at 200-300 mmHg and then deflated to induce reactive hyperemia, which is measured from arterial flow velocity obtained by pulsed Doppler. Reactive hyperemia increases shear stress and consequently induces NO-dependent dilation in the brachial artery[23], which is maximal after approximately one minute[22] (Figure 2).

The major problems with this method concern its very low reproducibility[24], which can be increased by utilizing a mechanical arm to secure the probe firmly in position and a computerized system to analyse changes in brachial artery diameter[25-27].

The degree of response to an endothelial stimulus is usually considered to be the marker of endothelial function. As a consequence, if a given study population shows blunted endothelium-dependent vasodilation as compared to another study population, this finding is considered an index of endothelial dysfunction. However such a statement is true only if it can be demonstrated that the vasodilating effect of an endothelium-independent agonist (usually nitrates such as sodium nitroprusside or nitroglycerin) does not differ between the two study populations.

Another dangerous inference lies in considering the response to an endothelial agonist as a marker of NO-dependent vasodilation. The endothelial response is an integrated mechanism and a number of different pathways and mediators account for endothelium-dependent vasodilation. Thus in the human forearm of healthy subjects the vasodilating effect of acetylcholine or bradykinin is mediated by NO, since the response to the agonist can be inhibited by L-NMMA[28]. But in hypertensive patients the response to both agonists is not only impaired as compared to healthy controls, but it is also resistant to L-NMMA blockade, demonstrating that a different pathway is responsible for the endothelial response in this study population[28,29]. It is very likely that hyperpolarization accounts for endothelium-dependent vasodilation in presence of impaired NO availability[29]. In line with this interpretation, when a treatment is shown to increase a depressed endothelial response, such a result cannot automatically be interpreted as an augmentation of NO production. Only if compounds such as L-NMMA are tested can the NO-dependent component of endothelium-dependent vasodilation be correctly quantified.

What is the best method for evaluating endothelial function? There is no answer to this question. The endothelium is an autocrine/paracrine system. Therefore endothelial responses are valid only for the vascular district under examination. Available evidence and unpublished observations demonstrate a very low correlation between endothelial function studied in different vascular districts of the same subjects. Preliminary results from our laboratory demonstrate an r = 0.38 correlation between the response to acetylcholine in the forearm circulation and brachial artery flow-mediated dilation recorded in the same subjects. This low correlation may depend not only on the different vascular district, but also on the different kind of stimulation (receptor-operated agonist and increase in shear stress). It is however interesting that although there is a low correlation between brachial artery flow-mediated dilation and the
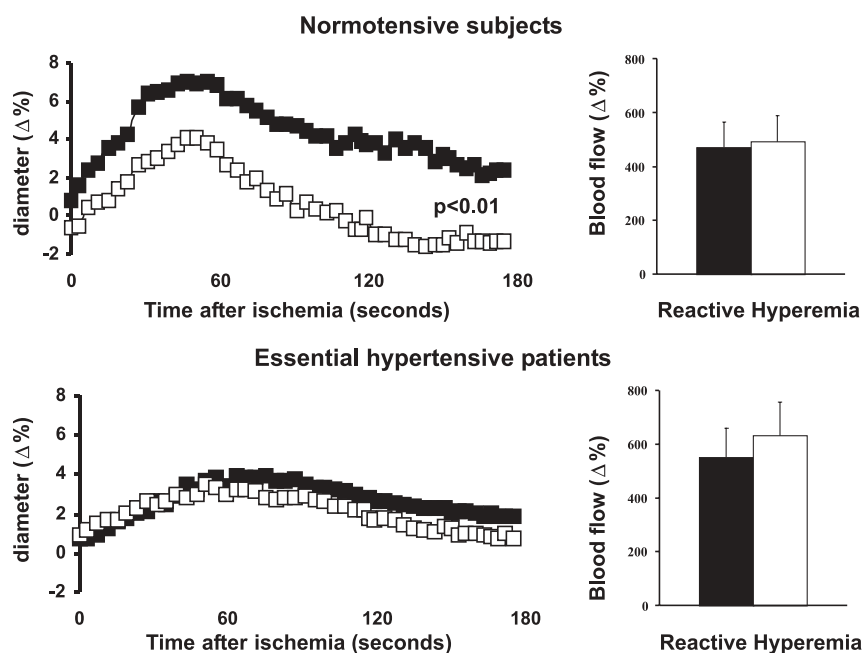
Figure 2 – Line graphs indicate flow mediated dilation (expressed as percent (%) increase in diameter), and bars (expressed as percent (%) increase in blood flow) indicate reactive hyperemia, in the brachial artery in the absence (black) or presence of NG-monomethyl-L-arginine (L-NMMA, white).

