The Role of Zofenopril in Myocardial Protection During Cardioplegia Arrest: An Isolated Rat Heart Model

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ABSTRACT ***Background:*** **Zofenopril has beneficial effects in acute myocardial infarction, and improves the functional recovery after ischemia and reperfusion.** ***Aim of the study:*** **The aim of this study was to investigate the cardioprotective effects of zofenopril, when added to a standard cardioplegic solution or when orally administered as pretreatment.** ***Methods:*** **A Langendorff model for isolated rat hearts was employed: three groups of eight hearts each were used, respectively, with plain St. Thomas cardioplegia as control (group A and C), and the same solution added with 12.5 mg of zofenopril (group B). The third group (C) was pretreated for 7days with oral administration of zofenopril (6.5 mg/day). The hearts had a baseline perfusion for 30 minutes with Krebs-Henseleit solution at 37°C, cardioplegia administration for 3 minutes, then 30 minutes of ischemia without any perfusion, and finally 30 minutes of reperfusion with Krebs-Henseleit solution at 37°C.** ***Results:*** **Left ventricle developed pressure was significantly higher in the reperfusion period only in the pretreated group (group C) with respect to groups A and B ($p = 0.016$). Similar results were obtained regarding dP/dt curves ($p = 0.020$). No differences were demonstrated between groups for cellular viability expressed as creatine phospho-kinase ($p =$ ns) and lactate dehydrogenase release ($p =$ ns).** ***Conclusions:*** **Zofenopril as oral pretreatment showed protective effects in an isolated model of cardioplegic arrest, although improvements in myocardial viability (enzymatic release) could not be demonstrated. Further experimental and clinical evaluations are necessary to assess the direct cardioprotective effect of zofenopril, modifying the length of treatment and the dosage of the drug.** doi: 10.1111/j.1540-8191.2006.00167.x *(J Card Surg 2006;21:44-49)*

Angiotensin-converting enzyme (ACE) inhibition improves the cardioprotective properties of cardioplegic solutions, preserving myocardial function during post-cardioplegic reperfusion.[1,2] The underlying mechanism involves modulation of myocardial oxygen consumption[3] and glucose metabolism.[4]

Zofenopril is an ACE inhibitor with interesting properties, namely a favorable tissue distribution, and with the presence of a sulfhydryl group in its active metabolite, zofenoprilat.[5] It has beneficial effects in acute myocardial infarction,[6-8] decreasing infarct size, and improves the functional recovery after ischemia and reperfusion.[9-15] The presence of the sulfhydryl group is a crucial issue in zofenopril effects.[16,17] The oxidation of protein sulfhydryl group, probably caused by reactive oxygen species, plays a major role in the pathophysiology of myocardial injury produced by ischemia and reperfusion.[16,17]

In the present work, we used an isolated rat heart model of ischemia-reperfusion in order to investigate the cardioprotective effects of zofenopril, when added to a standard cardioplegic solution or when orally administered as pretreatment.

MATERIALS AND METHODS

Langendorff isolated rat heart model

Experimental procedures were approved by the ethical committee of the Varese University Hospital. The investigation complies with the Italian guidelines for laboratory animals and the European Communities Directive of November 1986 (86/609/EEC).

Male Wistar rats (Charles River Italia, Calco, Italy), weighing 250 ± 20 g, were anesthetized by intraperitoneal injection of ketamine and xylazine; 1000 IU of sodium heparin was also administered. The chest was opened through a midline sternotomy.

