Cilnidipine Lowers Plasma Leptin of Patients with Obese Hypertension Associated with Cerebrovascular Disorder

Masahiko Watanabe1*, Akira Tamaka3, Yukio Morishita2 and Masayuki Noguchi3

1Department of Neurology, University of Tsukuba, Tsukuba, Ibaraki, Japan
2Department of Pathology, University of Tsukuba, Tsukuba, Ibaraki, Japan

Abstract

Background: Since cilnidipine suppresses not only the L-type but also N-type calcium channels found on peripheral sympathetic nerve endings which control noradrenaline release, it is thought to be a Ca antagonist with low sympathetic nerve activation when lowering blood pressure (dual blocking action). As activation of sympathetic nervous system is suggested to contribute to hypertension in obesity, dual blocking action of cilnidipine may contribute to patients with obesity-hypertension.

Methods: The effects of cilnidipine on plasma leptin levels and blood pressure were studied. Twenty three patients with obesity-hypertension associated with a past history of stroke or ischemic changes confirmed by brain magnetic resonance imaging were enrolled. Cilnidipine (10 mg/day) was administered orally once a day after breakfast. The duration of the study treatment was 12 weeks. Retroperitoneal adipose tissues around the kidney of both fat and lean cadaver and neuronal and non-neuronal tissues of rat were used for immunohistochemical examination.

Result: The mean circulating leptin level decreased from 11.8 ng/ml to 10.8 ng/ml that reached statistical significance. Multiple regression analysis revealed no significant effects of covariates except for baseline leptin level. An immunohistochemical study revealed that N-type calcium channel protein was expressed on the cell membrane of adipocytes. We speculate that a blockade of calcium current through the N-type channel, instead of the L-type channel may suppress leptin secretion.

Conclusion: The dual blocking action of cilnidipine may help control both arterial hypertension and leptin-induced atherosclerosis in patients with obese hypertension associated with cerebrovascular disorder.

Keywords: Cilnidipine; Leptin; Obesity; Sympathetic nervous system; Cerebrovascular disorder

Introduction

Stroke is a major cause of death in developed countries. Its prevalence and disability burden are expected to increase in the future due to population aging [1]. Obesity is a precursor of hypertension [2,3] which plays an important role in the epidemiology of stroke; moreover, it is also an independent risk factor for ischemic stroke [4].

Obese hypertensive subjects have increased sympathetic activities in their muscles [5] and kidneys [6,7]. Activation of the sympathetic nervous system in obesity may be due, in part, to hyperleptinemia [8]. Leptin, a 167-amino acid peptide hormone produced by white adipose tissue, is primarily involved in the regulation of food intake and energy expenditure. The plasma leptin concentration is proportional to body adiposity and is markedly increased in obese individuals [9]. Recent studies suggest that hyperleptinemia may play an important role in obesity-associated cardiovascular diseases including atherosclerosis [10].

Cilnidipine is a unique Ca2+ channel blocker that can inhibit both vascular L-type and sympathetic N-type Ca2+ channels, and is used to treat primary hypertension in Japan [11]. Although intracellular calcium regulates leptin secretion, calcium uptake and leptin secretion are not affected by inhibitors of L-type calcium channels [12].

Optimal pharmacological management of obesity-hypertension needs antihypertensive agents that ameliorate obesity-associated metabolic disorders, beyond lowering blood pressure values. The purpose of the present study was to evaluate the effects of cilnidipine on the blood pressure and hyperleptinemia among patients with obesity-hypertension associated with cerebrovascular disorders.

Methods

Patients

We recruited 23 patients with obesity-related hypertension that was unresponsive to lifestyle changes and was associated with a past history of stroke or ischemic changes confirmed by brain magnetic resonance imaging. Subjects with diabetes, liver or kidney diseases, secondary hypertension, or known hypersensitivity to cilnidipine were excluded, as were those with conditions that may have caused metabolic alterations within the past year (pregnancy, abdominal surgery, weight gain or loss of more than 3 kg). Informed consent was obtained from each patient. This study protocol was approved by the ethics committee of Tsukuba University Hospital.

