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Comparative Analysis of the Effects of Omeprazole and Ischemic Preconditioning in Protection against Ischemia and Reperfusion Myocardial: Experimental Study in Isolated Rat Hearts

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Abstract

Introduction: Myocardial protection against ischemic injury has been a major focus of studies of cardiovascular sciences worldwide. In 1986, Murry et al. became pioneers by showing a special ischemic preconditioning technique in hearts of dogs which motivated researches for better understanding of endogenous protective mechanisms of the human heart. The aim of this study was to evaluate the effects of omeprazole on protection of functional recovery of isolated rat hearts subjected to ischemia-reperfusion with and without ischemic preconditioning (IP).

Methods: In five groups of eight Wistar breed rats, the hearts were removed after anesthesia and perfused with Krebs-Henseleit solution (95% O_2, 5% CO_2, 37ºC). The GI was a control group. The GII, GIII, GIV and GV, hearts were submitted to ischemia (20 min) and reperfusion (30 min). In GIV and GV, preconditioning was performed with 5 min of ischemia and 5 min of reperfusion before 20 min of the ischemia period induction. In GIII and GV Omeprazole 200 µg was done before a 20 min-period of ischemia induction. Heart Rate (HR), Coronary Flow (CF), Systolic Pressure (SP), +dP/dt and−dP/dt were registered before (t0) and after reperfusion (t30). Kruskal-Wallisand Mann - Whitney ($p<0.05$) test were used.

Results: The CF analysis showed that the Groups II, III, IV and V, had a similar behavior analyzed over time ($p=0.316$). Group I presented the averages 18.6, 17.5 and 16.6 at t0, t15' and t30' respectively, with significant changes in the pattern of behavior analyzed over time ($p<0.001$). There were no significant differences in the HR, SP, $+dP/dt_{max}$, and $-dP/dt_{max}$ between Groups I, III, IV and V results.

Conclusion: Omeprazole conferred preconditioning characteristics to isolated rat hearts subjected to ischemic injury. There was no greater efficacy of protection shown in relation to existing methods of ischemic preconditioning. There was no synergism in the use of omeprazole in conjunction with the methods of IP.

Keywords: Omeprazole; Ischemic preconditioning; Myocardial ischemia

Introduction

The myocardial protection refers to the strategies used to mitigate or prevent post-ischemic myocardial dysfunction, a major degenerative diseases of the heart and constant concern of the objectives of all clinical and surgical cardiology since its origins.The ischemic preconditioning (IP) is an endogenous protective mechanism, first described in dog hearts [1] and subsequently demonstrated in humans [2]. Considering the therapeutic possibilities of IP, it is appropriate to expand the knowledge on the effects of drugs against it, not to damage or even enhance its protective mechanism. The pump inhibitors, H^+/K^+ ATPase, are widely prescribed drugs in clinical medicine for ordinary people. The pioneering studies by Lindberg et al. [3] in 1986, on the action of inhibitors of proton pump H^+/K^+, showed a new line of research and, in 1994, Nagashima et al. [4] was able to prove the existence of active sites of proton pumps, H^+/K^+ ATPase in the myocardium of guinea pigs [5]. In 1998, studies by Hotta et al. [6], using fluorometry and magnetic resonance imaging, in hearts of guinea-pigs, showed ionic and also pH alterations during ischemia and reperfusion. The pump inhibitors including omeprazole used in this study showed myocardial protection by blocking acute ionic changes in the cardiomyocytes. Recently, in 2008, studies by Budzynski et al. [7], showed the beneficial effects of omeprazole in the protection of angina attacks in coronary patients undergoing an exercise stress test.

New experimental studies have recently shown the influence of these drugs on ischemic preconditioning of the heart [8,9]. This may cause a major impact in the clinical and surgical cardiovascular area. However, there are still no specific experimental demonstrations showing the comparative analysis of the effects of omeprazole and ischemic preconditioning in protection against ischemia-reperfusion myocardial.The aim was to analyze the effects of omeprazole in the protection of the functional recovery of isolated rat hearts subjected to ischemia-reperfusion with and without ischemic preconditioning.

Materials and Methods

The guidelines of the Brazilian College of Animal Experimentation (COBEA) [10], were respected when conducting this experimental study. The Ethics Committee of the local institution also approved all the experimental project.

