Inhibitory Action of Benidipine on Balloon Catheterization-Induced Intimal Thickening of the Carotid Artery in Rats

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Received January 14, 1994 Accepted March 18, 1994

ABSTRACT—We examined the effect of benidipine, a long lasting Ca^{2+} channel blocker, on balloon catheterization-induced intimal thickening of the carotid artery in rats. In the carotid arteries of vehicle-treated rats, neo-intima formation and elevation of DNA content was observed at 14 days after the surgery. Benidipine (5 mg/kg, p.o., b.i.d.) halved the intimal thickening and suppressed the elevated DNA-content in the balloon catheter-injured artery. These results suggest that benidipine may be useful for the treatment of vascular proliferative diseases like restenosis following percutaneous transluminal angioplasty.

Keywords: Benidipine, Intimal thickening

It has been documented that a Ca^{2+} channel blocker inhibits athrogenesis in the cholesterol-fed animal (1) and the neo-intima formation after endothelial injury (2). The antiatherogenic effect of the Ca^{2+} channel blocker is probably due to the prevention of migration of vascular smooth muscle cells (VSMCs) from the media to the intima and proliferation of intimal VSMCs (3). In the present study, we examined the effects of benidipine, a long lasting Ca^{2+} channel blocker (4), on neo-intima formation after the endothelial injury induced by a balloon catheter in rats.

Male Sprague-Dawley rats (Japan SLC, Inc., Shizuoka), weighing 350-370 g, were used. Endothelial injury was induced by the method described by Clowes et al. (5). In brief, the animal was anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and then subjected to an injury of the left carotid artery with a 2F Fogarty balloon catheter passed across 3 times at 2 atm via the left external carotid artery. Benidipine or its vehicle (0.3% sodium carboxymethylcellulose (CMC)) was orally administered at 2 hr before and twice daily for 14 days after the endothelial injury. The dose (5 mg/kg, p.o., b.i.d.) of benidipine examined is the minimum effective dose to inhibit arterial DNA synthesis in the balloon-injured aorta in rats (6). Benidipine at 5 mg/kg (p.o., b.i.d.), but not at 2.5 mg/kg (p.o., b.i.d.), significantly prevented the DNA synthesis (6). Fourteen days after the surgery, the rat was sacrificed by an excessive dose of anesthetic; and thereafter, the left and right carotid arteries were excised. Both the intact (right) and the injured (left) carotid artery were divided transversely into two segments; two samples, one from each artery, were used for the measurement of total DNA and the other two samples were used for the histological study.

Total DNA was measured according to the method described by Labarca and Paigen (7). Briefly, two 10-mm segments from both carotid arteries were cut and stored in DNA buffer (0.05 M $NaPO_4$, pH 7.4). The segment was homogenized in DNA buffer and incubated with a DNA binding-dye, Hoechst 33258, and the fluorescence was measured by a spectrofluorometer (F-3000; Hitachi, Ltd., Tokyo). For histological examination, the specimen was fixed with 1% paraformaldehyde and then embedded in paraffin. Semithin sections (3 μm) were stained with hematoxylin-eosin. Morphometric analysis of the cross-section of the injured left carotid artery was performed using a computerized digitizer to perform planimetry on the surface area of the neo-intima and the media. The data are expressed as means $\pm$ S.E. and were statistically analyzed using Student's *t*-test. P values of 0.05 or less were considered to be statistically significant.

Figure 1 shows the representative cross-sections of the balloon catheter-injured carotid artery in the rat, either treated with the vehicle or benidipine. Thickening of the neo-intima in the injured artery was prominent, and the area of the neo-intima amounted almost to that of the media (Fig. 1a). Treatment with benidipine (5 mg/kg, b.i.d.) inhibited thickening of the neo-intima in the denuded

