Calcium antagonist isradipine improves abnormal endothelium-dependent vasodilation in never treated hypertensive patients

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Abstract

Objective: To examine whether middle (two months) and long-term (six months) isradipine sustained-release treatment improves endothelium-dependent vasodilation in never treated hypertensive patients. Methods: The responses of the forearm vasculature to acetylcholine (7.5, 15 and 30 μg/min) and sodium nitroprusside (0.8, 1.6, 3.2 μg/min) were evaluated in 12 normotensive controls (seven men and five women, aged 25 to 49 years), and in 12 hypertensives (eight men and four women, aged 20 to 47 years) at baseline and after two and six months of isradipine sustained-release treatment. Drugs were infused into the brachial artery, and forearm blood flow was measured by strain-gauge plethysmography. Results: At baseline, the response to acetylcholine was significantly lower in hypertensives vs controls: at the highest dose (30 μg/min), forearm blood flow was 28.6±2.4 ml/100 ml of tissue per min in the controls vs 8.9±1.0 ml/100 ml of tissue per min in hypertensives (p<0.0001). Similarly, vascular resistance was significantly (p<0.0001) higher in hypertensives: 4.8±0.5 units (controls) vs 15.1±1.7 units (hypertensives). After isradipine treatment, the forearm blood flow in hypertensive patients changed from 8.9±1.0 ml/100 ml of tissue per min to 16.0±1.2 ml/100 ml of tissue per min (two months; p<0.0001) and 15.2±1.4 ml/100 ml of tissue per min (six months; p<0.0001). Isradipine treatment did not modify the vasodilating effect of sodium nitroprusside. Conclusions: Our data demonstrate for the first time that the calcium antagonist isradipine improves acetylcholine-induced vasodilation in hypertensives. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Calcium channel blocker; Acetylcholine; Endothelium; Hypertension; Isradipine; Antihypertensive therapy

1. Introduction

The normal endothelium plays a key role in the local regulation of the vascular tone by producing and releasing contracting and relaxing factors [1]. One of these factors is the endothelium-derived relaxing-factor (EDRF), identified as nitric oxide (NO) [2–6]. NO is released after stimulation of endothelial cells by some physical stimuli, such as shear stress and blood flow [7], as well as some agonists such as acetylcholine (ACh), bradykinin, substance P, and serotonin [6]. On the contrary, sodium nitroprusside (SNP) is an endothelium-independent vasodilator compound that produces a vasodilation by direct activation of guanylate cyclase in vascular smooth-muscle cells by providing an inorganic source of NO [8]. The role of NO in human essential hypertension was investigated by comparing the vasodilator response to ACh and to SNP in the brachial or coronary arteries [9–11].

Endothelium-dependent vasodilation has been show to occur in most mammalian species [2], and in humans by in vitro studies using arterial preparations [12,13]. Subsequent, human studies have confirmed these experimental findings, and have demonstrated that this regulatory action of the endothelium is exerted on resistance vessels also [14]. Moreover, this endothelial function is impaired in the presence of different risk factors [9,10,15–21]; in particular, in hypertensive patients these studies, with the exception of Cockcroft and co-workers [22], have reported a reduced endothelium-dependent vasodilation to ACh when compared to normotensive control subjects.

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Experimental evidences suggest that neither calcium-channel blocker (CCB) nifedipine [23] nor ACE-inhibitors [24,25], with the exception of Hirooka [26] may improve endothelium-dependent vascular relaxation in hypertensive patients. I isradipine is a potent CCB of the dihydropyridine class and has been shown to be an effective first-line monotherapeutic antihypertensive drug [27–29]. Moreover, it was reported that isradipine treatment partly prevented endothelial dysfunction induced by hypercholesterolemia, and reduced structural and biological changes of atherosclerosis [30].

The aim of this study was to evaluate whether middle (two months) and long-term (six months) treatment with CCB isradipine improves endothelium-dependent vasodilation in the forearm resistance vessel of patients with essential hypertension.

2. Methods

2.1. Study population

2.1.1. Hypertensive patients

Twelve never treated outpatients, eight men and four women, aged 20 to 47 years (mean±SEM=37.1±1.8 years), with well documented history of primary hypertension were recruited for the study. All patients underwent physical examination and review of their medical histories before entering the trial. Causes of secondary hypertension were excluded in all patients. None of the patients had a history of diabetes, hyperlipidemia, peripheral vascular disease, coagulopathy, or any disease predisposing them to vasculitis or Raynaud’s phenomenon. Diagnosis of essential hypertension was obtained in all patients for the first time when they were recruited for the study. None of the participants were taking antihypertensive therapy.