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Forty male albino hearts of the Wistarbreed rats were isolated and after sedation by inhalation of ethyl ether and anesthesia with an intra-abdominal injection of 10 milligrams of ketamine and 2 milligrams of xylazine, the hearts were removed and maintained in antegradeperfusion with Krebs-Henseleit solution (95% O_2, 5% CO_2, 37°C and 110-120 mmHg perfusion pressure and diastolic pressure of 8 mm Hg) using the modified and disposable Langendorffsystem, model FCSFA-ServCor (COMEX Ind & Com Ltda - Belo Horizonte - MG) (Figure 1).

The 3-wire technique was used to ensure isolation of the ascending aorta [11]. The first wire pulls and lifts the aortic root to protect the aortic valve, the second wire pulls and lifts the edge (top) of the distal ascending aorta, and the third wire is passed between the first two and promotes the ligature of the aortic cannula set, immediately after cannulation of the aorta (Figure 2). A perfusion cannula was introduced cautiously and secured in the ascending aorta to prevent damage to the aortic valve leaflets (Figure 3). Later on, an incision was made in the left atrium, and the heart was transfixed by a multiperforated cannula which used the apex of the left ventricle as outflow (Figure 4). Another incision was made in the pulmonary artery for the purpose of draining the right ventricle. Then, the hearts were removed, 3 minutes after the procedure at most, and after 10 minutes of reperfusion, the multiperforated cannula was replaced by a balloon catheter to better evaluation of ventricular function.

The specimens were randomly divided into five groups of eight hearts each. Initially, all isolated hearts were subjected to a stabilization period of fifteen minutes. Then the specimens in Group I were perfused for thirty minutes counted from the end of the stabilization. Groups II and III were subjected to twenty minutes of ischemia followed by thirty minutes of reperfusion, and the Group III received 200 micrograms (μg) of omeprazole before ischemia. The isolated hearts of Groups IV and V were subjected to IP, one cycle consisting of a 5-minutes ischemia and a 5-minutes reperfusion, followed by an ischemia prolonged period of 20 minutes, on the other hand, the Group V received 200 μg of omeprazole immediately prior to prolonged ischemia (Figure 5). The variables Heart Rate (HR), Coronary Flow (CF), Systolic Pressure (SP), Positive

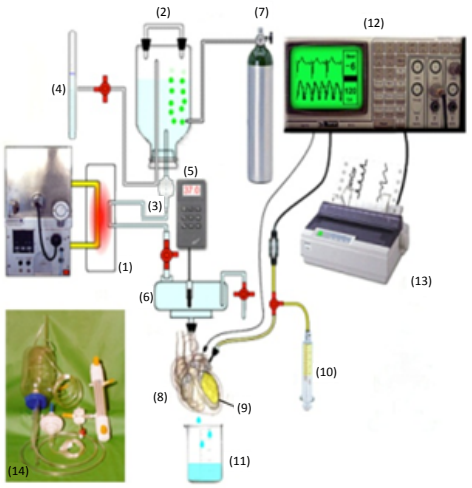

Figure 1: Isolated Heart System Diagram – 1.Terminal exchanger. 2. Perfusate reservoir. 3. Microfilter (20 i). 4. Gauge/Manometer. 5. Telethermometer. 6. Chamber. 7. Carbogen (95% O2 + 5% CO2). 8. Heart. 9. Intraventricular balloon. 10. Drug injector. 11. Flow Collector 12. ECG and Ventricular Pressure monitor. 13. Printer. 14. Disposable set

Figure 2: Ascending aorta isolated (3-wire technique).

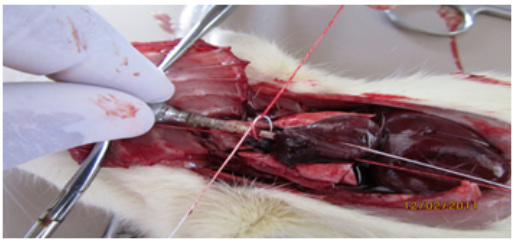

Figure 3: Aorta cannula fixation.

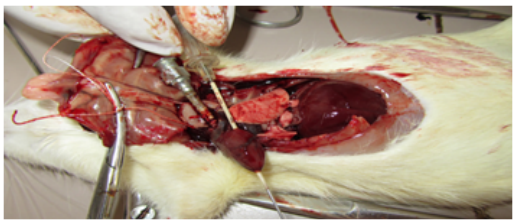

Figure 4: Multiperforated cannula through the left ventricule apex.