Cilnidipine (10 mg/day) was administered orally once a day after breakfast. The duration of the study treatment was 12 weeks. The patients' demographic characteristics, including sex, age, Body Weight (BW), Body Mass Index (BMI) and Waist Circumference (WC) were obtained, and the patients were observed for any changes in Systolic

*Corresponding author: Masahiko Watanabe, Department of Neurology, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, Japan, Tel: +81-29-853-3199, Fax: +81-29-853-3199, E-mail: masa-wat@md.tsukuba.ac.jp

Received August 16, 2012; Published November 24, 2012


Copyright: © 2012 Watanabe M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Heart Rate (HR), BW, BMI, WC, plasma leptin, triglyceride, and HDL cholesterol. The blood pressure was measured at each clinical visit.

Statistical analysis
Summary statistics for the continuous variables are presented as arithmetic means with standard deviations. Changes in each variable before and after antihypertensive treatment were investigated by a paired t test.

Leptin levels at baseline were compared with those at week 12 by means of Wilcoxon signed-rank test (two-sided).

Animal and human autopsy tissues
One 8-week-old female F344/DuCrj rat was obtained from Oriental Bioservice Kanto Inc. (Ibaraki, Japan). Care of the rat was in accordance with institutional guidelines. The rat was anesthetized with ethyl ether. The rat was transcardially perfused with 4% paraformaldehyde in PBS. After perfusion, the tissues were washed in Ringer’s solution, dissected under a surgical microscope (Zeiss OPM 19, Carl Zeiss, Oberkochen, Germany), and fixed by immersion in the same fixative overnight for light microscopy and immunohistochemistry.

Autopsy specimens were obtained from one fat male cadaver and one lean male cadaver. Tissues used for our investigation were, periadrenal white adipose tissues, renal glomeruli, renal arterioles, and brain tissue.

Antibodies and immunohistochemistry
Polyclonal rabbit antibodies to CACNA1B (Cav2.2), N-type Ca++ channel protein were obtained from Novus Biologicals (Littleton, CO, USA). Immunohistochemical demonstration of Cav2.2 was performed by the avidin-biotin peroxidase (ABC) method. Dewaxed sections (3 μm) were processed through
- 0.3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase;
- 0.02 M glycine for 10 min and normal goat serum 1:75 for 20 min to reduce nonspecific background staining;
- Polyclonal rabbit antibodies against Cav2.2 that was diluted 1:12000 in PBS, overnight at 4˚C;
- Biotinylated secondary antibodies; goat anti-rabbit IgG 1:200 for 30 min (Invitrogen Corp. (Molecular Probes Inc.), Eugene, OR, USA);
- ABC complex for 1 h (Vextastain ABC kit, Vector Labs, Burlingame, CA, USA);
- Histochemical visualization of peroxidase using 3',3'diaminobenzidine hydrochloride as the chromogen (Sigma, St. Louis, MO). Retroperitoneal adipose tissues around the kidney of both fat and lean cadaver were used for immunohistochemistry. Neuronal and non-neuronal tissues of rat were also used for immunohistochemical examination.

Results
Patient demographic characteristics
Eleven men and twelve women were enrolled in the study. The mean age was 70.9 ± 7.5 years. As shown in table 1, the mean body weight was 66.7 ± 9.0 kg. The average BMI was 27.4 ± 4.1 kg/m². The mean waist circumference was 95.9 ± 7.4 cm. The mean systolic and diastolic blood pressures were 157.8 ± 10.6 mmHg and 80.2 mmHg ± 15.2 respectively. The mean triglyceride was 193.2 ± 80.8 mg/dl. The mean HDL-cholesterol was 47.6 ± 12.5 mg/dl. The mean fasting blood glucose was 146.4 ± 52.8 mg/dl.

The presence of metabolic syndrome was determined according to the NCEP-ATPIII definition (three or more of the following criteria: hypertriglyceridemia, low High-Density Lipoprotein (HDL) cholesterol, hypertension, hyperglycemia, and BMI>25 kg/m²) [12]. Ten out of twelve women met the criteria, while seven out of eleven men met the criteria.

