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Original article

Cardioprotective Effect of Ranolazine in the Process of Ischemia-reperfusion in Adult Rat Cardiomyocytes

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ABSTRACT

Introduction and objectives: Ranolazine is used as a complementary treatment for angina in symptomatic patients who are inadequately controlled with first-line antianginal therapies. Ranolazine inhibits sodium voltage-dependent channels, suggesting their possible involvement in the reperfusion process by preventing the sodium and calcium overload that occurs during ischemia. In this study, we characterized the effect of ranolazine on calcium homeostasis in isolated adult cardiac myocytes from rats subjected to a simulated ischemia and reperfusion protocol.
Methods: The effects of ranolazine on changes in intracellular calcium concentration were evaluated at different times using field electrostimulation. The study of intracellular calcium was performed using microfluorimetry with the fluorescent indicator, Fura-2, and by confocal microscopy with the indicator, Fluo-3.
Results: We found that cardiomyocytes subjected to ischemia-reperfusion showed an increase in the diastolic calcium concentration and a decrease in the amplitude of intracellular calcium transients. The application of ranolazine during ischemia significantly improved intracellular calcium handling, preventing intracellular calcium overload, decreasing the diastolic calcium concentration, increasing the sarcoplasmic reticulum calcium load, and preserving the amplitude of the intracellular calcium transient, which was reflected by successful recovery in the process of excitation-contraction coupling during reperfusion. However, these effects of ranolazine did not occur when it was applied during reperfusion or when applied in both ischemia and reperfusion.
Conclusions: Ranolazine shows beneficial effects in cardiomyocytes exposed to ischemia/reperfusion but only when applied during ischemia. This effect is achieved through its improvement of calcium handling during ischemia.

Efecto cardioprotector de la ranolazina en el proceso de isquemia-reperfusión en cardiomiocitos de rata adultos

Palabras clave:
Isquemia-reperfusión
Cardioprotección
Ca^{2+}
Ranolazina

RESUMEN

Introducción y objetivos: La ranolazina se emplea como tratamiento complementario de la angina en pacientes sintomáticos insuficientemente controlados con los tratamientos antianginosos de primera línea. La ranolazina inhibe los canales de sodio operados por voltaje, lo cual indica su posible intervención en el proceso de reperfusión al prevenir la sobrecarga de sodio y calcio que se produce durante la isquemia. En este estudio, se ha caracterizado el efecto de la ranolazina en la homeostasis del calcio en miocitos cardiacos adultos de ratas a las que se aplicó un protocolo de isquemia y reperfusión simuladas.
Métodos: Se evaluaron los efectos de la ranolazina en los cambios de la concentración de calcio intracelular en diferentes momentos empleando electroestimulación de campo. El estudio del calcio intracelular se llevó a cabo mediante microfluorimetría utilizando el indicador fluorescente Fura-2 y por microscopia confocal utilizando el indicador Fluo-3.
Resultados: Se observó que los cardiomiocitos a los que se aplicaba la isquemia-reperfusión mostraban un aumento de la concentración de calcio diastólica y una disminución de la amplitud de los transitorios de calcio intracelular. La aplicación de la ranolazina durante la isquemia mejoró significativamente la regulación del calcio evitando la sobrecarga de calcio intracelular, reduciendo la concentración de calcio

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diastólica, aumentando la carga de calcio en el retículo sarcoplásmico y preservando la amplitud del transitorio de calcio intracelular, lo cual se reflejaba en una recuperación satisfactoria en el proceso de acoplamiento de excitación-contracción durante la reperfusión. Sin embargo, estos efectos de la ranolazina no se produjeron cuando el fármaco se aplicó solo durante la reperfusión o cuando se aplicó tanto en la isquemia como en la reperfusión.

Conclusiones: La ranolazina muestra unos efectos favorables en los cardiomiocitos expuestos a isquemia-reperfusión, pero solo cuando se aplica durante la isquemia. Este efecto se alcanza mejorando la regulación del calcio durante la isquemia.

