Effect of long-term irbesartan treatment on endothelium-dependent vasodilation in essential hypertensive patients

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Accepted: 26 August 2003

ABSTRACT

Endothelial dysfunction plays a pivotal role in the development of essential hypertension and its complications. The purpose of this study is to assess the effect of antihypertensive treatment with the angiotensin receptor blocker irbesartan on endothelial function in a group of essential hypertensive patients. Thirty-two untreated hypertensives are examined at baseline and at the end of a six-month period of irbesartan treatment. Endothelium-dependent and -independent responses are determined by measuring changes in forearm blood flow (FBF) by strain gauge plethysmography in response to intrarterial infusions of acetylcholine (endothelium-dependent vasodilation [EDV]), sodium nitroprusside (endothelium-independent vasodilation [EIV]), with and without the addition of the nitric oxide (NO) synthase inhibitor L-NMMA. Plasma endothelin, plasma and urinary nitrates and nitrites, and cyclic GMP are measured at baseline and at the end of treatment. Irbesartan promoted a significant increase in EDV (from 433±147% to 488±75%; $P=0.027$) and EIV (from 442±130% to 495±104%; $P=0.041$). L-NMMA-induced vasoconstriction was significantly enhanced after irbesartan treatment (relative decrease of FBF from 33.4±9.5% to 39.5±5.6%; $P=0.001$). Plasma concentrations of endothelin fell significantly after irbesartan treatment (from 5.78±1.86 to 4.16±1.52 pg/mL; $P=0.001$). We concluded that long-term irbesartan treatment enhances both endothelium-dependent and -independent vascular vasodilation capacity. In addition to this non-specific effect, irbesartan restores the vasoconstriction capacity of NO synthase inhibitors, suggesting a direct effect on tonic NO release, and decreases endothelin production. These actions may play an important role in the vascular protecting effects of irbesartan.

KEY WORDS: Angiotensin II. Endothelins. Endothelium. Hypertension. Nitric oxide.

Introduction

Cardiovascular diseases due to atherothrombosis are the leading cause of death and disability in developed countries. Endothelial dysfunction plays a pivotal role in the initiation and development of atherosclerotic lesions and is associated with risk factors such as hypertension, smoking, diabetes and dyslipidaemia.[1]

Endothelial dysfunction in essential hypertension is characterised by impaired endothelium-dependent vasodilation,[2,3] probably linked to a reduction in the availability of NO, which may be caused either by decreased NO production[4] or by excessive oxidative stress.[5] This reduced availability promotes the pathological processes that lead to atherosclerosis and thrombosis.

The activity of the renin–angiotensin system can directly affect endothelial function. Angiotensin II can promote endothelial dysfunction, both by decreasing NO availability and by increasing endothelin production. It has been reported that angiotensin II increases the production of oxygen free radicals that rapidly degrade NO, thus reducing its availability and impairing endothelial vasodilation capacity.[6,7] Moreover, in cultured endothelial cells, angiotensin II activates endothelin synthesis and release – an action mediated through the type 1 receptor.[8]

Numerous studies have examined the effect of antihypertensive treatment on endothelial function and have produced conflicting results.[9-21] Endothelium-dependent vasodilation (acetylcholine- or metacholine-induced) has been reported to increase after antihypertensive treatment in most[13,15,18,20,21] but not all[9-12] studies. Some have shown differences that depend on the type of drug used[14,16,19] and the duration of treatment.[15]

One study comparing the ACE inhibitor captopril against the calcium channel blocker nifedipine showed a greater effect for captopril on endothelium-dependent vasodilation,[14] whereas another comparing the calcium channel blocker lacidipine against the betablocker atenolol showed a greater effect for lacidipine.[19]

Two studies comparing angiotensin receptor blockers (losartan or irbesartan) against atenolol showed a greater effect for losartan[16] and no differences between irbesartan and atenolol.[20] Finally, antihypertensive treatment has been

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shown to increase endothelium-independent responses (nitroprusside- or nitroglycerin-mediated).[15]

The effect of antihypertensive treatment on vascular responses may be a reflection of the reduction in blood pressure or the result of counteracting the direct actions of angiotensin II on endothelial cells, mediated through either the blockade of AT_1 receptors or the stimulation of AT_2 receptors. Reductions in blood pressure can affect both endothelium-dependent and -independent responses, but a direct action on angiotensin–endothelium interactions must affect both endothelium-dependent vasodilation and the vascular blood flow response to L-NMMA.

