Myocardium functional recovery protection by omeprazole after ischemia-reperfusion in isolated rat hearts

Proteção da recuperação funcional do miocárdio pelo omeprazol após isquemia-reperfusão em corações isolados de ratos

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Abstract

Objective: To evaluate the myocardium contractility alterations of isolated hearts of rats, submitted to ischemia and reperfusion with and without administration of the omeprazol.

Methods: Twelve Wistar breed rats with 270g mean body weight were studied. After anaesthesia by intraperitoneal injection of ketamine 10mg and xilazine 2mg, their hearts were removed and perfused with Krebs-Henseleit solution (95% of O_2 and 5% of CO_2, 37°C, 110-120mmHg perfusion pressure, 8 mmHg ventricular diastolic pressure) in the São Francisco de Assis disposable Langendorff system model Comex Ltda, MG. The six hearts of Group I (GI) and of the Group II (GII) were submitted to 20 min ischemia and 30min reperfusion. In GII hearts, intracoronary injection of omeprazole 200mcg was done immediately before the ischemia period induction. The following parameters were registered after the stabilization period (t0), and after the reperfusion period (t30): heart rate (HR), coronary flow (CoF), systolic pressure (SP), +dP/dt and -dP/dt. The Kruskal-Wallis test (*P<0.05*) was applied to statistical analysis.

Results: There were no significant differences (*P>0.05*) between groups among HR and CoF values. Differences (*P<0.05*) occurred between groups I e II after the reperfusion period (t30) regarding sistolic pressure reduced for 28.0±3.6% in the control group GI and for 79.0±5.9% in GII; The +dP/dtmax declined to only 31.0±5.6% in GI, preserving 99.4±11.2% values in GII (*P<0.05*). The t30 -dP/dtmax values were GI 26.0±7.3% and GII 82.0±2.2% (*P<0.05*).

Conclusion: The omeprazole administration before ischemia induction significantly protected the myocardium function recovery.

Descriptors: Omeprazole. Myocardial infarction. Ischemic preconditioning, myocardial.

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Resumo

Objetivo: Analisar efeitos do omeprazol na proteção da recuperação funcional de corações isolados de ratos submetidos à lesão de isquemia-reperfusão.

Métodos: Foram estudados 12 ratos Wistar, peso corpóreo médio de 280g. Após anestesia com injeção intra-abdominal de 10mg de cetamina e 2mg de xilazina, os corações foram removidos e mantidos em perfusão com solução Krebs-Henseleit (95%O_2 e 5% CO_2, 37°C, 110-120mmHg de pressão de perfusão e pressão diastólica de 8 mmHg) em sistema Langendorff, modificado, descartável, modelo FCSFA-ServCor (Comex Ltda.). Os seis corações do Grupo I (GI) e os seis do Grupo II (GII) foram submetidos a 20 minutos de isquemia e 30 minutos de reperfusão. Nos corações do Grupo II, imediatamente antes da isquemia, foram administrados via perfusão coronária 200mcg de omeprazol. Foram controlados frequência cardíaca (FC), fluxo coronário (FCo), pressão sistólica (PS), +dP/dt e -dP/dt, após estabilização (t0) e no final da reperfusão (t30). Empregou-se método não paramétrico de Kruskal-Wallis (*P*<0,05) para análise estatística dos dados.

Resultados: Não ocorreram diferenças (*P*>0,05) entre os valores de FCo e FC nos dois grupos. No final do período de reperfusão (t30), foram significantes (*P*<0,05) as variações da PS reduzida para 28,0±3,6% do valor inicial (t0) no Grupo I e para 79.0±5,9% no Grupo II; a +dP/dtmax declinou para 31,0±5,6% no GI, mantendo-se em 99,4±11,2% (*P*<0,05) no GII e a -dP/dtmax declinou para 26,0±7,3% no GI, mantendo-se em 82,0±2, 2% no GII (*P*<0,05).

