Hemodynamic Effects of Benazeprilat in the Anesthetized Dog with Acute Left Ventricular Failure

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ABSTRACT—To examine the hemodynamic effects of benazepril, an angiotensin converting enzyme inhibitor, in left ventricular failure, its active metabolite benazeprilat was administered during acute ischemic left ventricular failure in anesthetized open chest dog induced by repeated injections of plastic microspheres into the left coronary artery. The coronary embolization with microspheres resulted in a moderate and stable left ventricular pump failure characterized by increased left ventricular end-diastolic pressure (LVEDP) and decreased cardiac output (CO). Benazeprilat (30 µg/kg) administered intravenously after a stabilization period lowered LVEDP and maintained CO. The total peripheral resistance was reduced with benazeprilat. The oxygen consumption and the coronary blood flow were reduced with benazeprilat because of a decrease in wall tension and afterload. These results suggest that benazeprilat (benazepril) has beneficial effects for the treatment of acute left ventricular failure.

Angiotensin converting enzyme inhibitors (ACE inhibitors) are potent vasodilators with abilities to modify electrolyte balance (1). Several clinical studies have shown the efficacy of these agents in chronic (2, 3) and acute cardiac failure (4).

Although there are as yet no animal models that can reproduce the clinical syndrome of heart failure exactly, using the experimental models of heart failure, one can address specific questions not easily answered in patients. An acute left ventricular failure (ALVF) induced by repeated injections of plastic microspheres into the coronary artery has been used for evaluating the effects of ACE inhibitors on heart failure (5–7). ACE inhibitors were found to produce a significant improvement of hemodynamic parameters, especially the left ventricular end-diastolic pressure (LVEDP) (5, 7).

Benazepril is a newly synthesized orally active and long acting ACE inhibitor with no SH group (8–12). Like enalapril or other recently found ACE inhibitors, the biological action of benazepril is considered to be exerted through benazeprilat, its active metabolite, because benazepril had a very low inhibitory activity on ACE (9). As regards to ACE inhibition, benazeprilat is nearly equipotent to enalaprilat in vitro or in vivo when injected intravenously (10).

This study evaluates the effects of benazeprilat, an active metabolite of benazepril, on ALVF induced by micro-embolization of the left coronary artery in the anesthetized dog. An effective decrease of LVEDP was found, indicating that benazeprilat may also be efficacious for the treatment of acute heart failure in patients.
MATERIALS AND METHODS

Animal preparation

Sixteen beagle dogs weighing 7 to 9.5 kg (9.0 ± 0.2 kg, n = 16) were premedicated with morphine (1.5 mg/kg, s.c.) and anesthetized with intravenous injection of a mixture of a-chloralose and urethane (60 mg/kg and 600 mg/kg, respectively). The dogs were placed in a left lateral position and artificially respired by means of a respirator (Takashima-Shoten, Tokyo, Japan) through a cuffed endotracheal tube with air and supplemented with oxygen to maintain the arterial Pot, Pco2 and pH levels (around 120 mmHg, 30 mmHg and 7.4, respectively) in the physiological range.

