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Original Article

Ivabradine Enhances its Positive Impact on the Heart Muscle/ (Myocardial Infarction) by Modulating Myocardial Nitric Oxide and ATP-dependent K^+ Channels but not by Adenosine and Bradykinin Levels

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ABSTRACT

Background: This study aimed to explore the cardioprotective effects of ivabradine and examine potential secondary mechanisms beyond the established role of If-HCN channel inhibition demonstrated in cellular studies. **Objectives:** Our hypothesis centered on the idea that Ivabradine could induce responses similar to ischemic preconditioning involving nitric oxide, adenosine, bradykinin, and ATP-dependent potassium channels. **Materials and Methods:** Ischemia-reperfusion injury was established using Langendroff's technique. 20 min ischemia and 40 min reperfusion to coronary artery to isolated heart was model of myocardial infarction. There were following groups: Control (Ischeamia-Reperfusion), Ischemic preconditioning, ivabradine (10 µmol/L), ivabradine+L-NAME (30 µmol/L) and ivabradine+Aminoguanidine (30 µmol/L), ivabradine+Theophylline (50 µmol/L), ivabradine+Aminophylline (50 µmol/L), ivabradine+Enalapiril (100 µmol/L), ivabradine+Losartan (50 µmol/L), ivabradine+Gliclazide (30 µmol/L), ivabradine+glimepiride (50 µmol/L) in perfusat**.** **Results:** Ivabradine has demonstrated the potential for providing cardiovascular defence. (Infarct Size: 5.334±0.422%; LDH: 101.500±1.147 I.U.; CK-MB: 100.167±1.302 I.U.) Ischemic preconditioning presents significant potential for enhancing cardioprotective effects. Infarct Size: 5.1667±0.478% LDH: 101.667±2.789 I.U.; CK-MB: 97.167±1.721 I.U.). Ivabradine+L-NAME (Infarct size: 64.167±0.872%; LDH: 154.667±1.256 I.U.; CK-MB: 200.167±1.537 I.U.). Ivabradine+Aminoguanidine (Infarct Size: 64.500±0.885%; LDH: 154.833±1.138 I.U.; CK-MB: 198.333±1.145 I.U.). Ivabradine+Theophylline (Infarct Size: 5.333±0.422%; LDH: 102.5±2.923 I.U.; CK-MB: 97.167±1.721 I.U.). Ivabradine+Aminophylline (Infarct Size: 5.667±0.333%; LDH: 102.833±1.515 I.U.; CK-MB: 100.833±1.250 I.U.). Ivabradine+Enalapril (Infarct Size: 5.500±0.428%; LDH: 102.667±1.498 I.U.; CK-MB: 100.667±1.429 I.U.). Ivabradine+Losartan (Infarct Size: 5.333±0.667%; LDH: 102.167±1.751 I.U.; CK-MB: 101.833±1.400 I.U.). Ivabradine+Gliclazide (Infarct Size: 63.833±1.352%; LDH: 154.667±1.054 I.U.; CK-MB: 201.833±1.815 I.U.). Ivabradine+Glimepiride (Infarct Size: 63.667±0.989%; LDH: 155.833±1.352 I.U.; CK-MB: 199.833±1.579 I.U.). **Conclusion:** Ivabradine and ischemic preconditioning exhibited cardioprotective effects by reducing infarct size, along with Lactate Dehydrogenase isoenzyme (LDH) and Creatine Kinase isoenzyme (CK-MB) levels. In contrast, treatments with L-NAME, Aminoguanidine, Gliclazide, and Glimepiride led to increased infarct size, LDH, and CK-MB levels. Meanwhile, Theophylline, Aminophylline, Enalapril, and Losartan contributed to a reduction in infarct size, LDH, and CK-MB levels. These findings suggest that ivabradine-induced cardioprotection does not involve nitric oxide, adenosine, or bradykinin pathways.

Keywords: Cardioprotection, Nitric oxide, KATP channels, Myocardial infarction, Ivabradine, animal models.

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INTRODUCTION

Cardiovascular diseases claim the lives of approximately 17.5 million individuals annually, taking into consideration around a third of each fatality worldwide (WHO, 2016). The non-invasive techniques for diagnosis to invasive procedures like heart replacement and Coronary Artery Bypass Graft surgery (CABG), cardiac discipline has advanced significantly over the nineteenth century. Cardiovascular illnesses persist to be the leading ground of ill health and fatality globally, irrespective of these developments, imposing a substantial financial burden on healthcare systems. The optimal treatment for Acute Coronary Syndrome (ACS) is myocardial reperfusion. Yet, Ischemia/ Reperfusion Injury (IRI), a situation where wounded tissue from preceding ischemia is exacerbated, can occur when blood flow is abruptly restored. One explanation for this phenomenon is the

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imbalance between vasodilator and vasoconstrictor molecules during reperfusion.[1]

Research into novel cardiac protection strategies has been driven by the need to treat and prevent cardiovascular injury. Currently, major focus areas include protecting against ventricular dysfunction, which can lead to heart failure, and preventing lasting damage to the heart muscle. In general, "cardio protection" refers to methods and practices that aim to reduce or even reverse myocardial degeneration, helping to maintain heart health.[2] Unfortunately, many findings from animal studies have not been successfully replicated in human settings, likely due to differences in risk factors and comorbidities across species.[3,4]

Over the past few decades, extensive research has unveiled that cardiac cells possess various defence mechanisms aimed at mitigating the damage driven by perfusion and ischemia. These mechanisms combat both the extended action of cardiac arrest and the immediate aftermath of ACS, which extends beyond the myocardium to areas proximal and distal to the heart. Examples of such protection include myocardial cells' protection measures before and after conditioning in response to ischemia/reperfusion incidents. Despite their significance, the precise cellular mechanisms governing these phenomena remain elusive, although they are likely intricate and may involve factors originating from extracardiac sources.[5]

Ivabradine is used as an add-on treatment for symptomatic patients when primary antianginal therapies do not sufficiently control angina symptoms. Many cardiovascular diseases have demonstrated the cardioprotective effects of Ivabradine (Iva), which serves as an effective heart rhythm-lowering drug by particularly inhibiting the pacemaker I(f) ionic current. Additionally, autophagy is a vital metabolic process that has been preserved throughout evolution, playing a crucial role in maintaining cardiac stability and health. This study seeks to explore the function of autophagy in the cardiovascular benefits of Iva in rodent models of Myocardial Infarction (MI). Study find that Iva therapy (po, 10 mg/kg/day) improved the blood flow and hemodynamics in rats that had had a MI. The analysis incorporates important metrics of left ventricular function, specifically the left atrial systole force, left atrial end-diastole force, and the maximum proportion of change in left atrial force. These measures are essential for understanding cardiac performance and optimizing clinical assessments. It also shown that Iva treatment greatly decreased the size of the infarct, halted myocardial cell death, and in rats with a MI, the cytokines that promote inflammation lost their potency. The Iva therapy improved autophagy and impaired the PI3K/AKT/mTOR/p70S6K channel in MI rodents. Concurrently, finding suggestive of both Iva and the autophagy enhancer rapamycin (administered intraperitoneally at 10 mg/kg/day) exhibited identical cardioprotective outcomes. Iva's therapeutic benefits were eliminated when the autophagy inhibitor 3-methyladenine (10 mg/kg/day) was added intraperitoneally, showing that autophagy promotion reduced post-MI cardiac damage. These findings showed that Iva reduced MI in rats by increasing autophagy.[6]

In recent decades, significant advancements have been made in pharmacological and interventional strategies for managing myocardial injury.[7,8] Among these innovations, Iva stands out as a target inhibitor of particular ion channels associated with the current that is activated by hyperpolarization called I(f), capturing interest.[9] Iva is the initial therapeutically utilized antagonist of hyperpolarization-activated cyclic nucleotide-gated transmit 4, effectively addressing long standing stable angina and persistent heart failure.[10] Research indicates that Iva effectively lowers pulse rate by declining the angle of diastolic depolarization in pacemaker nerve impulse, all while preserving cardiac inotropy and total peripheral resistance.[11,12] Growing evidence supports ivabradine's effectiveness and safety in managing various cardiac conditions, including problematic sinus tachyarrhythmia and ventricular arrhythmias.[13] Still, Iva guidelines for acute cardiac arrest are possible. Early administration of Iva, together with traditional therapy like β-blockers, may minimize left ventricular remodeling in individuals with acute ST-segment cardiac arrest, according to preclinical and clinical trials.[14] Apart from, Iva has shown promise in promoting maturation and mitigating coronary gigantism in mouse models of MI.[15] Despite this, further investigation into the effect of Iva in the MI is essential for full recognition of its potential.