Conclusão: A administração do omeprazol antes da indução da isquemia coronária protegeu significantemente a recuperação funcional do miocárdio.

Descritores: Omeprazol. Infarto do miocárdio. Precondicionamento isquêmico miocárdico.

INTRODUCTION

Lindberg et al. [1] in 1986 demonstrated the inhibitory effect of the H/K pump in the gastric parietal cells in reducing the acidity of gastric juice and in the treatment of peptic ulcer. Later, other H/K pump inhibitors have been developed, including the Lansoprazole, Pantoprazole, Rabeprazole, and Tenatoprazole [2]; the most frequent prescribed one being the omeprazole.

Although they have been developed with specific therapeutic intent to treat gastric hyperacidity, in recent years proton pump inhibitors were associated with benefits in the treatment of angina pectoris and myocardial ischemia, considering that chest pain may mimic the gastroesophageal reflux symptom from coronary artery disease, and the esophagitis pain may also be enough to motivate stress ischemia and cardiac pain in patients suffering from both diseases. That is why it has been described that the treatment of gastroesophagitis has reduced the frequency of angina attacks [3-5].

Other important evidence suggesting the protective effect of omeprazole on coronary ischemia is the fact that its administration has reduced the frequency of both anginal attacks and electrocardiographic signs of myocardial ischemia during exercise treadmill tests in studies of coronary heart disease patients [6]. However, there are still no specific experimental demonstrations showing the clinical hypotheses of its protective effect on coronary ischemia-reperfusion.

Another very important evidence suggesting the protective effect of omeprazole on the purpose of this research constitutes analyze the effects of omeprazole in protecting myocardial contractility in isolated rat hearts subjected to ischemia-reperfusion.

The aim of the present study was to analyze the effects of omeprazole on the protection of myocardial contractility of isolated hearts of rats submitted to the ischemia-reperfusion lesion.

METHODS

The study was approved by the Local Institutional Ethics Committee. We studied 12 Wistar rats without distinction of sex with an average body weight of 280 g. After anesthesia ketamine and xylazine in a 10 mg and 2 ml intra-abdominal dose intravenous, respectively, is administered. The animals underwent a wide range median thoracotomy and 500 IU of heparin were regularly injected into the right atrium. For safety and quickness in the procedure preparation, the ascending aorta was isolated with the 3-wire technique [7]. The first wire pulls and lifts the aortic root to protect of the cusps and to prevent ventricular cannulation; the second wire pulls and lifts the cranial (superior) end of the ascending aorta; and the third wire is passed between the first two, to tie off the aorta on the cannula after it was inserted (Figure 1A and B). A perfusion cannula was introduced and secured in the ascending aorta, preventing injury to the aortic cups to ensure adequate coronary perfusion; then the left atrium was opened and a multiperforated cannula inserted and externalized our push out through the left ventricle apex. Finally, the pulmonary artery was opened at its origin to facilitate the drainage of the right ventricle.

The hearts were removed, at a maximum of a 3-minute

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time after the procedure. They were reperfused with Krebs-Henseleit solution (aered/gassed with 95% O_2 and 5% CO_2, at 37°C, using a modified, disposable Langendorff system, model-FCSFA ServCor, Comex Ind. & Com Ltda) (Figure 2) with circulation system and water heating and telethermometer models (Braile Biomédica Ltd), biomonitor BESE®, and a matricial printer Epson®. After ten minutes of coronary reperfusion, the transventricular catheter was removed, and a pre-calibrated balloon catheter was introduced into the left ventricle.