The chest was opened by left thoracotomy, removing the 4th, 5th and 6th ribs to enable a wide view for the operation, and the heart was cradled in the opened pericardium. The ascending aorta was dissected free from the adjacent tissues to place an electromagnetic flowmeter probe (Nihon Kohden, Tokyo, Japan) for measurement of the aortic flow. A Judkins’ 7 French coronary artery catheter (Cordis Corporation, FL, U.S.A.) was introduced through the left carotid artery under fluoroscopy, which was verified with a small amount of contrast medium. Aortic pressure was recorded through a catheter introduced into the aortic arch from the femoral artery. The catheter was connected to a Statham P50 pressure transducer (Gould Statham, CA, U.S.A.) and a carrier amplifier (NEC San-ei, Tokyo, Japan). A catheter tip transducer (Millar P350, Millar, TX, U.S.A.) was inserted into the left ventricle from the femoral artery to measure left ventricular pressure (LVP) and LVEDP. The first derivative of LVP (dP/dt) was measured with a differential amplifier (NEC San-ei, Tokyo, Japan). The heart rate was measured by means of a cardio-tachometer (NEC San-ei, Tokyo, Japan) triggered by LVP pulses. After intra-venous injection of 500 U/kg of heparin, a Morawitz cannula was inserted into the coronary sinus through the right auricle to measure the coronary sinus outflow by means of an electromagnetic flowmeter (Nihon Kohden, Tokyo, Japan). A part of the coronary venous blood and the arterial blood were led to an AV-ox system (AV-ox Systems Inc., TX, U.S.A.) by a Multiperipex pump (2115, LKB, Bromma, Sweden) to measure the difference between arterial and venous oxygen content. All hemodynamic variables were recorded on a thermostyus recorder (Lectocorder 8z, NEC San-ei, Tokyo, Japan and Linearocorder Mark VII, Graphtech, Tokyo, Japan). After operation, the pericardium was closed with cotton threads.

The following parameters were calculated: total peripheral resistance (TPR, mmHg × min/l): mean aortic pressure divided by cardiac output (CO), left ventricular myocardial oxygen consumption (ml/min/100 g): coronary sinus outflow multiplied by A-V oxygen difference.

Induction of ALVF

After a stabilization period of 1 hr, ALVF was induced by subsequent intra-coronary injection of dextran-saline solution of plastic microspheres (51.92 ± 2.15 μm, 3 M Company, MN, U.S.A.) according to the method of Smiseth and Mjos (13). The microspheres were suspended with 9.9 ml of the dextran saline (Rheomacrodex® in saline, Midori Jugi, Osaka, Japan) and 0.1 ml of Tween 80 (Difco Laboratories, MI, U.S.A.) to make a stock solution of 100 mg/ml. The stock solution was diluted to 1 mg/ml with the dextran saline solution and sonicated before injection. The solution contained approximately 12000 microspheres/ml. Initially, 10 ml of the solution was injected into the left main coronary artery. Then, at about 5 min intervals, 2 to 5 ml of the solution was injected until LVEDP rose to around 11 mmHg. Six out of 16 dogs that were injected with microspheres died because of severe heart failure. The remaining 10 dogs were divided into two groups: the control (vehicle treated, n = 5) and the benazeprilat-group (30 μg/kg, n = 5).

Forty minutes after the final injection of microspheres, 30 μg/kg of benazeprilat or 0.1
ml/kg of vehicle (0.5% of NaHCO₃) was injected intravenously for 10 sec. The total volumes of injected microspheres were not significantly different between the vehicle and the benazeprilat group (5.5 ± 1 ml/kg and 4.3 ± 0.6 ml/kg, respectively).

Throughout the study, all animals were dealt with in a humanitarian way in accordance with recognized guidelines on animal experimentation.

Chemicals

Benazeprilat was obtained from Ciba-Geigy Co., Ltd. (Hyogo, Japan). All other chemicals were obtained from Wako Chemical Co., Ltd. (Osaka, Japan).

Data analyses

All values were represented as means ± S.E.M. Two-way analysis of variance (ANOVA) for repeated measure was used for comparison between the vehicle and the benazeprilat group. The paired- and unpaired-Student's t-test was employed for the comparison within the group and between the vehicle and the benazeprilat group before administration. A P value of less than 0.05 was used as the criteria for statistical significance.