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The heart was removed, cooled in oxygenated ice-cold modified Krebs-Henseleit solution during preparation of the aorta, and then mounted on the glass cannula of a Langendorff perfusion apparatus (Radnoti Glass Technology, Inc., Monrovia, CA). Retrograde perfusion of the aorta, according to the Langendorff method, was immediately started, with a modified Krebs-Henseleit solution. This standard perfusion buffer included (mmol/L): NaCl 136.9, KCl 4.7, $MgCl_2$ 1.0, $NaHCO_3$ 24.9, NaH_2PO_4-H_2O 0.42, $CaCl_2$-$2H_2O$ 1.8, and Glucose 5.55. The perfusate was kept between 36.8 and 37°C by a constant temperature bath/circulator (Julabo USA, Inc., Allentown, PA), and the pH was 7.4. The solution was continuously oxygenated with a mixture of O_2 (95%) and CO_2 (5%). The heart was perfused at a constant pressure of 45 mmHg and suspended in water-jacketed chamber to maintain its temperature at 37°C. A latex fluid-filled balloon on the end of a PE-100 catheter was inserted into the left ventricle through the left atrium. This balloon was inflated to achieve an end-diastolic pressure between 7 and 11 mmHg, the inflation remained constant during the whole experiment. Three electrodes were placed on the epicardium to record electrocardiographic data. Data recording and analysis were performed using Power Lab® hardware and software systems (AD Instruments Pty Ltd., Castle Hill, Australia).

Experimental protocol

After 30 minutes of stabilization (perfusion period), cardioplegic solution was administered for 3 minutes at the constant pressure of 45 mmHg, followed by 27 minutes of ischemic arrest without any perfusion (ischemic period). During ischemia, the water-jacketed chamber was removed. At the end of the ischemic period, the normal perfusion was resumed for 30 minutes (reperfusion period).

Twenty-four animals were randomly assigned to three groups: control group (A) received St. Thomas Hospital cardioplegic solution (Galenica Senese, Monteroni d'Arbia, Italy); group B received St. Thomas solution (100 mL) added with 12.5 mg zofenopril (Istituto Lusofarmaco d'Italia, Italy). The third group (C) was pretreated for 7 days with oral administration of zofenopril (6.5 mg/day) added to the usual drinking water, checking the body weight, and daily water intake. The daily dose of Zofenopril and the added dose to the cardioplegic solution were chosen in order to produce pronounced and long-lasting myocardial ACE inhibition. In group C, ischemic arrest was obtained by St. Thomas Hospital cardioplegic solution only.

Hemodynamic and biochemical measurements

Pressure gradient between systolic and diastolic pressure (left ventricle developed pressure, LVDP), and the left ventricle dP/dt was analyzed in order to evaluate the heart contractile efficiency. The variables were continuously recorded, and measurements were registered every 5 minutes.

Myocardial creatine phospho-kinase (CPK) and lactate-dehydrogenase (LDH) release as sensitive indicators of membrane integrity were collected to evaluate cellular viability. Coronary effluent was collected in graduated bechers as 1-minute samples in order to measure the coronary flow during the last 5 minutes of stabilization, and again during the first 10 and last 5 minutes of reperfusion. These samples were analyzed to measure CPK and LDH activities by a spectrophotometer (Spotchem® system 1, A. Menarini diagnostics, Firenze, Italy).

Statistical analysis

In all the experiments, differences among control and treated groups were evaluated for statistical significance using analysis of variance (ANOVA). A value of $p < 0.05$ was considered significant. In the depicted figures, results are expressed as means ± standard deviation (SD).

RESULTS

Hemodynamic data

Figure 1 shows LVDP. In the perfusion period, the three groups presented a similar curve shape. No statistical significance was detected among groups in the perfusion period ($p = ns$).

In the reperfusion period, significant differences were demonstrated among groups ($p = 0.016$), statistical significance was reached between groups C and A ($p = 0.015$) and groups C and B ($p = 0.019$). In group C, better LV performance was demonstrated after ischemic period, probably reflecting the effectiveness of myocardial protection guaranteed by the 1-week zofenopril pretreatment. No significant differences were observed between groups A and B ($p = ns$).

The dP/dt curves expressing LV performance displayed similar results to LVDP (Fig. 2). In the perfusion period, all the three curves demonstrated a linear increase without differences among groups ($p = ns$). However, in the reperfusion period the pressure gradient was raised from the basal level of the ischemic period to plateau in all groups with significance among groups ($p = 0.020$). In group C, a significantly higher dP/dt level was reached with respect to groups A and B ($p = 0.025$ and $p = 0.019$, respectively). The two other groups demonstrated almost equal lower findings ($p = ns$).