We hypothesise that the angiotensin receptor blocker irbesartan could restore endothelium-dependent relaxation and increase the effect of L-NMMA on endothelium-derived responses by blocking the interaction between angiotensin II and endothelial cells. Thus, the aim of the present study is to evaluate, in an open-label fashion, the effect of long-term (six months) antihypertensive treatment with the angiotensin type 1 receptor antagonist irbesartan on endothelium-dependent and -independent responses in a group of essential hypertensive patients.

Materials and methods

Patient selection

The study population included 40 (21 men, 19 women; mean age: 43 years; range 29–69 years) stage I or II essential hypertensive patients consecutively recruited from the hypertension unit of the Department of Internal Medicine, Hospital Clinic, Barcelona, Spain. The diagnosis of essential hypertension was considered if seated arterial blood pressure (BP; after 10 min rest) measured by a mercury sphygmomanometer three times at one-week intervals was repeatedly higher than 140/90 mmHg.

Secondary forms of hypertension were excluded by routine diagnostic procedures. Subjects with hypercholesterolaemia (total cholesterol >6.5 mmol/L [250 mg/dL]), diabetes mellitus, impaired renal function (serum creatinine >132 μmol/L [1.5 mg/dL]), or previous history of coronary or cerebrovascular diseases were excluded, as were patients who smoked more than five cigarettes a day and/or consumed more than 40 g pure ethanol per day, as well as women taking oral contraceptives or oestrogen replacement therapy.

Study design

Endothelium-dependent and -independent responses, as well as biochemical determination of endothelium-derived products, were measured at baseline. Most patients were recently diagnosed and were not receiving any treatment. In eight patients receiving antihypertensive drugs (not angiotensin receptor blockers or ACE inhibitors), procedures were performed after a four-week washout period. The protocol was approved by the hospital ethics committee and written informed consent was obtained from all participants.

Following completion of the baseline procedures, all patients received antihypertensive treatment with irbesartan (150 mg/day). Patients were visited monthly and irbesartan was titrated to 300 mg/day, with the addition of hydrochlorothiazide (12–25 mg/day) if necessary, in order to maintain BP below 140/90 mmHg.

At the end of the six-month treatment period, the baseline procedures were repeated in 32 patients (18 men, 14 women). Eight patients failed to complete the study due to adverse reactions (n=2), lost to follow-up (n=3) or withdrawal of consent (n=3).

Laboratory measurements

A venous blood sample was obtained at baseline and after the six-month treatment period, after 12 h fasting and 1 h of bed rest, and with the patient in the recumbent position, in order to measure plasma nitrates and nitrites (NO_x), cyclic GMP (cGMP) and endothelin. A prolonged fasting period of at least 12 h has been shown to be necessary for a meaningful measurement of plasma NO_x concentration.[22]

To measure serum and 24-hour urine NO_x concentrations, samples were ultrafiltered (PL-10 Ultrafree-MC centrifugal filter units, Millipore Corporation, Bedford, MA, USA) at 14,000 rpm to removal proteins before analysis. NOx concentration in the filtered samples was then determined by the fluorimetric method of Misko *et al*.[23] The fluorescent signal was measured in a fluorimeter (Perkin Elmer, Foster City, CA, USA) at excitation and emission wavelengths of 365 nm and 425 nm, respectively. Intra-assay and inter-assay coefficients of variation (CV) were 8.4 and 13.8%, respectively.[24]

