Role of Ivabradine in Vivo Myocardial Ischemia and Reperfusion Injuries Induced in Rats

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Abstract

Background and Aim: Higher heart rate may induce or exacerbate myocardial ischemia that is why rate-slowing drugs are considered to be the cornerstone of anti-ischemic therapy. Ivabradine is the representative of a novel class of drugs that exclusively reduce heart rate through selective inhibition of If current.

The present study aiming to investigate the effects of ivabradine on the Ischemia and Reperfusion-induced cardiac infarction in an in vivo rat model and to compare its protective effect to that of propranolol. As well as the possible mechanisms of infarction reduction by ivabradine were studied.

Animals and Methods: The study was carried out on male albino rats for in vivo study and rabbits for in vitro study. To produce cardiac damage, the left anterior descending coronary artery (LAD) was occluded for 90min followed by 120min reperfusion in a urethane 25% anesthetized rats. Ivabradine and propranolol were given prophylactically orally daily for 15 days before ligation of the LAD and ivabradine therapeutically was injected intraperitoneal during ischemia 15min after ligation and 5 minutes before reperfusion of the LAD. The changes in the ECG as regard the heart rates (bpm) and ST segment (mv) levels and their percentage reduction (in relation to controls) were recorded. Also the hearts were biopsied, stained with Hematoxylin-Eosin stain and examined under the light microscope.

The effects of gradually increasing doses of ivabradine on the inotropic and chronotropic properties of the heart were recorded from an isolated perfused rabbit heart as well as dose response for phenylephrine was recorded and the effect of cumulative doses of ivabradine on top of the submaximal dose of phenylephrine were measured and recorded on a power Lab from isolated rabbit aorta.

Results: The heart rate, ST segment level and the infarction size were significantly reduced in the ivabradine and propranolol prophylactic groups. The decrease in heart rate was more with ivabradine than with propranolol prophylaxis. Also in the ivabradine treated either given after ligation of the LAD or before reperfusion, all parameters were progressively reduced. Ivabradine in cumulative doses produced relaxant effect on phenylephrine induced rabbit aortic contraction.

Conclusion: Both ivabradine and propranolol showed equal protection against ischemic injury. Long term prophylaxis by ivabradine is more effective than ivabradine treatment against ischemic injury. Ivabradine attenuated the reperfusion injury. Cumulative doses of ivabradine showed antispasmodic effect on isolated rabbit aorta.

Key Words: Ischemia/reperfusion – Infarction – Heart rate reduction – If-channel – Ivabradine.

Introduction

MYOCARDIAL ischemia comes about when the myocardia fail to take in sufficient blood and oxygen to function correctly. This inadequate perfusion of blood and the resulting reduced delivery of oxygen and nutrients are directly correlated to blocked or narrowed blood vessels. The myocardium can tolerate brief periods of severe and even total myocardial ischemia without resultant cardiomyocyte death. Such transient periods of ischemia are encountered in the clinical situations of angina, coronary vasospasm, and balloon angioplasty, and are not associated with concomitant myocyte cell death [1].

With increasing duration and severity of ischemia, however, greater cardiomyocyte damage can develop, with a predisposition to a spectrum of reperfusion-associated pathologies, collectively called reperfusion injury [2]. Ischaemia followed by reoxygenation is associated with oxygen free radical generation, which is one of the major factors responsible for arrhythmias and myocardial damage accompanying ischaemia and reperfusion syndrome [3].

Heart rate is an important predictor of cardiovascular mortality. Elevated resting heart rate is associated with increased cardiovascular mortality and morbidity. An increase in heart rate results not only in an increase in myocardial oxygen demands, but also a potential impairment of supply resulting...
from a reduction of collateral perfusion pressure and collateral flow [4]. This imbalance may promote ischemia, arrhythmias and ventricular dysfunction, as well as acute coronary syndromes, heart failure or sudden death.

Lowering heart rate is therefore one of the most important therapeutic approaches in the treatment of myocardial ischemia [5].

