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The Long-Acting Ca^{2+}-Channel Blocker Azelnidipine Prevents Left Ventricular Remodeling After Myocardial Infarction

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Abstract. Long-acting Ca^{2+}-channel blockers have been reported to be effective in treating ischemic heart disease. However, their effects on cardiac remodeling after myocardial infarction (MI) are still unclear. We performed this study to examine the effect of azelnidipine on left ventricular (LV) remodeling, including systolic and diastolic dysfunction, in rats with MI. MI was induced by ligation of the left anterior descending artery. The rats were then separated into 3 groups: a sham-operated group (n = 9), untreated MI group (n = 10), and azelnidipine-treated MI group (n = 10). Four weeks after MI, hemodynamic measurements and Doppler echocardiographic assessment were performed. LV weight and LV end-diastolic dimension were significantly higher in the untreated MI group than in the sham-operated group. Azelnidipine significantly prevented the increases in these parameters. Azelnidipine also improved the ejection fraction ($42 \pm 3\%$, $P<0.05$) and the E wave to A wave ratio (3.2 ± 0.5, $P<0.05$), compared with the untreated MI group ($31 \pm 3\%$ and 5.3 ± 0.8, respectively). In conclusion, azelnidipine can prevent LV remodeling and improve systolic and diastolic function after MI. Administration of long-acting Ca^{2+}-channel blockers after MI is an effective strategy for treating MI.

Keywords: calcium channel blocker, myocardial infarction, cardiac remodeling, ventricular function, gene expression

Introduction

The morbidity and mortality following myocardial infarction (MI) are high and are associated with progressive post-MI ventricular remodeling and dysfunction (1). Recent experimental and clinical studies have demonstrated that angiotensin-converting enzyme inhibitors, AT1-receptor blockers, and beta-blockers, which are used to treat heart failure, have inhibitory effects on cardiac remodeling. Ca^{2+} antagonists are commonly prescribed for the treatment of angina pectoris and hypertension, and they have reliable hypotensive effects with few adverse effects and are particularly effective in preventing stroke. However, because it has been found that short-acting Ca^{2+} antagonists increase the risk of ischemic heart disease (2, 3), the use of long-acting Ca^{2+} antagonists is generally recommended instead of the former (4). We have previously shown that amlodipine, a long-acting Ca^{2+} antagonist, prevents the left ventricular (LV) remodeling process accompanied by systolic and diastolic dysfunction and inhibits abnormal cardiac gene expression following MI (5). However, the effects of long-acting Ca^{2+} antagonists on cardiac remodeling after MI remain unclear.

Azelnidipine is a long-acting Ca^{2+} antagonist newly developed by Ube Industries, Ltd. (Yamaguchi) and Sankyo Co., Ltd. (Tokyo). Its plasma half-life is about 8 h, and comparable to that of conventional dihydropyridine Ca^{2+} antagonists (6). Because this new Ca^{2+} antagonist is highly lipid-soluble (7), it is retained in the vascular wall after clearance from the blood and continues to exhibit hypotensive effects (8). In addition, some clinical studies on azelnidipine have also demonstrated that it does not increase heart rate in association

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with lowering of blood pressure (9, 10). In a 36-year follow-up of the Framingham Study, heart rate was related to death from all causes, cardiovascular disease, and coronary disease in the group of persons with hypertension (11). Treatment of cardiovascular disease with azelnidipine might thus be more beneficial than that with heart rate-increasing Ca^{2+} antagonists. In the present study, we examined the effects of azelnidipine on LV remodeling and mRNA expression related to remodeling in rats with MI.

Materials and Methods

Animals and experimental design

This investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 8523, revised 1996).

MI was induced in male Wistar rats weighing 290 – 310 g (Clea Japan, Osaka) as previously described (12). Briefly, the rats were anesthetized by injection of pentobarbital sodium (35 mg/kg, i.p.) and a left thoracotomy was performed under volume-controlled mechanical ventilation (tidal volume, 3.0 ml; respiratory rate, 60 cycles/min). Ligatures were then placed around the left anterior descending coronary arteries. Similar surgery was performed in sham-operated rats but without coronary artery ligation (SH: n = 9). One day after the surgery, the rats with MI were randomly divided into untreated MI (MI: n = 10) and azelnidipine-treated MI (CCB: n = 10) groups. Azelnidipine was provided by Sankyo Co., Ltd. (Tokyo). Azelnidipine at 5 mg/kg per day was administered to rats after MI (CCB: n = 10). The rats in the untreated MI group were administered vehicle (0.9% NaCl solution) in the same fashion.

