Effects of Benidipine, a Long-Lasting Dihydropyridine-Ca$^{2+}$ Channel Blocker, on Cerebral Blood Flow Autoregulation in Spontaneously Hypertensive Rats

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Received April 26, 2006; accepted August 4, 2006; published online August 15, 2006

Chronic hypertension shifts cerebral blood flow (CBF) autoregulation towards higher blood pressure. We examined whether or not benidipine, a long-lasting dihydropyridine calcium channel blocker (CCB), improves the CBF autoregulation in spontaneously hypertensive rats (SHRs). CBF was analyzed by laser-Doppler flowmetry during stepwise hypotension by controlled bleeding. The lower limit of CBF autoregulation was calculated as the mean arterial blood pressure at which CBF decreased by 10% of the baseline. Mean arterial blood pressure and cerebral vascular resistance in SHRs were higher than those in normotensive Wistar rats. Oral administration of benidipine (3 mg/kg) for 8 d lowered the mean arterial blood pressure and cerebral vascular resistance, which were equivalent to the effects of amlodipine (3 mg/kg), another CCB, or candesartan (1 mg/kg), an Angiotensin II type-1 receptor blocker. The lower limit of CBF autoregulation in SHRs (142±4 mmHg) was significantly shifted to a higher-pressure level compared with Wistar rats (59±2 mmHg). The lower limit of CBF autoregulation was significantly lower in the benidipine-treated group (91±4 mmHg) than that in the control SHRs, and similar to that of the amlodipine group (97±6 mmHg). Benidipine reduced the lower limit of CBF autoregulation more effectively than candesartan (109±4 mmHg). In conclusion, benidipine shifted the limit of CBF autoregulation towards lower blood pressure in SHRs under hypotensive conditions by hemorrhage. These results suggest that benidipine may be useful for the treatment of hypertensive patients with the elderly or cerebrovascular disorders, in whom autoregulation of CBF is impaired.

Key words benidipine; cerebral blood flow; autoregulation

Vascular autoregulation ensures the ability of brain to maintain a constant blood flow within wide limits of arterial pressure. To keep cerebral blood flow (CBF) at a constant level, cerebral arterioles constrict when arterial blood pressure increases whereas they dilate when blood pressure decreases. Aging progressively elevates arterial blood pressure accompanied with alternations of cerebral arterioles. Persistent hypertension and aging shifts the limits of CBF autoregulation towards higher blood pressure level. In such cases, reduction of blood pressure would lower CBF if the pressure would be below the lower limit of CBF autoregulation. Anti-hypertensive therapy itself might induce a risk of ischemic brain complications through reduction of CBF. Accordingly, it is important to examine whether or not anti-hypertensive drug could sift the limit of CBF autoregulation toward lower blood pressure.

Benidipine, a long-lasting dihydropyridine calcium channel blocker (CCB), which predominantly elicits vasodilatation of coronary and peripheral artery by blockade of Ca$^{2+}$ influx via L-type voltage-dependent calcium channels (VDCCs), is used for the treatment of hypertension and angina pectoris. Moreover, benidipine has pleiotrophic pharmacological actions, such as up-regulation of endothelial NO synthase and anti-oxidative property based on chemical structure, distinct from VDCC blockade. These favorable pleiotropic actions can offer additional benefits in clinical usage of benidipine. However, the effects of benidipine on CBF autoregulation remain unclear.

It is known that blockade of renin-angiotensin system with angiotensin-converting enzyme (ACE) inhibitor or Angiotensin II type-1 receptor blocker (ARB), which are also widely used for the treatment of hypertension, normalizes dysautoregulation of cerebral circulation by vasodilatation of large cerebral arterioles. Previous studies with respect to CCBs showed that CBF autoregulation is impaired by classical types of CCB, such as nimodipine and nitrendipine. On the other hand, amlodipine, which is a long-lasting CCB with pleiotrophic pharmacological actions like benidipine, is reported to improve the lower limit of CBF autoregulation. This discrepancy indicates that effects of CCBs on CBF autoregulation may be due to the specific pharmacological property of them. It has been shown that benidipine exerts protective effects in experimental cerebral ischemia rat model. Moreover, benidipine protects cerebrovascular injury in salt-loaded stroke-prone spontaneously hypertensive rats, as assessed by magnetic resonance imaging. Therefore, in the present study, we examined the effects of benidipine on CBF autoregulation in spontaneously hypertensive rats (SHRs), as compared with those of amlodipine, another CCB, and candesartan, an ARB.

