Cardiovascular Drugs and Therapy 1998;12:449–455

Combination of a Calcium Antagonist, Verapamil, with an Angiotensin Converting Enzyme Inhibitor, Trandolapril, in Experimental Myocardial Ischemia and Reperfusion: Antiarrhythmic and Hemodynamic Effects of Chronic Oral Pretreatment

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Summary. **The combination of a calcium antagonist with an angiotensin-converting enzyme (ACE) inhibitor is increasingly used in the therapy of hypertension, but there are no experimental data supporting the use of this combination in acute myocardial ischemia and reperfusion. We tested the effects of oral pretreatment in a pig model, paying special attention to arrhythmias and adverse hemodynamic effects. Pigs received verapamil 240 mg + trandolapril 4 mg, verapamil 240 mg, or placebo orally once daily for 10 days, after which a coronary artery was ligated for 20 minutes and then allowed to reperfuse. The ventricular fibrillation threshold (VFT) was measured during ischemia to assess the vulnerability of the heart to ventricular fibrillation, whereas spontaneous tachyarrhythmias were monitored during reperfusion. Regional left ventricular (LV) blood flow was measured with radioactive microspheres. *During the ischemic period*, both the combination of verapamil plus trandolapril, and verapamil alone, prevented a fall in the VFT, indicating antiarrhythmic activity. The combination maintained LV contractile activity and cardiac output (CO) at preligation levels, whereas verapamil alone decreased cardiac output. *During reperfusion*, verapamil plus trandolapril prevented spontaneous ventricular tachyarrhythmias and increased blood flow in the reperfused zone. In contrast, verapamil was not antiarrhythmic and decreased CO. Thus the addition of the ACE inhibitor trandolapril to the calcium antagonist verapamil resulted in antiarrhythmic activity during ischemia and reperfusion, and produced a better hemodynamic profile.**

Cardiovasc Drugs Ther **1998:12**

Key Words. **acute myocardial ischemia, reperfusion, calcium antagonist, ACE inhibitor, verapamil, trandolapril, myocardial blood flow**

Patients with hypertension are predisposed to myocardial ischemia and acute infarction (AMI). Calcium antagonists and angiotensin-converting enzyme (ACE) inhibitors may be effectively combined in the treatment of hypertension because of additive vasodilatory mechanisms [1,2]. This combination is increasingly used in clinical practice [3], and currently no less than five combinations of a calcium antagonist and an ACE inhibitor are registered for use in the United States. There are few or no data on the efficacy and safety of such combinations in myocardial ischemia and reperfusion.

Limited evidence suggests that the dihydropyridine calcium antagonist nifedipine may have adverse effects when given in high doses in acute ischemic syndromes. In the DAVIT studies that assessed the effects of verapamil on cardioprotection, the first study showed no benefit, possibly due to an adverse effect of this agent in early-phase AMI [4]. In contrast, in the second study [5], in which verapamil was started some days after the onset of AMI, there was protection against reinfarction. On the other hand, ACE inhibitors have been tested with benefit in early-phase AMI [6–8]. Based on these data, it could be predicted that the combination of verapamil and an ACE inhibitor might be more desirable in early-phase AMI than verapamil alone, especially because of additive beneficial properties, which include the antiischemic and antiarrhythmic effects of both agents, and the indirect antiadrenergic effect of ACE inhibition. We therefore studied the effects of chronic oral pretreatment with verapamil (240 mg) alone or in combination with the ACE inhibitor trandolapril (4 mg) on arrhythmias, hemodynamics, and myocardial blood in a pig model of ischemia and reperfusion.

Methods

Forty male pigs (Large White crossed with Landrace), weighing 27–30 kg, were randomized into three

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groups. Each group received either verapamil 240 mg plus trandolapril 4 mg (V + T), verapamil (V) 240 mg alone, or placebo once daily. The administration was done in a blinded manner. All three preparations were in the form of sugar-coated tablets, as supplied by Knoll AG, (Ludwigshafen, Germany). Tablets were given orally to the pigs before the morning feed for 10 days. Two hours after the administration of the final dose on day 10 (without a meal), the pigs received a premedication of ketamine 10 mg/kg IM and diazepam 1 mg/kg. Anesthesia was induced by the administration of thiopentone sodium 10 mg/kg IV. The animals were ventilated with a nitrous oxide/oxygen mixture (ratio 4:6) to which isoflurane 1.0–1.5% was added. The pO_2 of arterial blood was controlled between 120 and 160 mmHg, and pH between 7.43 and 7.48. Standard limb lead electrocardiograms and arterial pressure were monitored continuously.