2.1.2. Normotensive subjects

Twelve normal volunteers, seven men and five women, aged 25 to 49 years (mean±SEM=37.7±1.7 years), were selected as a control group. Each patient underwent clinical history, physical examination, standard electrocardiogram, routine chemical analyses, and chest X-rays. None had evidence of present or past hypertension, cardiovascular disease, hyperlipidemia, or any other systemic condition, and none were taking drugs at the time of the study.

The local ethical committee approved the study, and all participants gave written informed consent for all procedures.

2.2. Study protocol

At first, both endothelium-dependent and endothelium-independent vasodilation was evaluated in normotensive subjects and hypertensive patients with a dose-response to intra-arterial ACh and SNP infusions, respectively.

Successively, after the first evaluation in hypertensive patients we administered isradipine sustained-release (5 mg/day) for six months. Clinical evaluations were scheduled every four weeks to evaluate BP values and/or possible adverse effects.

Finally, in hypertensive patients the vascular function has been re-evaluated after two (middle term) and six months (long-term) of isradipine once-a-day administration. To assess the reproducibility of endothelium-dependent and endothelium-independent vasodilation, the vascular function in the normotensive group was reevaluated after two months again.

2.3. Blood pressure measurements

Clinical BP measurements were obtained in the morning between 8:00 a.m. and noon. BP was measured three times with a mercury sphygmomanometer. The mean of the last two measurements was used. Hypertension was defined as a systolic BP>160 mmHg, a diastolic BP>95 mmHg, or both. A physician made measurements with the subject seated for at least 5 min.

Ambulatory BP monitoring (ABPM) was recorded using an A&T TM 2420 recorder, model 7 (Takeda, Tokyo, Japan), validated in accordance with the protocol of the British Society of Hypertension [31]. The portable BP recorder employs a dual microphone system in a sphygmomanometer cuff to record the Korotkoff sounds. Recordings were taken every 10 min during the day (from 07.00 a.m. to 11.00 p.m.) and every 20 min during the night (from 11.00 p.m. to 7.00 a.m.). The patients were invited to observe these periods of rest and activity closely. When the device was operating, patients were asked to remain motionless and keep their arm still.

Mean values of heart rate (HR), systolic, diastolic and mean BP were calculated in each patient hour by hour, and averaged throughout the 24-h, and the day- and night-time periods.

2.4. Measurements of forearm blood flow

All studies were performed at 9:00 a.m. after overnight fasting, with the subjects lying supine in a quiet air-conditioned room (22° to 24°C). The subjects were instructed to continue their regular diet; caffeine and alcohol were all prohibited 24 h before the study. Forearm volume was evaluated according to the water-displacement method. Under local anesthesia and sterile conditions, a 20-gauge polyethylene catheter (Vasculon 2) was inserted into the brachial artery of the nondominant arm (left, in most cases) of each subject for evaluation of BP (Baxter Healthcare) and for drug’s infusion. This arm was slightly elevated above the level of the right atrium, and a mercury-filled silastic strain-gauge was placed on the widest part of
the forearm [32]. The strain-gauge was connected to a plethysmograph (model EC-4, D.E. Hokanson, Issaquah, WA) [33] calibrated to measure the percent change in volume; this was connected to a chart recorder to obtain the forearm blood flow (FBF) measurements. A cuff placed on the upper arm was inflated to 40 mmHg with a rapid cuff inflator (model E-10 Hokanson, Issaquah, WA) to exclude venous outflow from the extremity. A wrist cuff was inflated to BP values 1 min before each measurement to exclude the hand blood flow. The FBF of the opposite arm was also evaluated during the study.

The FBF was measured as the slope of the change in the forearm volume. The mean of at least three measurements was obtained at each time point. Forearm vascular resistance, as expressed in units (U), was calculated by dividing mean BP by FBF. BP was recorded directly from the intra-arterial catheter immediately before each measurement.