To date, β-blockers and some calcium-channel antagonists reduce heart rate, but their use may be limited by adverse reactions or contraindications [6-8].

Heart rate is determined by spontaneous electrical pacemaker activity in the sinoatrial node controlled by If currents [9]. Selective and specific inhibition of cardiac pacemaker If current, can lower heart rate without compromising myocardial contractility, hemodynamic status, or the electrophysiological properties of the heart.

Ivabradine is a specific and selective If inhibitor [10]. The inhibition of the If channel is current-dependent meaning that the higher the heart rate the more effective is ivabradine [11]. Inhibition of the cardiac pacemaker If current by ivabradine induces pure heart rate reduction, exerts anti-ischemic properties and protects against myocardial stunning and infarction [12,13].

In the present study, the prophylactic effect of ivabradine on the Ischemia induced by coronary artery ligation in an in vivo rat model was tested and its protective effect was compared to that of propranolol. Also, the therapeutic effect of ivabradine on the ischemia and reperfusion induced myocardial injury was studied as well as the mechanisms of infarction reduction.

**Material and Methods**

This study was approved by our institution’s (Kasr El-Eini Hospital) Animal Care Committee and the guidelines were strictly adhered to.

**Drugs:**
- Ivabradine (Procoralan) powder (Servier-France).
- Propranolol powder (Sigma Chemical Company).
- Phenylephrine powder (Sigma Chemical Company).

Powders were dissolved in isotonic saline.

**Chemicals:**
- Ringer’s solution for isolated mammalian heart (g/liter) [Sodium chloride 8.2, Potassium chloride 0.2, Calcium chloride 0.2, Magnesium chloride 0.2, Sodium bicarbonate 1.0 and Glucose 2.0].
- Krebs’ solution for Isolated aortic strip of rabbit (g/liter) [Sodium chloride 6.8, Potassium chloride 0.35, Magnesium chloride 0.29, Calcium chloride 0.28, Sodium bicarbonate 2.1, Sodium acid phosphate 0.16 and Glucose 2.0].
- Solutions of hematoxylin-Eosin stain: For determination of myocardial infarction size.

Powders and solutions were purchased from Sigma Chemical Company.

**Instruments:**
- Recording ECG.
- Monitoring and recording signals from the isolated rabbit’s heart connected to the Langendorff’s coronary perfusion set.
- Monitoring and recording signals from isolated rabbit aorta.

**Animals:**
- Laboratory bred male adult albino rats weighing between 150-250g were used for in-vivo experiments. They were maintained under standard laboratory conditions at 25°C, normal photoperiod (12 hours dark/12 hours light) and standard chow diet.
- Adult rabbits of either sex with average weight 1.5-2.5 kg were used for in-vitro experiments.

**Experimental protocols:**

**In vivo protocol:**

About 70 rats were randomly allocated into three main groups and, each subgroup included 10 rats:

1. **Prophylaxis group (30 rats):** The rats were given medications p.o. via gastric gavage daily for 15 days before ligation of the LAD.
   - Group 1: Control prophylactic: The rats were given saline 5ml/kg.
   - Group 2: Ivabradine prophylactic: The rats were given ivabradine 5mg/kg dissolved in 5ml saline [14].
   - Group 3: Propranolol prophylactic: The rats were given propranolol 5mg/Kg dissolved in 5ml saline [14].

2. **Treated group (20 rats):** The rats were given medications intraperitoneal 5min after ligation of the LAD.
Group 4: Control treated: The rats were given saline 1ml/kg.

Group 5: Ivabradine treated: The rats were given ivabradine 1mg/kg dissolved in 1ml saline [15].

3- Pre-reperfusion group (20 rats): The rats were given medications intraperitoneal 5 minutes before reperfusion.

Group 6: Control Pre-reperfusion: The rats were given saline 1ml/kg.

Group 7: Ivabradine Pre-reperfusion: The rats were given ivabradine 1mg/kg dissolved in 1ml saline [13].