response to acetylcholine in epicardial coronary arteries, all patients who show endothelial dysfunction in the peripheral circulation have the same alteration at coronary artery level[30].

Thus it is very likely that endothelial dysfunction diagnosed at the level of the peripheral circulation can be representative of other more important vascular districts.

Endothelial dysfunction in essential hypertension

Endothelial dysfunction is now recognised as a characteristic of patients with essential hypertension[17,18][19]. Reduced FMD is found in the brachial artery of patients with essential hypertension as compared to normotensive subjects[26]. It is worth noting that reactive hyperemia and response to nitrates are similar in the two groups. Preliminary data from our laboratory obtained in a population of 200 hypertensive patients and 150 healthy controls show that over 50% of hypertensive patients had FMD values within the 90% confidence limits of healthy subjects. This finding highlights the low power of discrimination of the technique and therefore the need for adequate power size calculations in clinical studies[22,24].

Available evidence concurs in indicating that endothelial dysfunction associated with essential hypertension is characterized by impaired NO availability. In essential hypertensive patients L-NMMA infusion does not significantly blunt FMD in the brachial artery (Figure 2) or the response to agonists such as acetylcholine or bradykinin as compared to healthy controls[19,28,29]. Taken together these results indicate the presence of impaired stimulated NO-release in arteries of essential hypertensive patients.

Inquiry 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[31]. These reactive oxygen species, mainly superoxide anions, combine and destroy NO producing peroxynitrates, which have several negative effects on vascular function and structure[2]. The role of oxidative stress is supported by the evidence that vitamin C, an oxygen free radical scavenger, can increase the response to acetylcholine in the peripheral circulation and in the coronary epicardial artery of essential hypertensive patients[19,32]. It is interesting that vitamin C, administered orally at the dosage of 2 g, improved FMD in the brachial artery of patients with coronary artery disease[33], but was ineffective in patients with essential hypertension[34], where much higher concentrations of the antioxidant are needed to improve endothelium-dependent vasodilation[19,35].

Moreover, an interaction between the NO-system and endothelial vasoconstrictor substances, mainly ET-1 and angiotensin II, can participate in the pathogenesis of endothelial dysfunction[36].

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

The clinical relevance of the presence of endothelial dysfunction in hypertension is attributable to the fact that NO and EDCFs not only exert an opposite effect on vascular tone but also respectively inhibit and activate mechanisms such as platelet aggregation[37], vascular smooth muscle cell proliferation[38], and migration[39], monocyte adhesion[40] and adhesion molecule expression[41] which exert an important role in the genesis of thrombosis and atherosclerotic plaque. In effect, endothelial dysfunction is 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
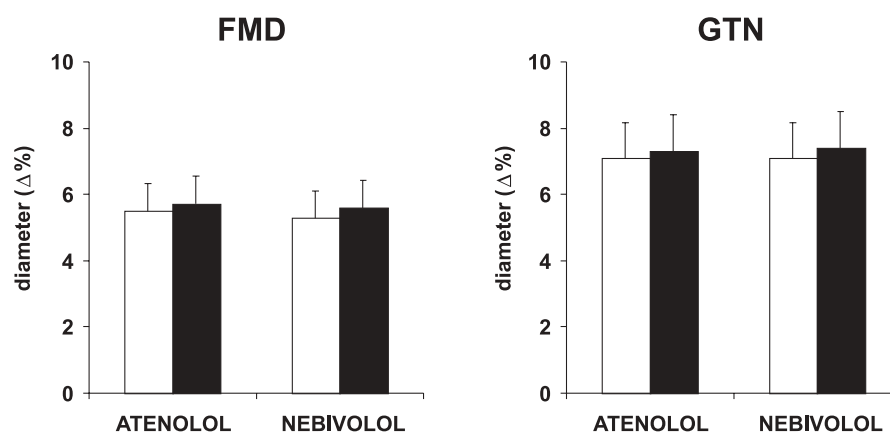
Figure 3 – Bars indicate flow mediated dilation (FMD) and response to 25 µg of sublingula glyceril trinitrate (GTN), expressed as percent (%) diameter increase, in the brachial artery of essential hypertensive patients at baseline (white) or after 6-month treatment (black) with atenolol (50-100 mg) or nebivolol (5-10 mg/daily).