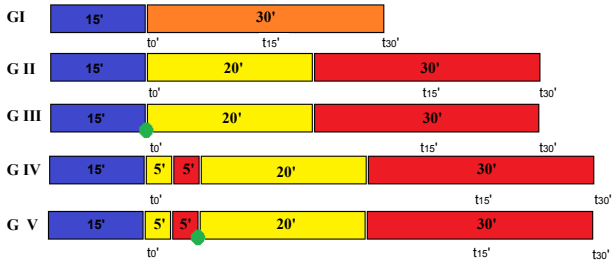

Figure 5: Stabilization Perfusion Ischemia Reperfusion Administration of 200 μg of omeprazole antegrade. Algorithm of different groups studied I- Control; II- Ischemia; III- Ischemia + 200 μg of omeprazole; IV- Preconditioning; V- Preconditioning + 200 μg of omeprazole. t0 = First collect the corresponding hemodynamic variables after the stabilization period. The times t15't30' and represent the collection times of the variables of each group.

First Time Derivative of the Left Ventricular Pressure ($+dP/dt_{max}$) and Negative First Time Derivative of the Left VentricularPressure ($-dP/dt_{max}$) were obtainedat three time points: t0, t15' and t30'.

Statistical methods

The statistical methods to analyze the effect of group was the Kruskall-Wallis test followed by Mann-Whitney was applied in

specific comparisons of groups. A p-value <0.05 was considered to be statistically significant.

Results

Groups I, III, IV and V showed a pattern of similar behavior (p=0.05) regarding HR. In each group the corresponding hemodynamic variables were measured at equal time intervalst0, t15' and t30' respectively (Table 1, Chart 1). The CF analysis showed that the Groups II, III, IV and V, had a similar behavior analyzed over time (p=0.316). Group I showed a pattern different from the others, with statistical significance for all the analyzed time (p<0.001) (Table 2, Chart 2).

In the study of SP,the groups I, III, IV and V, showed the same behavior at t0, t15' and t30' (p=0.345). Group II showed significant alteration of SP throughout the observed stages (p<0.001) (Table 3, Chart 3).

The corresponding hemodynamic variable statistic $+dP/dt_{max}$, concluded that there was the same pattern of behavior (p=0.511) between Groups I, III, IV and V. The Group II differs from other groups and presents significant change in $+dP/dt_{max}$ evaluated over time (p<0.001) (Table 4, Chart 4).

The evaluation of $-dP/dt_{max}$, showed similarity in behavior (p = 0.634) in Groups II and III. Groups II andIII showed significant alteration of $-dP/dt_{max}$ throughout the evaluations (p<0.001). Groups I, IV and V showed significant differences in analyzed mean times (p=0.005) (Table 5, Chart 5).

Discussion

Myocardial ischemia can cause serious damage to cardiomyocytes and sometimes the damage can be irreversible. The myocardial reperfusion also causes damage, which may result in fatal arrhythmias. The magnitude of reperfusion injury is directly proportional to the prior ischemic injury [12]. In 1986, Murry et al. [1] showed an important technique for myocardial protection from ischemic events, which he called ischemic preconditioning. The pump inhibitors H^+/K^+ pioneer studies used in Lindberg et al. [3], opened a new line of research, and Nagashima's et al. [4] work, in 1994, showed the presence of proton pump H^+/K^+ ATPase in human heart cells. Studies by Moffat and Karmazyn [13] were able to show good results in myocardial protection against ischemia and reperfusion through drugs inhibiting the Na^+/H^+ ATPase. This research resulted in multicenter studies involving the so-called drugs amiloride and cariporida (channel blocking $Na^+/$

GROUP	t_0	$t_{15'}$	$t_{30'}$
I	281,6	289	284,7
II	255,5	213	186,3
III	272,5	250,6	266,8
IV	274,3	268,1	267,5
V	287,5	283,7	283,1

Table 1: Change Average Heart Rate (bpm).