Clinical evidence strongly indicates that both ivabradine and ischemic preconditioning confer cardioprotection. Our research aims to investigate whether the cardioprotective effects induced by ivabradine mirror those elicited by ischemic preconditioning. If our hypothesis is validated, ivabradine could potentially serve as a pharmacological alternative to the application of ischemic preconditioning as a surgical technique that can significantly enhance interventional procedures, such as CABG and heart transplantation.

Animals

Wistar Albino Rats were provided by the Animal House at NIMS University Rajasthan, Jaipur. The selected rats weighing between 125 and 150 grams, regardless of their gender, for this study. Ad libitum a link to tap water and an established laboratory rat diet were given to ensure the subjects' well-being throughout the study. The Institutional Animal Ethics Committee (IAEC, 2022) of NIMS University Rajasthan authorized the research procedure under registration number 1203/PO/Re/S/09/CPCSEA. This approval follows CPCSEA norms, confirming our commitment to conducting research ethics.

MATERIALS AND METHODS

Drugs and Chemicals

The pharmaceuticals and substances utilized in the experiment are listed below in Table 1.

The biomarkers CK-MB and LDH

Cardiac-associated CK-MB and LDH are elevated in serum following myocardial infarction. Surgical manipulation of the atrium postoperatively can also elevate CK-MB levels due to similar CK-MB content in the atrial and ventricular myocardium. LDH isoenzyme distribution differs between atrial and ventricular myocardium.[17,18] Elevated serum enzyme levels due to myocardial necrosis are indicative of myocardial infarction. In this research, animals without myocardial infarction symptoms who were diagnosed based on clinical, hemodynamic, and electrocardiographic results were evaluated for fluctuations in the level of isoenzyme concentrations following bypass graft installation and atriotomy for surgical repair. There were high CK-MB and LDH values that suggested perioperative MI.[16,17]

Infarct Size Measurement

Using the volume approach, the infarct size was determined.[18,19]

Procedures for Surgery

Apparatus

Langendorff's apparatus (INCO, Ambala, India); ECG (BPL CARDIAART 108TDIGI, New Delhi, India).

Animal *ex vivo* pharmacological and physiological research use the Langendorff heart test. Heart rate and cardiac muscle force can be measured using this method without the complexities associated with investigating whole organisms. The heart is excised from the animal in the Langendorff apparatus by severing the blood vessels. A nutrient-rich, oxygenated solution, such as Krebs-Henseleit solution, is then used to retrogradely perfuse he heart through the aorta.[18,19]

Procedure

After opening the chest, rat was anaesthetized with ketamine (100 mg/kg) and installed a limb lead II ECG. Heparin was administered (500 IU, i.p) approximately 15 min before the animal's sacrifice. After pericardium removal, the excised heart was attached on the apparatus. The retrograde circulation of the aorta was initiated using Krebs-Henseleit buffering solution while maintaining a steady 70 mmHg pressure and 95% O_2. The heart was kept up at 37°C by circulating hot water and encased in a double-walled coat. After 20 min of ischemia induction, resuscitation took place for 40 min. Silver sensors were affixed

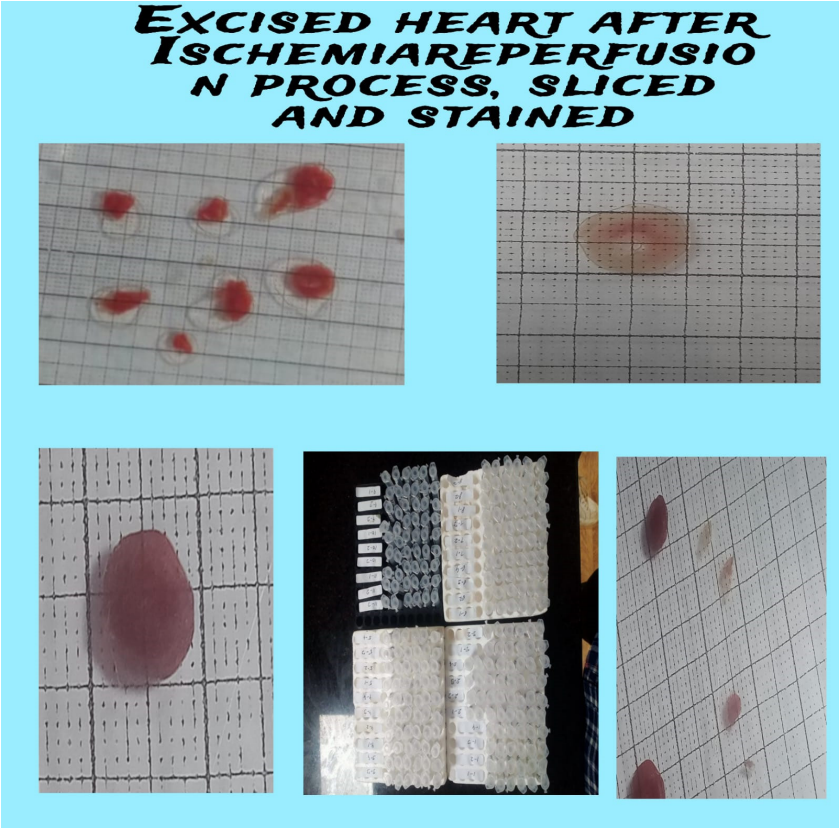

Figure 1: Heart muscle was carefully excised after the ischemia-reperfusion process, sliced, and stained using Evan's Blue and TTC.

to particular spots on the heart at different intervals during the study to carry out Electrocardiography (ECG). Following 20 min of ligation of the Left Anterior Descending coronary artery (LAD), reperfusion was executed to induce ischemia. Following reperfusion, exudate of myocardium was collected to quantify CK-MB and LDH levels. The injured heart muscle was carefully excised after the ischemia-reperfusion process, sliced, and stained using Evan's Blue and TTC (Triphenyl Tetrazolium Chloride) as shown in Figure 1. We employed both weight and volume methods to accurately assess the infarct size. To further investigate the mechanisms of action of the medications, we utilized pre-treatment in the context of ischemia-reperfusion injury.[18,19]

Procedure for the experiment

This study used 66 Wistar Albino Rats, divided into 6 sets of 6 animals each (n=6) as shown in Table 2.

Group I: Ischemia-reperfusion group for control

Firstly, ECG was implanted, and the rat was anaesthetized with 100 mg/kg of ketamine. Fifteen minutes before excision, heparin was administered. An incision was made to expose the heart by removing the pericardium. The heart was then mounted on Langendorff device. The LAD was occluded with a 4/0 silk suture in a shoelace knot form for 20 min to induce ischemia. Subsequently, reperfusion was carried out for 40 min after the 20-min ischemic period.

Group II: Group for ischemic preconditioning

In this experimental group, before the ischemia-reperfusion procedure, the heart was subjected to an ischemic preconditioning regimen.

Group III: Pre-treatment of Ivabradine drug

In this particular group, preceding the ischemia-reperfusion process, Ivabradine (at a concentration of 10 µmol/L).[20,21] was pre-incorporated into the Physiological Salt Solution (PSS).

Group IV: Ivabradine and L-NAME

In this group, before ischemia-reperfusion, Ivabradine (10 µmol/L) and L-NAME drug (30 µmol/L) were added in PSS earlier.

Group V: Iva+AG

In this experimental group, before the induction of ischemia-reperfusion, both Ivabradine (10 µmol/L) and Aminoguanidine (30 µmol/L) were included in the PSS in advance.

Group VI: Iva+Theo

In this particular group, preceding the ischemia-reperfusion phase, Ivabradine (10 µmol/L) and Theophylline drug (50 µmol/L) were both introduced into the PSS.

Group VII: Iva+Amino

In this specific group, before the ischemia-reperfusion procedure, Ivabradine (10 µmol/L) and Aminophylline (50 µmol/L) were included in the PSS.

Group VIII: Iva+Enal

In this group, before ischemia-reperfusion, Ivabradine (10 µmol/L) and A dose of enalapril (100 µmol/L) were previously added to the PSS.

Group IX: Iva+Los

In this group, before ischemia-reperfusion, Ivabradine (10 µmol/L) and Losartan (50 µmol/L) drugs were added to the PSS.

Group X: Iva+5-HD

In this group, before ischemia-reperfusion, Ivabradine (10 µmol/L) and 5-Hydroxydecanoate drug (30 µmol/L) were added in the PSS.

Group XI: Iva+Glim

In this group, before ischemia-reperfusion, Ivabradine (10 µmol/L) and Glimepiride (50 µmol/L) drug were added to the PSS.

