Nilvadipine, a New Calcium Channel Blocker, Reduces Ischemic Brain Injury in Rats

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ABSTRACT—The effects of nilvadipine, a dihydropyridine type calcium channel blocker, on cerebral infarction induced by focal brain ischemia was studied in rats. The area of infarction was measured 24 hr after occlusion of the middle cerebral artery (MCA) in spontaneously hypertensive rats using triphenyltetrazolium chloride. Nilvadipine, given immediately after MCA occlusion, reduced the area of infarction significantly at doses of 0.32 mg/kg (i.p.) and 3.2, 10 and 32 mg/kg (p.o.). Nicardipine suppressed the area of infarction at a dose of 32 mg/kg (p.o.). The results suggest that nilvadipine is effective against ischemic brain injury.

Ischemic cerebrovascular disease remains the most prevalent neurologic disorder. Calcium channel blockers have received attention as possible neuroprotective agents in ischemia both because of their potent cerebral vasodilatory activity (1, 2) and because they may protect neurons by preventing the accumulation of intracellular calcium (3, 4) which may serve as a trigger of irreversible cellular injury (5, 6). Transient occlusion of the middle cerebral artery (MCA) has been reported to cause brain calcium accumulation (7), and calcium channel blockers might therefore be expected to reduce the resulting ischemic injuries in the brain.

Nilvadipine, a novel dihydropyridine-type calcium channel blocker, has selective and long lasting effects on cerebral arteries (1). It has also been reported that nilvadipine exhibits a wide distribution into various tissues including the brain (8). However, in general pharmacological tests, nilvadipine caused few effects on the central nervous system; for example, it had no activity on the general behavior in conscious dogs (T. Ono et al., unpublished data). These results prompted us to study the effect of nilvadipine on experimental cerebral infarction in rats. In this study, we examined the effects of nilvadipine on the size of the infarction in the MCA occluded rat. MCA occlusion in SHR has been reported to be the most efficient for the production of large and uniform infarction (9), and this strain was used in the present study.

Spontaneously hypertensive male rats (SHR), weighing 230 to 310 g, obtained from Charles River, were used. Anesthesia was induced with inhalation of 4% halothane and maintained with 2% halothane in oxygen. The method of left MCA occlusion has been described by Shiino et al. (9). The left infratemporal fossa of the rat lying in the right lateral position was reached transorbitally. Using an operating microscope, a small craniectomy, ca 2.5 mm in diameter, was made, and the left MCA was exposed. It was occluded

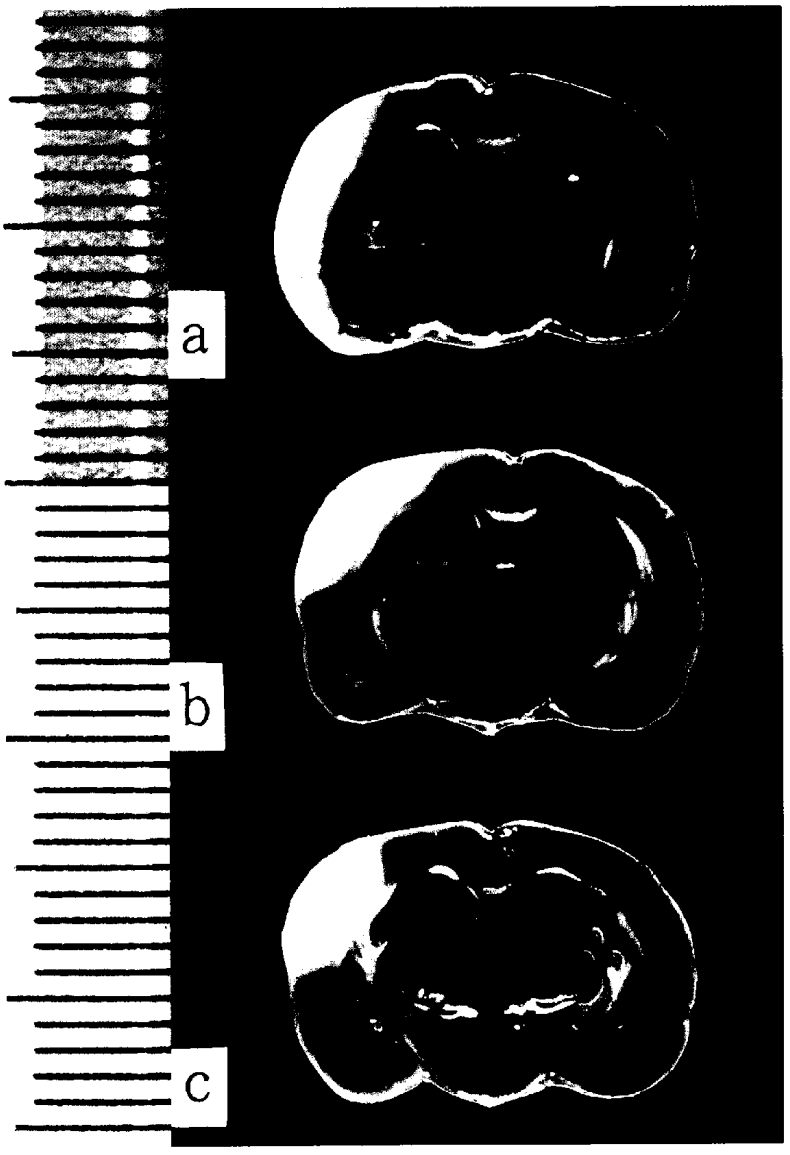

Fig. 1. Coronal section of TTC-stained rat brain 24 hr after the middle cerebral artery occlusion. The site of the section is B − 4. a: Control; b: nilvadipine 3.2 mg/kg, p.o.; and c: nicardipine 32 mg/kg, p.o. Infarct areas (white zone) are distinguishable from the non-infarct areas with TTC staining.

with a bipolar coagulator and was also transected at the lateral margin of the olfactory tract. Drugs were given orally (p.o.) or intraperitoneally (i.p.), immediately after MCA occlusion. The total duration of anesthesia and operation did not exceed 20 min. Nilvadipine was prepared in our laboratories and nicardipine hydrochloride (nicardipine) was obtained from Sigma. They were dissolved in polyethyleneglycol 400 (PEG 400). Rats in the control group were injected with PEG 400.