2.5. Vascular function

All hypertensive patients and normotensive subjects underwent measurement of FBF and BP during intra-arterial infusion of saline, and increasing doses of ACh and SNP. All participants rested at least 30 min after artery cannulation to obtain a stable baseline before data collection; FBF and vascular resistance were repeated every five minutes until stable.

Endothelium-dependent vasodilation was evaluated by dose response curve to intra-arterial ACh infusions (7.5, 15 and 30 µg/min, each for 5 min). Endothelium-independent vasodilation was assessed by the dose-response curve to intra-arterial SNP infusions (0.8, 1.6, 3.2 µg/min, each for 5 min). The sequence of administration of ACh and SNP was randomized to avoid any bias related to the order of drug infusion. The drug infusion rate was adjusted for the forearm volume of each subject. The infusion rate for each drug was 1 ml/min.

2.6. Drugs

ACh (Sigma, Milan, Italy) was diluted with saline immediately before infusion. SNP (Malesci, Florence, Italy) was diluted in 5% glucose solution immediately before each infusion and protected from light with aluminum foil. Isradipine capsules were provided from Sandoz (Milan, Italy).

2.7. Statistical analysis

Differences between means were compared by paired and unpaired Student’s t-test, as appropriate. The responses to SNP and ACh were compared by ANOVA for repeated measurements and when analysis was significant, the Tukey’s test was applied. All calculated p values are two tailed. Significant differences were assumed to be present at $p<0.05$. All group data are reported as mean±SEM.

3. Results

All participants completed the protocol study. Baseline demographic, hemodynamic, and humoral characteristics are summarized in Table 1. There were no significant differences in plasma cholesterol, glycemia, body mass index and FBF between control subjects and hypertensive patients. Vascular resistance in normal subjects and in hypertensive group was significantly different ($p<0.05$): 28.1±1.6 U and 33.3±1.4 U, respectively. According to inclusion criteria, systolic and diastolic BP values were significantly ($p<0.0001$) lower in control subjects in comparison with hypertensive patients (130/80±3/1 mmHg vs 169/99±5/2 mmHg).

All participants were never smokers.

3.1. Reproducibility of endothelium-dependent vasodilation

The FBF and vascular resistance, re-evaluated in normal subjects two months later, were not significantly different to baseline values as reported in Table 2. Similarly, the BP and HR did not change.

<table>
<thead>
<tr>
<th>Table 1 Baseline demographic, hemodynamic, and humoral characteristics of study population</th>
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</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
</tr>
<tr>
<td>Body mass index (Kg/m²)</td>
</tr>
<tr>
<td>Systolic and diastolic BP (mmHg)</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
</tr>
<tr>
<td>Glycemia (mmol/l)</td>
</tr>
<tr>
<td>FBF (ml/100 ml of tissue per min)</td>
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<tr>
<td>VR (U)</td>
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</tbody>
</table>
Table 2
Forearm blood flow and vascular resistance in normotensive subjects in baseline and two months later

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=12)</th>
<th>Two months (n=12)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forearm blood flow (ml/100 ml of tissue per min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>4.0±0.2</td>
<td>3.9±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>ACh (30 μg/ml/min)</td>
<td>28.6±3.4</td>
<td>28.3±2.5</td>
<td>NS</td>
</tr>
<tr>
<td>SNP (3.2 μg/ml/min)</td>
<td>11.8±0.7</td>
<td>11.5±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Vascular resistance (units)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>28.1±1.6</td>
<td>27.9±1.4</td>
<td>NS</td>
</tr>
<tr>
<td>ACh (30 μg/ml/min)</td>
<td>4.8±0.5</td>
<td>4.8±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>SNP (3.2 μg/ml/min)</td>
<td>10.5±0.7</td>
<td>10.3±0.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

3.2 Control subjects versus hypertensive patients

The forearm vasodilation induced by ACh was significantly increased in normotensive subjects (increments from base: 2.4 ml/100 ml of tissue per min, 8.9 ml/100 ml of tissue per min, 24.6 ml/100 ml of tissue per min) compared with hypertensive patients (increments from base: 1.0 ml/100 ml of tissue per min, 1.9 ml/100 ml of tissue per min, 5.3 ml/100 ml of tissue per min). At the highest dose (30 μg/min), FBF was 28.6±3.4 ml/100 ml of tissue per min in the control subjects, and 8.9±1.0 ml/100 ml of tissue per min in hypertensive patients (p<0.001) (Fig. 1). Forearm vascular resistance during ACh infusions significantly decreased in both normotensive (decrements from basal: −9.3 U, −16.5 U, −23.3 U) and hypertensive subjects (decrements from basal: −8.1 U, −11.8 U, −18.2 U). At the highest dose, vascular resistance was 4.8±0.5 U in control subjects and 15.1±1.7 U (p<0.0001) in hypertensive patients (Fig. 2). Incremental ACh infusions did not change baseline BP or HR values in either group.