To prevent contamination by endogenous phosphodiesterases, cGMP in plasma was assessed after acetylation. Plasma samples were brought to 0.5 mmol/L isobutylmethylxanthine (IBMX), cooled in an ice bath and assayed immediately for cGMP concentration. Urine measurements were performed on non-acetylated samples at a dilution of 1 in 100. The concentration of cGMP was determined by radioimmunoassay (Biomedical Technologies Inc, Stoughton, MA, USA). Intra-assay and inter-assay CVs were 3.9 and 11.2 %, respectively.[24]

Endothelin (ET) concentration was measured by radioimmunoassay (Nichols Institute Diagnostics, Wijchen, The Netherlands) after extraction on Sep-Pack C 18 cartridges (Waters Associates, Milford, MA, USA). Plasma samples (1 mL) were acidified with 4% acetic acid (4.5 mL) and applied to cartridges preactivated with methanol, distilled water, and 4% acetic acid. The cartridges were then washed with distilled water and 25% ethanol, and immunoreactive ET (irET) was eluted twice with 1 mL 4% acetic acid in 86% ethanol.

Eluted ET was then concentrated to dryness (Speed Vac concentrator; Savant Instruments Inc., Framingdale, NY, USA) and reconstituted for radioimmunoassay. The recovery rate for the extraction procedure was 85%, as determined by the addition of labelled ET-1 (3500 cpm) to plasma. Cross-reactivity of the antiserum for ET-1, -2, -3, and big ET was 100%, 52%, 96% and 7%, respectively. Intra-assay and inter-assay CVs were 6.9% and 12.1%, respectively.[24]

Measurement of forearm blood flow

After overnight fast and with the subject lying supine in a quiet air-conditioned room (22–24°C), a polyethylene cannula (Becton Dickinson, Madrid, Spain) was inserted into the brachial artery under local anesthesia (2% lidocaine) and connected through stopcocks to a pressure transducer for systemic mean BP (one-third pulse pressure plus diastolic pressure) and heart rate monitoring (Siemens, SC5000, Danvers, MA, USA) and for intra-arterial infusions. Forearm

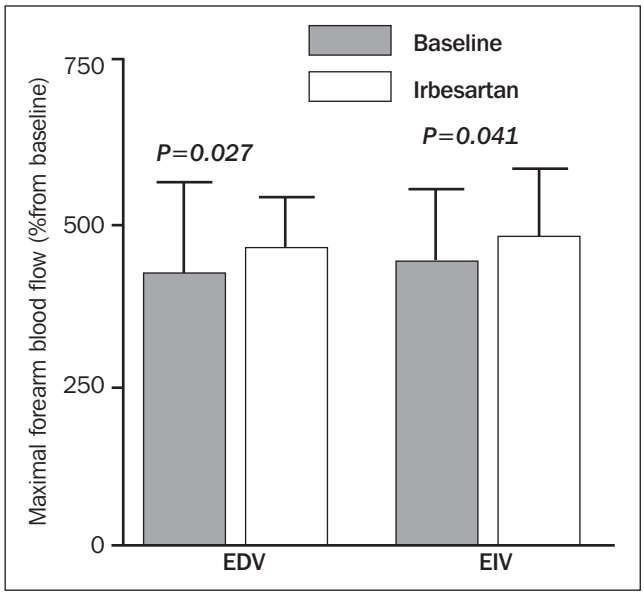

Fig. 1. Endothelium-dependent (EDV) and -independent (EIV) vasodilation (relative increase in FBF at maximal acetylcholine or sodium nitroprusside infusion) at baseline and after the six-month treatment with irbesartan. Irbesartan promoted a significant increase in both EDV and EIV.