is not specific to essential hypertension. Rather, it is a common alteration of the major cardiovascular risk factors, including aging[42,43], menopause[44], hypercholesterolemia[31], smoking[45], diabetes[25], and hyperhomocysteinemia[46,47]. It is conceivable that such an alteration may not be a mechanism participating in the pathogenesis of high blood pressure values. It is more likely to be a common pathogenetic mechanism leading to cardiovascular events in patients with cardiovascular risk factors.

Evidence is mounting that the presence of endothelial dysfunction is associated with markers of vascular damage and with cardiovascular events. In essential hypertensive patients impaired forearm response to acetylcholine is correlated with intima-media thickening of carotid arteries, an index of atherosclerosis[48]. Moreover in epicardial coronary arteries of normotensive subjects the response to acetylcholine shows an inverse correlation with intramural plaque as detected by intravascular ultrasounds[49]. Finally, in epicardial coronary arteries of patients with cardiac transplantation, endothelial dysfunction is a predictor of the subsequent development of arteriolosclerosis[50].

It is worth noting that the presence of endothelial dysfunction in the coronary circulation has been associated with the occurrence of cardiovascular events in longitudinal studies in patients with mild coronary artery disease[51,52]. Furthermore, the presence of endothelial dysfunction in peripheral large arteries (FMD in the brachial artery) has also been associated with increased coronary events[53].

Although these studies may be biased by low numerosity in the study population, concordant evidence is accumulating to suggest that endothelial dysfunction acts as a pathogenetic mechanism causing cardiovascular disease.

Effect of antihypertensive drugs

The above line of reasoning suggests that although impaired endothelium-dependent vasodilation is probably not involved in the pathogenesis of increased blood pressure values, in essential hypertensive patients it could act as a promoter of the atherosclerotic lesions which are one of the most serious complications of essential hypertension[13]. Such a hypothesis raises the issue that reversing impaired endothelium-dependent vasodilation could constitute an important goal for antihypertensive therapy.

Awareness that mere blood pressure normalization is not sufficient to normalize response to agonists[54,55] or FMD[26] in essential hypertensive patients is of crucial importance. It implies that antihypertensive drugs must be endowed with the ability to restore endothelial function, a specific property which goes far beyond blood pressure reduction. Antihypertensive drugs must therefore be reconsidered in terms of specific efficacy on endothelial function. Experimental studies indicate that the majority of available compounds have the potential to improve endothelium-dependent relaxations[56]. Drugs can act by different mechanisms including activation of NO-synthase, a scavenger activity on oxidative stress, or by decreasing the production of oxygen free radicals (Table 1). However, when the same compounds have been tested in a clinical setting, positive animal evidence has not always been confirmed.

This review will now examine available evidence documenting the effect of antihypertensive treatment on conduit artery endothelial function in essential hypertensive patients. However, several preliminary issues must be taken into account. First, when discussing the effect of treatment in essential hypertensive patients, 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 will see later that some drugs are effective in atherosclerotic patients, but not in essential hypertensive patients. The question of duration of treatment must also be addressed. Very often results obtained after acute drug administration are not confirmed by studies performed under chronic 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. Furthermore, 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. 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. When no experimental demonstration is given (for instance by utilization 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.