In vivo myocardial ischemia/reperfusion:

Myocardial ischemia and reperfusion injury was performed as previously described [16]. After opening the chest, the left main coronary artery was occluded for one and half hour, then the occlusion was released and reperfusion was allowed for two hours. Coronary artery occlusion was verified by epicardial cyanosis and by observing the ischemic ECG change recorded on the PowerLab.

The changes in the ECG as regard the heart rates and ST segment levels were recorded at the onset of each experiment, 5 minutes, 15 minutes and 1.5 hours after ligation of the LAD in the ischemic groups; in the reperfusion groups, measurements were done at the start of the experiment, 1.5 hours after ligation of LAD artery and 20 minutes after reperfusion.

At the end of each experiment, the heart was removed as a whole and put on a filter paper, separated from adjacent tissues and blood vessels and placed in 10% neutral formalin. After 24 hours, the heart was removed from formalin. The ventricles were sectioned 1mm below the site of ligation, in a plane parallel to the atroioventricular groove. Sections of the heart were cut at a thickness of 5-6 µm stained with Hematoxylin-Eosin stain and examined under the light microscope [17].

In-vitro protocol:

About 20 rabbits were used for determining the direct actions of ivabradine on the isolated perfused mammalian heart and aortic strip of rabbit; each experiment was repeated six times.

Isolated perfused mammalian heart:

The rabbit heart was mounted as by the method described by Burn (1952) [18].

Threads were attached to the most contractile area of the ventricle by a small clip and contractions were recorded on a PowerLab. The effects of gradually increasing doses of ivabradine on the inotropic and chronotropic properties of the heart were recorded.

In vitro aorta study:

Smooth muscle contractility was studied in rings of thoracic aorta isolated from rabbits according to the method described [19].

Ivabradine was tested for its spasmogenic effect on the isolated rabbit aortic ring. Dose response for phenylephrine was recorded and the relaxant effect of cumulative doses of ivabradine on top of the submaximal dose of phenylephrine were measured. The response was recorded on a PowerLab.

Statistical methods:

Quantitative Data were statistically described in terms of mean ± standard deviation (mean ± SD) qualitative data were described in percentage. A probability value (p-value) less than 0.05 was considered statistically significant. Group mean values were compared by 1-tail Student’s t-test or one-way analysis of variance, as appropriate. All statistical calculations were done using Computer package SPSS 9.0.

Results

In-vivo:

When ivabradine and propranolol were given prophylactically daily for 15 days before ligation of the LAD, they resulted in significant reduction in the heart rate (bpm), ST segment level (mv) measured at the onset of each experiment, 5 minutes, 15 minutes and 1.5 hours after ligation of the LAD in the ischemic groups; in the reperfusion groups, measurements were done at the start of the experiment, 1.5 hours after ligation of LAD artery and 20 minutes after reperfusion.

Also in the ivabradine treated either given intraperitoneal 5min after ligation of the LAD or before reperfusion, when measured at the start of the experiment, 1.5 hours after ligation of LAD artery and 20 minutes after reperfusion, the heart rate (bpm), ST segment level (mv) (Table 1, Figs. 1, 2) and size of myocardial infarction were progressively reduced (p<0.05) (Figs. 3,4).
Ivabradine given prophylactically daily for 15 days before ligation of the LAD was more effective than ivabradine treatment given 5 min after ligation of the LAD in reducing the heart rate, ST segment level elevation as well as reduction in myocardial infarction size after 1.5 hours from ischemia \( (p < 0.05) \) (Table 1, Figs. 1-4).

*In-vitro*:

Ivabradine in the studied doses did not show neither negative nor positive inotropic effects in the Langendorf’s coronary perfusion isolated rabbit's heart. Ivabradine dose dependent (0.5-8 ug/ml) decrease in heart rate amounting to 15.2%, 23.9%, 37.1%, 45.5% and 45% respectively (Fig. 5).