Factors associated with baseline leptin levels
The mean plasma leptin concentration was significantly higher among female patients (17.5 ± 10.9 ng/ml) than that of male patients (5.5 ± 2.2 ng/ml) (Figure 1). Linear regression analysis of plasma leptin and BMI was performed by the least square method. For all twenty three patients there was a significant positive correlation between leptin and BMI (r=0.796; p<0.000) (Figure 2).

Alterations in clinical and laboratory parameters of patients
Changes in the clinical and laboratory parameters of the patients are presented in table 1. Body weight and BMI decreased slightly but not significantly. Waist circumference and triglyceride did not change significantly. Cilnidipine significantly decreased the systolic and diastolic blood pressures from 157.8 ± 10.6 mmHg to 137.7 ± 15.2 mmHg and 90.2 ± 7.3 mmHg to 80.2 ± 10.5 mmHg, respectively. Cilnidipine significantly decreased the pulse rate from 76.7 ± 8.1 bpm to 73.5 ± 7.4 bpm. HDL cholesterol increased slightly but not significantly.

Table 1: Mean Values at baseline and after treatment with cilnidipine.
Effects of cilnidipine treatment on changes in leptin levels

As shown in table 1, the average plasma leptin level decreased from 11.8 ng/ml to 10.8 ng/ml, which reached statistical significance. Therefore, we employed multiple regression analysis to evaluate the effect of antihypertensive treatment on differences in the leptin level, body weight, BMI, waist circumference, systolic blood pressure, diastolic blood pressure, pulse rate and HDL cholesterol. However, there were no significant effects of covariates, with exception of the effect of baseline leptin level on Δleptin.

Immunohistochemical findings of N-type Ca++ Channel protein

Fat cadaver: All mature adipocytes had a thin rim of cytoplasm that was positive for Cav2.2 (Figure 3A).

Lean cadaver: Most adipocytes were also positive for Cav2.2, but the positive signals were a little weaker than those of the fat cadaver (Figure 3B).

Rat tissue: All soma of the neurons in the cerebral cortex showed intense staining with Cav2.2 antibody (Figure 3C). Axons in the white matter were also positive for Cav2.2, while glial cells were negative (data not shown).

Discussion

We report two new findings in this paper. First, cilnidipine lowered the plasma leptin levels in patients with obesity hypertension and cerebrovascular disorder. Second, N-type calcium channel proteins are expressed in white adipose tissue.

As shown in table 2, some antihypertensive agents lower the plasma leptin levels, while others do not. The current evidence indicates that obesity activates the Rennin-Angiotensin System (RAS) in adipose tissue as well as in cardiovascular organs [13]. In addition, adipocytes are known to contain a fully functional local RAS and angiotensin II, which has been shown to have a stimulatory effect on leptin secretion in in vivo studies [14]. However, it is difficult to explain the inconsistent results obtained from drugs that belong to the RAS modulation category, such as angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers [15-17].

Among the β-blockers, two different compounds, pindolol and celiprolol, showed marked ability to lower the plasma leptin levels in patients with essential hypertension [18,19], but the effect of beta antagonists in vivo on leptin secretion remains opposite to that in the in vitro studies, which showed that adrenergic stimulation with either isoproterenol or noradrenaline inhibited leptin release in white adipocytes in culture [20,21]. In addition, infusion of isoproterenol produced a dose-related decrease in plasma leptin in lean, healthy human volunteers [22,23], but a chronic increase in circulating noradrenaline levels in pheochromocytoma patients failed to suppress leptin secretion [24]. These apparent contradictions in the literature can be reconciled if we hypothesize that acute rather than chronic adrenergic stimulation is able to suppress leptin release from adipocytes.