Abbreviations

I/R: ischemia/reperfusion
I_{NaL}: late Na^+ current
NCX: Na^+/Ca^{2+} exchanger

INTRODUCTION

Ischemic heart disease is the leading cause of death in the western world.[1] The most devastating expression of this disease is ST-segment elevation myocardial infarction, which is due to acute coronary artery occlusion known to cause ischemic myocardial cell death. After ST-segment elevation myocardial infarction, rapid myocardial reperfusion by thrombolytic therapy or primary percutaneous coronary intervention is the most effective strategy to reduce myocardial infarct size and thus improve clinical outcome.[2] Reperfusion therapy has a substantial impact on early mortality after ST-segment elevation myocardial infarction. However, a high percentage (20% to 30%) of patients develops adverse remodeling in our hospital.[3] Early and effective reperfusion limits the extent of myocardial necrosis by reducing the incidence of left ventricular remodeling and dysfunction, but even with the best reperfusion therapy, paradoxically, a proportion of cardiomyocytes dies due to restoration of blood flow.[4] This phenomenon is called ischemia/reperfusion (I/R) injury.[5]

Oxygen deprivation and calcium overload during cardiac ischemia and reactive oxygen species production during reperfusion cause cardiomyocyte death by necrosis and apoptosis.[6] The major adverse changes that occur in the ischemic myocardium consist of an increase of intracellular Na^+ concentration due to failure of the sarcolemma Na^+/K^+ pump in the absence of energy and acidification of cytosol by anaerobic glycolysis. The cell attempts to solve this intracellular Na^+ increase through the Na^+/Ca^{2+} exchanger (NCX) acting in the reverse mode, extruding Na^+ and introducing Ca^{2+} inside the cells. This leads to an intracellular Ca^{2+} overload and a mishandling of Ca^{2+} by the cells.[7]

Ranolazine is a piperazine derivative with a novel mechanism of action that was first approved by the Food and Drug Administration in 2006 for the symptomatic treatment of patients with chronic angina. Due to its pharmacological properties, it is able to block the late Na^+ current (I_{NaL}) in cardiomyocytes and steer the oxidation of fatty acids toward glucose oxidation, making oxygen use more efficient in the heart.[8,9] However, the mechanism of action of ranolazine is not yet precisely known. The beneficial effects of ranolazine reside in its action of reducing the Na^+ influx into myocardial cells through Na^+ channels, which in pathologic situations fail during their inactivation or else they open again.[10] Ranolazine has also been suggested to decrease calcium overload in myocardial cells during ischemia by blocking the I_{NaL} current.[9] At therapeutic plasma concentrations ($\leq$ 10-21 mmol/L), ranolazine selectively inhibits I_{NaL}, reduces intracellular accumulation of Na^+ and subsequent Na^+-induced Ca^{2+} overload, as well as mechanical, electrical, and metabolic abnormalities in ischemic or insufficient myocardium.[11] However, at this concentration, ranolazine does not alter the peak of Na^+ current responsible for step 0 of the action potential, the input current of Ca^{2+}, or the activity of NCX and Na^+/H^+ exchanger.[12]

Nowadays, the use of ranolazine has been approved as an adjunctive therapy for symptomatic angina in patients who are inadequately controlled with first-line antianginal therapies.[12] The development of a substance capable of inhibiting or reducing the deleterious effects of a pathological increase in intracellular Ca^{2+} concentration in cardiomyocytes during ischemia processes would be a hugely important clinical and therapeutic contribution. In this study, we hypothesized that ranolazine might have a novel action in reperfusion procedures, preventing the Na^+ and Ca^{2+} overload that occurs in ischemic hearts and helping cells to improve Ca^{2+} handling at reperfusion.

METHODS

Animals were handled in accordance with the recommendations of the Royal Decree 53/2013 in agreement with Directive 2010/63/EU of the European Parliament. The study was approved by the local Ethics Committee on Human Research of the Virgen del Rocío University Hospital of Seville and the Animal Research Committee of the University of Seville.

Isolation of Ventricular Myocytes

We used adult male Wistar rats weighing approximately 250 g to 350 g, which were previously heparinized (4 IU/g intraperitoneally) and anesthetized by intraperitoneal administration of sodium thiopental (1 mL/250 g). The heart was quickly removed and mounted on a Langendorff perfusion system with a constant flow. Ventricular myocytes were isolated by perfusion using type II collagenase (251 IU/mL, Worthington Biochemical; Lakewood, New Jersey, United States).[13] Cardiomyocytes were maintained in the Tyrode solution (mM): 140 NaCl, 4 KCl, 1.1 $MgCl_2$, 10 HEPES, 10 glucose, 1.8 $CaCl_2$ (pH 7.4), supplemented with 1.8 mM $CaCl_2$. All experiments were conducted on rod-shaped cells at room temperature (24 °C to 26 °C).

Intracellular Ca^{2+} Measurement With Microfluorimetry

Intracellular Ca^{2+} transients were recorded using the imaging system Incyt hight speed Im2 (Intracellular Imaging Inc.; Imsol, United Kingdom) in freshly isolated cardiomyocytes loaded with the fluorescence Ca^{2+} dye, Fura-2AM. During experiments, cells