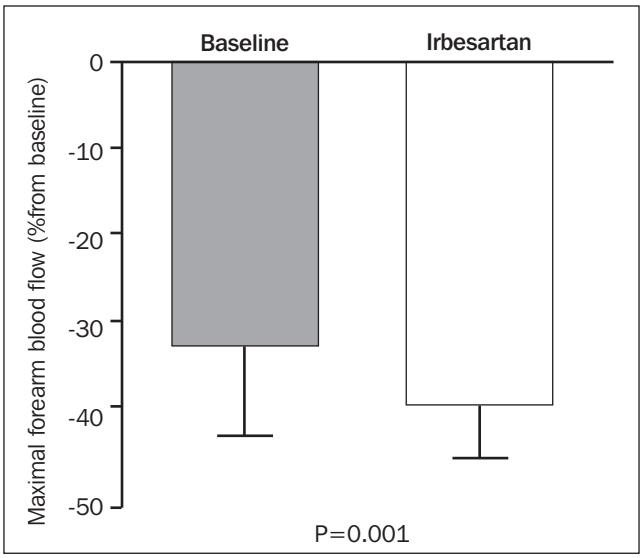

Fig. 2. Effect of L-NMMA on resting forearm blood flow at baseline and at the end of irbesartan treatment.
The vasoconstrictor effect of L-NMMA on resting FBF was more pronounced after irbesartan treatment.

blood flow (FBF) was measured in both experimental and contralateral forearms by strain-gauge venous plethysmography (EC5R-Hokanson, Bellevue, WA, USA). Circulation to the hand was excluded 1 min before each FBF measurement by inflating a pediatric cuff around the wrist at suprasystolic BP.

Baseline measurement of FBF was obtained after the infusion of 0.9% saline during 5 min at 1 mL/min. After this baseline measurement, endothelium-dependent vasodilation (EDV) and endothelium-independent vasodilation (EIV) were determined in random order. EDV was assessed by infusing intra-arterial acetylcholine (Laboratorios Cusi, Barcelona, Spain) at 15 μg/100 mL forearm tissue per min for 5 min. EIV was assessed by infusing intra-arterial sodium nitroprusside (Laboratorios Fides, Valencia, Spain) at 4 μg/100 mL forearm tissue per min for 5 min.

Doses of acetylcholine and sodium nitroprusside were selected to ensure maximum vasodilation according to previously reported data using dose-response curves.[25] The measurement of both EDV and EIV was separated by a 30 min rest to permit forearm blood flow to return to baseline values.

After a further 30-min rest, the same procedure was repeated with the addition of the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine, (L-NMMA; Alexis Biochemicals, Läufelfingen, Switzerland) at a constant infusion rate of 100 μg/100 mL forearm tissue per min for 5 min, and continued in the presence of acetylcholine and sodium nitroprusside. All the drugs were obtained from commercially available sources and freshly diluted to the desired concentration with normal saline. Sodium nitroprusside was dissolved in 5% glucose solution and protected from light by aluminum foil.

Statistical analysis

Values are expressed as mean ± standard deviation (SD) or median (interquartile range) for variables that deviated from normal distribution. EDV and EIV, as well as the effect of L-NMMA on both baseline blood flow and on maximal acetylcholine-induced vasodilation are presented as per cent increase in FBF above the baseline. Changes in parameters between baseline and the end of the six-month irbesartan treatment were analysed using the paired Student´s t-test or the Wilcoxon rank sum test, when appropriate.

Results

Blood pressure response

As shown in Table 1, BP decreased significantly during irbesartan treatment, with no significant changes in heart rate. The 32 patients that completed the study received irbesartan 150 mg/day (n=2), irbesartan 300 mg/day (n=18), irbesartan 300 mg/day plus hydrocholorothiazide 12.5 mg/day (n=9) and irbesartan 300 mg/day plus hydrochlorothiazide 25 mg/day (n=3).