Significant increases in FBF (Fig. 1) as well as decreases in forearm vascular resistance (Fig. 2) were observed in both normotensive and hypertensive subjects during SNP infusion, although no significant differences were found between groups. At the highest dose (3.2 μg/min), FBF increased to 3.8±0.1 ml/100 ml of tissue per min. A significant (p<0.05) decrease was observed in baseline vascular resistance (from 33.3±1.4 U to 27.2±1.3 U).

The isradipine treatment significantly increased the vasodilator response to incremental doses of ACh (Fig. 1).

3.3 Pre-treatment versus post-treatment patients (two months)

Isradipine treatment significantly (p<0.0001) lowered clinical systolic and diastolic BP from 169/99±5/2 mmHg to 138/85±3/1 mmHg; in Table 3, ambulatory BP values before and after pharmacological treatment are reported.

Baseline mean FBF did not change after isradipine administration (3.6±0.1 ml/100 ml of tissue per min vs 3.8±0.1 ml/100 ml of tissue per min). A significant (p<0.05) decrease was observed in baseline vascular resistance (from 33.3±1.4 U to 27.2±1.3 U).

The isradipine treatment significantly increased the vasodilator response to incremental doses of ACh (Fig. 1).

![Fig. 1. Left panels. Forearm blood flow (FBF) responses to acetylcholine (ACh) and sodium nitroprusside (SNP) infusions in 12 normal control subjects (●) and 12 hypertensive patients (●) are reported. Endothelium-dependent vasodilation was significantly lower in hypertensives in comparison with normotensives. Right panels. FBF responses to ACh and SNP in hypertensive patients (●) after two (▲) and six (■) months of isradipine treatment are graphically reported. Pharmacological treatment significantly improves the ACh-induced vasodilation without significant differences between two treatment periods. On the contrary, long-term oral isradipine treatment did not modify the vasodilating effect of SNP. Data are expressed as mean±SEM.](image-url)
Increases of vascular resistance (Fig. 2) were observed in hypertensive patients after isradipine treatment during SNP infusions. However, no significant differences with the pretreatment group were found at the different time points.

3.4. Long-term (six months) versus middle-term (two months) treatment

After six months of isradipine administration, systolic and diastolic BP remained lowered from baseline values. No significant differences were observed in mean 24-h BP values after two and six months of antihypertensive therapy (Table 3).

Similarly, after six months of isradipine treatment, the improvement of FBF (Fig. 1) and decrease of vascular resistance (Fig. 2) during incremental doses of ACh and SNP infusions were not significantly different from the values obtained after two months of antihypertensive therapy.

3.5. Side effects

No side effects were observed during the study. No patient had to discontinue treatment because of adverse reactions.

4. Discussion

In the present study, we evaluated the middle and long-term effect of CCB isradipine on endothelium-dependent vasodilation in never treated hypertensive patients. Our data confirm previous studies [9,10] showing that patients with essential hypertension have an impaired response to ACh infusions compared with normotensive control subjects. Similarly, the observation that there were no differences in the vasodilator effect of SNP between hypertensive patients and normotensive subjects excludes a nonselective defect in responsiveness of vascular smooth muscle cells to vasodilators in essential hypertension.

The new and very interesting findings of our study are the demonstration that middle-term (two months) and long-term (six months) antihypertensive therapy with the CCB isradipine improves endothelium-dependent vasodilation in hypertensive humans. This beneficial effect of isradipine on endothelial function is probably specific and unrelated to BP decrease. The demonstration that hypertension-associated endothelial dysfunction may be reversed by a

Table 3
Ambulatory blood pressure (BP) values before and after isradipine treatment in hypertensive patients

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Two months</th>
<th>Six months</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP 24-h (mmHg)</td>
<td>152/80±5/1</td>
<td>133/73±1/1</td>
<td>131/73±1/1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BP day (mmHg)</td>
<td>157/82±4/1</td>
<td>138/76±1/1</td>
<td>136/74±1/2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BP night (mmHg)</td>
<td>142/76±6/1</td>
<td>123/68±1/1</td>
<td>120/68±2/1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
pharmacological treatment is a very important clinical finding. In fact, our data suggest that the improvement of endothelial function may represent a new target for pharmacological treatment of essential hypertension.