The area of infarction was determined 24 hr after MCA occlusion (10). Rats were sacrificed by decapitation, and the brain was quickly removed. To determine a presumptive size of infarction, several serial coronal slices, 2 mm in thickness, were perpared both anteriorly and posteriorly to the bregma. Brain slices

Table 1. Effects of nilvadipine and nicardipine on ischemic brain injury after MCA occlusion in rats

Treatment	Dose (mg/kg)	n	Area of infarction (%)				
			B + 2	B	B − 2	B − 4	B − 6
i.p.							
Control	0	5	29.5 ± 2.6	36.1 ± 2.1	32.5 ± 1.9	24.3 ± 3.2	13.8 ± 4.3
Nilvadipine	0.03	7	33.4 ± 1.9	31.9 ± 0.7	30.9 ± 2.0	20.6 ± 1.6	10.0 ± 1.3
	0.1	8	25.5 ± 2.5	28.0 ± 2.6	25.0 ± 2.4	17.1 ± 2.4	5.5 ± 2.1
	0.32	7	20.5 ± 3.6	22.5 ± 3.5**	16.9 ± 3.6**	5.9 ± 3.5**	2.9 ± 2.1*
p.o.							
Control	0	7	22.8 ± 2.5	24.9 ± 1.1	25.3 ± 1.3	20.1 ± 2.0	7.4 ± 2.7
Nilvadipine	1	7	16.5 ± 2.9	22.9 ± 1.8	23.9 ± 2.1	17.1 ± 3.3	6.3 ± 2.1
	3.2	7	19.6 ± 2.8	18.1 ± 3.4	14.9 ± 2.8*	6.6 ± 2.6**	2.3 ± 1.4
	10	7	17.9 ± 5.2	22.7 ± 4.0	16.8 ± 3.2	9.0 ± 2.8*	3.0 ± 1.5
	32	7	10.1 ± 3.1*	15.4 ± 4.1	12.5 ± 3.6**	5.2 ± 2.0**	1.2 ± 0.7
Nicardipine	3.2	7	21.2 ± 1.8	27.3 ± 1.3	25.0 ± 1.4	17.4 ± 2.4	8.6 ± 1.9
	10	7	17.3 ± 2.7	22.3 ± 1.9	21.1 ± 1.6	14.0 ± 2.6	4.0 ± 1.7
	32	7	10.0 ± 3.2*	18.6 ± 3.9	18.1 ± 3.3	4.1 ± 2.2**	0.9 ± 0.7*

Mean ± S.E. Five serial coronal slices, 2 mm in thickness, were prepared at the bregma (B) and anteriorly (B + 2) and posteriorly to the bregma (B − 2, B − 4, B − 6). *,**: $P < 0.05$, $P < 0.01$ compared with the control (Dunnett's type multiple comparisons).

were incubated in 2% TTC solution at 37°C for 40 min. Photographs of the slices were then taken. For each slice, the area of infarction was measured, and the ratio (%) of the area of infarction to the whole area of the corresponding cerebral hemisphere was calculated using a computerized image analysis system. The data were expressed as the mean ± standard error (S.E.) and analyzed with Dunnett's type multiple comparisons.

Figure 1 shows a typical example of cerebral infarction in this study at 4 mm posterior to the bregma (B − 4). Infarction was found in the cerebral cortex and rarely extended into subcortical tissue. The area of infarction was almost always largest in the slice through the bregma in all groups (Table 1) and was gradually reduced as the distance from the bregma increased. The effects of nilvadipine and nicardipine on the area of infarction is shown in Table 1. Both drugs reduced the area of infarction in a dose-related fashion at each site. Nilvadipine, at doses of 3.2 mg/kg, p.o. and higher, significantly reduced the area of infarction at several sites. Nilvadipine in a dose of 0.32 mg/kg, i.p. also reduced the area of infarction significantly at the bregma and at posterior sites. Nicardipine also reduced the area of infarction; however, a higher dose was required than nilvadipine. The effects of nilvadipine on cerebral arteries are more selective and long-lasting than those of nicardipine (1).

Nilvadipine has been reported to be distributed well into the brain (8), whereas the distribution of nicardipine into the brain might be less effective because of its water-solubility (11). Brain concentrations of nilvadipine are indeed much higher than those of nicardipine after administration of equivalent doses (T. Fujiwara et al., unpublished data). These differences may explain the differences of potencies between nilvadipine and nicardipine. Calcium has been proposed as a trigger of ischemic cell death (5, 6). The neuroprotective effect of drugs tested in this study may therefore reflect not only their vasodilatory effects, but also their ability to inhibit calcium influx.

The MCA occlusion model in rats has been widely used to evaluate the effect of some types of drugs (10, 12–14). Reduction of the size of infarction may be the most important and fundamental of brain protective effects of drugs against cerebral ischemia. In conclusion, nilvadipine seems to be more potent than nicardipine and may be more effective as a neuroprotective agent in animal models of focal cerebral ischemia.

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