Technique for staining

After being removed from the apparatus, the heart was refrigerated for a day. Following this, 1 mm slices of the frozen heart were prepared. Following the staining of these cardiac sections with Evan's Blue and TTC, they were kept in a BOD incubator

Figure 2: Determination of CK-MB and LDH (VITREOUS 5600).

for 10 min. Subsequent to incubation, the slices were extracted, and the dimensions of the infarct were evaluated, followed by an extensive water rinse.[22,23]

Determination of LDH and CK-MB

LDH-Assay

The aid of the integrated VITROUS system 5600 instruments, LDH was quantified in the coronary fluid. LDH catalyzes the transformation of lactate and Nicotinamide Adenine Dinucleotide (NAD) into pyruvate and Nicotinamide Adenine Dinucleotide (NADH), which is the fundamental idea. In an alkaline environment, the resultant pyruvate combines with 2,4-Dinitrophenylhydrazine to generate the equivalent hydrazone, which gives the mixture a brown hue. This color's intensity is measured spectrophotometrically at a wavelength of 440 nm and is directly proportional to the LDH activity.

Creatine Phosphokinase (CPK) Assay

Using the VITROUS system shown in Figure 2, measurement of CPK in the coronary fluid was done. CPK is essential in catalyzing the conversion of ADP and creatine phosphate into ATP and creatine, particularly at a physiological pH of 7.4, where it promotes the forward reaction effectively. In an alkaline environment, the resultant creatine reacts with diacetyl and naphthol, resulting in a pink hue that can be quantitatively assessed spectrophotometrically at a wavelength of 520 nm. The intensity of this colour directly reflects the enzyme's activity. To halt the reaction and inactivate the enzyme, we introduce p-chloromercuric benzoate after adding activators such as Mg^{2+} and cysteine.[19,20]

Statistical Analysis

The infarct size and enzymatic data are shown as the mean±standard error of the mean (SEM). The statistical assessment was done using One-way ANOVA. Dunnett's *post hoc* test was utilized for comparing results with the control group, whilst Student's t-test was performed for comparisons in different groups. All statistical evaluations were performed utilizing SigmaPlot v15 software.

RESULTS

Effect of the IPC group and the control group (ischemia-reperfusion) on MI, LDH and CK-MB (Figures 1, 2 and 3); (Tables 3, 4 and 5)

The size of the myocardial lesion was considerably low in the IPC group versus the control (ischemia-reperfusion) group. The control group's myocardial infarction was measured at 65.667±0.558%., whereas in the ischemic preconditioning group, it was significantly lower at 5.1667±0.478% ($p<0.05$). Control group LDH was measured at 155.500±0.958 I.U., while in the ischemic preconditioning group, LDH levels were notably lower at 101.667±2.789 I.U. ($p<0.05$). Similarly, CK-MB in the control group were 198.500±1.803 I.U., whereas in the ischemic preconditioning group, they were significantly reduced to 97.167±1.721 I.U. ($p<0.05$).

Impact of Control and Iva treatment group on MI, LDH and CK-MB

Myocardial infarcts were substantially less in the Iva group than in the control group. Specifically, the control group's MI size was measured at 65.667±0.558%. Whereas in the Iva treatment group, it was notably lower at 5.334±0.422% ($p<0.05$). Control group LDH was measured at 155.500±0.958 I.U., whereas in the Ivabradine treatment group, LDH levels were significantly decreased to 101.500±1.147 I.U. ($p<0.05$). Similarly, level of CK-MB in the control group were 198.500±1.803 I.U., whereas, in the Ivabradine treatment group, they were notably reduced to 100.167±1.302 I.U. ($p<0.05$).

Effect of Ivabradine and Ivabradine+L-NAME on MI, LDH and CK-MB

Myocardial infarct size increases when Ivabradine is used with L-NAME, as opposed to when Ivabradine is used alone. In the Ivabradine+L-NAME group, the myocardial infarction size was 64.167±0.872%, but in the Ivabradine therapy group, it was substantially less at 5.334±0.422% ($p<0.05$). In contrast to the Ivabradine treatment group, which had significantly reduced LDH levels at 101.500±1.147 I.U. ($p<0.05$), the Ivabradine+L-NAME group had LDH levels of 154.667±1.256 I.U. Similarly, CK-MB levels were significantly lower at 100.167±1.302 I.U. ($p<0.05$) in

Table 2: Number of Animals in Various Groups.

Sl. No.	Group	No. of animals
1	Control (Ischaemic-reperfusion)	6
2	Ischaemic preconditioning (IPC)	6
3	Ivabradine	6
4	Iva+L-NAME	6
5	Iva+AG	6
6	Iva+Theo	6
7	Iva+Amino	6
8	Iva+Enal	6
9	Iva+Los	6
10	Iva+5-HD	6
11	Iva+Glim	6

Ivabradine and L-NAME, Iva+AG-Ivabradine and Aminoguanidine, Iva+Theo-Ivabradine and Theophylline, Iva+Amino-Ivabradine and Aminophylline, Iva+Enal-Ivabradine and Enalapril, Iva+Los-Ivabradine and Losartan, Iva+5-HD- Ivabradine and 5-hydroxydecanoate, Iva+Glim-Ivabradine and Glimepiride

the Ivabradine treatment group than in the Ivabradine+L-NAME group, which had higher levels at 200.167±1.537 I.U.

Effect of Ivabradine and Ivabradine+Aminoguanidine on MI, LDH and CK-MB

The combination of Ivabradine and Aminoguanidine, a nitric oxide synthase inhibitor, appears to lead to an increase in myocardial infarct size compared to the use of Ivabradine alone. Specifically, the myocardial infarction size in the Ivabradine+Aminoguanidine group was measured at 64.500±0.885%, which contrasts significantly with the 5.334±0.422% observed in the Ivabradine-only therapy group (p<0.05). Moreover, the higher levels of LDH in the Iva+Aminoguanidine group at 154.833±1.1377 I.U., compared to the significantly reduced levels of 101.500±1.147 I.U. in the Ivabradine group (p<0.05). Similarly, the CK-MB levels were elevated in the combination group at 198.333±1.145 I.U., whereas the Iva treatment group exhibited lower CK-MB levels of 100.167±1.302 I.U. (p<0.05).

Effect of Ivabradine and Ivabradine+Theophylline on MI, LDH and CK-MB

Theophylline and Ivabradine increase myocardial infarct size compared to ivabradine alone. The MI size in the Ivabradine+Theophylline group was measured at 5.333±0.421%, while in the Ivabradine treatment group; it was at 5.334±0.422% (p > 0.05, ns). Higher LDH level was recorded in Ivabradine+Theophylline group at 102.500±2.929 I.U., whereas Ivabradine therapy group had considerably decreased LDH levels (101.500±1.147 I.U.) (p>0.05, ns). Similarly, elevated level of CK-MB was seen in the Ivabradine+Theophylline group at 97.167±1.721 I.U., they were found lower in the Ivabradine group at 100.167±1.302 I.U. (p>0.05, ns).

Effect of Ivabradine and Ivabradine+Aminophylline on MI, LDH and CK-MB

The combination of Aminophylline with Ivabradine results in a larger myocardial infarct size compared to Ivabradine alone. The MI size in the group treated with Ivabradine and Aminophylline was measured at 5.667±0.333%, while the size in the Ivabradine-only group was significantly lower at 5.334±0.422% (p>0.05, ns). LDH levels in the Ivabradine and Aminophylline group were recorded at 102.833±1.515 I.U., in contrast to the Ivabradine group, where LDH levels were significantly lower at 101.500±1.147 I.U. (p>0.05, ns). Likewise, CK-MB levels in the Ivabradine+Aminophylline group were higher at 100.833±1.249 I.U., whereas in the Ivabradine group, they were considerably lower at 100.167±1.302 I.U. (p>0.05, ns).

Effect of Ivabradine and Ivabradine+Enalapril on MI, LDH and CK-MB

Compared to treatment with Ivabradine alone, there is no discernible increase in the extent of myocardial infarcts when Enalapril, a bradykinin enhancer, is added. The MI size in the Ivabradine+Enalapril group was measured at 5.500±0.428%, while in the Ivabradine treatment group, it was comparable at 5.334±0.422% (p>0.05, non-significant). LDH levels in the Ivabradine+Enalapril group were recorded at 102.667±1.498 I.U., whereas in the Ivabradine treatment group, LDH levels were similar at 101.500±1.147 I.U. (p>0.05, non-significant). The Ivabradine+Enalapril group had CK-MB levels of 100.667±1.429 I.U., while the Ivabradine therapy group had similar levels of 100.167±1.302 I.U. (p>0.05, non-significant).

Table 3: Dimensions of Myocardial Infarcts in Various Animal Species.