Diuretics

Only one study has reported the effect of diuretic treatment on FMD in conduit arteries. Muiesan et al.[57] evaluated the effect of 2 months of monotherapy with either nifedipine or hydrochlorothiazide in the brachial artery of 20 patients with essential hypertension. FMD did not change in patients receiving hydrochlorothiazide. Therefore additional studies are needed to determine the effect of diuretics, and in particular of classes other than thiazides, such as aldosterone antagonists, on endothelial function in conduit arteries.

ß-adrenergic receptor antagonists

The selective β-1 antagonist atenolol has frequently been employed as a control treatment in studies designed to assess the effectiveness of different compounds, including calcium antagonists or ACE-inhibitors. In subcutaneous arterioles[58] and in the forearm microcirculation[59] treatment with atenolol did not improve the impaired endothelium-dependent vasodilation of essential hypertensive patients, while nebivolol, a selective ß1-adrenoceptor antagonist with NO-mediated vasodilating[60] enhanced endothelial function in the forearm microcirculation of 12 hypertensive patients[61]. In this study patients were randomized in a double-blind, crossover fashion to 8-week treatment periods with either 5 mg of nebivolol with 2.5 mg of bendrofluazide or 50 mg of atenolol with 2.5 mg of bendrofluazide. Nebivolol/bendrofluazide and atenolol/bendrofluazide each lowered clinic blood pressure to the same extent, but the vasodilatory response to acetylcholine was significantly increased only with nebivolol/bendrofluazide. The response to sodium nitroprusside was not different between treatments, suggesting that the endothelium-independent pathway was unaffected.

In a prospective, randomized, parallel group study[62], FMD was assessed in the brachial artery of 168 hypertensive patients before and after 6-month treatment with randomly assigned different antihypertensive treatments, including nebivolol (5 to 10 mg, n = 28) and atenolol (50 to 100 mg, n = 29). FMD and response to GTN were not modified by therapy with the two ß-adrenergic receptor antagonists (Figure 4), suggesting that these drugs cannot improve endothelium dependent vasodilation in the conduit arteries of essential hypertensive patients. Interestingly, plasma markers of oxidative stress were unchanged after treatment with either nebivolol or atenolol[62].

A compound which potentially could restore endothelial function in essential hypertensive patients is carvedilol, a ß1 selective adrenergic antagonist with additional α1-blocker properties and, importantly, an elevated antioxidant effect. However data are available only in patients with coronary artery disease, in whom carvedilol increased FMD in the brachial artery[63].

Calcium antagonists

A positive effect of this class of drugs (mainly of the dihydropiridine type) on endothelial function in different vascular beds has also been documented in the epicardial arteries[64,65], in gluteal subcutaneous resistance-size small arteries of essential hypertensive patients[58] and above all in the forearm microcirculation[36,59,66-69].

The potential mechanism through which calcium antagonists may 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[70]. Experimental evidence suggests that calcium antagonists exert an antioxidant effect and therefore could protect endothelial cells against free radical injury[71,72]. Thus they may 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 nifedipine GITS treatment (30-60 mg/die for 3 months) restored NO availability, preventing the potentiating effect of vitamin C, and decreased oxidative stress in essential hypertensive patients[68]. In a double blind, randomized trial comparing the effect of three-month treatment with lacidipine and atenolol on vasodilation to acetylcholine and bradykinin, despite a similar antihypertensive effect, lacidipine, but not atenolol, increased the response to acetylcholine and bradykinin, restoring NO availability and reducing plasma markers of oxidative stress[59]. Finally, similar results were obtained also with lercanidipine[69].

In the peripheral macrocirculation the study by Muiesean cited earlier[73] demonstrates that nifedipine treatment, but not the diuretic hydrochlorotiazide, can improve flow mediated dilation in the brachial artery of 10 essential hypertensive patients.

This positive study is at variance with the results of our prospective, randomized, parallel group study, conducted in 168 previously untreated hypertensive patients before and after 6-month treatment with different drugs assigned randomly, including nifedipine GITS (30 to 60 mg, n = 28) and amlodipine (5 to 10 mg, n = 28). Treatment with two different

dihydropiridine calcium antagonists did not improve FMD in brachial artery dilation (Figure 4), despite a reduction in plasma markers of oxidative stress[62]. A possible explanation for this discrepancy could reside in the marked difference in sample size between the two studies, a circumstance that plays an important role given 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 BANFF study[74]. Although the latter study was not conducted in essential hypertensive patients, but rather in patients with coronary disease, two-month treatment with amlodipine at 5 mg daily failed to increase FMD[74].