All hearts studied presented normal parameters of myocardial contractility (after a 10-minute recovery all hearts presented systolic pressure less than or equal to 110 mmHg, diastolic pressure of 8 mmHg. The control register (t_0) was obtained at the 15th minute of recovery. The six hearts of

the group I (GI) underwent 20 minutes of ischemia and 30 minutes of reperfusion. In six hearts of group II (GII), immediately before ischemia, 200 mcg of omeprazole and H/K ATPase pump blocker of more frequent clinical use in our environment was administered through coronary perfusion (Figure 3).
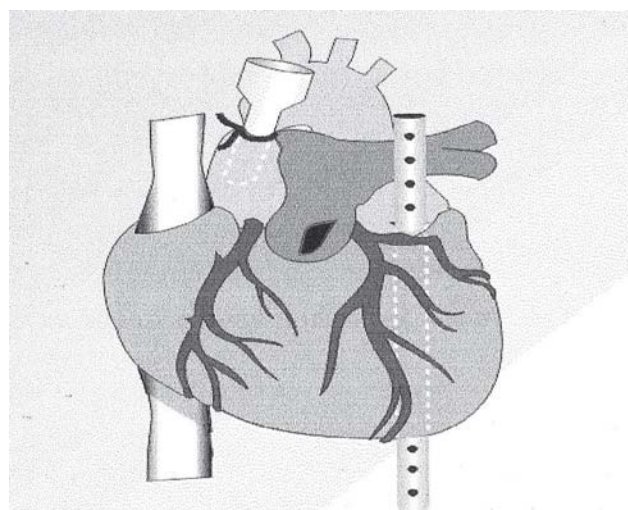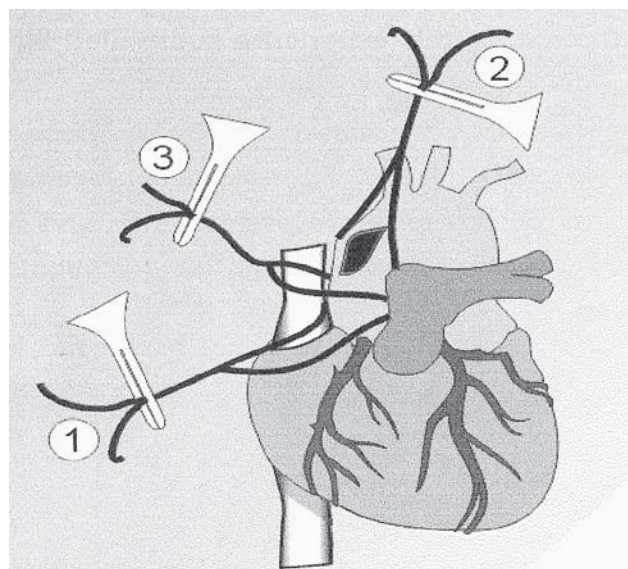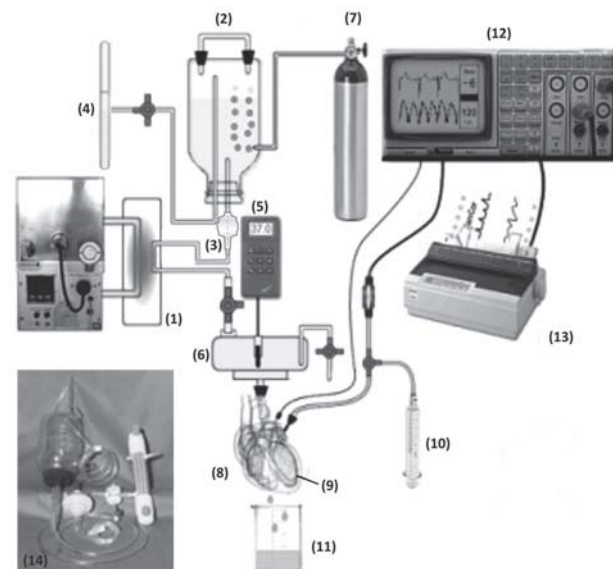