Ivabradine showed no spasmogenic effect on isolated rabbit aortic ring. In cumulative doses up to 64 ug/ml ivabradine produced relaxant effect on phenylephrine induced rabbit aortic contraction (Fig. 6).

Fig. (1): Percentage reduction in the heart rate and the ST segment level in the studied groups. \( p < 0.05 \) against ivabradine prophylactic.
Prophylactic groups: Group 3-Propranolol subgroup.

Treated group: Control group 4.

Treated group: Ivabradine group 5.

Pre-reperfusion groups: Control group: Group 6.

Pre-reperfusion groups Ivabradine group 7.

Fig. (2): ECG changes in the groups studied.
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Fig. (3): Percentage reduction in myocardial infarction size in comparison to controls. p < 0.05 against ivabradine prophylactic.

Fig. (4): Pathological changes in the myocardium: a) Normal myocardium stained uniformly pink with blue nuclei (H & E x100). b) Infarcted myocardium showing cytomegaly and karyopyknosis (H & E x100).

Fig. (5): Ivabradine dose dependent (ug/ml) decrease in heart rate in the Langendorff’s coronary perfusion isolated rabbit’s heart.

Fig. (6): Effect of cumulative doses of Ivabradine on the submaximal contraction induced by phenylephrine in isolated aortic ring of rabbit.
Table (1): Effect of ivabradine and propranolol on the heart rate (bpm) and the ST segment level (mv) as well as their percentage reduction in relation to controls in the studied rats.

<table>
<thead>
<tr>
<th>Groups Base</th>
<th>Heart rate (bpm) and ST segment level (mv) Mean ± S.D. and % Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
</tr>
<tr>
<td>Control prophylactic</td>
<td>313.29±7.91</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
</tr>
<tr>
<td>Ivabradine prophylactic</td>
<td>251.20±2.5</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
</tr>
<tr>
<td>Propranolol prophylactic</td>
<td>248.00±2.44</td>
</tr>
<tr>
<td>Group 4</td>
<td></td>
</tr>
<tr>
<td>control treated</td>
<td>323.50±6.025</td>
</tr>
<tr>
<td>Group 5</td>
<td></td>
</tr>
<tr>
<td>Ivabradine treated</td>
<td>310.83±8.68</td>
</tr>
<tr>
<td>Group 6</td>
<td></td>
</tr>
<tr>
<td>Control Pre-reperfusion</td>
<td>313.83±5.4</td>
</tr>
<tr>
<td>Group 7</td>
<td></td>
</tr>
<tr>
<td>Ivabradine Pre-reperfusion</td>
<td>310.83±4.40</td>
</tr>
</tbody>
</table>

*p<0.05 against controls. Ap<0.05 against ivabradine prophylactic.
**Discussion**

The present study provides guide on the beneficial effects of pure heart rate reduction in the prophylaxis against ischemia, post-infarcted and reperfused myocardium. Ivabradine-induced heart rate reduction protects against myocardial infarction and reperfusion by reducing significantly ST segment level and the infarction size which was more with ivabradine prophylactic than with propranolol prophylactic or ivabradine treatment. Ivabradine in the studied doses did not show neither negative nor positive inotropic effects. Ivabradine showed no spasmogenic effect on isolated rabbit aortic strip. While in cumulative doses it produced relaxant effect on phenylephrine induced rabbit aortic contraction.

Large-scale epidemiological studies, such as the Framingham Heart Study, showed that high resting heart rate correlates with increased all-cause and cardiovascular mortality [20]. Several mechanisms can explain this phenomenon. Accelerated heart rate is involved in the progression of atherosclerosis through mechanical and metabolic processes. Increased wall stress, resulting from tachycardia, may induce endothelial injury and easier penetration of lipids into the vessel wall, a mechanism which could also explain the higher incidence of atherosclerotic plaque disruption and new acute coronary events in patients with high resting heart rate [21,22]. Elevated heart rate usually reflects activation of the sympathetic nervous system with its attendant deleterious metabolic effects resulting in accelerated atherosclerosis. Reduction in HR under these circumstances is therefore highly beneficial.