In conclusion, calcium antagonists are compounds active on endothelial dysfunction. They exert this activity on different vascular beds, including the coronary macrocirculation and peripheral microcirculation. However conflicting results have been presented concerning the effect of dihydropiridine compounds on peripheral large arteries, where available evidence on the beneficial effect of these drugs is not concordant.

But it should be kept in mind that calcium antagonists can improve endothelial function by restoring NO availability, an effected probably related to antioxidant properties.

Angiotensin converting enzyme (ACE) inhibitors.

ACE-inhibitors have been extensively studied as they are potentially able to improve endothelial function. They are known to increase the plasma concentration of bradykinin, an endothelium-dependent vasodilator, by inhibiting degradation of the peptide. Moreover, angiotensin II can cause endothelial dysfunction by inhibiting NO-synthase activity or by inducing oxidative stress through activation of membrane $NAD(P)H$-oxidase[75]. It follows that considerable interest focuses on the action of ACE-inhibitors.

In the forearm microcirculation treatment with Ace-inhibitors failed to improve endothelial function[55,76,77] and only prolonged (two/three-year, though not one-year) treatment with cilazapril improved the blunted response to acetylcholine in the subcutaneous microcirculation of essential hypertensive patients[78-80].

As stated earlier, we performed the first prospective study designed to perform a comparative assessment of the effects of pharmacological treatment with the main drug classes on endothelial dysfunction in the conduit arteries of essential hypertensive patients[62]. The study was conducted according to a randomized, single blind, parallel group design, while conduit artery endothelium-dependent vasodilation was assessed as FMD of the brachial artery.

The original result of the study was that administration of the ACE-inhibitor perindopril (2-4 mg/daily) was the only treatment able to improve FMD (Figure 5). Since reactive hyperemia and response to GTN did not change after treatment, this suggested that perindopril can improve endothelium-dependent vasodilation in the brachial artery of essential hypertensive patients.

The effect of the ACE-inhibitor was independent of the blood pressure lowering effect, inasmuch as blood pressure values were similarly reduced in the different study groups after treatment[62]. Moreover, it was shown that acute blood pressure reduction, even obtained with another ACE-inhibitor, did not modify FMD in the brachial artery of essential hypertensive patients[26].

Our finding is in agreement with previous evidence demonstrating that acute intra-venous administration of perindoprilat can reverse impaired FMD in the epicardial coronary artery free from overt atherosclerosis of essential hypertensive patients[81].

The effectiveness of ACE-inhibitors in improving endothelial function in conduit arteries is confirmed in epicardial[82] and brachial arteries of patients with coronary artery disease[74,83]. In the BANFF study[74] treatment with quinapril (20
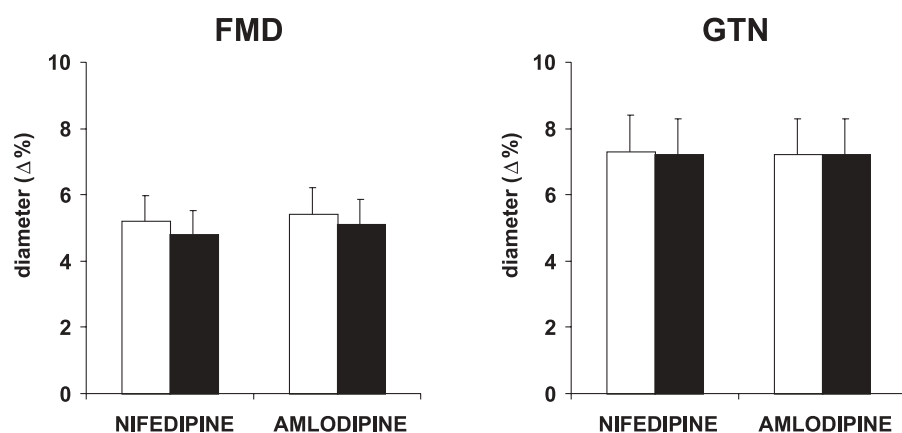
Figure 4 – Bars indicate flow mediated dilation (FMD) and response to 25 μg of sublingula glyceril trinitrate (GTN), expressed as percent (%) diameter increase, in the brachial artery of essential hypertensive patients at baseline (white) or after 6-month treatment (black) with nifedipine GITS (30-60 mg) or amlodipine (5-10 mg/daily).