Endothelium-derived factors

Table 2 shows changes in plasma and 24-hour urinary excretion of NO_x and cGMP, as well as in plasma endothelin from baseline to the end of the six-month irbesartan treatment. Plasma endothelin significantly decreased after treatment (from 5.78 ± 1.86 to 4.16 ± 1.52 pg/mL; P=0.001). Although both plasma and urine NOx and cGMP tended to increase after treatment, changes were not statistically significant.

Forearm blood flow

As mentioned above, EDV and EIV were estimated as the per cent increase in FBF in response to the drugs. We found a significant increase in both EDV (from 433 ± 147% to 488 ± 75%; P=0.027) and EIV (from 442 ± 130% to 495 ± 104%; P=0.041) at the end of irbesartan treatment (Fig. 1).

Figure 2 shows the effect of L-NMMA infusion on resting FBF at baseline and after the six-month irbesartan treatment.

Table 1. Mean values (±SD) of blood pressure and heart rate in essential hypertensive patients (n=32) at baseline and after six months irbesartan treatment.

	Baseline	End of treatment	*P*
Systolic blood pressure (mmHg)	165.3 ± 12.3	142.9 ± 11.2	<0.001
Diastolic blood pressure (mmHg)	96.7 ± 5.2	87.0 ± 5.6	<0.001
Heart rate (beats/min)	79.8 ± 6.6	79.1 ± 5.6	0.400

Irbesartan significantly increased the vasoconstrictor effect of the NO synthase inhibitor, with the per cent change in FBF after L-NMMA infusion falling from 33.4 ± 9.5% to 39.5 ± 5.6% (P=0.001).

In contrast, the inhibitive effect of L-NMMA on maximum EDV (acetylcholine-induced) was not significantly modified after irbesartan treatment (from a decrease of 95 ± 130% at baseline to a decrease of 146 ± 96% at the end of the study period; P=0.095).

Discussion

This study showed that six-months treatment with irbesartan improved both endothelium-dependent and -independent responses, and increased the effect of NO synthase inhibitors on forearm blood flow in a group of essential hypertensive patients. Long-term irbesartan treatment also tended to restore the equilibrium between vasodilators and vasoconstrictor endothelium-derived products. We found a significant decrease in plasma endothelin concentrations and a non-significant increase in NO_x and cGMP production.

Acceptance of the pivotal role of endothelial dysfunction in the development of essential hypertension and its complications[1-3] has led investigators to examine the effect of BP reduction or antihypertensive treatment on endothelium-dependent responses. In addition, the interaction between angiotensin II and endothelial function suggests that angiotensin blockade may directly ameliorate endothelial dysfunction.

The first experimental studies performed in animals with primary hypertension demonstrated that antihypertensive treatment improved EDV[26,27] However, studies in essential hypertensive patients have produced inconsistent results. While some studies found antihypertensive treatment to have no effect on endothelium-dependent vasodilation,[9-12] others have shown a positive effect,[13-21] and some studies that compared different antihypertensive drug classes found either equivalence[20] or superiority[14,16,19] of one class over another.

Methodological differences may affect these results. EDV and EIV responses have been examined in most studies[9-16,18-21] by changes in FBF in response to intra-arterial agonist infusions (usually acetylcholine or methacholine and sodium nitroprusside) or non-invasively by measuring FBF in response to reactive hyperaemia and sublingual nitroglycerin.[17,28]

Some studies also evaluated FBF in response to NO synthase inhibitors, as well as the effect of these compounds on maximum acetylcholine-induced vasodilation.[15,18,19] The effect of antihypertensive treatment has also been examined, both in the short- and long-term. One study, using the angiotensin receptor blocker candesartan[15] showed a significant effect on L-NMMA-induced vasoconstriction, but no effect on EDV after a two-month treatment period. However, the same patients re-examined after 12 months of treatment exhibited both increased L-NMMA-induced vasoconstriction and acetylcholine-induced vasodilation.

Table 2. Mean values (± SD) of endothelium-derived factors in essential hypertensive patients at baseline and after six months irbesartan treatment.