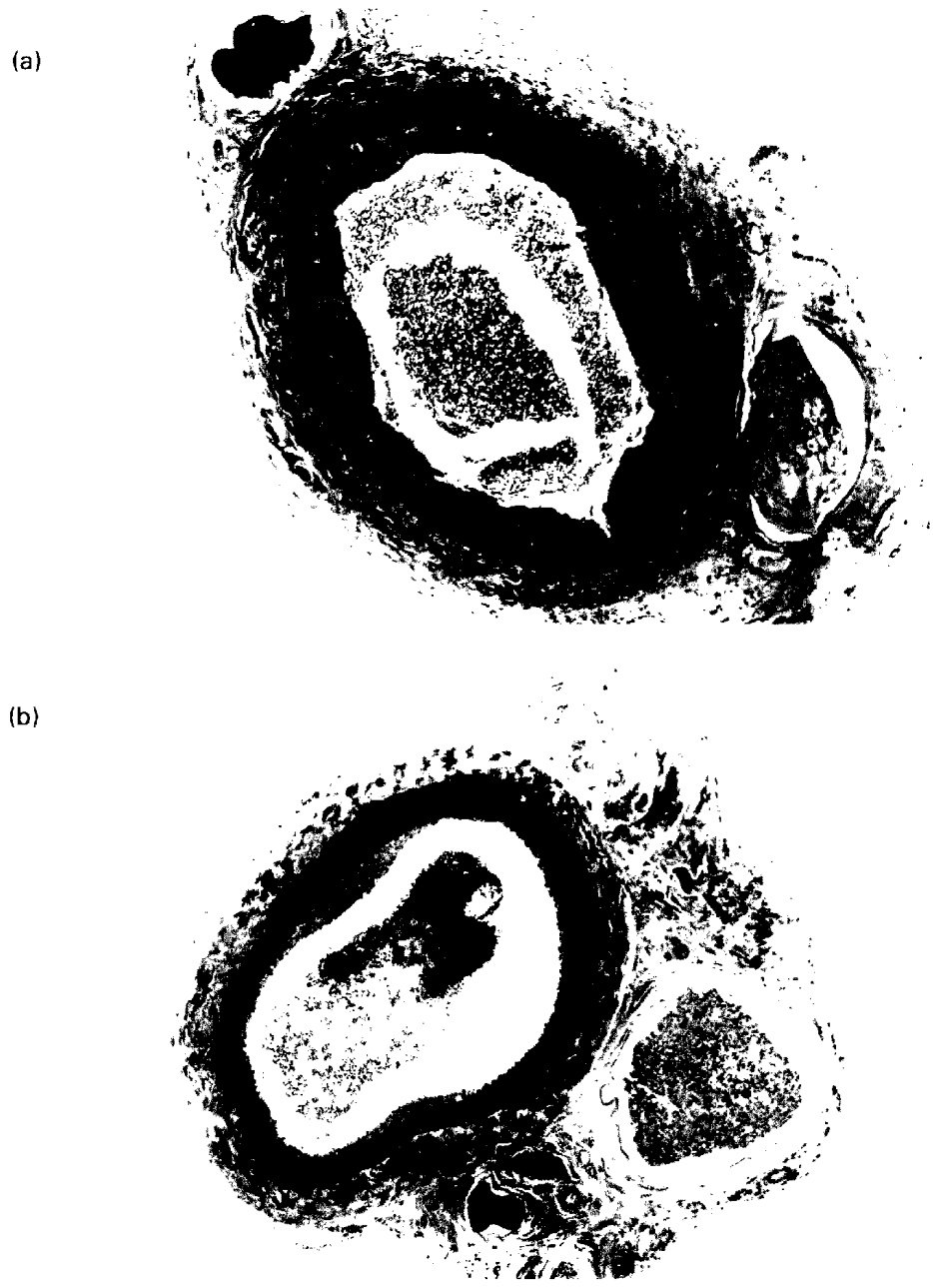

Fig. 1. Representative hematoxilin/eosin stained cross-sections of the rat carotid arteries 14 days after balloon-catheter injury. a: vehicle (0.3% CMC, 5 ml/kg, b.i.d.), b: benidipine (5 mg/kg, b.i.d).

part (Fig. 1b). Tables 1 and 2 summarize the effects of benidipine on the neo-intima formation and the tissue DNA content in the artery. Benidipine significantly suppressed both the intimal thickening and the elevation of tissue DNA content in the injured carotid arteries.