Creatine phospho-kinase and lactate dehydrogenase release

The effects of zofenopril on myocardial release of CPK and LDH levels are depicted in Figs. 3 and 4.

CPK release after reperfusion onset was slightly lower in the control group (A) compared to both groups B and C, without reaching statistical significance ($p = ns$) (Fig. 3).

LDH release after reperfusion onset was lower in the control group (A), compared to both groups B and C. This difference did not reach the statistical significance ($p = ns$) (Fig. 4).

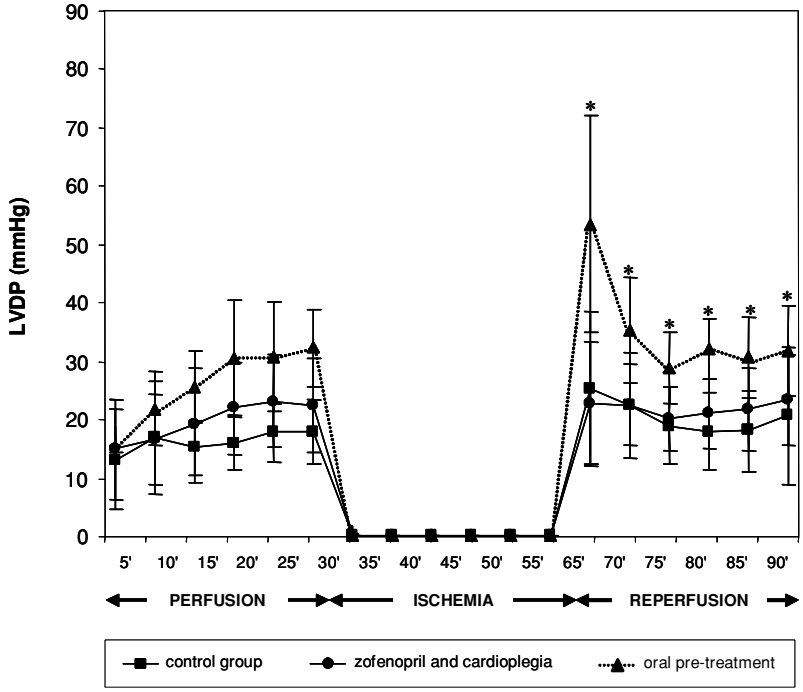

Figure 1. *Left ventricle developed pressure (LVDP) in isolated perfused rat hearts. All hearts were equilibrated in aerobic perfusion (30 min), made temporarily ischemic (27 min) by cardioplegic arrest (3 min), and reperfused (30 min) (mean values, error bars represent SD). * Pretreatment group versus group A and B (p = 0.016).*

CONCLUSIONS

ACE inhibitors have therapeutic effects in treating hypertension and congestive heart failure.[18,19] The ACE inhibitor pretreatment also decreases infarct size and improves functional recovery after ischemia and reperfusion.[9-12]

The cardioprotective effects are related to the reduction of endogenous bradykinin degradation and to the synthesis of Angiotensin II from Angiotensin I.[18,19] In