MATERIALS AND METHODS

Animals Male SHRs (20—23 weeks of age, Japan SLC, Shizuoka, Japan) and weight-matched Wistar rats (11 weeks of age, Japan Charles River, Kanagawa, Japan) were used in this study. Rats were housed under controlled temperature (19—25 °C) and humidity (30—70%) conditions. They were given access to commercial chow (FR-2, Funabashi Farms, Chiba, Japan) and water ad libitum under a 12-h light–dark cycle. All rats received human care in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, formulated by the Japanese Pharmacological Society, and the protocol was approved by the Bioethical Committee.
The lower limit of cerebral blood flow autoregulation was defined as the mean arterial blood pressure at which cerebral blood flow decreased by 10% of the baseline. Values represent means±S.E. of 6 animals. **p<0.01 vs. the control group. *p<0.05 vs. the benidipine group.

### Statistical Analysis
The results were expressed as the means±S.E. Statistical analysis was performed using statistical analysis software (SAS, version 9.1.3, SAS Institute, Cary, NC, U.S.A.). Probit test (logistic model) was used for the estimation of the lower limit of CBF. The Aspin–Welch test or Student’s t-test following the F-test was used for analysis of differences between two groups. p values <0.05 were considered significant.

### RESULTS
Under resting conditions, the values of arterial gasses and pH in rats were in the range of PaCO₂ 31.4—34.6 mmHg, PaO₂ 82.1—90.7 mmHg and pH 7.37—7.39. There were no significant differences in these parameters among groups.

Baseline arterial blood pressure in SHRs (184±2 mmHg) was higher than that in Wistar rats (97±1 mmHg), as shown in Table 1. Benidipine (3 mg/kg) as well as amlodipine (3 mg/kg) and candesartan (1 mg/kg) were selected to exert similar anti-hypertensive effects in SHRs. SHRs were orally administrated with these drugs or vehicle once a day for 8 d.

Cerebral Blood Flow Autoregulation
Rats were anesthetized with amobarbital (100 mg/kg, i.p., Nippon Shinyaku, Kyoto, Japan) and given artificial respiration with mechanical ventilator (SN-480-7, Shinano, Tokyo, Japan) about 2.5 h after the final administration with above drugs or vehicle. Both femoral arteries were cannulated for recording of arterial blood pressure and sampling of blood for stepwise hypotension by controlled bleeding, respectively. Rats were mounted on stereotaxic head-holder (Summit Medical, Tokyo, Japan). Body temperature was maintained at 37 °C with heating pad (Model CMA/150, Carnegie Medicin, Stockholm, Sweden). A hole was made in the parietal bone with electric power drill (C-201, Urawa Denki, Saitama, Japan) and laser-Doppler needle probe was inserted for measurement of CBF according to the Paxinos and Watson ATLAS of rat brain in stereotaxic coordinate. CBF in the parietal cortex was measured by laser-Doppler flowmetry (ALF-2100, Advance, Tokyo, Japan). The location of inserted laser-Doppler probe was 1.5 mm posterior and 1 mm lateral to the bregma, and 1.5 mm ventral to the top of skull. Mean arterial pressure and CBF were continuously recorded (TYPE3066, Yokogawa Electric, Tokyo, Japan). After confirmation of stabilization of CBF and mean arterial blood pressure, arterial blood was withdrawn from the femoral artery to decrease arterial blood pressure in a step-wise manner (10 mmHg/step). Cerebral vascular resistance was calculated as the mean arterial blood pressure/CFB ratio. The lower limit of CBF autoregulation was defined as the mean arterial blood pressure at which CBF decreased by 10% of the baseline value. Arterial pH and gasses (PaO₂ and PaCO₂) were measured with laptop hemanalysis system (AVL-OPTI-CCA, Sysmex, Hyoyo, Japan).

### Statistical Analysis
The results were expressed as the means±S.E. Statistical analysis was performed using statistical analysis software (SAS, version 9.1.3, SAS Institute, Cary, NC, U.S.A.). Probit test (logistic model) was used for the estimation of the lower limit of CBF. The Aspin–Welch test or Student’s t-test following the F-test was used for
cant differences between the benidipine- (91±4 mmHg) and amlodipine-treated groups (97±6 mmHg). The determined value in the benidipine group was significantly lower than that in the candesartan group (109±4 mmHg).