RESULTS

Time course of changes in hemodynamic parameters in ALVF induced by coronary embolization

Changes in LVEDP and CO are depicted in Fig. 1. The microsphere-induced micro-embolization of the left coronary artery caused a significant increase in the LVEDP from the control value of 3.7 ± 1.0 mmHg to 11.0 ± 1.1 mmHg (just after embolization) and 13.2 ± 1.7 mmHg (40 min after embolization). LVEDP was about 13 mmHg during the entire course of the experiment (Fig. 1). As shown in Fig. 1, CO decreased from the control value of 1.02 ± 0.05 l/min to 0.93 ± 0.10 l/min (just after embolization) and 0.85 ± 0.12 l/min (40 min after embolization). It decreased further to reach a value of 0.74 ± 0.09 l/min at 70 min after embolization (P < 0.05, Fig. 1). These changes in hemodynamic parameters indicate the establishment of a moderate left ventricular failure.

Heart rate, mean aortic pressure, LVP, max dP/dt, coronary blood flow, oxygen consumption and TPR are listed in Table 1. Coronary embolization caused no significant changes in heart rate, TPR and coronary blood flow. In contrast, mean aortic pressure, LVP, max dP/dt and oxygen consumption were reduced

![Fig. 1. Effects of coronary embolization on the left ventricular end-diastolic pressure (LVEDP) and the cardiac output (CO) in the anesthetized dog (n = 5). *P < 0.05, **P < 0.01 vs. before embolization.](image-url)
by coronary embolization (Table 1).

**Hemodynamic effects of benazeprilat in the anesthetized dog with ALVF**

The hemodynamic parameters of the vehicle and the benazeprilat group after 1 hr stabilization and after induction of ALVF were not significantly different from each other as shown with the values just before administration (at 40 min after embolization) in Table 2. Therefore, the effects of benazeprilat on ALVF were evaluated using percent changes from the pre-administration values.

Effects of benazeprilat on LVEDP and CO are depicted in Fig. 2. The end-diastolic pressure was significantly lowered during the 30 min period after administration of benazeprilat ($P < 0.01$, ANOVA, 15% reduction at 10 min after embolization). CO of the benazeprilat group was slightly but significantly higher than that of the vehicle group ($P < 0.05$, ANOVA).

Significant and sustained decreases of the aortic pressure (30%) and TPR (30%) were observed in the benazeprilat group (Fig. 3). Changes in coronary blood flow and oxygen consumption are shown in Fig. 4. Both parameters were significantly reduced by benazeprilat treatment (26 and 30% reduction, respectively). The reduction of oxygen consumption

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**Table 1. Effects of coronary embolization on the hemodynamic parameters in control animals**

| Parameter                  | Before | 10 min | 40 min | 50 min | 60 min | 70 min |
|----------------------------|--------|--------|--------|--------|--------|--------|
| HR (beats/min)             | 166±13 | 155±9  | 158±9  | 156±10 | 154±10 | 154±10 |
| AP (mmHg)                  | 101±5  | 83±5** | 79±5** | 78±5** | 78±5** | 75±4** |
| LVP (mmHg)                 | 127±8  | 109±6* | 102±6**| 100±7**| 98±7** | 98±7** |
| max dP/dt (mmHg/sec)       | 3014±483 | 1958±244* | 1926±206* | 1942±253* | 1946±232* | 1890±230* |
| CBF (ml/min/100 g)         | 64±15  | 70±21  | 70±21  | 70±22  | 67±21  | 68±23  |
| O₂C (ml/min/100 g)         | 6.6±1.3 | 4.9±1.2* | 4.9±1.3* | 4.7±1.3* | 4.4±1.2* | 4.3±1.3* |
| TPR (mmHg × min/l)         | 92±7   | 75±9   | 77±8   | 82±9   | 86±8   | 88±7   |

HR: Heart rate, AP: mean aortic pressure, LVP: left ventricular pressure (systolic), CBF: coronary blood flow, O₂C: myocardial oxygen consumption, TPR: total peripheral resistance. Before: the values before embolization.
*P < 0.05, **P < 0.01 vs. before embolization (paired t-test, n = 5).