The heart was exposed by midsternal thoracotomy. The anterior descending coronary artery was dissected free for about 5 mm at a point about halfway between the origin and its apical termination. It was abruptly ligated by tightening thin polyvinyl tubing around a 2-cm length of rigid tubing placed alongside the artery. This procedure was used to minimize tissue damage and to facilitate reperfusion after 20 minutes of ischemia.

We measured the ventricular fibrillation threshold (VFT) to assess the vulnerability of the heart to ventricular fibrillation (VF), by using the "train" method. An electrical stimulus consisting of a train of 10 square-wave pulses was triggered by the R wave and distributed over 210 ms during the T wave. The stimulus was passed between two platinum electrodes sutured onto the anterior wall of the left and right ventricle, 2.5 cm apart. The anode was placed across the border of the expected ischemic zone in the left ventricle, and the cathode over the nonischemic zone of the right ventricle. Pulses were generated using a Grass S88 stimulator (Grass Instruments, Quincy, MA). The stimulus current was progressively increased in 2mA steps starting at 3 mA until VF [9] occurred. The VFT was taken as the lowest current required to produce VF. Care was taken to avoid the late portion of the T wave, as previously described [10,11]. Epicardial direct current shock was applied usually within 4 seconds of the onset of VF and repeated if necessary. If defibrillation could not be accomplished within 60 seconds, the experiment was terminated. The VFT was measured before ligation as well as 5 minutes thereafter. Twenty minutes after ligation, the ligature was released to effect reperfusion. During reperfusion a high incidence of spontaneous ventricular arrhythmias is evident in this model. We therefore did not measure the VFT during this period. Ventricular tachycardia (VT) was defined as more than four consecutive uniform or multiform ventricular premature systoles. Runs of VF or VT were considered terminated when they were followed by at least three normally conducted sinus beats. Sustained VT in our model is accompanied by a severe decrease in arterial pressure. This definition of VT enables us to distinguish between sustained and nonsustained VT.

Left ventricular pressures and the maximal rate of pressure development of the left ventricle ($LV_{max}dP/dt$) were measured by advancing a tip catheter transduce through the apex into the LV cavity. The catheter was connected to a Cardiomax computerized system (Columbus Instruments, OH). Cardiac output was measured by thermodilution, using a Swan-Ganz catheter. The Cardiomax system gives real-time displays of digital and analogue signals. Each parameter is measured over at least three consecutive cardiac cycles at a time when no arrhythmias are evident. Each data point represents the mean of two readings.

Regional left ventricular blood flow was measured before ligation, 18 minutes after ligation, as well as 18 minutes after reperfusion, using the sample reference method. The radionuclides utilized were gadolinium-153, strontium-85, and tin-113 (New England Nuclear, Boston, MA). The microspheres (mean size 15 μM) were prepared in a 0.9% saline solution containing Tween-80 0.01% and were administered according to the details and precautions previously described [12, 13].

Size of the underperfused zone

Thirty minutes after the onset of reperfusion, the anterior descending artery was again ligated at the same site and the underperfused zone was delineated by injection of 5 mL of patent blue 7.5% solution (May & Baker, UK) in saline into the left atrium. The heart was then excised and arrested. The left ventricle was dissected and the well-defined underperfused zone isolated. The size of this zone was then expressed as a percentage of total left ventricular mass. When the underperfused zone was less than 26% or more than 34%, the results were not processed. This exclusion criterion had been decided on before the start of the study because links between eventual infarct size and arrhythmias have been well described [14, 15].

Plasma ACE determination

Plasma ACE levels were determined according to a modification of the method of Saveedra et al. [16] previously published [17].

Statistics

All values are expressed as means ± SEM. Student's *t*-test (paired) was used for within-group comparisons. Analysis of variance was used for between-group comparisons. Bonferroni's correction was applied for multiple comparisons [18]. The Fisher exact test was used for comparing incidences of arrhythmias. *P* values of less than 0.05 were considered significant.

Results

One experiment was terminated prematurely because VF induced during the assessment of the VFT could not be reverted to sinus rhythm. The results represent data obtained from 13 pigs in each of the three groups.