Sl. No.	Groups	Groups Description	Infarct Size
1.	I	Control (Ischemia-Reperfusion)	65.667±0.558
2.	II	Ischemic preconditioning	5.1667±0.47***a
3.	III	Ivabradine	5.334±0.422 ***b
4.	IV	Ivabradine+L-NAME	64.167±0.872***c
5.	V	Ivabradine+Aminoguanidine	64.500±0.885 ***d
6	VI	Ivabradine and Theophylline	5.333±0.421 (p>0.05, non-significant) e
7	VII	Ivabradine and Aminophylline	5.667±0.333 (p>0.05, non-significant) f
8	VIII	Ivabradine and Enalapril	5.500±0.428 (p>0.05, non-significant) g
9	IX	Ivabradine and Losartan	5.333±0.667 (p>0.05, non-significant) h
10	X	Ivabradine and Gliclazide	63.833±1.352***i
11	XI	Ivabradine and Glimepiride	63.667±0.989***j

Here the *** represents extreme significant which means p<0.01-0.001 in comparison to Ivabradine control. The statistical assessment was done using One-way ANOVA. Dunnett's post hoc test was utilized for comparing results with the control group, whilst Student's t-test was performed for comparisons in different groups. All statistical evaluations were performed utilizing SigmaPlot v15 software

Effect of Ivabradine and Ivabradine+Losartan on MI, LDH and CK-MB

Compared to treatment with Ivabradine alone, there is no noticeable rise in the size of myocardial infarcts when Losartan, a bradykinin enhancer, is added. The myocardial infarction size was 5.333±0.667% in the Ivabradine+Losartan treatment and 5.334±0.422% in the Ivabradine therapy group ($p>0.05$, non-significant). LDH levels in the Ivabradine+Losartan group were recorded at 102.167±1.751 I.U., In contrast, the Ivabradine group had similar LDH levels at 101.500±1.147 I.U. ($p>0.05$, non-significant). The Ivabradine+Losartan group had CK-MB levels of 101.833±1.400 I.U., while the Ivabradine therapy group had similar levels at 100.167±1.302 I.U. ($p>0.05$, non-significant).

Effect of Ivabradine and Ivabradine+Gliclazide on MI, LDH and CK-MB

When Gliclazide, an ATP-dependent K^+ channel inhibitor, is combined with Ivabradine, there is a significant rise in myocardial infarct size in contrast to Ivabradine-only therapy. The MI size in the Ivabradine+Gliclazide group was measured at 63.833±1.352%, while in the Ivabradine group, it was significantly lower at 5.334±0.422% ($p<0.05$). LDH levels in the Ivabradine+5-Hydroxydecanoate group were recorded at 154.667±1.054 I.U., whereas in the Ivabradine treatment group, LDH levels were significantly lower at 101.500±1.147 I.U. ($p<0.05$). Similarly, CK-MB levels in the ivabradine+5-Hydroxydecanoate group were elevated at 201.833±1.815 I.U., whereas in the Ivabradine treatment group, they were notably lower at 100.167±1.302 I.U. ($p<0.05$).

Effect of Ivabradine and Ivabradine+Glimepiride on MI, LDH and CK-MB

Ivabradine+glimepiride significantly enhance the size of myocardial infarcts in comparison with Ivabradine therapy alone. The MI size in the Ivabradine+Glimepiride group was measured at 63.667±0.989%, while in the Ivabradine group, it was significantly lower at 5.334±0.422% ($p<0.05$). LDH levels in the Ivabradine+Glimepiride group were recorded at 155.833±1.352 I.U., whereas in the Ivabradine group, LDH levels were significantly decrease at 101.500±1.147 I.U. ($p<0.05$). Similarly, CK-MB levels in the Ivabradine+Glimepiride group were elevated at 199.833±1.579 I.U., whereas in the Ivabradine group, they were notably lower at 100.167±1.302 I.U. ($p<0.05$) as presented in Table 3.

Rat Heart Infarct Size

The numbers shown are the mean±SEM of eleven studies, each including six subjects. In terms of significance, a value is considered non-significant if $p>0.05$, significant if $*p\leq0.05$, very significant if $**p<0.01$, and highly significant if $***p<0.001$. Notations such as a, b, c, d, e, f, g, h, i, and j indicate comparisons between different groups, where a comparison marked with a letter denotes statistical significance ($p<0.05$).

All combined treatments show significant differences from Ivabradine alone ($p<0.05$) and are not different from the control ($p>0.05$) except for treatment VI (Ivabradine+Theophylline) vs Group III (Ivabradine), which shows no significant difference ($p>0.05$, non-significant), and treatment IX (Ivabradine+Aminophylline) vs Group III (Ivabradine), which also shows no significant difference ($p>0.05$, non-significant). VIII (Enalapril) vs Group III (Ivabrdaine), which shows no significant difference ($p>0.05$, non-significant), and treatment

Table 4: LDH release in the isolated rat heart's coronary outflow at 40-min reperfusion.

Sl. No.	Groups	Groups Description	LDH at 40-min reperfusion
1.	I	Control (Ischemia-Reperfusion)	155.500±0.957
2.	II	Ischemic preconditioning	101.667±2.789***a
3.	III	Ivabradine	101.500±1.147***b
4.	IV	Ivabradine+L-NAME	154.667±1.256***c
5.	V	Ivabradine+Aminoguanidine	154.833±1.1377***d
6	VI	Ivabradine and Theophylline	102.500±2.929***e
7	VII	Ivabradine and Aminophylline	102.833±1.515***f
8	VII	Ivabradine and Enalapril	102.667±1.498 ($p>0.05$, non-significant) g
9	IX	Ivabradine and Losartan	102.167±1.751 ($p>0.05$, non-significant) h
10	X	Ivabradine and Gliclazide	154.667±1.054***i
11	XI	Ivabradine and Glimepiride	155.833±1.352***j

The statistical assessment was done using One-way ANOVA. Dunnett's post hoc test was utilized for comparing results with the control group, whilst Student's t-test was performed for comparisons in different groups. All statistical evaluations were performed utilizing SigmaPlot v15 software

IX (Ivabradine+Losartan) vs Group III (Ivabradine), which also shows no significant difference ($p>0.05$, non-significant) Figure 3.

Group comparisons are as follows:

Group II (Ischemic preconditioning) showed a significant difference compared to Group I (Control-ischemia-reperfusion) ($p<0.05$).

Group III (Ivabradine) showed a significant difference compared to Group I (Control-ischemia-reperfusion) ($p<0.05$).

Group IV (Ivabradine+L-NAME) showed a significantly distinct compared to Group III (Ivabradine) ($p<0.05$).

Group V (Ivabradine+Aminoguanidine) showed a significantly distinct compared to Group III (Ivabradine) ($p<0.05$).

Group VI (Ivabradine+Theophylline) did not show a significantly distinct compared to Group III (Ivabradine) ($p>0.05$, non-significant).

Group VII (Ivabradine+Aminophylline) did not show a significantly distinct compared to Group III (Ivabradine) ($p>0.05$, non-significant).

Group VIII (Ivabradine+Enalapril) did not show a significantly distinct compared to Group III (Ivabradine) ($p>0.05$, non-significant).

Group IX (Ivabradine+Losartan) did not show a significantly distinct compared to Group III (Ivabradine) ($p>0.05$, non-significant).

Group X (Ivabradine+Gliclazide) showed a significantly distinct compared to Group III (Ivabradine) ($p<0.05$).

Group XI (Ivabradine+Glimepiride) showed a significantly distinct compared to Group III (Ivabradine) ($p<0.05$).

All combined treatments were significantly different from Ivabradine alone ($p<0.05$) and not different from the control ($p>0.05$) except for Group VI (Ivabradine+Theophylline), Group VII (Ivabradine+Aminophylline), Group VIII (Ivabradine+Enalapril) and Group IX (Ivabradine+Losartan), which did not show significant differences compared to Group III (Ivabradine) Table 4.

LDH Release in Coronary Outflow of isolated Rat Heart at 40-Min Reperfusion

These values are the mean±SEM of eleven experiments, each including six participants. In terms of significance, a value is considered non-significant if $p>0.05$, significant if $*p\leq0.05$, very significant if $**p<0.01$, and highly significant if $***p<0.001$. Comparisons between groups are represented by symbols such as a, b, c, d, e, f, g, h, i, and j; a comparison with a letter indicates statistical significance ($p<0.05$).