Ivabradine therefore offers a valuable approach to lowering heart rate exclusively and provides an attractive alternative to conventional treatment for a wide range of patients with confirmed stable angina.

As opposed to a number of other pharmacological agents, ivabradine expresses high selectivity and specificity for its target. Ivabradine exerts a unique action on cardiac pacemaker activity, based on its block of the hyperpolarization-activated, cyclic nucleotide-gated channels that pass the pacemaker current, If. In doing so, it suppresses but does not stop the sinoatrial pacemaker’s rate of firing. Investigation has shown that block by ivabradine requires open If-channels, is use dependent, and is affected by the direction of current flow [14].

Both ivabradine and β-blockers have favorable effects on the myocardium, e.g., they enhance exercise tolerance and inhibit ischemia [23-25]. Unlike β-blockers, ivabradine does not have negative inotropic or negative lusitropic effects [26]. However, several studies, including the present study, have documented advantages of ivabradine over β-blockers. Ivabradine shares with β-blockers the property of decreasing heart rate and oxygen demand from the ischemic heart, which is presumably fundamentally important in mediating anti-ischemic effects. Colin et al. [27] compared the effects of ivabradine, atenolol, and placebo on oxygen consumption and supply during exercise in dogs. For a given heart rate, left ventricular ejection time was longer with atenolol than with ivabradine because of the negative inotropic effects of atenolol. As a consequence, the increase in diastolic time and coronary blood flow was greater with ivabradine than with atenolol. Furthermore, in contrast to β-blockers, ivabradine has also been shown not to limit the decrease in coronary resistance induced by exercise [28].

A small double-blind study, comparing the effect of ivabradine and propranolol on cardiac hemodynamic at rest and during exercise was conducted by Joannides et al. [29] with nine healthy volunteers receiving ivabradine or propranolol or placebo. This study demonstrated that administration of ivabradine reduced myocardial oxygen demand at the same degree as propranolol but without negative inotropic effect.

Michal and Urszula [30] showed that metoprolol and ivabradine, in doses producing a similar heart rate reduction in the post-MI rats, result in comparable preservation of LV systolic function.

Patients with acute myocardial infarction generally benefit from heart rate reduction both through a decrease in myocardial oxygen requirement and a lengthening in the duration of diastole and myocardial perfusion. However, the negative inotropic and hypotensive effects of β-blockers contraindicate their use in patients with pulmonary congestion, borderline blood pressure, overt pulmonary edema, or cardiogenic shock. Ischemic left ventricular dysfunction is also often accompanied by diastolic dysfunction. Because excessive tachycardia has deleterious consequences on diastolic function, heart rate reduction is important to achieve. Such patients who require heart rate reduction would clearly benefit from the lack of this side effect with ivabradine [31].
BEAUTIFUL results (morBidity-mortality EvaluaTion of the If inhibitor ivabradine in patients with CAD and left ventricular dysfunction) with ivabradine can be explained by its well documented ability to relieve myocardial ischemia in patients with chronic stable angina [32]. New research has demonstrated that ivabradine improves endothelial dysfunction [33] and prevents the progression of atherosclerosis.

Despite all the advances, the World Health Organization reports [34] that till 2030 coronary artery disease will remain the leading healthcare problem worldwide. Ivabradine would help to reduce this burden because as shown by the BEAUTIFUL study, ivabradine reduce the risk of myocardial infarction and revascularization. Half of the CAD patients have a resting heart rate more than 70 bpm. These patients can now benefit from a treatment that will greatly reduce their chances of having another heart attack or needing further surgery [32].