Ventricular arrhythmias (Table 1)

During ischemia: Coronary ligation caused a fall in the VFT in placebo group. V+T as well as V prevented the fall in VFT during ischemia, indicating antiarrhythmic activity. The incidences of spontaneous ventricular tachyarrhythmias were low in all three groups.

During reperfusion: The incidence of ventricular tachyarrhythmias (VF and/or VT) was high in both the placebo and verapamil groups. V+T was markedly antiarrhythmic.

Left ventricular pressures, arterial pressure, cardiac output, and heart rate (Table 2)

Arterial pressure and *LV systolic pressure (LVSP)* were not decreased in the preligation phase, following pretreatment. This finding is to be expected because the pigs were not hypertensive. During ischemia these pressures were decreased in the placebo and verapamil groups compared with preligation values, but not in the V+T group. During reperfusion arterial pressure and LVSP returned to preligation levels in the placebo group. In both treatment groups, these were decreased compared with preligation levels.

$LV_{max}dP/dt$ was similar in all three groups after pretreatment and before coronary ligation. In the placebo group, LV_{max}dP/dt was decreased during ischemia and reperfusion. Both V+T and V prevented this decrease during ischemia, but not during reperfusion. *Cardiac output* in all three groups was similar after chronic oral pretreatment and before ligation. In the verapamil group, cardiac output was decreased throughout ischemia and reperfusion, when compared with preligation values. This decrease is probably related to a decrease in stroke volume, at least during the ischemic period.

Table 1. *Ventricular arrhythmias*

	Before ligation	During ischemia	During reperfusion
Ventricular fibrillation threshold (mA)			
Placebo	19.8 ± 2.1	14.4 ± 1.7[a]	Not measured
V+T	18.8 ± 1.3	16.2 ± 1.5	Not measured
Verapamil	18.3 ± 1.9	16.2 ± 1.5	Not measured
Spontaneous tachyarrhythmias (VF and/or VT)			
Placebo	None	4/13	7/13
V+T	None	0/13	1/13[b,c]
Verapamil	None	2/13	10/13

[a]$P < 0.01$ vs. before ligation; [b]$P < 0.05$ vs. placebo; [c]$P < 0.01$ vs. verapamil.
V+T = verapamil + trandolapril; VF = ventricular fibrillation; VT = ventricular tachycardia.

Table 2. *Left ventricular pressures, arterial pressure, cardiac output, and heart rate*

	Before ligation	Ischemia 10 minutes	Reperfusion 10 minutes
Arterial systolic pressure (mm Hg)			
Placebo	96 ± 3	83 ± 3[b]	87 ± 3
V + T	103 ± 4	95 ± 4	88 ± 4[b]
Verapamil	108 ± 5	94 ± 4[b]	93 ± 4[a]
Arterial diastolic pressure (mmHg)			
Placebo	59 ± 2	53 ± 3[b]	56 ± 3
V + T	64 ± 3	58 ± 5	53 ± 3[b]
Verapamil	64 ± 4	58 ± 3[a]	54 ± 2[a]
Left ventricular systolic pressure (mmHg)			
Placebo	91 ± 6	82 ± 3[a]	89 ± 5
V + T	95 ± 5	86 ± 5	84 ± 4[a]
Verapamil	105 ± 6	89 ± 5[a]	85 ± 5[b]
LV_{max}dP/dt (mmHg/s)			
Placebo	1154 ± 124	873 ± 63[a]	812 ± 98[a]
V + T	1056 ± 84	1004 ± 83	827 ± 55[a]
Verapamil	945 ± 59	952 ± 68	753 ± 44[a]
Cardiac output (L/min)			
Placebo	3.0 ± 0.5	2.6 ± 0.4	2.8 ± 0.4
V + T	3.0 ± 0.4	3.0 ± 0.3	2.5 ± 0.2
Verapamil	3.1 ± 0.2	2.3 ± 0.1[a]	2.3 ± 0.2[a]
Stroke volume (mL/beat)			
Placebo	28 ± 3	24 ± 2	26 ± 3
V + T	33 ± 4	32 ± 4	31 ± 4
Verapamil	35 ± 5	25 ± 2[a]	27 ± 3
Heart rate (beats/min)			
Placebo	105 ± 8	108 ± 8	104 ± 5
V + T	92 ± 4	96 ± 5	90 ± 7
Verapamil	91 ± 6	91 ± 5	92 ± 6

[a]$P < 0.05$ vs. before ligation.
[b]$P < 0.005$ vs. before ligation.
LV_{max}dP/dt = maximal rate of pressure development of the left ventricle;
V + T = verapamil plus trandolapril.

