

ROLE OF CALCIUM CHANNEL BLOCKERS IN MYOCARDIAL PRECONDITIONING

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ULOGA BLOKATORA KALCIJUMSKIH KANALA U PREKONDICIONIRANJU SRCA

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ABSTRACT

Coronary heart disease is the leading cause of mortality and morbidity worldwide. The effects of coronary heart disease are usually attributable to the detrimental effects of acute myocardial ischaemia-reperfusion injury. Newer strategies such as ischaemic or pharmacological preconditioning have been shown to condition the myocardium to ischaemia-reperfusion injury and thus reduce the final infarct size. This review investigates the role of calcium channel blockers in myocardial preconditioning. Additionally, special attention is given to nicorandil whose mechanism of action may be associated with the cardioprotective effects of preconditioning. There are still many uncertainties in understanding the role of these agents in preconditioning, but future research in this direction will certainly help reduce coronary heart disease.

Keywords: Preconditioning, Calcium channel blockers, Nicorandil

SAŽETAK

Koronarna bolest srca je vodeći uzrok mortaliteta i morbiditeta širom sveta. Efekti koronarne bolesti srca se najčešće pripisuju štetnim efektima akutnog infarkta, odnosno ishemijsko-reperfuzionij povredi. Novije strategije, kao što su ishemijsko i farmakološko prekondicioniranje, su pokazale da uticajem na ishemijsko-reperfuzione povrede srca konačna veličina infarkta može biti smanjena. Ovaj pregledni članak ispituje ulogu blokatora kalcijumskih kanala u prekondicioniranju srca. Takođe, posebna pažnja je posvećena nikorandilu, čiji bi mehanizam dejstva mogao biti povezan sa kardioprotektivnim efektima prekondicioniranja. Još uvek postoji mnogo nejasnoća u razumevanju uloge ovih lekova u prekondicioniranju ali buduća istraživanja u ovom pravcu bi svakako doprinela smanjenju koronarne bolesti srca.

Ključne reči: Prekondicioniranje, Blokatori kalcijumskih kanala, Nikorandil



ABBREVIATIONS

ATP - Adenosine triphosphate
CCBs - Calcium channel blockers
CHD - Coronary heart disease
I/R - Ischaemia-reperfusion

IPC - Ischaemic preconditioning
K(ATP) - ATP-sensitive K⁺
PKC - protein kinase C
ROS - reactive oxygen species

INTRODUCTION

Coronary heart disease (CHD) is the leading cause of mortality and morbidity worldwide. According to the World Health Organization, 12.8% of all deaths result from CHD. The effects of CHD are usually attributable to the detrimental effects of acute myocardial ischaemia-reperfusion (I/R) injury (1, 2). This injury includes different clinical manifestations such as myocardial necrosis, arrhythmia,

myocardial stunning and endothelial and microvascular dysfunction. Depending on the severity of the condition, the patient may be treated with medications, surgery or both. The treatment includes the use of thrombolytic agents, beta-antagonists, angiotensin-converting enzyme inhibitors, calcium channel blockers (CCBs), coronary artery bypass surgery, angioplasty and stenting (3-5). Despite



several therapeutic advances, both medical and surgical, there is still no effective therapy for preventing myocardial I/R injury.

In the past few decades, it has become clear that the myocardial response to I/R injury can be reduced. Based on recent studies, a universally accepted potential endogenous strategy for protecting the heart against acute ischaemia-reperfusion injury is preconditioning. Research into the mechanisms of preconditioning has revealed multiple receptors, pathways, and end effectors. Recent studies suggest that certain pharmacological agents may stimulate these mechanisms. Our understanding of the complex mechanism of preconditioning is still incomplete, and its disclosure could contribute to the treatment of acute myocardial infarction.