Doppler echocardiography and hemodynamic measurements

Four weeks after MI, we performed transthoracic echocardiography on each rat (13). The rats were lightly anesthetized with an injection of ketamine hydrochloride (25 – 50 mg/kg, i.p.) and xylazine (5 – 10 mg/kg, i.p.). Echocardiograms were performed with an echocardiography system equipped with a 7.5-MHz phased-array transducer (SONOS 5500; Philips Medical System, Best, The Netherlands). Two-dimensional short-axis views of the left ventricle and M-mode tracings were recorded through the anterior and posterior LV walls at the papillary muscle level to measure LV end-diastolic dimension (LVDd) and LV end-systolic dimension (LVDs). LV ejection fraction (EF) was measured by the modified Simpson's method, with use of a 4-chamber view. Pulsed-wave Doppler spectra of mitral inflow were recorded from the apical 4-chamber view, with the sample volume placed near the tips of the mitral leaflets in maximal opening with laminar flow pattern.

Blood pressure and heart rate of the conscious rats were measured using the tail-cuff method. The method of hemodynamic measurement used was previously described in detail (12). In brief, LV pressure was recorded by inserting a polyethylene-tubing catheter (0.58-mm internal diameter, PE-50) into the right carotid artery and advancing it into the left ventricle. Water-filled catheters were connected to the tubing, which was in turn connected to a water-filled pressure transducer. The pressures were recorded on a physiological recorder, while rats were allowed to breathe spontaneously. LV end-diastolic pressure (LVEDP) was obtained by averaging the values for 10 beats. Myocardial infarct size was measured as previously described. Rats with an infarct size of <10% were excluded from the analysis. After determination of infarct size, the heart was immediately excised and septal myocardium was dissected as non-infarcted myocardium.

Histological examination

Transverse myocardial sections (5-μm-thick) obtained four weeks after MI were stained with collagen-specific sirius red stain. Each field of non-infarcted myocardium was digitized and the area of interstitial fibrosis was calculated as the ratio of the sum of total area of interstitial fibrosis to the sum of total connective tissue area and cardiomyocyte area in all LV fields of the section. Perivascular areas were not included in this analysis.

RNA preparation and Northern blot analysis

All procedures were performed as previously described in detail (13). In brief, total RNA was isolated from each heart by the guanidium thiocyanate-phenol-chloroform method. Twenty microgram samples of total RNA were subjected to 1% agarose gel electrophoresis, transferred to nylon membrane, and hybridization was carried out with (^{32}P)-dCTP-labeled cDNA probes for brain natriuretic peptide (BNP), collagen types I and III, monocyte chemoattractant protein-1 (MCP-1), plasminogen activator inhibitor-1 (PAI-1), and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The densities of individual mRNA bands were measured using a bioimaging analyzer (BAS-2000; Fuji Photo Film, Tokyo). For all RNA samples, the density of an individual mRNA band was divided by that of the GAPDH mRNA band to correct for differences in RNA loading and/or transfer.

Statistical analyses

All results are expressed as the mean ± S.E.M. The statistical significance of differences was determined using ANOVA and the Student-Newman-Keuls test. Statistical significance was assumed at $P<0.05$.

Results

Effects of azelnidipine on ventricular weight, hemodynamics, and infarct size

As shown in Table 1, there was no significant difference in heart rate among the groups at 2 and 4 weeks after the surgery. MI (n = 10) did not change mean blood pressure, compared with sham-operated rats (n = 10). However, azelnidipine (n = 10) significantly reduced mean blood pressure at 4 weeks, compared with sham-operated rats ($P<0.05$). As shown in Table 2, MI (n = 10) significantly increased LVEDP ($P<0.01$) and the LV weight/body weight ratio ($P<0.01$). Azelnidipine (n = 10) significantly prevented increase in weight of the left ventricle ($P<0.05$) and reduced LVEDP ($P<0.05$) compared with rats with untreated MI. Infarct size in the azelnidipine-treated group did not differ from that in the group with untreated MI.