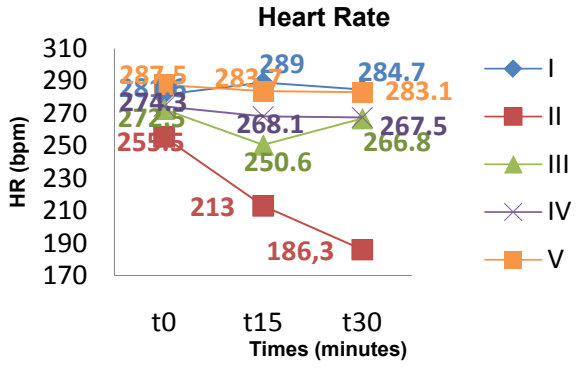

Chart 1: Change Average Heart Rate (bpm)

GROUP	t_0	$t_{15'}$	$t_{30'}$
I	18,6	17,5	16,6
II	18,2	14,5	13,5
III	18,8	15,5	15,7
IV	19,7	16,3	15,5
V	19,3	16,3	15,3

Table 2: Change Average Coronary Flow (CF).

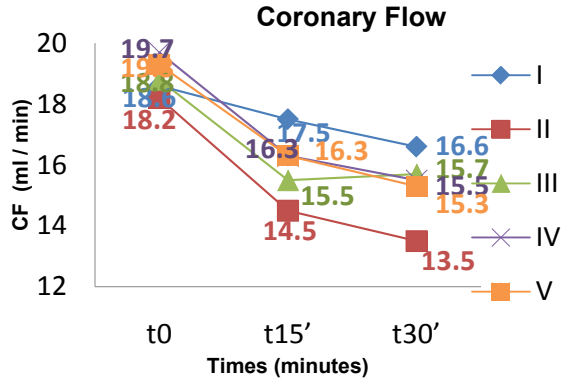

Chart 2: Change Average Coronary Flow (CF)

GROUP	t_0	$t_{15'}$	$t_{30'}$
I	127,8	123,5	114,5
II	134,3	47,1	50,3
III	162,8	131,7	128,0
IV	149,7	124,5	118,7
V	152,8	129,1	129,1

Table 3: Change Average Coronary Flow (CF)

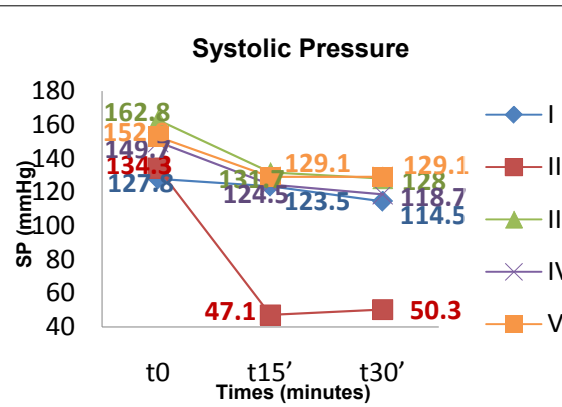

Chart 3: Change Average Systolic Pressure (SP)

H^+), which confirmed good protection of cardiomyocytes. These good results can be associated with studies using ischemic preconditioning and also the benefits from the use of omeprazole, an inhibitor pump H^+/K^+ ATPase initially used for reduction of gastric hyperacidity [8,17-20]. The explanation for the action of omeprazole in the myocardium may be related with the changes of transmembrane H^+/K^+ and flags of ischemic electrocardiographic tracings which action is expressed in the morphological changes of the T wave [21].

Recent experimental study demonstrated the protective pump inhibitor for H^+/K^+ ATPase against ischemia and reperfusion [8]. Subsequently, a new experimental study with pump inhibitor H^+/K^+ ATPase pantoprazole conferred preconditioning characteristics to isolated rat hearts subjected to ischemic injury [9]. There are no studies on the comparative analysis of omeprazole and ischemic preconditioning in the literature consulted.