Plasma ACE activity

ACE activity was 49.4 ± 6.7 in placebo group, 16.4 ± 2.8 in the V+T group ($P < 0.0002$), and 55.9 ± 5.6 mU/mL in the V group, indicating a reduction of between 60% and 80% in the concentration of plasma ACE by trandolapril.

Regional left ventricular blood flow (Fig. 1)

Blood flow rates in the three groups after pretreatment, but before ligation, did not show any differences: placebo 0.86 ± 0.03, V+T 0.93 ± 0.04, and V 0.86 ± 0.04 mL/g/min. Coronary ligation in this model resulted in

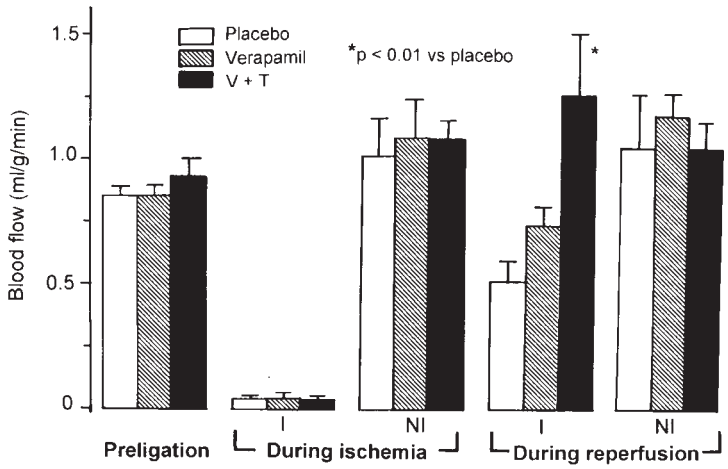

Fig. 1. *Regional left ventricular blood flow. During the ischemic period, blood flow in the ischemic zone (I) was decreased to less than 10% of preligation values in all three groups, whereas blood flow in the nonischemic zone (NI) remained unchanged. During the reperfusion period, blood flow in the previously ischemic zone of the combination group (V + T) was higher, compared placebo group. Blood flow in the nonischemic zone was once again unchanged. V + T = verapamil + trandolapril.*

severe ischemia, with more than 90% reduction in blood flow rates. The values in the three groups were as follows: placebo group, 0.04 ± 0.01; V+T group, 0.04 ± 0.01; and V, 0.05 ± 0.02 mL/g/min. Reperfusion partially restored blood flow rates to between 50% and 70% of preligation values in the placebo and V groups, respectively. V+T markedly increased blood flow to 1.26 ± 0.25 versus 0.51 ± 0.08 mL/g/min in the placebo group, $P < 0.01$. Blood flow rates in the uninvolved nonischemic zone of the left ventricle remained unchanged in all three groups for the duration of the experiments.

Size of underperfused zone

The sizes of the underperfused zones in the three groups were as follows: placebo, 29 ± 2%; V+T group, 30 ± 2%; and V group, 27 ± 1%.

Discussion

Chronic oral pretreatment with the calcium antagonist verapamil combined with the ACE inhibitor trandolapril resulted in important beneficial effects during acute myocardial ischemia and reperfusion: (1) The combination was antiarrhythmic during ischemia as well as during reperfusion. (2) No redistribution of LV blood flow occurred at the cost of the ischemic zone. (3) LV blood flow was increased during reperfusion. (4) LV contractile activity was not depressed. Verapamil alone resulted in antiarrhythmic effects during ischemia only, and cardiac output was decreased.

Verapamil and ischemic arrhythmias

The dosage and mode of verapamil administration appear to be important in the prevention of arrhythmias. We have previously studied the antiarrhythmic effects of a clinically relevant acute intravenous dose of verapamil in this pig model. Verapamil 0.2 mg/kg given 30 minutes before coronary ligation was not antiarrhythmic during ischemia [19]. In supraclinical doses (0.6 mg/kg) in the same study [19] and in another study [20], verapamil was antiarrhythmic, but at the cost of a depression of left ventricular contractile activity. Although just above the upper limit of clinical doses, the dose of verapamil (8 mg/kg orally) used in the present study prevented arrhythmias. LV contractile activity did not fall. However, verapamil decreased cardiac output and stroke volume during ischemia.