FMD

GTN
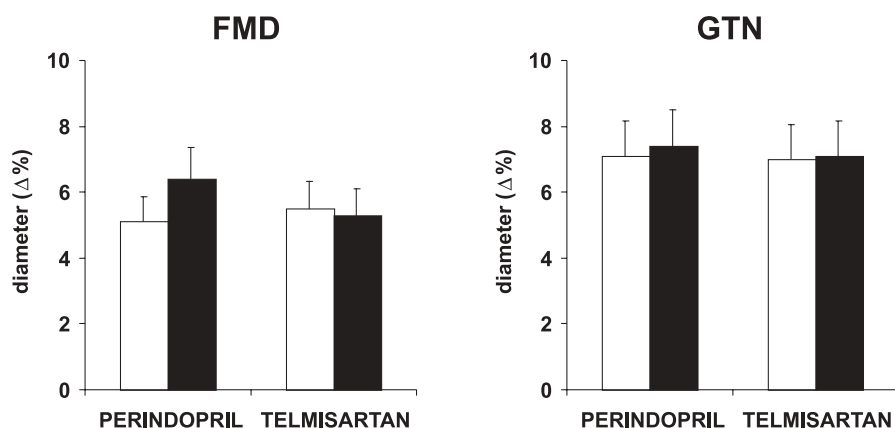
Figure 5 – Bars indicate flow mediated dilation (FMD) and response to 25 µg of sublingula glyceril trinitrate (GTN), expressed as percent (%) diameter increase, in the brachial artery of essential hypertensive patients at baseline (white) or after 6-month treatment (black) with perindopril (2-4 mg) or telmisartan (80-160 mg/daily).

act on endothelial function by mechanisms possibly independent from bradykinin[59,68,83].

In conclusion, ACE-inhibitors are compounds that can restore endothelial function above all in large coronary and peripheral arteries, while this effect is more difficult to obtain in the peripheral microcirculation. Clear information is lacking as to whether the beneficial effect of these compounds on the large arteries is related to restoration of NO availability. It should be kept in mind that in the peripheral microcirculation, selective potentiation of vasodilation to bradykinin is independent of the L-arginine-NO pathway and probably related to hyperpolarization.

Angiotensin II receptor antagonists

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[75], can promote NO synthesis in endothelial cells via AT-2 receptor stimulation[85]. It is possible, depending on the predominance of the activity of the two receptor subtypes or NO availability, that angiotensin II may deeply influence endothelial function or dysfunction. On this basis it could be hypothesised that AT1 receptor antagonists may restore NO availability by reducing this angiotensin II-mediated negative influence on endothelium.

A study exploring such a hypothesis found that in subcutaneous microcirculation one-year treatment with losartan was able to restore the vasodilating effect of acetylcholine in essential hypertensive patients[86]. In contrast, in the forearm microcirculation, up to one year antihypertensive treatment with candesartan (8-16 mg daily) did not

mg/daily), but not with enalapril (10 mg/daily), improved brachial artery FMD. It should be pointed out that a daily dose of enalapril at 10 mg is not equipotent, in terms of 24 hours duration of action, to a daily dose of quinapril at 20 mg. Finally, 4 week treatment with ramipril (10 mg daily) improved FMD of the brachial artery[83]. In these patients with coronary artery disease the potentiating effect exerted by the compound was blunted by L-NMMA, indicating that the treatment improves NO availability. Moreover, since ramipril administration prevents the facilitating activity of vitamin C on endothelium-dependent vasodilation, it is very likely that the ACE-inhibitor has antioxidant activity.