All combination treatments demonstrated significant differences compared to Ivabradine alone ($p<0.05$) but did not differ significantly from the control group ($p>0.05$), except for treatment VI (Ivabradine+Theophylline) versus Group III (Ivabradine), which showed no significant difference ($p>0.05$). Similarly, treatment IX (Ivabradine+Aminophylline) versus Group III (Ivabradine) showed no significant difference ($p>0.05$). Additionally, treatment VIII (Enalapril) versus Group III

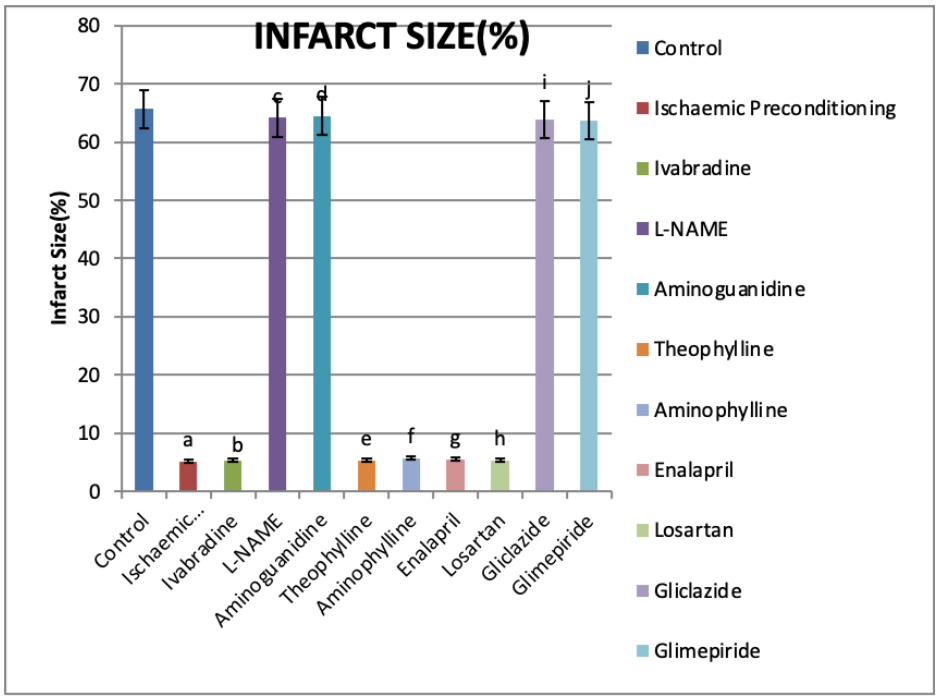

Figure 3: Infarct size of rat's heart.

(Ivabradine) and treatment IX (Ivabradine+Losartan) versus Group III (Ivabradine) also exhibited no significant difference ($p>0.05$), as illustrated in Figure 4.

Group comparisons are as follows:

Group II (Ischemic preconditioning) showed a significant difference compared to Group I (Control-ischemia-reperfusion) ($p<0.05$).

Group III (Ivabradine) showed a significant difference compared to Group I (Control-ischemia-reperfusion) ($p<0.05$).

Group IV (Ivabradine+L-NAME) showed a significantly distinct compared to Group III (Ivabradine) ($p<0.05$).

Group V (Ivabradine+Aminoguanidine) showed a significantly distinct compared to Group III (Ivabradine) ($p<0.05$).

Group VI (Ivabradine+Theophylline) did not show a significantly distinct compared to Group III (Ivabradine) ($p>0.05$, non-significant).

Group VII (Ivabradine+Aminophylline) did not show a significantly distinct compared to Group III (Ivabradine) ($p>0.05$, non-significant).

Group VIII (Ivabradine+Enalapril) did not show a significantly distinct compared to Group III (Ivabradine) ($p>0.05$, non-significant).

Group IX (Ivabradine+Losartan) did not show a significantly distinct compared to Group III (Ivabradine) ($p>0.05$, non-significant).

Group X (Ivabradine+Gliclazide) showed a significantly distinct compared Group III (Ivabradine) ($p<0.05$).

Group XI (Ivabradine+Glimepiride) showed a significantly distinct compared to Group III (Ivabradine) ($p<0.05$).

All combination treatments exhibited significant differences compared to Ivabradine alone ($p<0.05$) and showed no significant differences from the control group ($p>0.05$). However, Group VI (Ivabradine+Theophylline), Group VII (Ivabradine+Aminophylline), Group VIII (Ivabradine+Enalapril), and Group IX (Ivabradine+Losartan) did not show any significant difference when compared to Group III (Ivabradine), as presented in Table 5.

CK-MB release in coronary outflow of isolated rat heart at 40-min Reperfusion

The values displayed are the mean±SEM of eleven experiments, each with six participants. $^{**}p<0.01$ shows a very potential value, $^{***}p<0.001$ indicates an extremely vital value, $^{*}p\leq0.05$ indicates a significant value, and $p>0.05$ indicates a non-significant value. Comparisons between groups are indicated by notations like a, b, c, d and more; a comparison with a letter ($p<0.05$) shows statistical significance.

All combined treatments show significant differences from Ivabradine alone ($p<0.05$) and are indistinguishable from the control ($p>0.05$) except for treatment VI (Ivabradine+Theoplylline) vs Group III (Ivabradine), which shows no substantial difference ($p>0.05$, non-significant), and treatment IX (Ivabradine+Aminoplylline) vs Group III (Ivabradine), which also shows no substantial difference ($p>0.05$, non-significant). VIII (Enalapril) vs Group III (Ivabradine), which shows no substantial difference ($p>0.05$, non-significant), and treatment IX (Ivabradine+Losartan) vs Group III (Ivabradine), which also shows no substantial difference ($p>0.05$, non-significant) Figure 5.

Group comparisons are as follows:

Table 5: CK-MB Release in Coronary Outflow of Isolated Rat Heart after 40 Min of Reperfusion.

Sl. No.	Groups	Groups Description	LDH at 40-min reperfusion
1.	I	Control (Ischemia-Reperfusion)	198.500±1.803
2.	II	Ischemic preconditioning	97.167±1.721***a
3.	III	Ivabradine	100.167±1.302***b
4.	IV	Ivabradine+L-NAME	200.167±1.537***c
5.	V	Ivabradine+Aminoguanidine	198.333±1.145***d
6	VI	Ivabradine and Theophylline	97.167±1.721***e
7	VII	Ivabradine and Aminophylline	100.833±1.249***f
8	VII	Ivabradine and Enalapril	100.667±1.429 ($p>0.05$, non-significant) g
9	IX	Ivabradine and Losartan	101.833±1.400 ($p>0.05$, non-significant) h
10	X	Ivabradine and Gliclazide	201.833±1.815***i
11	XI	Ivabradine and Glimepiride	199.833±1.579***j

The statistical assessment was done using One-way ANOVA. Dunnett's post hoc test was utilized for comparing results with the control group, whilst Student's t-test was performed for comparisons in different groups. All statistical evaluations were performed utilizing SigmaPlot v15 software

Group II (Ischemic preconditioning) revealed significant variations from Group I (Control-ischemia-reperfusion) ($p<0.05$).

Group III (Ivabradine) revealed significant variations from Group I (Control-ischemia-reperfusion) ($p<0.05$).

Group IV (Ivabradine+L-NAME) was significantly different from Group III (Ivabradine) ($p<0.05$).

Group V (Ivabradine+Aminoguanidine) was significantly distinct from Group III (Ivabradine) ($p<0.05$).

Group VI (Ivabradine+Theophylline) did not show a significantly distinct compared to Group III (Ivabradine) ($p>0.05$, non-significant).

Group VII (Ivabradine+Aminophylline) did not show a significantly distinct compared to Group III (Ivabradine) ($p>0.05$, non-significant).

Group VIII (Ivabradine+Enalapril) did not show a significantly distinct compared to Group III (Ivabradine) ($p>0.05$, non-significant).

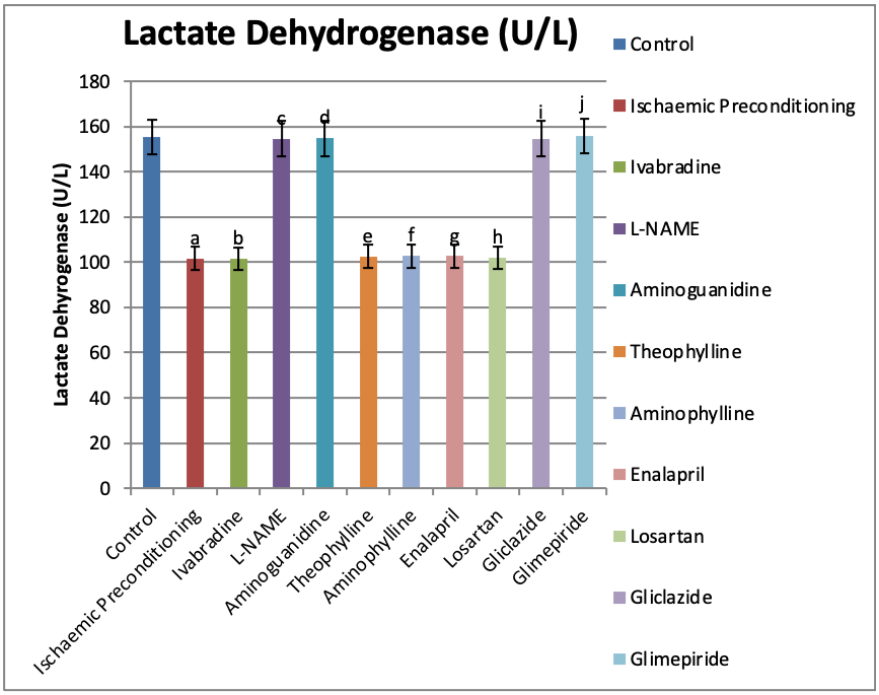

Figure 4: Release of LDH in the isolated rat heart's coronary effluent at 40 min after reperfusion.

