Effects of Olprinone on Myocardial Ischemia-Reperfusion Injury in Dogs

Kentaro SETOYAMA1), Ryozo KAMIMURA1), Makoto FUJIKI2), Kazuhiro MISUMI2), Kenkichi MIYAHARA3) and Hiroshi SAKAMOTO2)

1)Frontier Science Research Center, Kagoshima University, 8–35–1 Sakuragaoka, Kagoshima 890–8520, 2)Laboratory of Veterinary Surgery, Department of Veterinary Medicine, Faculty of Agriculture, Kagoshima University, 1–21–24 Korimoto, Kagoshima 890–0065 and 3)Division of Cardiology, Shinkyo Hospital, 3–41–1 Usuki, Kagoshima 890–0073, Japan

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ABSTRACT. We investigated the effect of olprinone on canine myocardial pump function and myocardial damage after ischemia-reperfusion injury. Three dogs of the experimental group were given olprinone (Olprinone group) and another 3 dogs were served as control (Intact group). All animals were occluded left anterior descending artery for 60 min, followed by 6 hr of reperfusion. In the experiment, hemodynamics, infarct area, creatine kinase and troponin-I were measured. Olprinone infusion induced significantly high cardiac output value and significantly low values in left ventricular end diastolic pressure and systemic vascular resistance index after reperfusion. Also, olprinone tend to attenuate the infarct area, creatine kinase and troponin-I.

KEY WORDS: canine, ischemia-reperfusion injury, olprinone.

In the field of veterinary clinics, cardiovascular surgery using cardiopulmonary bypass has become one of the promising treatments in recent years. However, open heart surgery sometimes induces myocardial ischemia-reperfusion injury and leads to myocardial dysfunction as well as myocardial infarction [4]. To prevent myocardial dysfunction, various agents such as Na+/H+-exchange inhibitor, oxygen-derived free radical scavenger and statins have been studied [10].

Phosphodiesterase (PDE) III inhibitors, a therapeutic agent for acute congestive heart failure, have both inotropic and vasodilatory effects. The pharmacological effect of PDE III inhibitors have been shown to be due to an increase in intracellular cyclic-adenosine monophosphate (cAMP) without beta-adrenergic pathway [1–3, 6, 7, 11]. Recently, it has been demonstrated that amrinone and milrinone decreased infarct size in isolated rabbit heart with ischemia-reperfusion injury [9]. Rechtman et al. also reported that amrinone suppressed creatine kinase and lactate dehydrogenase elevation, indicators of myocardial injury, in the isolated rat heart with ischemia-reperfusion injury [9]. In addition, our previous study showed milrinone increases myocardial blood flow in the hypoxic dogs [8]. From these results, we hypothesized that PDE III inhibitors may protect myocardial damage from ischemic or ischemia-reperfusion injury and it seems to have an interesting effect on treating myocardial dysfunction after cardiopulmonary bypass or myocardial infarction.

However, few reports have evaluated the effect of PDE III inhibitors on canine myocardial pump function after ischemia-reperfusion injury. In this study, we investigated the effect of olprinone on canine myocardial function and damage after ischemia-reperfusion injury. The experiment was carried out after permission from the committee on Animal Experimentation, Kagoshima University.

NOTE

We used 6 beagle dogs (2 years old, male) weighing 9.2 ± 0.5 kg (mean ± standard error). The dogs were healthy, as determined by pre-operative examinations, including physical examination, clinical laboratory evaluation and radiography of the thorax.

The dogs were premedicated with atropin sulfate (0.05 mg/kg subcutaneously) and administered ketamine hydrochloride (10 mg/kg) intravenously. After endotracheal intubation, the dogs were anesthetized with oxygen-isoflurane (2.0%) and paralyzed with pancuronium bromide.

During the experiment, the lungs were ventilated mechanically (Ace-300, Acoma Medical Industry Co., Ltd, Tokyo, Japan). The respiratory rate and tidal volume were kept at 10 times/min and 20 ml/kg, respectively.

To monitor left ventricular and arterial pressures, 5 Fr pig catheter and polyethylene catheter were inserted in left carotid and femoral arteries, respectively. Also, to monitor central venous pressure (CVP) and cardiac output (CO), 5 Fr Swan-Ganz catheter was placed through the left juglar vein to pulmonary artery. In the left femoral vein, polyethylene catheter was placed for olprinone (Eisai Co., LTD, Tokyo, Japan) or saline infusion. After catheterization, thoracotomy was performed and the left anterior descending coronary artery (LAD) was exposed.