Table 1. Heart rate and blood pressure

	Sham	MI	
		untreated	CCB
Heart rate (bpm)			
2 weeks	359 ± 23	339 ± 32	336 ± 26
4 weeks	297 ± 10	310 ± 14	270 ± 13
MBP (mmHg)			
2 weeks	125 ± 3	120 ± 4	126 ± 4
4 weeks	126 ± 5	113 ± 4	102 ± 5*

sham: sham-operated rats, MI: myocardial infarction, MBP: mean blood pressure. *$P<0.05$, compared with sham.

Echocardiographic assessment of LV

As shown in Table 2 and Fig. 1, LVDd and LVDs were significantly higher in the MI group (n = 10) than in the sham-operated group (n = 10) ($P<0.01$ and $P<0.01$, respectively). Azelnidipine (n = 10) prevented the increases in both LVDd and LVDs compared with the untreated MI group ($P<0.05$ and $P<0.05$). The MI group exhibited significant systolic dysfunction compared with the sham-operated group, as evidenced by decreased EF ($P<0.01$). Azelnidipine significantly prevented this decrease in EF ($P<0.05$). The E wave to A wave ratio (E/A ratio), a parameter of diastolic dysfunction, was significantly higher in the untreated MI group than in the sham-operated group ($P<0.01$). In the CCB group, the E/A ratio was significantly improved compared with the untreated MI group ($P<0.05$).

Histological and morphometric assessments

As shown in Fig. 2, sirius red staining revealed an 8.3-fold increase in the proportion of interstitial fibrosis in non-infarcted myocardium in rats with untreated MI (n = 10) compared with sham-operated rats (n = 10) ($P<0.01$). Azelnidipine (n = 10) significantly reduced the proportion of interstitial fibrosis (0.2-fold, $P<0.01$).

Table 2. Hemodynamics, ventricular weight, and Doppler echocardiographic measurements

	Sham	MI	
		untreated	CCB
LVEDP (mmHg)	8.7 ± 1.7	24.8 ± 3.5**	16 ± 0.5§
Body weight (g)	350 ± 16	302 ± 15	322 ± 15
LV/BW (g/kg)	1.87 ± 0.02	2.21 ± 0.04**	2.1 ± 0.02**§
RV/BW (g/kg)	0.51 ± 0.01	0.8 ± 0.08*	0.7 ± 0.08
MI size (%)	—	34 ± 2.2	31 ± 2.8
LVDd (mm)	7.6 ± 0.3	10 ± 0.4**	8.9 ± 0.3*§
LVDs (mm)	5.6 ± 0.4	8.8 ± 0.4**	7.5 ± 0.4**§
EF (%)	58.2 ± 2.8	30.7 ± 2.8**	42 ± 3.3**§
E wave deceleration time (ms)	48.3 ± 1.82	42.3 ± 2.8	53 ± 4.4
E/A	2.4 ± 0.2	5.3 ± 0.8**	3.2 ± 0.5*§

sham: sham-operated rats, MI: myocardial infarction, LVEDP: left ventricular end-diastolic pressure, LV/BW: left ventricle weight/body weight, RV/BW: right ventricle weight/body weight, LVDd: left ventricular dimension end diastole, LVDs: left ventricular dimension end systole, EF: ejection fraction. *$P<0.05$, **$P<0.01$, compared with sham; §$P<0.05$ compared with untreated MI.