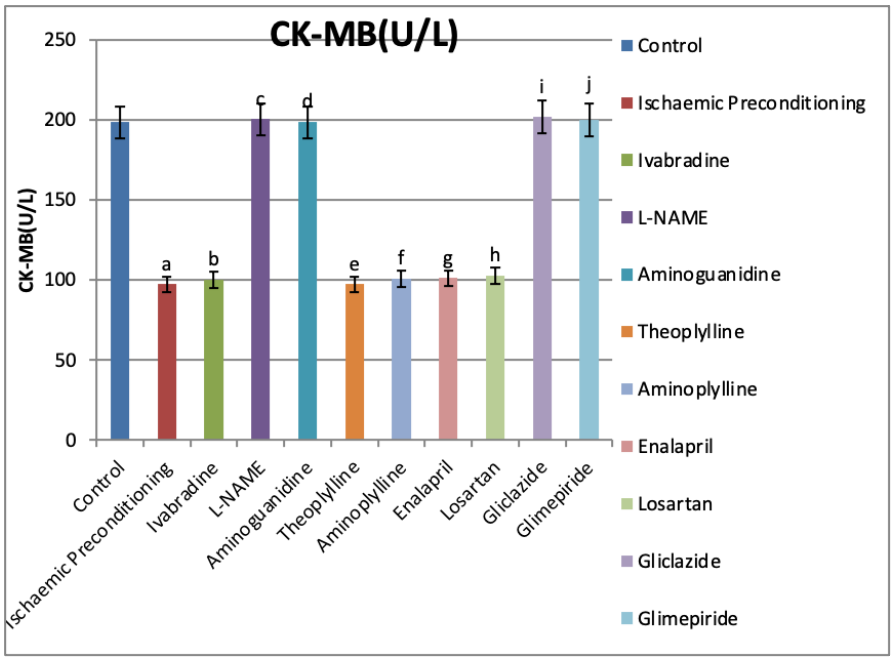

Figure 5: CK-MB Release in Coronary Outflow of Isolated Rat Heart at 40 min Revascularization.

Table 1: Acquisition of Pharmaceuticals and chemicals.

Sl. No.	Name
1.	Ivabradine
2.	Ketamine
3	N-nitro-L-arginine methyl ester (L-NAME)
4.	Aminoguanidine
5.	Theophylline
6.	Aminophylline
7.	Enalapril Maleate
8.	LOSARTAN POTASSIUM USP (1000272)
9.	Gliclazide
10.	Glimepiride
11.	Heparin
12.	Triphenyl tetrazolium Chloride
13.	$NaHCO_3$, NaH_2PO_4
14.	$MgSO_4$, $CaCl_2$, Diethyl ether, Carboxymethylcellulose
15.	KCl, Dextrose, NaCl
16.	Na_2HPO_4

Group IX (Ivabradine+Losartan) did not show a significantly distinct compared to Group III (Ivabradine) ($p>0.05$, non-significant).

Group X (Ivabradine+Gliclazide) showed a significantly distinct compared to Group III (Ivabradine) ($p<0.05$).

Group XI (Ivabradine+Glimepiride) showed a significantly distinct compared to Group III (Ivabradine) ($p<0.05$).

All combined treatments were quite different from Ivabradine alone ($p<0.05$) and not distinct from the control ($p>0.05$) except for Group VI (Ivabradine+Theophylline), Group VII (Ivabradine+Aminophylline), Group VIII (Ivabradine+Enalapril) and Group IX (Ivabradine+Losartan), which did not show significant differences compared to Group III (Ivabradine).

DISCUSSION

Mechanism of Action of Ivabradine

Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) channels play a vital role in regulating neural excitability and cardiac rhythm, particularly in response to hyperpolarization. During this process, intracellular cAMP enhances ion flow through HCN channels, which selectively permit the entry of potassium and sodium ions. Research utilizing pharmacological studies, transgenic models, and mutagenesis has underscored the importance of HCN channels in various physiological and pathological conditions. This has positioned HCN channels as potential targets for novel therapeutic interventions, particularly in the treatment of bradycardia, epilepsy, and pain management. Significantly, in 2005, ivabradine became the first HCN channel inhibitor approved for clinical use, paving the way for innovative treatments for chronic stable angina and heart failure.[20]

Alinidine, an imidazoline molecule developed from clonidine, was the first bradycardic drug with a proven ability to suppress the sino-atrial pacemaker current (If). The calcium channel inhibitor verapamil has been the source of further bradycardic medicines, including ivabradine, zatebradine, and cilobradine. An anti-ischemic drug called ivabradine (Procoralan®) blocks the If current with a notable degree of selectivity.[21] This is essential for sino-atrial node pacemaker control.[22] The BEAUTIFUL investigation concluded that ivabradine decreases coronary artery disease outcomes in a subgroup of people with heart rates exceeding 70 bpm.[23] Ivabradine reduces infarct size and enhances regional cardiac blood flow and function.[24] Ivabradine causes the hyperpolarization-activated current to be blocked (in a pronounced exponential use-dependent manner). At a high concentration (10 μM), ivabradine mildly reduces L-type calcium current through a use-dependent block while having no effect on T-type calcium current. Additionally, it decreases the slow outward potassium current in rabbit sinoatrial node cells.[25,26] Studies on ivabradine's impact on action potential repolarization have been conducted only in guinea pig papillary muscle and rabbit sinus node pacemaker cells, both of which are characterized by small heart sizes and high frequencies. At higher concentrations (50 μM), ivabradine exhibited frequency-dependent Vmax blockade in guinea pig apical muscle; however, at therapeutic concentrations, it did not alter action potential duration.[27,28]

Ivabradine has been shown in several human and animal investigations to be cardioprotective. In rats with acute non-reperfused myocardial infarction, ivabradine suppresses ventricular arrhythmias just as well as metoprolol.[6,29] Ivabradine inhibits Cyclophilin-A secretion in pigs with myocardial ischemia, providing cardio protection against MI.[30] Iva regulates heart rate, mitigates myocardial injury, enhances cardiac function and exercise capacity, and may lower the risk of significant cardiac events in persons who have undergone an Acute Myocardial Infarction (AMI) following Percutaneous Coronary Intervention (PCI).[31]

Ischemic Pre-conditioning (IPC) and its mediators

When blood flow in a tissue or organ is partially or completely blocked, it is referred to as ischemia. If treatment is not received, this situation can result in necrosis and cell death. Ischemia Reperfusion Injury (IRI) transpires when blood flow is restored following ischemia. In partial nephrectomy, renal transplant, aortic cross-clamping and shock, renal IRI causes injury to the kidneys. Hypothermia, IPC, hydrogen sulfide, SOD, apocynin, allopurinol, and remote ischemia preconditioning reduce IRI. Remote Ischemic Preconditioning (RIPC) has the potential to minimize kidney damage caused by Ischemia-Reperfusion Injury (IRI). Conditioning one organ can confer protection

to another against prolonged IRI. Brief periods of ischemia and reperfusion stimulate the production of antioxidants that neutralize oxygen-derived free radicals. Although the exact protective mechanisms of RIPC remain unclear, Nitric Oxide (NO) may play a role in enhancing the heart's resistance to IRI.[32] The participation of several variables, including NO, bradykinin, and ATP-dependent K+ channels, has been connected to the cardioprotective effects of ischemia preconditioning.[33-50]

Prior Research Regarding LDH and CK-MB in Cardioprotection

Despite being restricted as cardiac biomarkers, CK-MB and LDH are routinely employed to measure heart damage and ischemic-reperfusion injury. They are key indicators of the efficacy of cardioprotective treatments such prophylactic measures, medicines, and other therapies that reduce damage to tissues during ischemia episodes. In outcome, cardiovascular protection research requires different markers (CK-MB and LDH) of cellular and myocardial damage. Keeping an eye on their levels can help determine how different tactics and interventions that try to reduce damage from ischemia-reperfusion injury are working.[51,52] Prior studies indicate that ischemic preconditioning provides cardioprotection through mechanisms involving nitric oxide, adenosine, bradykinin, and ATP-sensitive K+ channels. This study seeks to explore whether Ivabradine utilizes these same pathways to exert its cardioprotective effects.

The study provides evidence supporting the participation of ATP-dependent K+ channels and nitric oxide in Ivabradine's cardioprotective action. This is drawn from the observation that inhibitors of these pathways, such as L-NAME and aminoguanidine, NO inhibitors, eliminate ivabradine's cardioprotective properties. Theophylline and aminophylline adenosine inhibitors do not eliminate the cardioprotective action of ivabradine. Gliclazide and Glimepiride abolish the cardioprotective action of Ivabradine. Conversely, enalapril and losartan, which increase bradykinin levels, enhance the cardioprotective effects of ivabradine.