After hemodynamics were stabilized, the control data was recorded and the animals were divided into the olprinone group (n=3) and intact group (n=3). The olprinone group was infused olprinone (10 µg/kg bolus over 5 min followed by 0.3 µg/kg/min infusion intravenously) and the intact group was infused saline instead of olprinone. Thirty minutes after infusion, LAD was occluded proximal to the first diagonal branch for 60 min, followed by 6 hr of reperfusion. Lidocaine (2 mg/kg) was administered intravenously in all dogs before reperfusion, since ventricular tachycardia and fibrillation was observed in the preliminary study.

Heart rate, mean arterial pressure, CVP, left ventricular end diastolic pressure (LVEDP) and left ventricular peak positive dp/dt (LV dp/dt) were recorded through the experiment. CO measurements were performed at control, 60 (Re-60), 180 (Re-180) and 360 (Re-360) min after reperfusion. CO was measured by thermodilution method. Cardiac index (CI), systemic vascular resistance index (SVRI) and double-product variables were calculated as follows.

\[
CI: \frac{(CO)}{\text{Body Surface Area}}
\]
\[
SVRI: \frac{(\text{mean arterial pressure} - \text{CVP}) \times 80}{CI}
\]
\[
\text{Double-product:} \quad \text{systolic arterial pressure} \times \text{Heart Rate}
\]

After 6 hr of reperfusion, LAD was reoccluded, and Evans blue (Sigma-Aldrich, Inc., St. Louis, U.S.A.) dye was injected via the pig catheter to determine the area at risk. After the dog was euthanized by phlebotomy under full anesthesia, the heart was removed and sliced 6 to 7 mm in width. The area at risk was separated from the nonischemic area and incubated for 15 min in 1% solution of triphenyltetrazolium chloride (Sigma-Aldrich, Inc., St. Louis, U.S.A.) at 37°C to differentiate necrotic from nonnecrotic area at risk (Fig. 1). Area of necrosis as a percent of the area at risk was calculated as \[\left(\frac{\text{weight of necrotic area in the area at risk}}{\text{weight of total area at risk}}\right)\times100\].

Creatine kinase and troponin-I were assayed to estimate myocardial damage. To measure these parameters, arterial blood samples were collected at Control, Re-180 and Re-360. Collected samples were centrifuged at 3,000 rpm, 15 min immediately and serum was stored at –70°C until the assay.

Creatine kinase was quantified by routine diagnostic method using auto dry chemistry analyzer (Spotchem, SP-4410, ARKRAY, Inc., Kyoto, Japan). Troponin-I assay was performed by using canine cardiac troponin-I ELISA kit (Life Diagnostic, Inc., Pennsylvania, U.S.A.). ELISA procedure has carried out with manufacture’s instructions.

All values in the figures and table are expressed as the mean ± standard error. Statistical differences obtained from each group were compared by means of unpaired t test. Differences in p value less than 0.05 were considered to be statistically significant.

Table 1 shows changes in hemodynamics after reperfusion. Between 2 groups, significant differences were not seen in heart rate and mean arterial pressure. As compared with intact group, hypotensive tendency was recognized at Re-60 and Re-180 in olprinone group. SVRI and LVEDP indicate significantly lower value at Re-60 in olprinone group (Table 1).

Changes of CI and double-product are shown in Fig. 2. As compared with intact group, CI was maintained at significant.

### Table 1. Changes in Heart rate (HR), mean arterial pressure (MAP), central venous pressure (CVP), systemic vascular resistance index (SVRI), left ventricular end diastolic pressure (LVEDP) and left ventricular peak positive dp/dt (LV dp/dt) after reperfusion. All values are expressed as the mean ± standard error. † shows p value less than 0.05 (Intact vs Olprinone)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Re-60</th>
<th>Re-180</th>
<th>Re-360</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>Intact</td>
<td>126 ± 9</td>
<td>136 ± 2</td>
<td>146 ± 7</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>Olprinone</td>
<td>130 ± 13</td>
<td>126 ± 7</td>
<td>132 ± 6</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>Intact</td>
<td>84 ± 6.8</td>
<td>83 ± 8.6</td>
<td>82 ± 10.9</td>
</tr>
<tr>
<td>SVRI</td>
<td>Olprinone</td>
<td>87 ± 1.2</td>
<td>71 ± 5.6</td>
<td>74 ± 6.5</td>
</tr>
<tr>
<td>LVEDP (dyne-sec-cm⁻³-m⁻²) Olprinone</td>
<td>2.6 ± 2.6</td>
<td>5.3 ± 0.5</td>
<td>5.9 ± 0.8</td>
<td>5.0 ± 0.5</td>
</tr>
<tr>
<td>LV dp/dt (mmHg) Olprinone</td>
<td>4.7 ± 0.8</td>
<td>4.1 ± 0.4</td>
<td>4.3 ± 0.3</td>
<td>3.7 ± 1.1</td>
</tr>
<tr>
<td>(mmHg/sec) Olprinone</td>
<td>2135 ± 11</td>
<td>1961 ± 176</td>
<td>1902 ± 249</td>
<td>1997 ± 311</td>
</tr>
</tbody>
</table>