Among the Calcium Channel Blockers (CCB), both amloidipine and felodipine failed to lower the leptin level [15,25]. These dihydropyridine-type Ca++ channel blockers have been widely used in the treatment of arterial hypertension. Their pharmacological and therapeutic properties were mainly attributed to the blockade of Ca++ influx through L-type Ca++ channels. Calcium is known to be essential for insulin to stimulate leptin secretion [12], but according to the results of the aforementioned

Table 1:

<table>
<thead>
<tr>
<th>Class of antihypertensives</th>
<th>Compound</th>
<th>Leptin lowering effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitor</td>
<td>enalapril</td>
<td>positive</td>
<td>Masuo et al. [15]</td>
</tr>
<tr>
<td></td>
<td>perindopril</td>
<td>negative</td>
<td>Ficek et al. [18]</td>
</tr>
<tr>
<td>Angiotensin II receptor</td>
<td>losartan</td>
<td>negative</td>
<td>Sonmez et al. [16]</td>
</tr>
<tr>
<td>blocker</td>
<td>valsartan</td>
<td>positive</td>
<td>Fogari et al. [17]</td>
</tr>
<tr>
<td>β blocker</td>
<td>celiprolol</td>
<td>positive</td>
<td>Sonmez et al. [16]</td>
</tr>
<tr>
<td></td>
<td>pindolol</td>
<td>positive</td>
<td>Ficek et al. [18]</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>felodipine</td>
<td>negative</td>
<td>Ficek et al. [18]</td>
</tr>
<tr>
<td></td>
<td>amloidipine</td>
<td>negative</td>
<td>Ersoy et al. [25]</td>
</tr>
<tr>
<td></td>
<td>felodipine</td>
<td>negative</td>
<td>Fogari et al. [17]</td>
</tr>
</tbody>
</table>

Among the β-blockers, two different compounds, pindolol and celiprolol, showed marked ability to lower the plasma leptin levels in patients with essential hypertension [18,19], but the effect of beta antagonists in vivo on leptin secretion remains opposite to that in the in vitro studies, which showed that adrenergic stimulation with either isoproterenol or noradrenaline inhibited leptin release in white adipocytes in culture [20,21]. In addition, infusion of isoproterenol produced a dose-related decrease in plasma leptin in lean, healthy human volunteers [22,23], but a chronic increase in circulating noradrenaline levels in pheochromocytoma patients failed to suppress leptin secretion [24]. These apparent contradictions in the literature can be reconciled if we hypothesize that acute rather than chronic adrenergic stimulation is able to suppress leptin release from adipocytes.

Among the Calcium Channel Blockers (CCB), both amloidipine and felodipine failed to lower the leptin level [15,25]. These dihydropyridine-type Ca++ channel blockers have been widely used in the treatment of arterial hypertension. Their pharmacological and therapeutic properties were mainly attributed to the blockade of Ca++ influx through L-type Ca++ channels. Calcium is known to be essential for insulin to stimulate leptin secretion [12], but according to the results of the aforementioned

Figure 2: There was a significant positive correlation between leptin and BMI ($r = 0.796$, $p = 0.00001$).
clinical trials using amlodipine and felodipine, calcium current through 
L-type channels seemed to have little importance.

Cilnidipine is a dihydropyridine CCB that is slow in onset and 
vasoselective, and has a weak direct dromotropic effect, a strong 
vaso depressor effect, and an arrhythmia-inhibiting effect. Furthermore, 
experimental evidence employing a whole-cell patch clamp method, using 
neither rat dorsal root ganglion neurons or rat sympathetic neurons of superior 
cervical ganglia and experiments using PC12 cells derived from 
phaeochromocytoma have clarified that cilnidipine has an N-type 
voltage-dependent Ca2+ channel inhibiting effect in addition to its 
action on the L-type channel [26-28]. Thus, we hypothesized that 
N-type channel instead of an L-type channel on adipocytes may have a regulatory function in leptin secretion, and we confirmed that the 
N-type channel proteins were expressed on the plasma membrane of 
adipocytes. Recently the N-type channel was also shown to be 
expressed in adrenocortical cells and to regulate corticosteroid synthesis 
[29]. N-type channels may work in non-neural tissue to regulate its 
endocrine function. However, further pharmacological studies using 
ω-conotoxin are necessary to elucidate the mechanism through which 
N-type channel regulates leptin secretion by adipocytes.

Acknowledgments

The professional aid of Mr. Shuichiro Furuya in calcium channel 
immunohistochemistry is appreciated.

References