In the current investigation no differences ($p>0.05$) were found between groups regarding HR results. The SP, $+dP/dt_{max}$, and $-dP/dt_{max}$ differences from t0, t15' t30' was significant ($p<0.05$) in all groups but regarding SP, $+dP/dt_{max}$ and $-dP/dt_{max}$ after the reperfusion period differences occurred between the results of Group II and Groups I, III , IV and V with SP averages reduced to 89% in the 30 minutes of reperfusion (t30') in GI, 37% in GII, 79% in GIII, 79% in GIV and 84% in GV. The $+dP/dt_{max}$ declined to 88% (t30') in GI; 29% (t30') in GII; 89% (t30') in GIII , 83% (t30') in GIVand 84% (t30') in GV. The $-dP/dt_{max}$ declined 96% (t30') in GI; 33% (t30') in GII; 73% (t30') in GIII, 87% (t30') in GIVand 81% (t30') in GV, without no significant differences ($p<0.05$) in the SP, $+dP/dt_{max}$, and $-dP/dt_{max}$ results between Groups I, III, IV and V.The corresponding hemodynamic variable CF, showed a pattern of behavior in the GI different from other groups ($p<0.05$), with CF averages reduced to 89% in the 30 minutes of perfusion (t30 ') in GI and declined to 74% (t30 ') in GII, 83% (t30') in GIII, 79% (t30 ') in GIV and 79% (t30') in GV.

GROUP	t_0	$t_{15'}$	$t_{30'}$
I	3196,8	3062,5	2806,2
II	3346,8	847,6	978,1
III	3476,1	3177,5	3113,1
IV	3631,8	3230,5	3033,8
V	3640,6	2918,1	3054,6

+ $dP/dt_{max\ mmHg.\ min^{-1}}$

Table 4: Change Average+ dP/dt_{max}

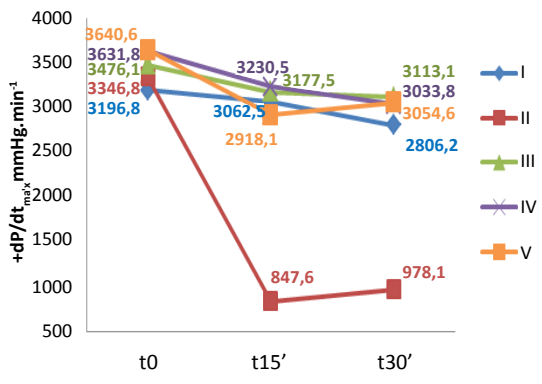

Chart 4: Positive First Time Derivative of the Left Ventricular Pressure

GRUPO	t_0	$t_{15'}$	$t_{30'}$
I	1084,1	1091,6	1040,3
II	1057,8	355,0	353,8
III	1963,6	1421	1434
IV	1435,6	1266,8	1251
V	1564,0	1297,2	1262,3

Table 5: Change Average-dP/dt_{max}

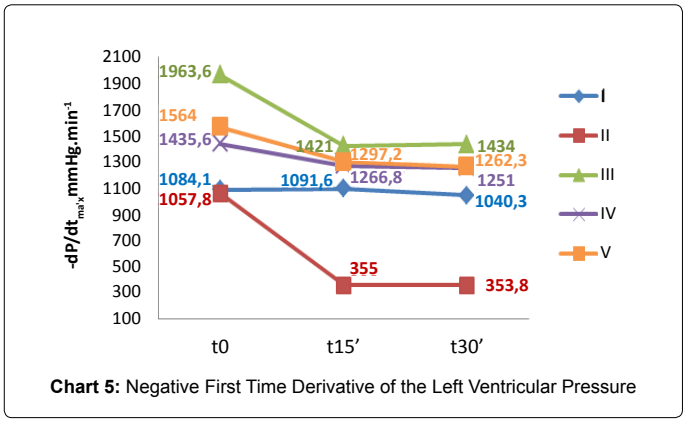

Chart 5: Negative First Time Derivative of the Left Ventricular Pressure

Conclusion

Inconclusion, omeprazole conferred preconditioning characteristics to isolated rat hearts subjected to ischemic injury. There was no greater efficacy of protection shown in relation to existing methods ofischemic preconditioning. There was no synergism in the use of omeprazole in conjunction with the methods of IP.The analysis of the corresponding variables Heart Rate, Systolic Pressure, Positive First Time Derivative of the Left Ventricular Pressure and Negative First Time Derivative of the Left Ventricular Pressure, showed the same behavior when it comes to timings evaluated in Groups I, III, IV and V. None of the authors has declared any conflict of interest in accordance with the results of this study.