PHENOMENON OF MYOCARDIAL PRECONDITIONING

Myocardial preconditioning is a process where myocardium cells or tissues are exposed to a sublethal stimulus to protect them from a subsequent normally lethal stress. Preconditioning can attenuate the subsequent prolonged or lethal tissue injury by increasing the cell tolerance to the stress (6). The term “preconditioning” was used for the first time in 1964 by Janoff to refer to the phenomenon of stress-induced endogenous tolerance against traumatic or endotoxic insults (7). The protective effect of preconditioning in the heart can be demonstrated by the reduction in infarct size and myocardial stunning, prevention of arrhythmias, or the acceleration of the recovery of myocardial function after ischaemia (8-10). Myocardium can be preconditioned by two basic techniques including ischaemic and pharmacological preconditioning.

Ischaemic preconditioning (IPC)

Ischaemic preconditioning was described for the first time in 1986 by Murry and colleagues when they found that a “preconditioned” heart in a canine model became resistant to ischaemia-induced infarction. In fact, infarct size and the degree of reperfusion injury were reduced by single or multiple cycles of ischaemia and reperfusion (11). The same beneficial effect has since been confirmed in every species studied, independently of both the presence of collaterals in the coronary circulation and the size of the animal model (12-14), and has more recently been confirmed in the human (15).

The underlying mechanisms of IPC are still a matter of debate. Protective effects of IPC on the heart can be a consequence of the reduction in reactive oxygen species (ROS) generation, delay in adenosine triphosphate (ATP) depletion, and the reduction of the infarct size, apoptosis and neutrophil accumulation. It is possible that adenosine, noradrenaline and bradykinin play a role in this mechanism (16-18). In addition,, activation of protein

kinase C (PKC), which is known to be a key player in numerous intracellular signal transduction pathways, is believed to be one of the main causative mechanisms in the protection of IPC across various species (19). Nevertheless, for many of the proposed mechanisms, a modulation of intracellular calcium ion ($[Ca^{2+}]_i$) homeostasis may be the final common pathway in the protection against ischaemic injury. A rise in intracellular free $[Ca^{2+}]_i$ has been postulated to be an important factor in ischaemic myocardial injury (20, 21).

Notably, there are many candidates for the mechanism of IPC. Recently it has been reported that the activation of PKC opens the ATP-sensitive K^+ ($K(ATP)$) channels which is currently thought to be the end-effector of many signal transduction systems related to the IPC mechanism. Therefore, it is thought that $K(ATP)$ channel openers are also effective in protecting against human ischaemic injury (22).

Myocardial ischaemia occurs when blood flow to the heart muscle (myocardium) is obstructed by a partial or complete blockage of a coronary artery. Coronary arteries can be occluded by thrombi, atherosclerotic plaques, vasoconstriction or inflammation (23). During myocardial ischaemia, the absence of oxygen and metabolic substrates in cardiomyocytes can cause functional, structural and metabolic diseases (24). As a result, the cells switch to anaerobic metabolism, which leads to the accumulation of lactate and a drop in intracellular pH. The main result is intracellular Na^+ overloading. Therefore, diminished intracellular concentrations of ATP and creatine phosphate cause decreased activity of adenosine triphosphate-reliant ion pumps, including the Na^+/K^+ ATP-ase pump, and the exacerbation of contractile function. This induces the Na^+-H^+ exchanger to extrude H^+ and results in intracellular Na^+ overload, which activates the $2Na^+-Ca^{2+}$ exchanger to function in reverse to extrude Na^+ and leads to intracellular $[Ca^{2+}]_i$ overload. These processes and the generation of ROS can lead to cell death induced by ischaemic episodes (20, 25-27).

Furthermore, with reperfusion, the restoration of blood flow after an ischaemic episode may result in paradoxical cardiomyocyte dysfunction caused by ROS, intracellular and mitochondrial Ca^{2+} overload and accumulation of inflammatory cells. The phenomenon called “reperfusion injury” occurs when prompt changes in intracellular ions and normalization of pH cause cell death and greater damage than what is induced by pre-reperfusion ischaemia (26, 28).