	Baseline	End of treatment	*P*
Plasma nitrates (nmol/mL)	36.5 [29.7-50.3]	39.0 [35.0-52.5]	0.456
24-hour urine nitrates (μmol/24h)	364 [185-508]	420 [200-602]	0.379
Plasma cGMP (nmol/L)	4.27 [3.00-6.94]	6.00 [4.30-10.1]	0.093
24-hour urine cGMP (nmol/24h)	439 [327-628]	479 [398-572]	0.572
Plasma endothelin (pg/mL)	5.8 ± 1.9	4.2 ± 1.5	0.001

Several studies that compared different drug classes showed a superiority of the renin–angiotensin system blockade on EDV with respect to other drugs. Millgard *et al*.[14] compared the acute effect of captopril and nifedipine administration on EDV in 23 essential hypertensives. With a similar effect on BP, only captopril significantly increased EDV one hour after administration. Schiffrin *et al*.[16] compared 12-months losartan and atenolol treatments on EDV. Only losartan improved EDV, an effect accompanied by a reduction in the wall:lumen ratio of resistance arteries. In contrast, a recent report by von zur Mühlen *et al*.[20] showed an increase in EDV after a three-month treatment period with irbesartan or atenolol, but with no significant differences between the two drugs.

An important limitation to the present study was the lack of a comparison or control group. Unlike other studies, however, the effect of irbesartan on vascular responses was examined with and without the addition of an NO synthase inhibitor and complemented by non-invasive assessment of different endothelium-derived products.

After six months of irbesartan treatment, we found an increase of both EDV and EIV. The first finding is consistent with most previously studies;[13-21] however, the increase in EIV was only significant in one previous study[15] but some non-significant increases were observed in others.[20,21] This parallel between the effect on EDV and EIV responses, as well as the fact that several antihypertensive drug classes restore EDV, seems more consistent with a general BP reduction effect than with a direct effect on NO production or degradation. Thus, we believe that the effect of irbesartan on EDV and EIV observed in the present study is probably mediated through its antihypertensive action or the restoration of arterial vascular structure,[16] rather than a direct effect of AT^1 blockade on NO production.

In contrast, we found a direct effect of irbesartan on

L-NMMA-induced vasoconstriction with respect to both baseline FBF and maximal acetylcholine-induced vasodilation. After six months of irbesartan treatment, the vasoconstrictor effect of L-NMMA on baseline blood flow was increased significantly. We also found that L-NMMA infusion resulted in more pronounced inhibition of the maximal acetylcholine-induced vasodilation, although the effect was of only borderline statistical significance ($P=0.095$).

As L-NMMA is a selective NO synthase inhibitor, the increased effect of L-NMMA added experimentally means that more NO is available at the vascular wall. Thus, the effect of irbesartan is probably due to a direct increase in tonic NO production or a decrease in NO degradation through reduced superoxide anion production. In this respect, it has been reported that oxygen free radical synthesis can be promoted by angiotensin II.[6,7]

Finally, as with other studies that used candesartan[15] or losartan,[29,30] the present one demonstrated an inhibiting effect for irbesartan treatment on plasma endothelin concentration, whereas plasma and urinary markers of NO production did not change significantly. However, it should be noted that plasma and urine levels of endothelium-derived products are not a good reflection of endothelial production, as only about 20% of this is secreted luminally.

In conclusion, six-months irbesartan treatment significantly improved EDV and EIV responses. Although merely speculative, there appears to be two aspects to this effect: generally better performance of the vascular bed (increases in EDV and EIV), and a direct effect on tonic NO release (increase in vasoconstrictor effect of NO synthase inhibitors). Moreover, either directly or by its BP reduction effect, irbesartan also reduces endothelin production by endothelial cells.

This work was supported in part by a grant from Sanofi-Synthelabo Laboratories and from the Fondo de Investigaciones Sanitarias (FIS 00/0435).

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