References

1. Murry CE, Jennings RB, Reimer KA (1986) Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74: 1124-1136.
2. Yellon D, Alkhulaifi A, Pugsley W (1993) Preconditioning the human myocardium. Lancet 342: 276-277.
3. Lindberg P, Nordberg P, Alminger T, Brändström A, Wallmark B (1986) The mechanism of action of the gastric acid secretion inhibitor omeprazole. J Med Chem 29: 1327-1329.
4. Nagashima R, Odashiro K, Morita S (1994) Evidence for the existence of myocardial H+-K+ATP and its electrophysiological effects. Jpn Heart J 35: 473-474.
5. Nagashima R, Tsuda Y, Maruyama T, Kanaya S, Fujino T, et al. (1999) Possible evidence for transmembrane K(+)-H+ exchange system in guinea pig myocardium. Jpn Heart J 40: 351-364.
6. Hotta Y, Fujita M, Nakagawa J, Ando H, Takeya K, et al. (1998) Contribution of cytosolic ionic and energetic milieu change to ischemia- and reperfusion-induced injury in guinea pig heart: fluorometry and nuclear magnetic resonance studies. J Cardiovasc Pharmacol 31: 146-156.
7. Budzyński J, Kłopocka M, Pulkowski G, Suppan K, Fabisiak J, et al. (2008) The effect of double dose of omeprazole on the course of angina pectoris and treadmill stress test in patients with coronary artery disease--a randomised, double-blind, placebo controlled, crossover trial. Int J Cardiol 127: 233-239.
8. Gomes OM, Magalhães Mde M, Abrantes RD (2010) Myocardium functional recovery protection by omeprazole after ischemia-reperfusion in isolated rat hearts. Rev Bras Cir Cardiovasc 25: 388-392.
9. Gomes OM, Magalhães Mde M, Abrantes RD, Kallás E (2011) Pantoprazole

provides myocardial protection similar to ischemic preconditioning: experimental study of isolated hearts of rats. Rev Bras Cir Cardiovasc 26: 433-439.

10. The Brazilian College of Animal Experimentation (COBEA) (2005) Ethical aspects when using animals in research. Braz oral res 19.
11. Gomes OM, Gomes ES, Carvalho JI, Faraj M (1999) Myocardium functional recovery protection by omeprazole after ischemia-reperfusion in isolated rat hearts. Rev Bras Cir Cardiovasc 9: 36-38.
12. Du XJ, Anderson KE, Jacobsen A, Woodcock EA, Dart AM (1995) Suppression of ventricular arrhythmias during ischemia-reperfusion by agents inhibiting Ins(1,4,5)P3 release. Circulation 91: 2712-2716.
13. Moffat MP, Karmazyn M (1993) Protective effects of the potent Na/H exchange inhibitor methylisobutyl amiloride against post-ischemic contractile dysfunction in rat and guinea-pig hearts. J Mol Cell Cardiol 25: 959-971.
14. Hurtado C, Pierce GN (2000) Inhibition of Na(+)/H(+) exchange at the beginning of reperfusion is cardioprotective in isolated, beating adult cardiomyocytes. J Mol Cell Cardiol 32: 1897-1907.
15. Mentzer RM Jr (2003) Effects of Na+/H+ exchange inhibition by cariporide on death and nonfatal myocardial infarction in patients undergoing coronary artery bypass graft surgery: The EXPEDITION.study. Circulation 108: 3M.
16. Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, et al. (2003) Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol 285: H579-588.
17. Saini HK, Dhalla NS (2006) Modification of intracellular calcium concentration in cardiomyocytes by inhibition of sarcolemmal Na+/H+ exchanger. Am J Physiol Heart Circ Physiol 291: H2790-2800.
18. Gomes OM, Valladares UF, Santos CH, Abrantes RD (2009) Preconditioning abolishion by midazolam in isolated hearts of rats. Acta Cir Bras 24: 173-176.
19. Pinheiro BB, Fiorelli AI, Gomes OM Efeitos (2009) Myocardium functional recovery protection by omeprazole after ischemia-reperfusion in isolated rat hearts. RevBrasCirCardiovasc 24: 31-37.
20. Oliveira DM, Gomes ES, Mussivand T, Fiorelli AI, Gomes OM (2009) Effects of n-acetylcysteine on ischemic preconditioning: study in isolated rat hearts. Rev Bras Cir Cardiovasc 24: 23-30.
21. Gomes OM, Gomes ES, Faraj M (2004) Myocardium stress diskinesia disease. RevBrasCirCardiovasc 19: 378-385.

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