Effects of Milrinone on Hemodynamics and Regional Blood Flow in the Hypoxic Dog

Kentaro SETOYAMA1), Hiromi OTA1), Naoki MIURA1), Makoto FUJIKI1), Kazuhiro MISUMI1) and Hiroshi SAKAMOTO1)

1) Laboratory of Veterinary Surgery, Department of Veterinary Medicine, Faculty of Agriculture, Kagoshima University, 1–21–24 Korimoto, Kagoshima 890–0065, Japan

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ABSTRACT. Milrinone, a therapeutic agent for acute congestive heart failure, has both inotropic and vasodilatory effects, but investigations of these effects of milrinone were almost all conducted under normoxia, and few reports have investigated how milrinone affects the hemodynamics and redistribution of regional blood flow under severe hypoxia. By using colored microspheres, we investigated how milrinone affects hemodynamics and the redistribution of regional blood flow under severe hypoxia. Twelve healthy mongrel dogs were divided into 2 groups. The milrinone group was infused with milrinone cumulatively at 25, 75 and 250 µg/kg for 5 min each. The intact group was infused with saline instead of milrinone. We measured the hemodynamics and cerebral, cerebellum and kidney blood flow in both groups. Both groups were inspired with 10% oxygen. Milrinone induced significant decrease in mean pulmonary artery and pulmonary vascular resistance, compared with the intact group. In both groups slight decreases in mean arterial pressure, systemic vascular resistance and double-product were seen. In regional blood flow, milrinone-induced increases in blood flow were seen in the cerebrum, cerebellum, and especially in the kidneys. Milrinone’s vasodilatory effects were sufficient even under hypoxia. And milrinone increased regional blood flow slightly in the cerebrum and cerebellum, and significantly in the kidneys. These results suggested that milrinone protects against hypoxia-induced organ damage especially in the kidneys. In addition, milrinone is very potent in improving severe congested hemodynamics which complicates hypoxic pulmonary vasoconstriction.

KEY WORDS: canine, colored microsphere, hypoxia, milrinone, regional blood flow.

FULL PAPER

Congestive heart failure is a critical disorder secondary to mitral valve regurgitation, pulmonary hypertension, vena cava syndrome, and so on in small-animal practice. Traditional medical treatment for severe congestive heart failure commonly included digitalis glycosides, vasodilators, and catecholamines, but these drugs have either only inotropic or only vasodilatory effects, and animals often develop tolerance to the drugs or experience arrhythmogenesis as a result of digitalis glycosides, vasodilators, and catecholamines. Milrinone, a therapeutic agent for acute congestive heart failure, has both inotropic and vasodilatory effects [2, 6, 8–10, 14, 16]. It is reported that the vasodilatory effect of milrinone is not only potent to the systemic vascular bed but also selective especially to the pulmonary vascular bed, and that it reduces pulmonary vascular resistance and pulmonary artery pressure [5, 11, 19, 20]. Tolerance to milrinone is rare, as is arrhythmogenesis as a result of its use [8, 17]. It is also reported that milrinone increases regional blood flow in the renal cortex, brain, endocardium and epicardium [15]. Nevertheless, investigations of these effects of milrinone were almost all conducted under normoxia, and few reports have investigated how milrinone affects the hemodynamics and redistribution of regional blood flow under severe hypoxia. In severe congestive heart failure, respiratory disorder complications have been common. In those cases, patients have often been in severe hypoxia and complicated pulmonary vasoconstriction, so that it is important to improve the hemodynamics and the distribution of regional blood flow as soon as possible. With colored microspheres, we investigated how milrinone affects the hemodynamics and the redistribution of regional blood flow under severe hypoxia.

MATERIALS AND METHODS

This study was carried out after permission from the committee on Animal Experimentation, Faculty of Agriculture, Kagoshima University.