DISCUSSION

Chronic hypertension results in adaptive changes that allow cerebral autoregulation to occur over a higher blood pressure.1–3 Under the condition of impaired CBF autoregulation, hypotension may involve the risk of reduction in CBF.1–3 Consideration of agent-specific effects on CBF autoregulation must be required for selection of appropriate antihypertensive therapy for hypertension with ischemic brain injury or elderly hypertension.1–3 In the present study, we confirmed that CBF autoregulation in SHR showed shift toward higher blood pressure compared with normotensive Wistar rats under hemorrhagic hypotensive conditions. Moreover, benidipine, a long-lasting dihydropyridine CCB, shifted the limits of CBF autoregulation in the direction of lower blood pressure in SHRs as well as amlodipine, another CCB, and candesartan, an ARB. Both amlodipine and candesartan have been shown to reduce risk of stroke in clinical studies.25,26 These results led us to postulate that benidipine would be safe in hypertensive patients with impaired CBF autoregulation, because it preserves the function of CBF autoregulation in the therapy of blood pressure control. In addition, normalization of dysautoregulation of CBF may be one of the important mechanisms underlying the protection by benidipine against cerebrovascular injury.22,23

In this study, benidipine significantly lowered the lower limit of CBF autoregulation more effectively than candesartan regardless of the equivalent hypotensive effect. Amlodipine also showed the same tendency of benidipine. In VALUE,27 stroke incidence in the amlodipine-treated group was lower than that in the group given with valsartan, an ARB. The normalization of cerebrovascular circulation by the treatment with CCB may be related to its protective effect against brain ischemia. Hence, CCB, such as benidipine or amlodipine, may have advantageous actions for improvement of deleterious changes in cerebral circulation.

The shift in the limits of CBF autoregulation is due to changes in the cerebral resistance vessels.1–3 Hypertension, aging, and other risk factors render structural and functional alterations of cerebral blood vessels, which contribute to increased resistance of cerebral arteries.28,29 Benidipine is reported to attenuate the wall thickness of the basilar artery and improve its endothelial function in SHRs.30 Moreover, benidipine increases CBF, and duration of effects of benidipine is longer than that of nifedipine.31 Besides the primary vasodilatation by VDCC blockade, benidipine as well as amlodipine enhances eNOS expression and increases in NO levels.8,20 Endothelium-derived NO plays crucial roles in vascular physiological function.32 The local NOS inhibition in the cerebral cortex raises the lower limit of CBF autoregulation, suggesting that NO is one of the vasodilators mediating the vasodilatation near the lower limit.33 Moreover, NO exerts anti-inflammatory, anti-thrombotic, anti-apoptotic and anti-proliferative actions. It has been already demonstrated that benidipine prevents intimal thickening of the carotid artery and increases CBF through increase in NO production with increment of eNOS levels.6–8 Stain, a hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, is one of well-known drugs that increase eNOS expression. Stains enhance CBF and improve stroke outcome in cerebral ischemic animal models via eNOS-dependent mechanism.34,35 Therefore, benidipine may protect the cerebral circulation by augmentation of endothelial NO production.

Superoxide anion in cerebral vessels also contributes to the impairment of CBF autoregulation, and gene therapy to reduce oxidative stress effectively ameliorates the impairment of CBF autoregulation.36 Thus, anti-oxidative property based on chemical structure of benidipine,10,11 one of the pleiotrophic actions, may be involved in the normalization of dysautoregulation of cerebral circulation.

Sympathetic nervous activity, which is enhanced during hypertension, contributes not only to the adjustment to circulatory stresses but also to sustained elevation in vascular resistance and arterial pressure.1–3 Some CCBs induce an increase in plasma catecholamine involved in sympathetic nervous system.37 In SHRs, oral administration of benidipine (2 mg/kg) did not affect plasma norepinephrine while the other CCBs (nifedipine, cilnidipine and amlodipine) elevated it despite the equal levels of anti-hypertensive dosage, suggesting that hypotensive baroreflex-induced sympathetic nerve activity by benidipine may be less than that by other CCBs.38

In conclusion, benidipine, a long-lasting dihydropyridine CCB, shifted the limit of CBF autoregulation towards lower blood pressure in SHRs, suggesting that benidipine has a favorable effect for the maintenance of CBF during acute reduction of blood pressure. Normalization of impaired cerebral circulation during hypertension would participate in protection by benidipine against cerebrovascular injury. On the other hand, nimodipine and nitrendipine are shown to defect CBF autoregulation.17–19 Thus, the above possible beneficial properties are not necessarily shared by all anti-hypertensive drugs. These results suggest that benidipine may be therapeutically useful for the treatment of hypertensive patients with cerebrovascular disorders.

Acknowledgements We are grateful to Drs. K. Nagashima and E. Tsukuda for critical reading of this manuscript. We thank Dr. I. Yoshitake for encouragement and support.

REFERENCES