Fig. 2 - Diagram of the Study System of an Isolated Heart. 1. Termal exchanger, 2. Perfusate reservoir. 3. Microfilter (20 ì), 4. Gauge/Manometer, 5. Telethermometer, 6. Chamber, 7. Carbogen (95% 02 +5% C02), 8. Heart, 9. Intraventricular balloon, 10. Drug injector, 11. Flow Collector, 12. ECG and Ventricular Pressure monitor, 13. Printer, 14. Disposable set
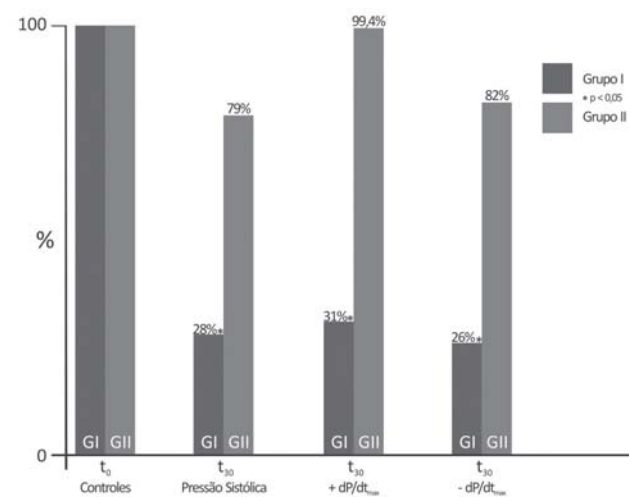

Fig. 1 – A: Aortic isolation for cannulation; B: Cannulationz of the aorta and left ventricle.

Fig. 3 – Percentage of systolic pressure variation, $+dP/dt_{max\ }$ $_{and}$ $-dP/dt_{max}$.

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The following parameters were controlled after the stabilization period (t_0) and after a 30-minute period past coronary reperfusion was initiated (t_{30}): heart rate (HR/bpm), coronary flow (CFo/ mL/min), systolic pressure (mmHg), +dP/dt$_{max}$ and -dP/dt$_{max}$ (mmHg/s).

We used the Kruskal-Wallis nonparametric statistical analysis. All *p* vales < 0.05 were considered statistically significant.

RESULTS

No differences ($P > 0.05$) were found between HR and CFo in both groups (Table 1). At the end of reperfusion period (t_{30}), the reduced SP changes were significant ($P < 0.05$) with averages ranging from 138 ± 14.7 mmHg (t_0) to 38 ± 14.4 mmHg (t_{30}). These were equivalent to the initial value ($28.0 \pm 3.6\%$) (t_0) of Group I, which ranged from 140 ± 15.6 mmHg (t_0) to 112 ± 25 mmHg ($79.0 \pm 5.9\%$) in Group II (t_{30}); the +dP/dt$_{max}$ declined to $31.0 \pm 5.6\%$ at t_{30} (t_0 and t_{30} were 3328 ± 996 and 1187.4 ± 449.6 mmHg/s, respectively) in GI, remaining at 99.4

$\pm 11.2\%$ ($P < 0.05$) in GII at t_{30} (t_0 3374 ± 1247 mmHg; t_{30} 3141 ± 1681.1 mmHg/s). The -dP/dt$_{max}$ declined to $26.0 \pm 7.3\%$ ($t_0 = 1859 \pm 203.9$; t_{30} 407 ± 284.1) in GI, remaining at $82.0 \pm 2.2\%$ in GII ($P < 0.05$) ($t_0 = 1859 \pm 203.9$ mmHg/s; $t_{30} = 1570 \pm 515.7$ mmHg/s) (Table 2 and Figure 3).

DISCUSSION

In 1994, Nagashima et al. [8] were also the first to demonstrate the existence of the proton pump H^+/K^+ ATPase in the myocardium as well. They studied their electrophysiological interference in the hearts of guinea pigs [9], motivating further research, such as the multicenter trials GUARDIA and ESCAMI. These trials have carefully analyzed inhibitors such as eniporide and cariporide – the proton pump Na^+/H^+ blockers –, emphasizing the protection against myocardial ischemia-reperfusion injuries, but with contrasting results and doubts about the prophylactic effect and indication of its therapeutic use [10]. More recently, Budzynski et al. [6], in 2008, described the beneficial effects of omeprazole in the protection of anginal attacks and ischemic electrocardiographic changes during the exercise test in patients with coronary heart diseases.