Animal preparation: We studied 12 healthy mongrel dogs weighing 7.0–14 kg. The dogs were anesthetized with sodium pentobarbital (25 mg/kg) and paralyzed with pancuronium bromide (0.08 mg/kg). After endotracheal intubation, the lungs were ventilated mechanically (Ace-300, Acoma Medical Industry Co., Ltd, Tokyo, Japan) with 21% oxygen and the respiratory rate was adjusted to maintain arterial carbon dioxide tension at approximately 35 mmHg to 40 mmHg (Table 1). Arterial blood pH was maintained at approximately 7.4. Sodium pentobarbital and pancuronium bromide were supplied as required.

Measurements and surgical procedure: We measured mean arterial pressure (MAOP) and mean right atrium pressure (MRAP) via a polyethylene catheter (Intramedic Polyethylene Tubing No.7430, Clay Adams Co., New Jersey) inserted in the right femoral artery and vein. Mean pulmonary artery pressure (MPAP) was measured via a 7 French gauge Swan-Ganz catheter (Baxter Health Care Co., Deerfield, Illinois) inserted in the left jugular vein. Left ventricular end diastolic pressure (LVEDP) and peak positive left ventricular dp/dt (LVdp/dt) pressure were measured via a 7 French gauge pig catheter inserted in the left carotid artery.
A colored microsphere (E-Z Trac, Interactive Medical Technologies, Los Angeles) was infused via a catheter inserted into the left carotid artery. Arterial blood gas samples and references were collected via a polyethylene catheter inserted into the left femoral artery. Milrinone (Milrila, Yamanouchi Co., Ltd., Tokyo, Japan) was infused via a polyethylene catheter inserted in the left carotid artery. Arterial blood gas samples of the hypoxia control were recorded, oxygen in the inspired gas was kept at 10%. Hand-exercise with the experiment when hemodynamics was stabilized after the 2nd dose for 5 min each. We measured the hemodynamics and sampled arterial blood gas at 3 min and 5 min after each dose was started. The intact group was infused with saline at 6 ml/kg/hr throughout the experiment. After the experiment, each dog was euthanized and its cerebrum, cerebellum, kidneys and heart were removed to measure regional blood flow.

**Experimental protocol:** We divided the animals into two groups. One group received milrinone (milrinone group) and the control group (intact group) did not. We started the experiment when hemodynamics was stabilized after the surgical procedure. After we recorded the normoxia control data, oxygen in the inspired gas was kept at 10%. With the hemodynamics stabilized, we recorded the hypoxia control data. After the hypoxia control data were recorded, milrinone was infused cumulatively 25 µg/kg (1st dose), 75 µg/kg (2nd dose) and 250 µg/kg (3rd dose) for 5 min each. We measured the hemodynamics and sampled arterial blood gas at 3 min and 5 min after each dose was started. The intact group was infused with saline at 6 ml/kg/hr throughout the experiment. After the experiment, each dog was euthanized and its cerebrum, cerebellum, kidneys and heart were removed to measure regional blood flow.

**Statistical analysis:** Data are expressed as the mean ± standard deviation (SD). Values obtained from each group were compared by means of factorial ANOVA. A P value <0.05 was considered significant.

**RESULTS**

**Hemodynamics:** Figure 1 shows the changes in PVR and MPAP. In both groups, MPAP and PVR were significantly higher in the hypoxia control than in the normoxia control. After milrinone was infused, a dose-related decrement was seen in the milrinone group compared with the hypoxia control. At the 3rd dose, a significant decrease was observed in the milrinone group (15.1 ± 2.28 mmHg, 149.7 ± 92.18 dyne.sec.cm⁻³.m⁻²), compared with the intact group (23.2 ± 4.15 mmHg, 302.6 ± 105.99 dyne.sec.cm⁻³.m⁻²). Figure 2 shows the changes in SVR and MAOP. A dose-related decrease was also seen at SVR and MAOP, but the decrease was not significant. HR and double-product are shown in Fig. 3. A dose-related increase was seen in HR, and a significant change was seen after the 2nd and 3rd doses. Double-product, an index of cardiac oxygen consumption, decreased slightly in both groups. As Table 2 shows, no significant change was seen in PAWP, CI, SI or LVdP/dt in the milrinone and intact groups during the study.

