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Protective effect of labedipinedilol-A, a novel dihydropyridine-type calcium channel blocker, on myocardial apoptosis in ischemia–reperfusion injury

Jhy-Chong Liang [a], Hen-Rong Chen [b], Chaw-Chi Chiu [c], Shu-Fen Liou [b], Ing-Jun Chen [b], Jwu-Lai Yeh [b,*]

[a] Department of Early Childhood Care and Education, Chin Min Institute of Technology, Miaoli County, Taiwan
[b] Department and Graduate Institute of Pharmacology, College of Medicine, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan, Republic of China
[c] Department of Cardiovascular Surgery, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

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Abstract

The effects of labedipinedilol-A, a novel dihydropyridine-type calcium channel blocker with α-/β-adrenoceptor blocking activities, on myocardial infarct size, apoptosis and necrosis in the rat after myocardial ischemia/reperfusion (45 min/120 min) were investigated. Ten minutes prior to left coronary artery occlusion, rats were treated with vehicle or labedipinedilol-A (0.25 or 0.5 mg/kg, i.v.). In the vehicle group, myocardial ischemia–reperfusion induced creatine kinase (CK) release and caused cardiomyocyte apoptosis, as evidenced by DNA ladder formation and terminal dUTP deoxynucleotidyltransferase nick end-labeling (TUNEL) staining. Treatment with labedipinedilol-A (0.25 or 0.5 mg/kg) reduced infarct size significantly compared to vehicle group (18.75±0.65% and 8.27±0.29% vs. 41.72±0.73%, $P<0.01$). Labedipinedilol-A also reduced the CK, CK-MB, lactate dehydrogenase (LDH) and troponin T levels in blood. In addition, labedipinedilol-A (0.5 mg/kg) significantly decreased TUNEL positive cells from 19.21±0.52% to 9.73±0.81% ($P<0.01$), which is consistent with absence of DNA ladders in the labedipinedilol-A group. Moreover, labedipinedilol-A pretreatment also decreased calcium content in ischemic–reperfused myocardial tissue. In conclusion, these results demonstrate that labedipindielol-A, through reduction of calcium overload and apoptosis, exerts anti-infarct effect during myocardial ischemia–reperfusion and would be useful clinically in the prevention of acute myocardial infarction.

Keywords: Ischemia–reperfusion; Apoptosis; Infarct; Calcium antagonist

Introduction

Ischemic heart disease is one of the clinical problems causing myocardial damage, arrhythmia and stunning (Saeki et al., 2002). Reperfusion through thrombolysis or percutaneous coronary angioplasty (primary PTCA) is standard treatment in impending acute myocardial infarction (Wang et al., 2002). A number of studies have shown that calcium overload in cardiomyocytes undergoing ischemia–reperfusion is one of the major factors that ultimately results in myocardial infarction (Bagchi et al., 1997). Calcium overload during reperfusion may occur by several mechanisms. One such mechanism is related to an increase of Ca^{2+}-influx through the Ca^{2+}-channels as well as a reduced Ca^{2+}-reuptake by the sarcoplasmatic reticulum (SR): this leads to accumulation of large quantities of Ca^{2+} during reperfusion of myocardial cells, which are irreversibly injured during ischemia (Allard et al., 1994; Wang et al., 2002). An increase in myocardial calcium concentration has been proposed to be the major mediator of the structural deterioration of the myocardium leading to cardiac necrosis and is implicated in pathogenesis of contractile dysfunction and arrhythmias in failing hearts. Moreover, recent basic experimental and clinical evidence has demonstrated that cytosolic and intra-mitochondrial calcium overload markedly enhances the apoptotic process during ischemia–reperfusion and leads to cardiomyocyte death (Eefting et al., 2004). Some studies have shown that calcium overloading also increases free radical generation (Bagchi et al.,

* Corresponding author. Tel.: +886 7 3121101x2139; fax: +886 7 3234686.
E-mail address: jwulai@kmu.edu.tw (J.-L. Yeh).

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1997), a well-defined apoptosis-inducing factor (Das et al., 1999). Free radicals are known to trigger cardiomyocyte apoptosis in myocardial infarction, ischemia–reperfusion injury, cardiomyopathy, atherosclerosis and heart failure (Hannunstetter and Izumo, 1998; Kumar and Jugdutt, 2003).