4.1. Endothelium-dependent vasodilation in hypertension

Hypertension is an important cardiovascular risk factor associated with myocardial infarction, heart failure and stroke [34–36]. Normal endothelium plays a very important role in the regulation of vascular tone through the release of different vasoactive substances. Results of recent investigations indicate that abnormal endothelial function, manifested as impaired vascular relaxation response to endothelium-dependent agonists, exists in some cardiovascular conditions [9,10,15–21]. It is well established that endothelium-dependent vasodilation is impaired in the experimental model of hypertension as well as in hypertensive patients. Nevertheless, some animal studies suggest that endothelial impairment is secondary to hypertension, while other studies indicate that in humans this alteration is primary to the hypertensive disease. This latter concept is based on previous studies in animal models of hypertension that have demonstrated the improvement of impaired vascular relaxation after BP normalization [37,38].

Basal formation of NO and the effect of ACh infusion on the release of NO were found to be reduced in hypertensive patients, [10,39,40] whereas flow-induced vasodilation was maintained [41]. However, this defect seems not to be related to a decreased availability of the natural substrate for NO production [39] nor is it a consequence of a specific defect of the endothelial cell surface receptor [42], but it is probably related to a more generalized abnormality of endothelial vasodilator function [43].

4.2. Antihypertensive treatment and vascular relaxation

Previous reports demonstrated that chronic ACE-inhibitors treatment failed to restore impaired endothelium-dependent relaxation in hypertensive patients [23–25]. On the contrary, Hirooka et al. [26] reported that the ACE-inhibitor captopril, but not CCB nifedipine, may acutely improve the impaired endothelium-dependent forearm vasodilation in hypertensives. In our study, chronic isradipine treatment improves endothelium-dependent vascular relaxation as is proven by the significant increase of FBF during ACh infusion (+79.7% after two months, and +70.7% after six months). For the first time, our findings demonstrate that antihypertensive treatment with a CCB may improve the endothelium-dependent vasodilation for a long-time in hypertensive humans. Contrary to other studies [24,25], our patients were all previously never treated and all received the same antihypertensive drug (isradipine, sustained-release 5 mg/day).

4.3. CCBs and vascular function

The results of our study do not provide direct insight into the mechanism by which CCB isradipine restores endothelial function. However it is probably not related to an interaction with surface endothelial receptors. In fact, previous reports have demonstrated that in hypertensive humans, endothelial dysfunction is not induced by an alteration in receptor or signal transduction pathways [42,43], but it is probably related to a primary or induced defect in the NO system [17,39,40].

Nevertheless, dihydropyridine CCBs seem to mediate part of their pharmacological effect by increasing endothelial NO production via a calcium-dependent mechanism [44]. In addition, the influence on endothelial NO production may be responsible for some concomitant actions of these vasodilators, such as their antiproliferative and antiatherosclerotic properties [45,46].

Finally, most recently, beneficial effects of ascorbic acid on endothelial dysfunction in forearm vessels in diabetic patients [47], cigarette smoking [48], ischemic heart disease [49], and in epicardial coronary arteries of hypertensive patients [50] have been reported. In fact, there is evidence from experimental studies that an excess of superoxide anions in the arterial vascular bed could account for the depressed ACh-induced vasodilation in hypertension. Grunfeld and co-workers reported in genetically hypertensive rats that the major cause of the decreased NO concentration is that although the rate of production is nearly normal, it is scavenged as it is produced by excess superoxide anions [51]. The CCBs are antioxidant compounds and their beneficial effects on endothelial function, as reported in our study, might be related to this mechanism.

5. Conclusions

The isradipine treatment is very effective in lowering for a long-time, the BP in patients affected by mild to moderate hypertension. Moreover, the CCB isradipine seems to be able to improve the impaired ACh-induced vasodilation in hypertensive patients. Nevertheless, further investigations are needed to identify the mechanism by which isradipine exerts this beneficial effect on vascular endothelium.

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