**Regional blood flow:** Regional blood flow change is shown in Figs. 4 and 5. Figure 5 shows regional blood flow as a percentage change compared to the hypoxia control. The hypoxia control is set up to 100%.

In the cerebrum, blood flow increases were seen in both groups. In the intact group, cerebral blood flow increased to 1.16 and 1.19 times at 5 and 8 min, respectively, compared to the hypoxia control. But in the milrinone group, increases of 1.32 and 1.41 times were seen at 5 and 8 min compared to the hypoxia control. In the cerebellum, 1.26- and 1.36-fold increases were seen in the intact group, cerebral blood flow increases were seen in both the milrinone and intact groups, though 1.07- and 1.16 times at 5 and 8 min, respectively, compared with the hypoxia control. But in the milrinone group, increases of 1.32 and 1.41 times were seen at 5 and 8 min compared to the hypoxia control. In the cerebellum, 1.26- and 1.36-fold increases were seen in the milrinone group, though 1.07- and 1.28-fold increases were seen in the intact group at 5 and 8 min (Figs. 4, 5).

In the kidneys, though a decrease in blood flow was seen in the intact group, an increase was seen in the milrinone group. And significant change was seen after 15 min in the milrinone and intact groups, but no significant percentage change was seen (Figs. 4, 5).

For myocardial blood flow, the same degree of increase was seen in both groups. And there was no significant difference between the milrinone and intact groups (Figs. 4, 5).

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### Table 1. Blood-gas data compared with group intact and group milrinone. All values are expressed as the mean ± SD

<table>
<thead>
<tr>
<th></th>
<th>Normoxia cont</th>
<th>Hypoxia cont</th>
<th>1st dose</th>
<th>2nd dose</th>
<th>3rd dose</th>
</tr>
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<tbody>
<tr>
<td><strong>PaO₂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Intact group</td>
<td>99.8 ± 12.79</td>
<td>30.8 ± 8.50</td>
<td>29.6 ± 6.89</td>
<td>26.3 ± 6.21</td>
<td>25.4 ± 4.29</td>
</tr>
<tr>
<td>Milrinone group</td>
<td>104.9 ± 12.69</td>
<td>26.3 ± 7.32</td>
<td>26.6 ± 4.42</td>
<td>24.4 ± 5.22</td>
<td>24.7 ± 3.44</td>
</tr>
<tr>
<td><strong>PaCO₂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact group</td>
<td>37.3 ± 4.62</td>
<td>38.8 ± 1.77</td>
<td>41.8 ± 5.63</td>
<td>41.4 ± 5.53</td>
<td>42.3 ± 4.33</td>
</tr>
<tr>
<td>Milrinone group</td>
<td>36.9 ± 3.38</td>
<td>39.1 ± 4.41</td>
<td>38.5 ± 6.96</td>
<td>38.9 ± 7.05</td>
<td>38.2 ± 9.06</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact group</td>
<td>7.397 ± 0.03</td>
<td>7.378 ± 0.02</td>
<td>7.340 ± 0.03</td>
<td>7.321 ± 0.03</td>
<td>7.290 ± 0.04</td>
</tr>
<tr>
<td>Milrinone group</td>
<td>7.40 ± 0.05</td>
<td>7.39 ± 0.04</td>
<td>7.378 ± 0.04</td>
<td>7.362 ± 0.04</td>
<td>7.332 ± 0.05</td>
</tr>
</tbody>
</table>
**EFFECTS OF MILRINONE IN THE HYPOXIC DOG**

**DISCUSSION**

It is reported that milrinone, a PDE3 inhibitor, has inotropic and vasodilatory effects [2, 6, 8–10, 14, 16]. The vasodilatory effect in particular is selective to the pulmonary vascular bed [5, 11, 19, 20]. These effects have been shown in many fundamental experiments. Tanaka et al. investigated these effects of milrinone under normoxia by using dogs with pulmonary hypertension [20]. Kato et al. also investigated the effects of milrinone in dogs under mild hypoxia (PO2 60 mmHg) [11].