It is generally accepted that reperfusion of the heart after a period of ischemia causes oxidative damage that is indicated by generation of free radicals, intracellular calcium overloading and loss of membrane phospholipids. At the onset of reperfusion, the mitochondrial respiratory rate is increased markedly and greater quantities of free radicals are generated. Cardiac myocytes, endothelial cells, and infiltrating neutrophils contribute to this enhanced reactive oxygen species (ROS) production. These radicals may exceed the capacity of the cellular intrinsic free radical scavenging systems and lead to cellular dysfunction and death [35].

Regarding the effects of ivabradine in the present study, one can conclude that part of its protective effects are directly related to heart rate reduction per se observed after the single administration of ivabradine intraperitoneal 5 minutes before reperfusion. Similar acute changes have been previously reported in the stunned myocardium [12,36]. However, one cannot rule out the possibility of pleiotropic properties of ivabradine beyond pure heart rate reduction. Indeed, a previous report has demonstrated in an established pig model of regional myocardial ischemia reperfusion that the protective effects of ivabradine on myocardial infarction were only partially reversed by atrial pacing [13,37]. Unfortunately, no data have been reported so far on the potential persistence of ivabradine’s beneficial effects on post-myocardial infarction remodelling when heart rate is not reduced [38].

The fact that Ivabradine was still protective when given before reperfusion pointed towards mechanisms involved in attenuation of reperfusion injury and post conditioning [37,39]. The mechanism(s) underlying the pleiotropic protection by Ivabradine were largely unclear; attenuation of damage by reactive oxygen species and reduced calcium exchange and ultimate reduction in calcium overload had been suggested [38,40].

A study was done by Couvreur and his coworker [41]. In their study rabbits underwent 20 minutes of coronary artery occlusion followed by 3 weeks of reperfusion. Throughout reperfusion, rabbits received ivabradine 10mg/kg/day or vehicle. The authors noted that chronic heart rate reduction protected the myocardium against ventricular dysfunction induced by myocardial ischaemia followed by 3 weeks of reperfusion. Beyond pure heart rate reduction, ivabradine improved global and regional systolic function of the reperfused heart through a dual mechanism involving a direct mechanical effect and a long-term adaptation in calcium handling.

The present study could prove that ivabradine produced significant anti-spasmodic effect on the pre-contracted isolated rabbit aortic strips. In the context of atherosclerosis and vascular disease in more general, the functional role of If-carrying channels and potential targets of ivabradine remain to be elucidated. Two recent studies have reported effects of ivabradine on vascular function. In dyslipidaemic mice, the impairment of Acetylcholine-induced, endothelium-dependent vasodilation of cerebral and renal arteries was restored by ivabradine, but not by metoprolol at equal heart rate reduction, suggesting that ivabradine's action was not related to heart rate reduction [42]. Again in dyslipidaemic mice, cholinergic endothelium-dependent vasodilation was restored by ivabradine; vascular NADPH oxidase activity and free radical production as well as atherosclerotic lesion formation were reduced. In this particular study, a direct effect of ivabradine on vascular function and free radical formation was not observed and thus ivabradine's action was related to attenuation of vascular shear stress along with heart rate reduction [43]. Overall, the findings of the previous two studies showed that long-term HR reduction with ivabradine improved endothelial function and reduced progression of atherosclerosis in mice models of dyslipidemia and atherosclerosis.

In conclusion, this experimental study supports the concept that pharmacological reduction of heart rate with the selective If current blocker ivabradine
is beneficial in limiting the progression of both ventricular and vascular dysfunctions.

Long-term studies are required to confirm whether pure HR reduction translates into decreased mortality in patients with angina, heart failure and other cardiovascular disease. More mechanistic analyses on the benefits from ivabradine in the setting of myocardial reperfusion must be carried out.

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References

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41- COUVREUR N., TISSIER R., PONS S., CHETBOUL V., GOUNI V., BRUNEUVAL P. and MANDET C.: Chronic heart rate reduction with ivabradine improves systolic fuction of the reperfused heart through a dual mechanism involving a direct mechanical effect and a long-term increase in FKB12/13.6 expression. European Heart Journal, 31 (12): 1529-37, 2010.