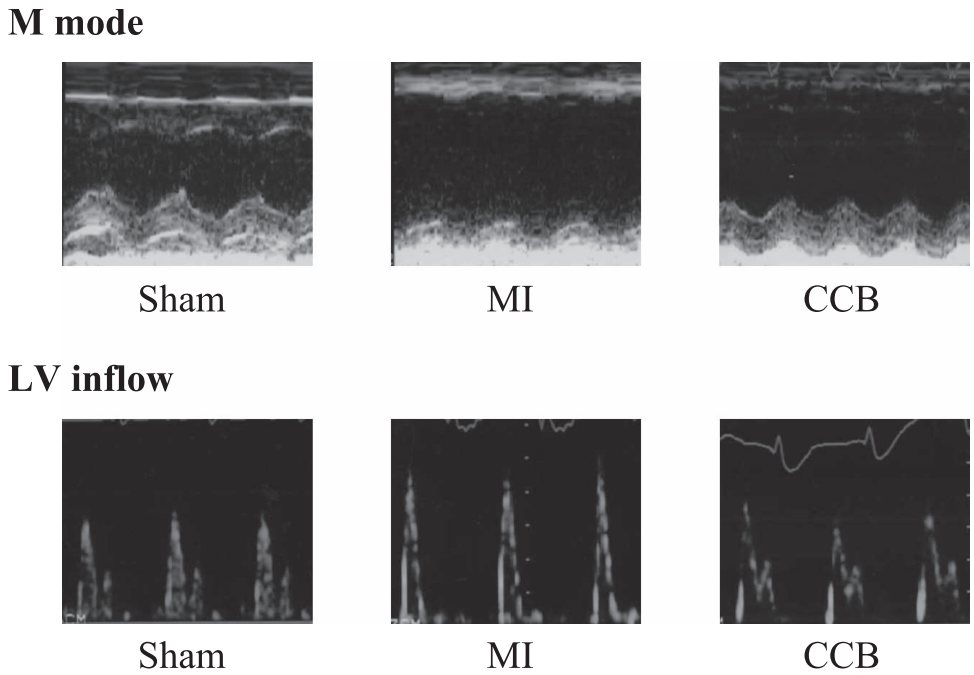

Fig. 1. Echocardiographic assessment 4 weeks after MI. Upper panels: LV M-mode echocardiograms (parasternal short-axis view) from a sham-operated rat (sham, n = 9), a rat with untreated MI (MI, n = 10), and infarcted rats treated with azelnidipine (CCB, n = 10). Lower panels: Examples of pulse-wave Doppler spectra of mitral inflow from a sham-operated rat (sham, n = 9), a rat with untreated MI (MI, n = 10), and infarcted rats treated with azelnidipine (CCB, n = 10).