Comparing our results with those authors’ results, we find that pulmonary arterial pressure and pulmonary vascular resistance decreased by almost the same degree that they described. Mean aortic pressure and systemic vascular resistance also decreased. Especially in pulmonary hemodynamics, increases in PAP and PVR were seen in the intact group during the study, though in the milrinone group PAP and PVR were decreased significantly by milrinone infusion. These results suggest that milrinone is sufficiently potent in acting on hypoxic pulmonary constriction and systemic vasoconstriction, which are caused by a vascular endothelial agents (endothelin, intrinsic catecholamines and EDCF). The mechanism of the vasodilatory effect of PDE3 inhibitor has been shown to be due to an increase in intracellular cyclic-AMP as a result of PDE3 inhibition. This increase activates cyclic-AMP dependent protein phosphorlyating enzyme and causes a decrease in the intracellular...
Ca level by facilitating processes such as Ca uptake in the sarcoplasmic reticulate [14, 17]. In amrinone, there are some reports that nitric-oxide (NO) is also related to these vasodilatory effects [13], but Lugnier and Komas reported NO was not related to the vasodilatory effect in milrinone [12]. The mechanisms by which milrinone causes these vasodilatory effects are still being studied.

In our present study, no increase in LVdp/dt, an index of cardiac constriction, was seen. It is considered that high stroke volume, which is caused by hypoxia, masked the inotropic effect of milrinone.

It is reported that, compared with catecholamines, milrinone does not increase myocardial oxygen consumption [4]. Also, in our study, double-product indices of myocardial oxygen consumption did not increase. It is considered that reductions in preload and afterload, which were caused by milrinone, decreased the myocardial load. As a result, myocardial oxygen consumption did not increase. In the present study, myocardial blood flow increased by the same degree in the milrinone and intact groups, and no significant

### Table 2. Hemodynamic indices before and after administration of milrinone. Comparison of group intact (n=6) and group milrinone (n=6). All values are expressed as the mean ± SD

<table>
<thead>
<tr>
<th></th>
<th>Normoxia cont</th>
<th>Hypoxia cont</th>
<th>1st dose</th>
<th>2nd dose</th>
<th>3rd dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAP (mmHg)</td>
<td>Intact group</td>
<td>0.8 ± 0.72</td>
<td>1.5 ± 0.83</td>
<td>1.4 ± 0.83</td>
<td>1.3 ± 0.86</td>
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<tr>
<td></td>
<td>Milrinone group</td>
<td>2.5 ± 1.85</td>
<td>3.2 ± 2.27</td>
<td>3.1 ± 1.88</td>
<td>2.9 ± 1.72</td>
</tr>
<tr>
<td>PAWP (mmHg)</td>
<td>Intact group</td>
<td>5.4 ± 1.52</td>
<td>9.2 ± 2.91</td>
<td>7.7 ± 2.05</td>
<td>7.6 ± 2.11</td>
</tr>
<tr>
<td></td>
<td>Milrinone group</td>
<td>5.4 ± 5.31</td>
<td>9.7 ± 6.18</td>
<td>9.5 ± 6.44</td>
<td>8.1 ± 4.62</td>
</tr>
<tr>
<td>CI (L/min/m²)</td>
<td>Intact group</td>
<td>4.1 ± 0.76</td>
<td>4.5 ± 0.97</td>
<td>4.4 ± 0.97</td>
<td>4.3 ± 0.87</td>
</tr>
<tr>
<td></td>
<td>Milrinone group</td>
<td>3.7 ± 1.37</td>
<td>4.3 ± 0.77</td>
<td>4.6 ± 1.03</td>
<td>4.4 ± 1.00</td>
</tr>
<tr>
<td>SI (L/min/m²)</td>
<td>Intact group</td>
<td>23.2 ± 4.76</td>
<td>25.8 ± 5.09</td>
<td>25.9 ± 4.88</td>
<td>24.9 ± 4.50</td>
</tr>
<tr>
<td></td>
<td>Milrinone group</td>
<td>22.4 ± 8.96</td>
<td>23.1 ± 5.48</td>
<td>23.0 ± 6.89</td>
<td>20.6 ± 5.36</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>Intact group</td>
<td>5.5 ± 3.92</td>
<td>6.2 ± 4.02</td>
<td>6.1 ± 4.14</td>
<td>7.4 ± 4.31</td>
</tr>
<tr>
<td></td>
<td>Milrinone group</td>
<td>2.4 ± 2.01</td>
<td>4.6 ± 2.77</td>
<td>4.5 ± 3.35</td>
<td>4.9 ± 4.03</td>
</tr>
<tr>
<td>LVdp/dt (mmHg/sec)</td>
<td>Intact group</td>
<td>5.854 ± 1.371</td>
<td>6.577 ± 1.296</td>
<td>6.750 ± 1.086</td>
<td>6.685 ± 1.253</td>
</tr>
</tbody>
</table>