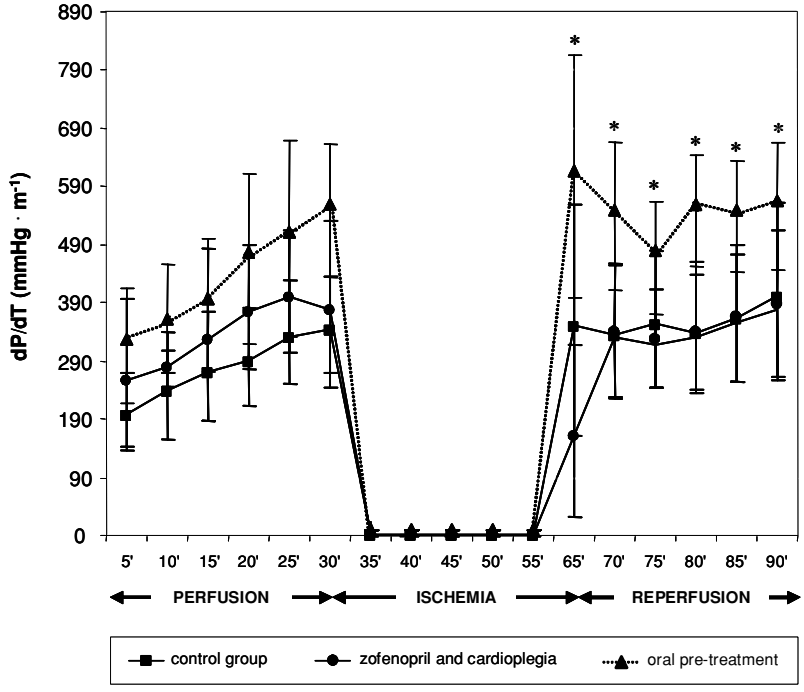

Figure 2. *dP/dt in isolated perfused rat hearts (mean values, error bars represent SD). * Pretreatment group versus group A and B (p = 0.020).*

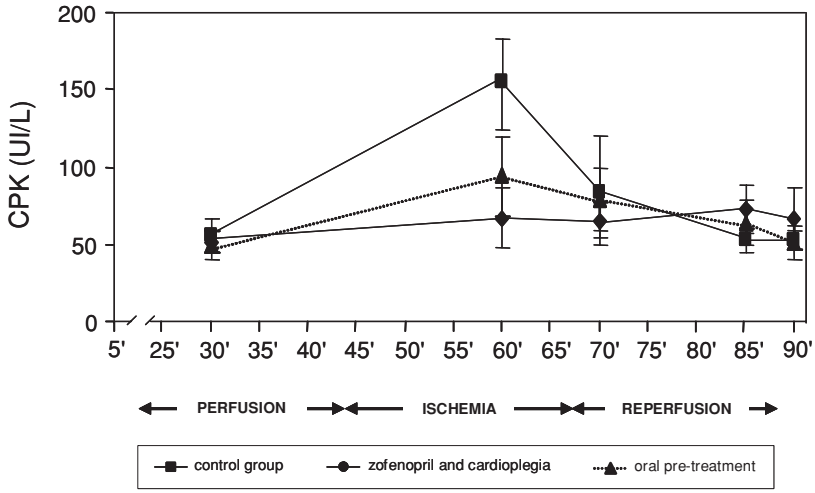

Figure 3. *Creatine phospho-kinase (CPK) release in coronary effluent (mean values, error bars represent SD). No differences were observed among groups (p = ns).*

addition, ACE inhibitors may alter the localized Angiotensin II or bradykinin concentrations in cardiac tissue.[20] Angiotensin II may induce cardiac myocyte necrosis and fibroblast proliferation, thereby exacerbating ischemia-reperfusion injury.[21] Conversely, kinins may induce an increase in coronary circulation and improve cardiac function in ischemia-reperfusion injury. This conclusion is corroborated by different investigations, showing that the protective effect of different ACE inhibition was abolished by bradykinin antagonists.[22-27] Moreover, bradykinin and other kinins may increase myocardial resistance to ischemia, probably by triggering a protein kinase C-dependent pathway, and may be implicated in the pathophysiology of ischemic preconditioning.[28]

Besides the common properties of other ACE inhibitors, zofenopril counts another interesting chemical property: the presence of a sulfhydryl group in its molecule (zofenoprilat). This is produced in vivo by cleavage of an esteric bond.[29,30]

Several studies have demonstrated that the presence of a sulfhydryl group in the molecule may be a crucial issue for cardioprotection.[16,17] Similar conclusions have been reached by Ferrari et al.,[31] who tested the effects of zofenopril and enalapril on oxidative stress. In endothelial cells, zofenoprilat reduced the formation of reactive oxygen species induced by different stimuli.[32] Moreover, clinical and experimental studies demonstrated the superior cardioprotective effectiveness of zofenopril compared with captopril.[16,31]