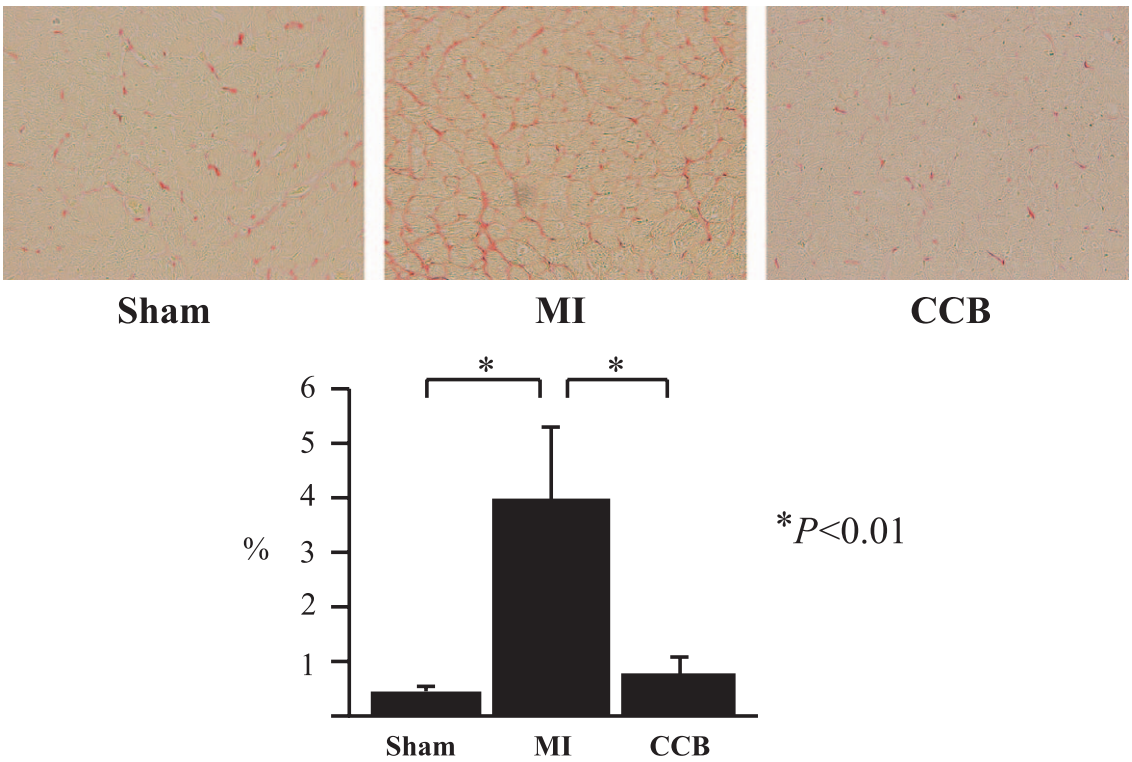

Fig. 2. Histopathologic analysis of non-infarcted myocardium of rat left ventricle. Photomicrographs show sirius red-stained cardiac sections from sham-operated rats (sham, n = 9), rats with untreated MI (MI, n = 10), and infarcted rats treated with azelnidipine (CCB, n = 10). A bar graph shows the fraction of interstitial fibrosis. Values are each a mean ± S.E.M.

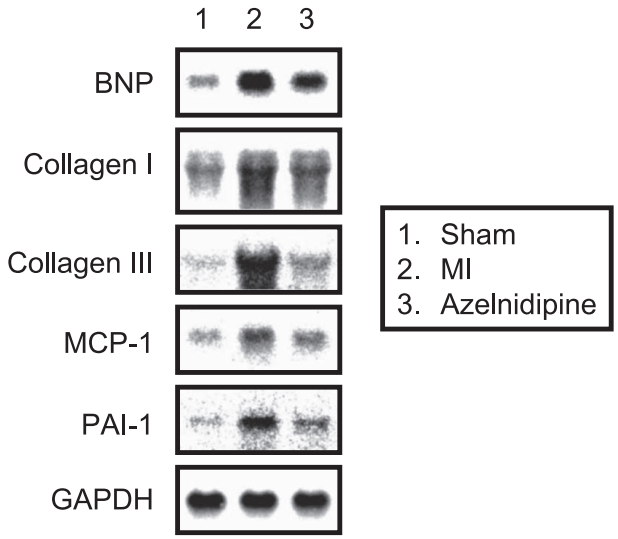

Fig. 3. Effects of azelnidipine on non-infarcted myocardial mRNA expression 4 weeks after MI. Myocardial mRNA expressions of brain natriuretic peptide (BNP), collagen types I and III, monocyte chemoattractant protein 1 (MCP-1), and plasminogen activator inhibitor 1 (PAI-1) were compared in sham-operated rats (sham), rats with untreated MI (MI), and infarcted rats treated with azelnidipine (CCB). Each panel shows a representative autoradiogram of Northern blot analysis of BNP, collagen I, collagen III, MCP-1, and PAI-1.

mRNA expression in non-infarcted myocardium

Figure 3 shows the results of analysis of cardiac gene expression. mRNA expressions of BNP, collagen types I and III, MCP-1, and PAI-1 were significantly increased 2.1-, 4.0-, 3.4-, 2.7-, and 3.2-fold, respectively, at four weeks after MI ($P<0.05$) in non-infarcted myocardium (n = 10). Azelnidipine (n = 10) significantly attenuated the increases in expression of BNP (67%, $P<0.05$), collagen I (53%, $P<0.05$), collagen III (54%, $P<0.05$), MCP-1 (55%, $P<0.05$), and PAI-1(52%, $P<0.05$) mRNA.

Discussion

In the present study, we found that azelnidipine prevents ventricular remodeling accompanied by cardiac dysfunction following MI. We also demonstrated that azelnidipine suppresses increases in mRNA expression and fibrosis related to cardiac remodeling in non-infarcted myocardium.

Azelnidipine did not change blood pressure and prevented cardiac remodeling in MI groups. It suggests that azelnidipine has preventive effects on cardiac remodeling beyond its antihypertensive effects. The mechanisms of the preventive effects may be partially related to suppression of the remodeling-related mRNA expression and inflammatory-related mRNA expression such as MCP-1 and PAI-1 by azelnidipine.