![Fig. 4. Changes in blood flow to organs compared to group hypoxia distribution. N: Normoxia control, H: Hypoxia control. S:P<0.05 compared with group hypoxia.](image1)

![Fig. 5. Percentage change in blood flow to organs compared to group hypoxia distribution. N: Normoxia control, H: Hypoxia control. S:P<0.05 compared with group hypoxia.](image2)
change was seen. This also suggests that the infusion of milrinone did not increase myocardial oxygen consumption. These results suggest that milrinone improves congested hemodynamics efficiently under hypoxia.

It is reported that regional blood flow is controlled by intrinsic and extrinsic regulatory systems. These systems are regulated by the central nervous system and by humoral factors. The coronary artery and brain are mainly controlled by the intrinsic regulatory system [18]. The intrinsic regulatory system is affected by systolic and diastolic pressure, PaCO₂, and sympathetic nervous activity. It has been suggested that PaCO₂ in particular plays an important role in regulating the blood flow in the cerebrum and cerebellum [3]. In the present study, no significant differences were seen between blood flow values in the cerebrum and cerebellum in the two groups. In our investigation, PaCO₂ was maintained at approximately 40 mmHg in both the intact and milrinone groups, and systolic and diastolic artery pressure did not change significantly. It is considered that these are the reasons why no significant change was seen in the blood flow in the cerebrum or cerebellum in either group.

It is reported that renal blood flow under hypoxia is affected by the degree of hypoxia, the duration of the animal’s exposure to hypoxia, and the species [21]. In our investigation, renal blood flow increased slightly in the hypoxia control from the normoxia control in both groups. This result is the same as that described by Adachi et al. [1]. In the hypoxia group, a slight decrease in renal blood flow was seen during the investigation, but an increase in renal blood flow was seen in the milrinone group. We suggest that this increase is caused by a vasodilatory effect of milrinone. Fujishima et al. reported an effect on renal blood flow and femoral artery blood flow when using amrinone, milrinone, and olprinone under normoxia [7]. In that investigation, milrinone and olprinone did not increase renal blood flow as much as amrinone did. These findings suggest that there is a different mechanism for vasodilation in amrinone than in milrinone and olprinone [7], but in our present study it is considered that milrinone is sufficiently potent to protect against renal dysfunction caused by hypoxia and circulatory collapse.

In conclusion, the vasodilatory and inotropic effects of milrinone are sufficiently potent under hypoxia, and regional blood flow is increased by milrinone injection. As a result, milrinone improves congested hemodynamics which complicates hypoxic pulmonary vasoconstriction and protects against organ dysfunction caused by hypoxia.

REFERENCES