These different cardioprotective qualities showed by zofenopril/zofenoprilat constitute the rationale for testing this drug in a myocardial ischemia-reperfusion model. Our experimental model was designed to mimic a clinical cardioplegic arrest. After the administration of cardioplegic solution, the water-jacket chamber was

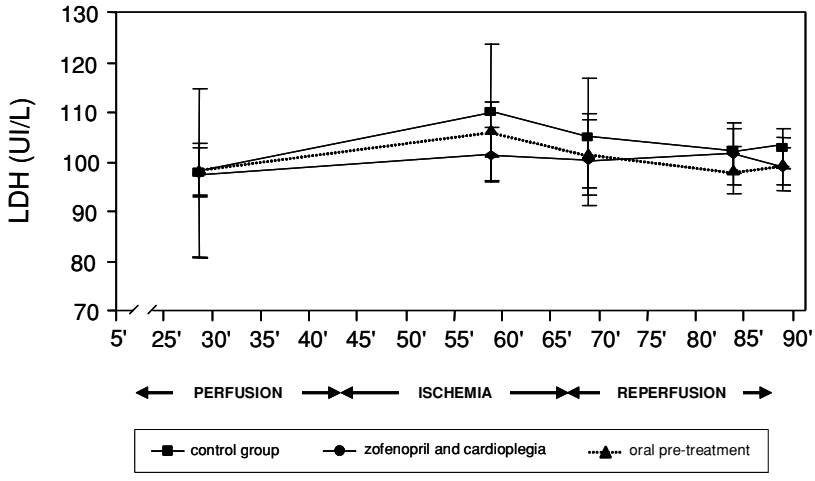

Figure 4. *Lactate-dehydrogenase (LDH) release in coronary effluent (mean values, error bars represent SD). No differences were observed among groups (p = ns).*

removed and the heart was exposed to room temperature (18 to 20°C). This condition is reasonably similar to that of a cross-clamped heart in the pericardial cradle of a patient assisted by moderately hypothermic cardiopulmonary bypass. However, as a limitation of the study, we decided to keep a constant volume of the intraventricular balloon in order to obtain a constant preload condition. This model is not as sophisticated as the constitution of pressure–volume curves, but it has the advantage of simplicity and reproducibility.

The orally pretreated group (C) demonstrated a significant improvement in mechanical performance during the reperfusion period. Zofenopril added to cardioplegia only (group B) showed just a slight improvement, without statistical significance. However, the release of CPK and LDH was unaffected by zofenopril administered in either way.

Our findings go with other reported data,[25,33] employing different sulfhydryl-group ACE inhibitors. Improved hemodynamic performances were achieved by oral pretreatment of captopril.[33] Moreover, a better myocardial recovery and viability were afforded by captopril added to a standard cardioplegic solution.[34]

In our study, zofenopril revealed improved cardiodynamics during reperfusion period only in the orally pretreated group. The cardioprotective mechanism of this drug was not evaluated in our experiment. A plausible explanation may be related to the above-mentioned properties of zofenopril.[9-12,29,30] One-week oral pretreatment may result in higher myocardial concentration of the drug, determining a direct cardioprotection and antioxidant action. These effects were not probably reached by a single zofenopril dose added to the cardioplegia. Moreover, when the pro-drug zofenopril is added to cold cardioplegia, the hydrolization process into zofenoprilat could be incomplete or ineffective due to cardioplegic constituents, hypothermia or both.

In contrast with other studies, we could not find any evidence of ameliorated myocardial viability.[16,31] Probably, at the dosage employed the drug could not reach an effective myocardial concentration level.

In conclusion, oral pretreatment with zofenopril seems to preserve myocardial contractile efficiency in an isolated rat heart model of cardioplegic arrest and reperfusion. No changes were observed when zofenopril was administered as a single dose in the cardioplegic solution. Further experimental and clinical evaluations are necessary to assess the direct cardioprotective effect of zofenopril, modifying the length of the treatment, and the dosage of the drug.

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