Fig. 1. a) The sliced heart (after Evans blue injection). Gray area indicates area at risk. Black area is nonischemic area. b) The sliced heart which stained by triphenyltetrazolium chloride stain. Arrow head indicates nonnecrotic area. Arrow indicate necrotic area.
significantly high level at Re-60, Re-180 and Re-360 (Intact group: 3.2 ± 0.1, 3.2 ± 0.2, 2.7 ± 0.3 L/min/m², Olprinone group: 4 ± 0.2, 3.7 ± 0.1, 4.2 ± 0.1 L/min/m², respectively).

Double-product, as an index of myocardial oxygen consumption, tended to be lower in olprinone group at Re-60 (Intact group: 13916 ± 1323 mmHg⋅bpm, Olprinone group: 11257 ± 1076 mmHg⋅bpm) and Re-180 (Intact group: 15292 ± 2456 mmHg⋅bpm, Olprinone group: 12192 ± 834 mmHg⋅bpm).

Figure 3 shows necrotic size in the both groups. Olprinone infusion tends to attenuate necrotic size (Intact group: 18.9 ± 2.2%, Olprinone group: 10.8 ± 2.1%). Creatine Kinase and Troponin-I elevation were also tended to attenuate by olprinone infusion though significant differences were not recognized (Fig. 4).

Myocardial dysfunction has often seen after myocardial ischemia-reperfusion injury. The depressed myocardial function is called “myocardial stunning” and in some cases, it continues for several days to weeks [4]. Improvement of myocardial pump function and myocardial damage will be the important strategies in this situation. Recent studies demonstrated that PDE III inhibitors attenuate myocardial damage and pump function after ischemia-reperfusion injury [9, 12, 13]. PDE III inhibitors are the therapeutic agents for acute congestive heart failure and they possess positive inotropic and vasodilatory action. In addition, unlike beta-adrenergic agents, PDE III inhibitors improve cardiac function without increment of myocardial oxygen consumption [5].

In our experiment, significant differences of myocardial contractility, one of the pharmacological actions, were not recognized. However, olprinone infusion induced high car-
diac output value after reperfusion 60, 180 and 360 min. In addition, significantly low values in LVEDP and SVRI were recognized at Re-60 and Re-180 by olprinone infusion. From these results, we indicated that olprinone can strengthen myocardial pump function without increasing myocardial burden even after myocardial ischemia-reperfusion. In the present study, we measured infarct area, creatine kinase and troponin-I as a marker of myocardial damage. Significant differences were not recognized in these parameters though olprinone tends to attenuate myocardial damage. This phenomenon suggests that PDE III inhibitors may attenuate myocardial damage after myocardial ischemia-reperfusion injury in dogs. In our previous study, increment of myocardial flow was recognized by olprinone infusion [8]. From these results, we speculate that the phenomenon which was seen in the present study may be due to increment of collateral flow by olprinone’s vasodilatory effect during myocardial ischemia. However, further investigations are required to clarify the cardioprotection effect of PDE III inhibitors during myocardial ischemia and after reperfusion.

Cardioprotection mechanisms of PDE III inhibitors are shown by a few investigations. Rechtman et al. demonstrated that amrinone, one of the PDE III inhibitors, attenuates myocardial damage in ischemia-reperfusion injured isolated rat heart [9]. They speculate that the reduction of Ca²⁺ overload in the myocyte due to amrinone induces the myocardial cardioprotection. Also, Sanada et al. reported that the transient activation of p38 MAPK which induced by PDE III inhibitors elicit cardioprotection [13]. However, the mechanisms of myocardial protection from ischemia-reperfusion injury by PDE III inhibitors are not clarified.

REFERENCES