The findings reveal that inhibitors of nitric oxide and ATP-dependent K+ channels, such as L-NAME, aminoguanidine, gliclazide, and glimepiride, lead to an increase in different levels in the Ivabradine treatment groups. On the other hand, theophylline, aminophylline, enalapril, and losartan result in a decrease in different levels.

In one of study *Kamisah, Y., et al* (2023).[53] concluded that ivabradine reduces heart rate and relieves symptoms related to heart failure by selectively inhibiting If current. In animal tests, it shows pleiotropic antiremodeling through a number of different pathways, including antifibrotic, anti-inflammatory, antioxidant, and antiapoptotic actions. Additionally, it increases the animals' autophagy and mitochondrial bioenergetics.[53] Further research is needed in animal models (*in vivo, in vitro*, or *ex vivo*) to explore Ivabradine's impact on cellular calcium regulation (e.g., transient receptor potential canonical channels), mitochondrial function and biogenesis (e.g., sirtuin 3), autophagy (e.g., FUN14 domain-containing 1 and BCL2-interacting protein 3-like), inflammation (e.g., calcineurin-nuclear factor of activated T-cells), and oxidative stress signaling pathways (e.g., AMP-activated protein kinase/Nur77). Additionally, its potential antifibrotic role in regulating the zinc finger transcription factor GATA-binding protein 4 (GATA4) should be investigated.[53]

Initially approved for the treatment of angina pectoris, the drug has been used since 2005 as an adjunct therapy for patients with stable symptomatic heart failure with reduced ejection fraction (HFrEF) who have a persistently elevated resting heart rate (>70 beats per min), a recognized independent risk factor for cardiovascular disease.[54,55]

The study's results highlight how nitric oxide and ATP-dependent K+ channels promote Ivabradine's cardioprotective effects, which are comparable to ischemia preconditioning. However, adenosine and bradykinin were not found to be mediators of Ivabradine-induced cardioprotection. Thus, Ivabradine may improve patient adherence as a pharmaceutical replacement for surgical ischemia preparation before heart transplantation and CABG by modulating nitric oxide and ATP-dependent K+ channels.

CONCLUSION

In the Ivabradine-treated groups administered with inhibitors targeting NO, adenosine, and ATP-dependent K+ channels, the percentage of MI, LDH, and CK-MB values were significantly increased. Conversely, theophylline and aminophylline, which inhibit adenosine levels, demonstrated a decrease in CK, LDH, and infarct size-MB, so adenosine does not release in ivabradine-induced cardioprotection. Enalapril and Losartan, which elevate bradykinin levels, demonstrated a decrease in infarct size, LDH and CK-MB in ivabradine-induced cardioprotection. Nitric oxide and ATP-dependent K+ channels act as secondary messengers in cardioprotection, similar to ischemia preconditioning, according to the study result. Adenosine and bradykinin are not typically considered key contributors to Ivabradine's cardioprotective effects in ischemic preconditioning. However, the study revealed that these mechanisms play a significant role in Ivabradine's cardioprotective action. This indicates that ivabradine could serve as an alternative to surgical ischemic preconditioning, potentially enhancing patient adherence and outcomes in chronic ischemic heart disease and preoperative settings such as CABG and heart transplantation. This effect is mediated through signaling pathways involving nitric oxide and ATP-dependent K+ channels as secondary messengers.

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ETHICAL APPROVAL

"Compliance with Ethical Standards"
"The Institutional Animal Ethics Committee (IAEC, 2022) has granted ethical approval for the experimental study protocol. Protocol No. 01, 02, 03, 05, and 08, NIMS University Rajasthan, Jaipur, Rajasthan, India, Registration No. 1203/PO/Re/S/09/CPCSEA, under the supervision of CPCSEA, Government of India, New Delhi, India."

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ATP: Adenosine Triphosphate; **CABG:** Coronary Artery Bypass Graft; **CK-MB:** Creatine Kinase Myoglobin Binding; **DCGI:** Drug Controller General of India; **ECG:** Electrocardiogram; **GCP:** Good Clinical Practice; **HCN:** Hyperpolarization-activated Cyclic Nucleotide-gated channel; **ICF:** Informed Consent Form; **IL-1β:** Interleukin-1 Beta; **LDH:** Lactate Dehydrogenase; **L-NAME:** N^ω^-nitro-L-arginine methyl ester; **MI:** Myocardial Infarction; **MMR:** Mismatch Repair; **NO:** Nitric Oxide; **PSS:** Physiological Salt Solution; **SEM:** Standard Error of the Mean; **TTC:** Triphenyl Tetrazolium Chloride; **TNF-α:** Tumor Necrosis Factor Alpha; **IPC:** Ischaemic preconditioning; **NO:** Nitric Oxide; **IRI:** Ischemia Reperfusion Injury.