Pharmacological preconditioning

As we mentioned above, in addition to IPC, preconditioning can be induced in the heart with pharmacological agents. However, it is still unclear whether the various forms of pharmacological preconditioning have the same molecular mechanisms as ischaemic preconditioning. In recent years, many investigators have studied myocardial



pharmacological preconditioning with various agents (29–32), but particular attention has been directed towards pharmacological agents that modulate Ca^{2+} (33, 34).

Ca^{2+} channel blockers are well known to be cardioprotective when taken after I/R injury (35, 36), but little is known about a possible preconditioning effect before ischaemia. Controversy remains about their ability to reduce infarct size or at least delay the necrotic process. With this in mind, the aim of this review was to examine the possible role of calcium channel blockers as a mediator of ischaemic preconditioning.

CALCIUM CHANNEL BLOCKING AGENTS

Calcium antagonists or calcium channel blockers (CCBs) were introduced into clinical medicine in the 1960s and were approved for the treatment of hypertension in the 1980s (37, 38). CCBs have been one of the mainstays in therapy for cardiovascular diseases, such as angina pectoris, paroxysmal supraventricular tachyarrhythmia, hypertrophic cardiomyopathy, Raynaud phenomenon, pulmonary hypertension, diffuse oesophageal spasm, and cerebral vasospasm, for many years (39). CCBs as a group are heterogeneous and, based on the chemical structure and functional distinctions, include 3 main classes: dihydropyridine, phenylalkylamine and benzothiazepine derivative (40, 41). The differences in chemical structures provide heterogeneity in the action of these agents. However, all CCBs inhibit calcium influx by binding to the α_1 subunit of calcium channels and inhibit cell excitability (42). Inhibition leads to the relaxation of vascular smooth muscle cells, vasodilation and a lowering of blood pressure. In cardiac muscle, contractility is reduced, and the sinus pacemaker and atrioventricular conduction velocities are slowed (43, 44). CCBs also reduce angiotensin II-mediated vasoconstriction and decrease the angiotensin II-stimulatory effect on adrenal biosynthesis and secretion of aldosterone (45). Unlike other vasodilators, calcium antagonists induce mild natriuresis and do not cause volume retention. Thus, calcium antagonists lower blood pressure mainly by reducing peripheral vascular resistance (46).

Furthermore, in many clinical trials, there is controversial data on taking CCBs and an increase in cardiovascular mortality (47, 48). Although several theories have been offered, the mechanism by which CCBs increase cardiovascular mortality is still unknown.

However, the hypothesis that cellular calcium overload may contribute to the onset of irreversible ischaemic cell injury suggested a possible role for CCBs in the protection of ischaemic myocardium (49).

Dihydropyridine CCBs in myocardial preconditioning

Dihydropyridine CCBs include amlodipine, felodipine, isradipine, lacidipine, lercanidipine, nicardipine, nifedipine, nisoldipine, and others (40). Experimental

data suggest that this group of CCBs binds to both dihydropyridine and nondihydropyridine binding sites. Dihydropyridines selectively inhibit calcium ion influx across cell membranes, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells (50). Dihydropyridine CCBs have variable effects on heart rate. Acutely, these drugs tend to induce a reflex tachycardia, but long-term studies have shown similar heart rates before and during therapy (51).

Higher doses of these drugs are generally associated with an increase in heart rate. A group of authors even suggested that the use of short-acting nifedipine in moderate to high doses causes an increase in total mortality. In patients with poor or no collateral flow, nifedipine reduced ischaemic episodes, while in patients with good collateral flow nifedipine significantly increased them (52). Nicardipine and isradipine show the same effects (53).