Hayashidani et al. (14) reported that MCP-1 expression contributes to LV remodeling and failure after MI, potentially through increased cardiac fibrosis via enhancement of transforming growth factor beta and matrix metalloproteinase 9 expression. They suggested that sustained MCP-1 expression can lead to sustained cytokine expression and inflammatory responses that in turn result in myocardial damage. In the present study, azelnidipine attenuated the increase in MCP-1 mRNA expression in non-infarcted myocardium, an effect that may be involved in inhibition of myocardial fibrosis and resultant LV failure.

PAI-1 regulates the plasminogen activator-plasmin system, which appears to be an important modulator during cardiac repair following MI. Regional PAI-1 induction may contribute to the progression of tissue fibrosis (15). In the present study, azelnidipine attenuated the increase in PAI-1 mRNA expression in non-infarcted myocardium, which may have contributed to inhibition of cardiac fibrosis.

Oxidative stress might play an important role in the progression of LV remodeling and failure that occur following MI. Kinugawa et al. (16) suggested that increase in myocardial reactive oxygen species (ROS) could contribute to the activation of matrix metalloproteinase and thus to the development of LV remodeling after MI. There is also growing evidence that ROS are increased in heart failure and may contribute to disease progression (17). Shinomiya et al. (18) reported that inhibition of 8-iso-$PGF_{2\alpha}$ was greater with azelnidipine than with other drugs when they evaluated antioxidant activity in cultured human arterial endothelial cells under oxidative stress. Similarly, azelnidipine inhibited tumor necrosis factor-α-induced IL-8 expression in HUVEC by blocking NADPH oxidase-mediated ROS generation and subsequent AP-1 activation (19). The antioxidant activity of azelnidipine might thus contribute to its beneficial effects on LV remodeling and failure.

Neurohumoral factors such as angiotensin II (Ang II), aldosterone, endothelin-1 (ET-1), and norepinephrine (NE) play pivotal roles in the development of LV remodeling. Hypotension after infarction activates the renin-angiotensin-aldosterone axis, catecholamine production by adrenal medulla, and spillover from sympathetic nerve terminals. Enhanced NE release contributes both directly and indirectly to the hypertrophic response. The activation of β_1-adrenoreceptors in the juxtaglomerular apparatus induces renin release, which enhances the production of Ang II. Ang II and NE may augment ET-1 release, and increased Ang II and ET-1 stimulate myocyte hypertrophy. In addition, stimulation of α_1-adrenoreceptors by NE leads to myocyte hypertrophy via the Gαq-dependent signaling

pathway (20). NE also induces remodeling processes such as collagen synthesis and apoptosis (21). Shokoji et al. (22) suggested that azelnidipine possesses sympathoinhibitory effects, which may be a reason why it has less pronounced effects on heart rate in hypertensive patients. In the present study, azelnidipine did not increase heart rate. This lack of increase in heart rate suggests that azelnidipine does not cause reflex sympathetic stimulation. Some studies also suggest that ROS are involved in the regulation of sympathetic nerve activity (23, 24). It is thus interesting to speculate that some of the sympathoinhibitory effects of azelnidipine are mediated via its antioxidative activity. These considerations together suggest that the sympathoinhibitory effects of azelnidipine following MI might be a reason for its anti-remodeling effects.

Kobayashi et al. (25) have reported that endogenous nitric oxide synthase (eNOS) expression may play an important role in the amelioration of myocardial remodeling. Their results demonstrated that chronic administration of celiprolol increased eNOS mRNA and protein expression, thereby increasing NOS activity, and improved myocardial remodeling. Kimura et al. (26) have reported that treatment with azelnidipine significantly increased eNOS expression levels in the brain, heart, and aorta, but did not alter neuronal nNOS or inducible iNOS expression levels. Cardiac remodeling after MI was prevented possibly due to increased eNOS expression in the LV.

Amlodipine and manidipine are reported to possess the anti-inflammatory and antioxidant activity via the augmentation of eNOS expression and to have vasoprotective effects beyond the blood pressure-lowering effects (27). Taken together with our results, a Ca^{2+} antagonist may potentially have preventive effects against cardiac remodeling after MI.

In conclusion, we found that azelnidipine prevented LV dysfunction and remodeling after MI. Our present study suggests that the anti-remodeling effect of azelnidipine could be of significant clinical benefit for patients after MI.

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