REFERENCES

1. Voronkov NS, Maslov LN, Vyshlov EV, Mukhomedzyanov AV, Ryabov VV, Derkachev IA, Kan A, Gusakova SV, Gombozhapova AE, Panteleev OO. Do platelets protect the heart against ischemia/reperfusion injury or exacerbate cardiac ischemia/reperfusion injury? The role of PDGF, VEGF, and PAF. Life Sciences. 2024:122617.
2. Dalimunthe A, Satria D, Sitorus P, Harahap U, Angela IF, Waruwu SB. Cardioprotective effect of hydroalcohol extract of andaliman (Zanthoxylum acanthopodium DC.) fruits on doxorubicin-induced rats. Pharmaceuticals. 2024;17(3):359.
3. Tantray J, Sharma AK, Singh S, Zaid M, Bhat M, Gill K, Sharma RK, Singh RP. Ranolazine Exert its Beneficial Effects in myocardial infarction like Ischemic preconditioning mediators by Increasing Myocardial Nitric oxide, Adenosine, Bradykinin and K+ ATPase Levels.
4. McCafferty K, Forbes S, Thiemermann C, Yaqoob MM. The challenge of translating ischemic conditioning from animal models to humans: the role of comorbidities. Disease models and mechanisms. 2014;7(12):1321-33.
5. Gattullo D, Linden RJ, Losano G, Pagliaro P, Westerhof N. Ischaemic preconditioning changes the pattern of coronary reactive hyperaemia in the goat: role of adenosine and nitric oxide. Cardiovascular research. 1999;42(1):57-64.
6. Dai Y, Chen Y, Wei G, Zha L, Li X. Ivabradine protects rats against myocardial infarction through reinforcing autophagy via inhibiting PI3K/AKT/mTOR/p70S6K pathway. Bioengineered. 2021;12(1):1826-37.
7. Wu Z, Zai W, Chen W, Han Y, Jin X, Liu H. Curdione ameliorated doxorubicin-induced cardiotoxicity through suppressing oxidative stress and activating Nrf2/HO-1 pathway. Journal of Cardiovascular Pharmacology. 2019;74(2):118-27.
8. Chen G, Song X, Lin D, Xu P. Isofraxidin alleviates myocardial infarction through NLRP3 inflammasome inhibition. Inflammation. 2020;43:712-21.
9. Koruth JS, Lala A, Pinney S, *et al.* The clinical use of Ivabradine. J Am Coll Cardiol. 2017;70:1777-1784.
10. Ide T, Ohtani K, Higo T, *et al.* Ivabradine for the treatment of cardiovascular diseases. Circ J. 2019;83:252-260.
11. Vilaine JP, Bidouard JP, Lesage L, *et al.* Anti-ischemic effects of ivabradine, a selective heart rate-reducing agent, in exercise-induced myocardial ischemia in pigs. J Cardiovasc Pharmacol. 2003;42(5):688-96.
12. Mathew ST, Po SS, Thadani U. Inappropriate sinus tachycardia-symptom and heart rate reduction with ivabradine: a pooled analysis of prospective studies. Heart Rhythm. 2018;15(2):240-7.
13. Marciszek M, Paterek A, Oknińska M, *et al.* Ivabradine is as effective as metoprolol in the prevention of ventricular arrhythmias in acute non-reperfused myocardial infarction in the rat. Sci Rep. 2020;10(1):15027.
14. Xu Y, Zhang W, Zhong X, *et al.* Effect of early use of ivabradine on left ventricular remodeling after primary percutaneous coronary intervention in patients with acute ST-segment elevation myocardial infarction: a pilot test. Ann Noninvasive Electrocardiol. 2021;26(2):e12816.
15. Wu X, You W, Wu Z, *et al.* Ivabradine promotes angiogenesis and reduces cardiac hypertrophy in mice with myocardial infarction. Anatol J Cardiol. 2018;20(5):266-272.
16. Nilsson BO. Biological effects of aminoguanidine: an update. Inflammation Research. 1999;48:509-15.
17. Wolf RE, Graeber GM, Burge JR, DeShong JL, MacDonald JL, Zajtchuk R. Evaluation of serum creatine kinase and lactate dehydrogenase in experimental myocardial infarction, atriotomies, and thoracotomies. The Annals of thoracic surgery. 1986;41(4):378-86.
18. Sharma, A.K., Munajjam, A., Vaishnav, B., Sharma, R., Sharma, A., Kishore, K, Involvement of adenosine and standardization of aqueous extract of garlic (*Allium sativum* Linn.) on cardioprotective and cardiodepressant properties in ischemic preconditioning and myocardial ischemia-reperfusion induced cardiac injury. Journal of Biomedical Research, 2012;26(1):24-36.
19. Dhein S. The langendorff heart. InPractical methods in cardiovascular research 2005 Mar 17 (pp. 155-172). Berlin, Heidelberg: Springer Berlin Heidelberg.
20. Ying Caoa, Jianxin Panga and Pingzheng Zhoua,* HCN Channel as Therapeutic Targets for Heart Failure and Pain Current Topics in Medicinal Chemistry, 2016, 16, 1-7
21. Vilaine JP, Thollon C, Villenueve N, Peglion JL. Procoralan, a new selective If current inhibitor. European Heart Journal Supplements. 2003;5(suppl_G):G26-35.
22. DiFrancesco D. The pacemaker current (I f) plays an important role in regulating SA node pacemaker activity. Cardiovascular research. 1995;30(2):307-8.
23. Fox K, Ford I, Steg PG, Tendera M, Ferrari R. Ivabradine for patients with stable coronary artery disease and left-ventricular systolic dysfunction (BEAUTIFUL): a randomised, double-blind, placebo-controlled trial. The Lancet. 2008;372(9641):807-16.
24. Heusch G, Skyschally A, Gres P, van Caster P, Schilawa D, Schulz R. Improvement of regional myocardial blood flow and function and reduction of infarct size with ivabradine: protection beyond heart rate reduction. European heart journal. 2008;29(18):2265-75.
25. Bois P, Bescond J, Renaudon B, Lenfant J. Mode of action of bradycardic agent, S 16257, on ionic currents of rabbit sinoatrial node cells. British journal of pharmacology. 1996;118(4):1051.
26. Thollon C, Bidouard JP, Cambarrat C, Lesage L, Reure H, Delescluse I, *et al.* Stereospecific *in vitro* and *in vivo* effects of the new sinus node inhibitor (+)-S 16257. European journal of pharmacology. 1997;339(1):43-51.
27. Thollon C, Cambarrat C, Vian J, Prost JF, Peglion JL, Vilaine JP. Electrophysiological effects of S 16257, a novel sino-atrial node modulator, on rabbit and guinea-pig cardiac preparations: comparison with UL-FS 49. British journal of pharmacology. 1994;112(1):37-42.
28. Perez O, Gay P, Franqueza L, Carron R, Valenzuela C, Delpon E, Tamargo J. Effects of the two enantiomers, S-16257-2 and S-16260-2, of a new bradycardic agent on guinea-pig isolated cardiac preparations. British journal of pharmacology. 1995;115(5):787-94.
29. Marciszek M, Paterek A, Oknińska M, Mackiewicz U, Mączewski M. Ivabradine is as effective as metoprolol in the prevention of ventricular arrhythmias in acute non-reperfused myocardial infarction in the rat. Scientific Reports. 2020;10(1):15027.
30. Hernandez I, Tesoro L, Ramirez-Carracedo R, Diez-Mata J, Sanchez S, Saura M, *et al.* Ivabradine induces cardiac protection against myocardial infarction by preventing cyclophilin-A secretion in pigs under coronary ischemia/reperfusion. International Journal of Molecular Sciences. 2021;22(6):2902.
31. He R, Li L, Han C, An W, Liu Z, Gao J. Effect of the combined use of ivabradine and metoprolol in patients with acute myocardial infarction early after percutaneous coronary intervention: A randomized controlled study. Heliyon. 2024;10(13).
32. La Padula PH, Etchegoyen M, Czerniczyniec A, Piotrkowski B, Arnaiz SL, Milei J, Costa LE. Cardioprotection after acute exposure to simulated high altitude in rats. Role of nitric oxide. Nitric Oxide. 2018;73:52-9.
33. Kanno S, Lee PC, Zhang Y, Ho C, Griffith BP, Shears LL, *et al.* Attenuation of myocardial ischemia/reperfusion injury by superinduction of inducible nitric oxide synthase. Circulation. 2000;101(23):2742-8.
34. Robertson NJ, Pang R, Hausenloy DJ. Remote ischaemic post conditioning in NE: A feasible and simple paradigm for self-protection in the brain?. Pediatric Research. 2025:1-3.

35. Liu GS, Thornton J, Van Winkle DM, Stanley AW, Olsson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart. Circulation. 1991;84(1):350-6.
36. Fan H, Sun B, Gu Q, Lafond-Walker A, Cao S, Becker LC. Oxygen radicals trigger activation of NF-κB and AP-1 and upregulation of ICAM-1 in reperfused canine heart. American Journal of Physiology-Heart and Circulatory Physiology. 2002;282(5):H1778-86.
37. Starkopf J, Bugge E, Ytrehus K. Preischaemic bradykinin and ischaemic preconditioning in functional recovery of the globally ischaemic rat heart. Cardiovascular research. 1997;33(1):63-70.
38. Pan HL, Chen SR, Scicli GM, Carretero OA. Cardiac interstitial bradykinin release during ischemia is enhanced by ischemic preconditioning. American Journal of Physiology-Heart and Circulatory Physiology. 2000;279(1):H116-21.
39. Wall TM, Sheehy R, Hartman JC. Role of bradykinin in myocardial preconditioning. Journal of Pharmacology and Experimental Therapeutics. 1994;270(2):681-9.
40. Sheng Z, Yao Y, Li Y, Yan F, Huang J, Ma G. Bradykinin preconditioning improves therapeutic potential of human endothelial progenitor cells in infarcted myocardium. PLoS One. 2013;8(12):e81505.
41. Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischemic'precondi tioning'protects remote virgin myocardium from subsequent sustained coronary occlusion. circulation. 1993;87(3):893-9.
42. Regoli D. Pharmacology of bradykinin and related kinins. Advances in experimental medicine and biology. 1983;156:569-84.
43. Carretero OA, Miyazaki SE, Scicli AG. Role of kinins in the acute antihypertensive effect of the converting enzyme inhibitor, captopril. Hypertension. 1981;3(1):18-22.
44. Hanley PJ, Mickel M, Löffler M, Brandt U, Daut J. KATP channel-independent targets of diazoxide and 5-hydroxydecanoate in the heart.
45. Gross GJ, Auchampach JA. Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. Circulation research. 1992;70(2):223-33.
46. Ali K, Morimoto M, Fukaya Y, Furukawa Y. Improvement of cardiac function impaired by repeated ischemic arrests in isolated rat hearts. The Annals of thoracic surgery. 1993;55(4):902-7.
47. Olders J, Turek Z, Evers J, Hoofd L, Oeseburg B, Kreuzer F. Comparison of Tyrode and blood perfused working isolated rat hearts. Oxygen Transport to Tissue XII. 1990:403-13.
48. L.S. Gettes, What are the effects of potassium on the electrophysiology of acute ischemia. Life-Threatening Arrhythmias During Ischemia and Infarction. New York, NY: Raven Press Publishers. (1987) 77-90.
49. Curtis MJ, Macleod BA, Walker MJ. Models for the study of arrhythmias in myocardial ischaemia and infarction: the use of the rat. Journal of molecular and cellular cardiology. 1987;19(4):399-419.
50. Schaper W, Binz K, Sass S, Winkler B. Influence of collateral blood flow and of variations in MVO2 on tissue-ATP content in ischemic and infarcted myocardium. Journal of molecular and cellular cardiology. 1987;19(1):19-37.
51. E. Carmeliet, Oral antidiabetics and hypoxic shortening of the cardiac action potential. Eur. Heart J. 8(Suppl 2) (1987) 315-27.
52. Amani M, Ahmadiasl N, Usefzade N, Zaman J. Effect of HEMADO on level of CK-MB and LDH enzymes after ischemia/reperfusion injury in isolated rat heart. BioImpacts: BI. 2012;3(2):101.
53. Kamisah, Y., and Che Hassan, H. H. Therapeutic use and molecular aspects of ivabradine in cardiac remodeling: A review. International Journal of Molecular Sciences, 2023;24(3):801.
54. Thorup L, Simonsen U, Grimm D, Hedegaard ER. Ivabradine: current and future treatment of heart failure. Basic and clinical pharmacology and toxicology. 2017;121(2):89-97.
55. Chen C, Kaur G, Mehta PK, Morrone D, Godoy LC, Bangalore S, Sidhu MS. Ivabradine in cardiovascular disease management revisited: a review. Cardiovascular Drugs and Therapy. 2021:1-2.

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