In vitro experiments on human atrial trabeculae indicate that patients using CCBs (amlodipine $n=7$, diltiazem $n=4$, verapamil $n=1$) were not functionally protected by transient ischaemia. However, a difference in functional performance after I/R between atrial trabeculae with and without CCB exposure was not detected (54).

Likewise, not all studies have demonstrated increased cardiovascular mortality with CCBs. Wallbridge and colleagues studied whether pretreatment with nisoldipine could modify cardioprotective effects of IPC in pigs. Their results indicate that continuous infusion of nisoldipine throughout the entire protocol until onset of reperfusion does not attenuate the potential protective mechanism of IPC; in fact, it may have even exerted a small additional cardioprotective effect (55). These findings are in accordance with an experimental study in guinea pig isolated hearts by Camara and colleagues. They suggest that nifedipine given before ischaemia induces a preconditioning effect as shown by improved left ventricular pressure and lower $[\text{Ca}^{2+}]$ on reperfusion after ischaemia (56).

Phenylalkylamine CCBs in myocardial preconditioning

Phenylalkylamine CCBs are relatively selective for myocardium and are often used to treat angina; they also reduce myocardial oxygen demand, reverse coronary vasospasm. Within this group are verapamil, gallopamil, and others (41), which inhibit the alpha and beta subunits of voltage-dependent calcium channels. Specifically, their effect on L-type calcium channels in the heart causes a reduction in inotropy and chronotropy, reducing heart rate and blood pressure. The most commonly used drug from this group is verapamil (57).

Miyawaki and colleagues have indicated that pretreatment with verapamil alone ($0.63 \mu\text{mol/L}$; 15 minutes before I/R) did not exert a significant effect on ischaemic injury in the Langendorff-perfused rat model compared with no pretreatment in ischaemic control hearts. On the other hand, verapamil administered during IPC, at the same dose, significantly attenuated the salutary effects of IPC



(33). In addition, another study by the same authors demonstrated that ATP contents were significantly higher and cell structure was better preserved in Ca^{2+} preconditioned hearts than in ischaemic control hearts. In other groups, Ca^{2+} influx during Ca^{2+} preconditioning was inhibited with low doses of verapamil (0.2 and 0.5 $\mu\text{mol/L}$), but verapamil did not influence the cardioprotection of Ca^{2+} preconditioning. On the contrary, in hearts treated with 2 $\mu\text{mol/L}$ verapamil, lactate dehydrogenase release was significantly increased, ATP content was reduced, mitochondria were swollen and partly disrupted, glycogen was depleted, and myofibrils were partly transformed into contraction bands (58).

The effects of verapamil preconditioning are controversial. Yu and colleagues found that verapamil preconditioning (20 $\mu\text{mol/L}$; 10 min) significantly improved diastolic and systolic functions and reduced the incidence of arrhythmias. One of the possible mechanisms for this effect is a reduction in the influx of $[\text{Ca}^{2+}]$, thereby stabilizing cardiomyocytes in myocardial stunning and avoiding the occurrence of Ca^{2+} -induced $[\text{Ca}^{2+}]$ release during I/R injury (59).

Benzothiazepine CCBs in myocardial preconditioning

This class of drugs is an intermediate class between phenylalkylamine and dihydropyridines in their selectivity for vascular calcium channels. By having both cardiac depressant and vasodilator actions, benzothiazepines are able to reduce arterial pressure without producing the same degree of reflex cardiac stimulation caused by dihydropyridines (41). The main representative of this group is diltiazem. Diltiazem is effective in the treatment of angina, and the longer-acting formulation is effective in the treatment of hypertension. It is less negatively inotropic than verapamil but should still be used cautiously with beta-blockers (60).

Okuda and colleagues have suggested that diltiazem (10 mg/kg) preconditioning leads to a reduction in the infarct area in the coronary artery of an adult mongrel dog (61). De Jong and colleagues studied the effects of diltiazem administered before or during myocardial ischaemia in the Langendorff-perfused rat heart. They observed that diltiazem decreases adenine nucleotide catabolism and presumably does not protect by negative inotropy during myocardial ischaemia. Myocardial function measured by the capacity to develop tension was decreased by diltiazem, and pretreated hearts did not show arrhythmias. Diltiazem also reduced myocardial oxygen demand, thereby diminishing the effect of flow impairment (62).