It is now well established that calcium channel blockers have cardioprotective effects, such as reduction of mortality after myocardial infarction in humans and animals, reduction of infarct size after ischemia–reperfusion injury and prevention of cardiac remodeling after myocardial infarction (Wang et al., 2002). Labedipinedilol-A (Fig. 1), like nifedipine, belongs chemically to a family of dihydropyridines, but it has additional α-/β-adrenoceptor blocking activities, which are lacking in nifedipine (Fig. 1). In our previous studies, intravenous labedipinedilol-A produced dose-dependent hypotensive and bradycardia responses, significantly different from nifedipine-induced hypotensive and reflex tachycardia activities in pentobarbital-anesthetized Wistar rats. Oral administration of labedipinedilol-A in SHR and DOCA-salt hypertensive rats demonstrated a long-acting antihypertensive and slight bradycardia effect. Labedipinedilol-A inhibited the protein kinase C-ε translocation and the formation of angiotensin II, endothelin-1 and atrial natriuretic peptide in DOCA-salt hypertensive rats (Liang et al., 2000; Yeh et al., 2003). In addition, labedipinedilol-A is an inhibitor of vascular smooth muscle cell proliferation induced by a broad group of mitogens, such as serum, platelet-derived growth factor and norepinephrine, and may thus have great potential in the prevention of progressive atherosclerosis (Liou et al., 2004, 2005). Labedipinedilol-A, as a calcium antagonist and α-/β-blocker, may possess these beneficial effects and have a protective against myocardial ischemia–reperfusion injury. Therefore, the aims of the present experiment were to investigate the cardioprotective effects of labedipinedilol-A in an open-chest anesthetized rat model of ischemia–reperfusion injury in Wistar rats and the possible mechanisms involved. It is now well accepted that DNA ladder formation is highly specific for apoptotic cell death (Kobara et al., 2003; Gao et al., 2001). However, some recent reports have questioned whether some of the apoptotic cells detected by TUNEL staining after ischemia and reperfusion may in fact be necrotic (Gottlieb and Engler, 1998; Gao et al., 2001; Zhao et al., 2003). In the present study, we analyzed myocardial apoptosis in the ischemic zone from a model of 45-min ischemia/2-h reperfusion, which may have included necrotic tissue. Some recent studies showed that the TUNEL predominantly detected apoptotic cells, rather than necrotic cells (Zhao et al., 2000). Therefore, we used both TUNEL and DNA laddering to improve accuracy and reliability of our results concerning myocyte apoptosis in this study.

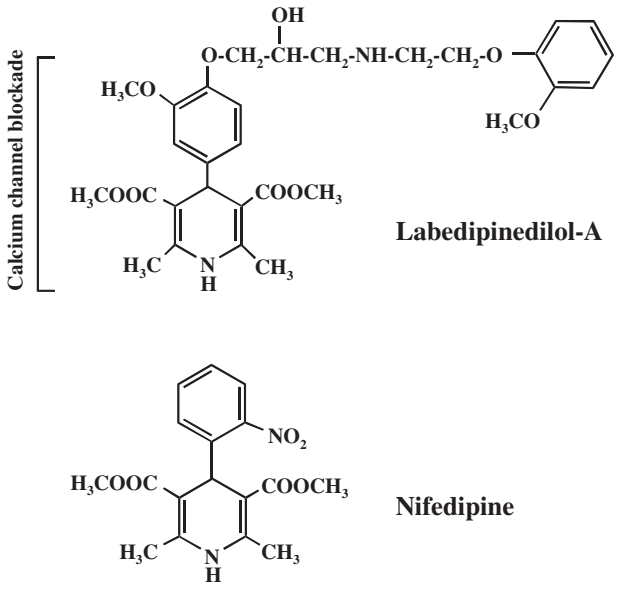

Fig. 1. Chemical structures of labedipinedilol-A and nifedipine.

Materials and methods

Animals

Wistar rats were from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). They were housed under conditions of constant temperature and controlled illumination (light on between 7:30 and 19:30). Food and water were available ad libitum. The study was approved by the Animal Care and Use Committee of the Kaohsiung Medical University.

Experimental preparation

The ischemia–reperfusion protocol was performed as previously described (Lee et al., 2001, 2002). In brief, male Wistar rats weighing 250–300 g were anesthetized with intraperitoneal pentobarbital sodium (40 mg/kg). Tracheotomy was performed and an intubating cannula was connected to a rodent ventilator. The animals were ventilated artificially with room air. Respiratory rate was synchronized with the rat's spontaneous rate (60–80 strokes/min, 10 ml/kg). Arterial blood pH and blood gases were maintained within normal physiological limits (pH: 7.35–7.45, PCO_2: 30–35 mm Hg, PO_2: 85–100 mm Hg) by adjusting the respiratory rate and tidal volume. The left femoral artery and vein were cannulated for the measurement of arterial blood pressure (ABP) and heart rate (HR) via a Statham pressure transducer and a Biotechnometer (AD Instruments, Mountain View, CA, USA), and for the administration of drugs, respectively.

After a left-side thoracotomy was performed at the fourth intercostals space, the pericardium was incised and the heart was exteriorized. A ligature (6/0 silk suture) was placed around the left main coronary artery close to its origin. The thread was then made into a knot as an occluder and another thread was tied to the first knot as a releaser. The ends of both threads were brought outside the thoracic cavity. Thus, the occlusion could be tightened or loosened by pulling the thread of the releaser. The coronary artery was occluded for 45 min, followed by 120 min of reperfusion.

Sham-operated animals underwent all the above-described surgical procedures, apart from the fact that the 6/0 silk,