In the present study, it was found a significant protection of omeprazole against ischemia-reperfusion in isolated rat hearts, with reduced +dP/dt and –dP/dt to $31 \pm 5.6\%$ and to 26 ± 7.3 %, respectively, on average after 30 minutes of postischemic reperfusion in Group I (control), and mean values of +dP/dt and –dP/dt of $99.4 \pm 11.2\%$ and $82 \pm 2.2\%$, respectively, in Group II with administration of 200 mcg of omeprazole pre-ischemia. These results associate the benefits of omeprazole with the results reported by studies addressing myocardial protection with ischemic preconditioning [11-15]. The pharmacokinetics basis may

Table 1.   Heart rate (HR) and coronary flow (CoF) variations.

Rato número	FC (bpm)				Fco (bpm)			
	GI		GII		GI		GII	
	t_0	t_{30}	t_0	t_{30}	t_0	t_{30}	t_0	t_{30}
1	300	250	310	305	21	12	18	17
2	300	260	300	290	19	12	23	18
3	300	285	320	300	17	10	19	16
4	277	265	300	290	22	15	20	15
5	288	276	300	300	20	11	21	12
6	295	245	288	285	21	10	17	16
Média	293	264	303	295	2,0	12	20	16
DP±	18.1	19.5	15.3	16.0	3.2	4.9	4.0	3.6

Table 2.   Systolic pressure variations, +dP/dt$_{max}$ and -dP/dt$_{max}$.

Rato número	PS mmHg				+dP/dt$_{max}$ (mmhg/seg)				-dP/dt$_{max}$ (mmhg/seg)			
	GI		GII		GI		GII		GI		GII	
	t_0	t_{30}	t_0	t_{30}	t_0	t_{30}	t_0	t_{30}	t_0	t_{30}	t_0	t_{30}
1	147	38	153	112	3675	950	3825	3021	1225	190	1912	1400
2	149	12	160	118	3725	625	4000	3840	1490	300	2000	1580
3	159	46	153	167	3975	1150	3825	4175	1942	383	1915	2090
4	120	35	120	98	2220	875	2220	1842	1685	485	1685	1516
5	120	60	125	72	3000	1500	3125	2500	1714	583	1785	1161
6	135	35	130	104	3375	875	3250	3466	2250	500	1857	1671
Média	138	38	140	112	3328	996	3374	3141	1718	407	1859	1570
DP±	14.7	14.4	15.6	20.5	1187.4	449.6	1247.0	1681.1	1025.9	284.1	203.9	515.7

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be related to changes in transmembrane H^+/K^+ gradients, the first markers of myocardial ischemia, responsible for the repolarization expressed in T-wave morphology of the electrocardiogram [16]. In 1998, the studies by Hotta et al. [17] in isolated hearts of guinea pigs, using an analytic method for detecting fluorescent compounds, the fluorometry and magnetic resonance imaging, indeed confirmed the acute ionic intracellular changes of the myocardial ischemia-reperfusion with changes in pH and concentrations of cytosolic N^+, H^+ and Ca^+, thus confirming the blockade and protection by omeprazole as well.

Additional studies may precisely determine the interaction of proton pump blockers with the phenomena of ischemic myocardial pre- and post-conditioning, with a potential favorable resolution for both clinical therapeutic and preventive use in coronary heart diseases as well as in the myocardial protection for cardiac surgeries. In this respect, the preconditioning by H^+/K^+ pump blockers preoperatively can optimize the cardiac protection, replacing intermittent occlusions of the coronary arteries or the aorta itself, with a parietal harmful potential whose efficacy had already been refuted [18], and differing from the conclusion of the present study, which establishes a significant protection of myocardial contractility

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