In our study in essential hypertensive patients[62], the possibility that endothelial dysfunction could be related to increased oxidative stress was indirectly explored by measuring plasma parameters of oxidative stress. Interestingly, administration of perindopril, telmisartan, nifedipine or amlodipine was able to reduce plasma markers of oxidative stress and increase markers of plasma antioxidant capability, confirming previous evidence that ACE-inhibitors, AT-1 antagonists and calcium antagonists can interfere with oxidative stress[59,68,83]. However, the beneficial effect of treatment on oxidative stress was not universally associated with improvement in endothelial function. Two possible explanations could be put forward to account for this discrepant effect. First, the drug activity on systemic markers of oxidative stress may not reflect the effect on intracellular oxidative mechanisms. Alternatively, the increase in endothelium-dependent vasodilation observed after perindopril treatment may not be determined by antioxidant activity. One peculiar effect of ACE-inhibitors is the accumulation of bradykinin, which can increase endothelium-dependent vasodilation by a pathway involving hyperpolarization[29,84]. Thus it could be hypothesized that the beneficial effect of perindopril on conduit artery endothelium-dependent vasodilation may be related to bradykinin-dependent mechanisms. This would be in line with the lack of effect observed with AT-1 antagonists or calcium antagonists, i.e. with compounds which, at least in humans,

improve the impaired response to acetylcholine or the lack of inhibition exerted by L-NMMA[87].

Data on large arteries are scanty since only one published study is available in essential hypertensive patients. In our laboratory we have observed that up to 6 month treatment with telmisartan (80-160 mg/daily) failed to improved flow mediated dilation in essential hypertensive patients (figure 5), although plasma markers of oxidative stress were reduced[62].

Moreover, conflicting results are available in patients with atherosclerosis. While the BANFF study[74] demonstrated that losartan treatment did not increase flow dependent dilation (although a non statistically significant positive trend was observed), Prasad et al.[88] demonstrated that 8 weeks of oral losartan treatment improved flow dependent dilation without effect on the nitroglycerin response. Similar results with the same compound were obtained in the above mentioned study by Horning et al.[83], in which 4-week losartan treatment in patients with coronary artery disease improved brachial artery flow mediated dilation by restoring NO availability. The degree of the beneficial losartan effect was similar to that exerted by ramipril treatment.

In conclusion, scant and discordant evidence is available concerning the effect of AT1 antagonists on endothelial function in essential hypertensive patients.

Relationship between restoration of endothelial function and better cardiovascular prognosis

At the present time no evidence is available to demonstrate that drug classes found to be effective in reversing endothelial dysfunction (such as calcium antagonists or ACE inhibitors) exert a greater beneficial effect than other drug classes with regard to morbidity and mortality trials in essential hypertensive patients[89]. But this observation is probably not pertinent since such trials last no longer than 5 years (usually around 3-4 years) and may not fully reflect the clinical situation of hypertensive patients treated over several decades. Trials have shown that in the very short term (3-5 years) the most important mechanism effective in reducing morbidity and mortality in essential hypertensive patients is blood pressure reduction, independently of the drug class employed[89]. 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.

On the other hand, it cannot be excluded that the mere demonstration that a certain drug increases endothelium-dependent vasodilation may not in itself be a valid surrogate marker for improvement in the entire complexity of endothelial function. 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 type 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 essential hypertensive patients.

Conclusions

Endothelial dysfunction occurs in essential hypertension and may be of particular clinical relevance since it can be a promoter of atherosclerotic and thrombotic damage, a typical complication of hypertension. Thus although not demonstrated incontrovertibly, the suggestion that antihypertensive pharmacological treatment could reverse endothelial dysfunction may be important. At the present time negative studies are available for diuretics and ß-adrenergic receptor antagonists.

Calcium antagonists can improve endothelium-dependent vasodilation in the microcirculation by restoring nitric oxide availability through a mechanism possibly related to antioxidant activity, but they fail to modify conduit artery endothelial dysfunction. ACE-inhibitors, on the other hand, seem to improve endothelial function in epicardial and conduit arteries, whereas they are less effective in the peripheral microcirculation of essential hypertensive patients. Finally, scant and discordant evidence is available concerning the effect of AT1 antagonists on endothelial function in essential hypertensive patients.

Therefore 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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