Migration and proliferation of VSMCs are induced by endogenous substances such as platelet-derived growth factor (PDGF) (8) and basic-fibroblast growth factor (9). It is reported that neo-intima formation induced by the balloon catheter injury was inhibited by an antibody to PDGF (10). We previously observed that benidipine (5 mg/kg, p.o., b.i.d.) inhibited arterial DNA synthesis in the balloon-injured aorta of the rat and, in addition, that benidipine suppresses PDGF-induced proliferation of porcine aortic smooth muscle cells (6), presumably due to the inhibition of Ca^{2+} entry into VSMCs. Therefore, the mechanism through which benidipine decreases the tissue DNA content and the neo-intima formation might be ascribed to the inhibition of PDGF-induced phenomena. On the other hand, benidipine at 5 mg/kg (p.o.) reduced

Table 1. Effects of benidipine on intimal thickening of the carotid artery 14 day after balloon-injury

	Intimal area (mm^2)	
	medie	neo-intima
Vehicle	0.125±0.008	0.106±0.010
Benidipine (5 mg/kg, b.i.d.)	0.107±0.003	0.048±0.018*

Results are expressed as means±S.E. (n=6). *P<0.05 vs. vehicle.

Table 2. Effects of benidipine on DNA-content in the carotid artery 14 day after balloon-injury

	DNA-content (μg/10 mm vessel)	
	left carotid	right carotid
Vehicle	15.6±1.7	9.1±0.6
Benidipine (5 mg/kg, b.i.d.)	8.6±1.1*	9.0±0.6

Results are expressed as means±S.E. (n=6). *P<0.05 vs. vehicle.

systolic blood pressure from 126.5±6.4 to 85.0±5.6 mmHg (mean±S.E., n=4) in rats (S. Ide et al., unpublished observation), suggesting an involvement of the hypotensive action in the antiproliferative effect. However, Handley et al. (11) have demonstrated that the calcium antagonists PN 200-110 and PY 108-068 produce similar reductions in neo-intima formation development in the balloon-injured rat carotid artery despite their disparate effects on blood pressure. It is, therefore, unlikely that the hypotensive effect of benidipine is responsible for the ameliorated neo-intima formation after arterial injury. Further studies are required to elucidate the precise mechanism involved in the anti-proliferative effect of benidipine.

In the clinical study, the usefulness of Ca^{2+} channel blockers for restenosis is controversial. The placebo-controlled clinical trial showed that the restenosis rate after coronary angioplasty was not decreased by the treatment with nifedipine at 10 mg, 4 times daily (12). On the other hand, treatment with a high-dose of verapamil (240 mg, twice daily) significantly reduced the restenosis rate (12). These results suggest that a higher dose of the Ca^{2+} antagonist, than the antihypertensive and antianginal doses, may be required to achieve the beneficial effect on restenosis. In fact, in the present animal study, the dose of benidipine that suppressed the neo-intima formation was higher than the hypotensive dose (4). Since the first generation calcium antagonist nifedipine has a relatively short plasma half-life, causes the rapid fall in blood pressure and precipitates a reflex tachycardia (13), this drug could not be administered at high doses. Moreover, the neurohumoral activation, associated with the first generation calcium antagonist, might aggravate the proliferation of VSMCs. In fact, the expression of growth-related genes was enhanced in VSMCs when the $alpha_1$-adrenoceptor was stimulated (14). On the other hand, the antihypertensive effect of benidipine is slow in onset and long lasting, and the tachycardia concomitant with the fall of blood pressure is less obvious (4). In addition, the negative inotropic action of benidipine is less prominent than those of nifedipine and verapamil (15) and, thus, benidipine could be administered at high doses. The clinical usefulness of benidipine for the restenosis following angioplasty awaits further investigation.

In summary, benidipine inhibited both the intimal thickening and the elevated DNA-content in the balloon catheter-injured rat carotid artery. These results suggest that benidipine may be useful for the treatment of vascular proliferative diseases like restenosis following percutaneous transluminal angioplasty.

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