Both the calcium-dependent inward current and the subsequent intracellular calcium overload have been implicated in ischemic arrhythmias [21–23]. Antiarrhythmic effects of verapamil during ischemia in the present and other studies [19,20] may have been the direct result of blockade of the L-type calcium channel (Fig. 2).

ACE inhibitors as antiarrhythmic agents during ischemia

Various ACE inhibitors are known to decrease early ischemic arrhythmias in rats [24–26] and dogs [27]. In our model the ACE inhibitor perindopril, given 30 minutes before coronary artery ligation, prevented ischemic ventricular arrhythmias [28]. However, captopril [29] and zofenopril [30] did not prevent ischemic arrhythmias in pigs. When trandolapril was given chronically to pigs, as in the present study, but in an oral dose about twice as high, antiarrhythmic effects could be obtained during ischemia [17]. These contradictory results may be related to the variable incidence of spontaneously occurring ventricular arrhythmias af-

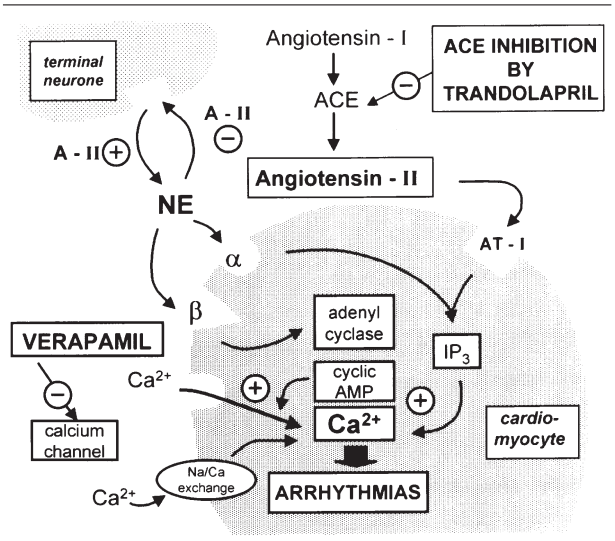

Fig. 2. *Proposed mechanisms for antiarrhythmic effects of verapamil and trandolapril. Apart from stimulating its own receptors, angiotensin II facilitates sympathetic responses in the myocardium through more than one pathway. Angiotensin II may also stimulate calcium release from the sarcoplasmic reticulum via the inositol trisphosphate pathway. In theory, the combination of an ACE inhibitor with a calcium antagonist should have additive antiarrhythmic effects. A-II = angiotensin II; NE = nor-epinephrine; α = alpha receptor, β = beta receptor; AT-I = angiotensin receptor; Ca^{2+} = calcium ions.*

ter coronary ligation in open-chest pigs. Various factors may underlie this phenomenon. We therefore preferred to measure the VFT in addition to monitoring the incidence of spontaneous arrhythmias during ischemia.

Acute myocardial infarction is associated with activation of the renin-angiotensin system [31]. Postjunctional stimulation of angiotensin II receptors may result in an increase in cytosolic calcium via the inositol trisphosphate pathway [32] (see Fig. 2). Angiotensin II, in addition, facilitates sympathetic activation in the myocardium by several mechanisms [33–35]. Angiotensin II may result in increased levels of norepinephrine for stimulation of postsympathetic adrenergic receptors [36–38]. Thus angiotensin II could, through various intracellular pathways, result in increased levels of calcium ions, which could trigger ventricular arrhythmias via the transient inward current, slow response action potentials, or cell uncoupling [21,39]. Trandolapril may have exerted antiarrhythmic effects by decreasing angiotensin II, with secondary inhibition of sympathetic outflow during ischemia. Second, the accumulation of bradykinin as a result of inhibition of ACE activity may have contributed to the antiarrhythmic effects [40,41]. Third, ACE inhibition prevented the action of norepinephrine on the transient inward current [42], a phenomenon that has been implicated in ventricular arrhythmias.