Nicorandil in myocardial preconditioning

As already mentioned, besides CCBs, K(ATP) channel openers are also effective in protecting against human ischaemic injury; therefore, we pay special attention to nicorandil.

Nicorandil is an antianginal drug whose properties lie between those of nitrates and K^+ channel openers. Activation of K(ATP) channels causes K^+ efflux, hyperpolarization of the smooth muscle cell membrane, and closure of voltage-gated Ca^{2+} channels. Closure of Ca^{2+} channels reduces intracellular levels of Ca^{2+} , resulting in relaxation of vascular smooth muscle and dilation of systemic and coronary arterioles. The nitrate moiety produces relaxation of vascular smooth muscle with dilation of systemic venous circulation and epicardial coronary arteries (63). This drug has been shown to be effective after oral administration in patients with stable angina and acute myocardial infarction (64, 65). Nicorandil allows for simultaneous dilation and relaxation of arterial and venous vasculature via its effect on smooth muscles (64).

Ohno and colleagues have shown that nicorandil preconditioning reduced the size of myocardial infarcts by opening the K(ATP) channels, and this effect was dependent on the plasma nicorandil concentrations immediately before the ischaemia induced in rabbits (66). This study corroborates the findings of Matsubara and co-authors where the preconditioning mechanism of nicorandil is explained by opening K(ATP) channels (67). Nicorandil reduces myocardial infarct size in various animal models. A chronic experiment on rabbits has shown that nicorandil (100 $\mu\text{g/kg}$ bolus + 30 $\mu\text{g/kg}$ –1 min–1 iv for 60 min) induces delayed cardioprotection against myocardial infarction (68). Similar findings were obtained in the study where rats were administered nicorandil (in oral dose; 3 or 6 mg/kg; 5 consecutive days). Rats were then subjected to myocardial I/R (40 min/10 min). Nicorandil was effective in attenuating the ischaemia/reperfusion-induced ventricular arrhythmias, creatine kinase-MB release, lactate accumulation and oxidative stress (69). Another possible mechanism of preconditioning is the upregulation in the expression of COX-2 and Bcl-2 as it occurs after IPC and nicorandil preconditioning (68, 70).

Several clinical studies have shown that nicorandil improves functional and clinical outcomes in patients with acute myocardial infarction (71, 72). Intravenous preadministration of nicorandil attenuates ST-segment elevation and improves lactate metabolism during coronary angioplasty, suggesting that pharmacological preconditioning is induced by nicorandil. Also a level of troponin T, one of the reliable metabolic markers of myocardial injury, is suppressed after coronary angioplasty as well as ST-segment elevation during coronary angioplasty (72). In the I-WIND trial, Kitakaze and colleagues randomized patients with an anterior ST-segment elevation myocardial infarction to receive intravenous nicorandil as a bolus or placebo after primary percutaneous coronary intervention. The overall morbidity and mortality were the same in both groups after 3 years. However, 61 patients continued on oral nicorandil after discharge, and in this group, the LV ejection fraction was better at the 6-month follow-up (73). Larger trials are needed, however, to examine the cardioprotective action of nicorandil.



Although many investigators have studied the role of calcium channel blockers in myocardial preconditioning, there are many open questions that future research should seek to answer. Nevertheless, the use of calcium channel blockers to mimic preconditioning in selected clinical settings may be a desirable future therapeutic goal. Based on current knowledge, we can say that nicorandil preconditioning is certainly worth investigation. More importantly, future studies should reveal simpler and even more effective therapeutic interventions for protecting the heart from ischaemia/reperfusion.

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