Reperfusion arrhythmias

One major mechanism thought to underlie reperfusion arrhythmias is the increased influx of calcium ions into the cytosol [43]. Verapamil decreased reperfusion arrhythmias in the isolated rat heart [21]. In the pig, verapamil at a high intravenous dose was also effective when administered shortly before coronary ligation and reperfusion [20]. The reason why chronic oral pretreatment by verapamil in the present study did not reduce reperfusion arrhythmias may be that insufficient levels of the agent remained in the biophase at the time of reperfusion.

Perfusion with angiotensin II increased reperfusion arrhythmias in isolated rat hearts [44]. The effectiveness of ACE inhibitors in the prevention of reperfusion arrhythmias in animal models is well documented [17,45,46]. The increase in blood flow in the reperfused zone by V + T in the present study during the reperfusion period may directly underlie the antiarrhythmic effects. A similar increase in blood flow, associated with antiarrhythmic activity, was evident during reperfusion when trandolapril was given as monotherapy at a dose of 9 mg daily [17].

Vasodilatory effects of calcium antagonists and ACE inhibitors

The vasodilatory effects of both calcium channel blockers and ACE inhibitors are well known. Calcium channel blockers facilitate the effects of endothelium-derived relaxing factors and prevent those of endothelium-derived contracting factors in smooth muscle. ACE inhibitors, on the other hand, are able to increase the activity of the L-arginine-nitric oxide pathway through their effects on bradykinin, in addition to their inhibitory effects on angiotensin II formation [2]. A recent article described the release of endothelium-derived nitric oxide by trandolaprilat [47]. In a dog model of variant angina, coronary blood flow increases were potentiated when verapamil was added to trandolapril [48].

In the present study, neither treatment regime changed LV blood flow during the ischemic period. A higher dose of trandolapril, given as chronic oral pretreatment in a previous study by our group, showed a redistribution of LV blood flow during the ischemic period: Blood flow in the nonischemic zone was increased, but at the cost of a decrease in blood flow in ischemic tissue [17]. This finding may suggest a coronary steal phenomenon. A similar decrease in blood flow in ischemic tissue after acute ACE inhibitor administration has also been described in animals [28] and in patients [49,50]. Thus the addition of a calcium channel blocker to an ACE inhibitor may be necessary to overcome this clinically important deleterious effect.

Left ventricular contractile activity

$LV_{max}dP/dt$ was used as an index of LV contractile activity in our study. In two previous studies done by

our group in this model, acute verapamil pretreatment, at doses that were antiarrhythmic during ischemia, depressed contractile activity [19,20]. It therefore appears that chronic oral pretreatment with verapamil in the present study has the important advantage that antiarrhythmic activity during ischemia can be obtained without a depression of contractile activity. In a previous study, a higher dose of trandolapril decreased $LV_{max}dP/dt$ during ischemia and reperfusion compared with the placebo group. In the present study, the combination of verapamil with a lower dose of trandolapril did not have deleterious effects on contractile activity during the ischemic period. Despite an increase in blood flow by V + T during reperfusion, the depression in LV contractile activity, which could be indicative of reperfusion stunning, could not be overcome.

In conclusion, the present study suggests that in, contrast to acute pretreatment, chronic oral pretreatment with verapamil at clinically relevant doses can result in antiarrhythmic effects during acute myocardial ischemia without suppression of contractile activity of the heart. Chronic oral treatment with verapamil combined with trandolapril can also protect against arrhythmias during ensuing reperfusion. In addition, results from previous studies [17,28,49,50] and from the present study suggest that a coronary steal phenomenon associated with ACE inhibition in patients and animal models of myocardial ischemia may be overcome by the addition of a calcium channel blocker.

Extrapolation from an anesthetized open-chest pig model to the clinical situation should be undertaken with considerable caution. Another reservation is that we studied a nondihydropyridine calcium channel blocker, so that our proposals may not apply to the commonly used dihydropyridine–ACE inhibitor combinations. Our data may suggest that patients treated with an ACE inhibitor combined with verapamil may be less prone to ventricular arrhythmias during ischemia and reperfusion. In a follow-up investigation to the DAVIT II study, Fischer Hansen and others [51] have shown that verapamil reduced cardiac event rates in post-AMI patients when added to the ACE inhibitor trandolapril and a diuretic. The combination of verapamil and trandolapril has recently been approved for clinical use in various countries.

Acknowledgments

The authors thank the South African Medical Research Council and the University of Cape Town for ongoing financial support. We also thank Knoll AG, Germany for additional support.

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