**Table 2. Hemodynamic parameters before drug administration**

| Parameter                  | Vehicle (n = 5) | Benazeprilat (n = 5) |
|----------------------------|----------------|----------------------|
| LVEDP (mmHg)               | 13.2 ± 1.7     | 12.9 ± 0.6           |
| CO (l/min)                 | 0.85 ± 0.12    | 0.79 ± 0.05          |
| HR (beats/min)             | 158 ± 9        | 157 ± 10             |
| AP (mmHg)                  | 79 ± 5         | 87 ± 6               |
| LVP (mmHg)                 | 102 ± 6        | 110 ± 5              |
| max dP/dt (mmHg/sec)       | 1926 ± 206     | 2134 ± 241           |
| CBF (ml/min/100 g)         | 70 ± 21        | 70 ± 22*             |
| O₂C (ml/min/100 g)         | 4.9 ± 1.3      | 5.4 ± 1.6*           |
| TPR (mmHg × min/l)         | 77 ± 8         | 112 ± 15             |

LVEDP: left ventricular end-diastolic pressure, CO: cardiac output, HR: heart rate, AP: mean aortic pressure, LVP: left ventricular pressure (systolic), CBF: coronary blood flow, O₂C: myocardial oxygen consumption, TPR: total peripheral resistance. *: n = 4. No significant difference was observed between the two groups (non-paired t-test).
means that the myocardial work load was reduced by benazeprilat. The max dP/dt and LVP were significantly lowered with benazeprilat treatment (data were not shown). The heart rates of both groups were stable during the experiment and were not significantly different from each other (data were not shown).

DISCUSSION

It was reported that ACE inhibitors were effective for the treatment of patients with heart failure (1, 14). The rationale for using ACE inhibitors under these conditions is the facilitation of ventricular emptying by reducing the peripheral vascular resistance. According to Cohn et al. (15), vasodilators are effective not only in chronic heart failure but also in acute heart failure following myocardial infarction. Moreover, it was demonstrated that several ACE inhibitors were effective in ALVF induced in experimental animals by coronary embolization (4–6). Therefore, using a similar animal model of ALVF, we evaluated the action of benazeprilat, a new potent and long acting ACE inhibitor with no direct inotropic effect (7, 11, 16), on acute heart failure. ALVF was induced by injecting microspheres into the left coronary artery of an anes-
Our data indicated that when injection of microspheres was terminated on attainment of LVEDP of around 11 mmHg, a sustained and marked depression of cardiac contractility as evidenced by reduction of CO could be induced. With the exception of a smaller rise in LVEDP, the degree of heart failure observed in the present experiment was similar to that reported from other laboratories using the same technique but in the closed chest animal (4-6, 13, 17). The smaller rise in LVEDP in our model may be due to the difference of experimental setup, i.e. open chest vs. closed chest and/or the intactness of pericardium.

In the present study, benazeprilat effectively and significantly reduced the elevated LVEDP (15%). Moreover, the CO in the benazeprilat group was slightly but significantly higher than that in the vehicle group, indicating the effectiveness of benazeprilat in acute left ventricular failure. Aortic pressure and TPR were effectively reduced with benazeprilat (30% reduction in both parameters). Sweet et al. (5) reported similar results (21% reduction of LVEDP and no change in CO) using 100 µg/kg of enalaprilat. In their experiments, TPR and aortic pressure were reduced to 76% and 81%, respectively. These results were in agreement with the potency of these two drugs as inhibitors of ACE assessed in vitro (10).

It is interesting to examine whether the hemodynamic effects of ACE inhibitors in the ALVF differ from those in the normal animal. Although, in the present study, no comparison was made between the hemodynamic effects of benazeprilat in the ALVF and those in the non-ALVF, Sweet et al. (5) have already shown that the effects of enalaprilat on arterial pressure, myocardial blood flow, TPR and CO in the ALVF were similar to those in the non-ALVF. Thus, it is likely that the effects of ACE inhibitors in the ALVF may be similar to those in the normal animal.

In conclusion, benazeprilat induced a definite reduction in both LVEDP and afterload in an animal model of ALVF, which may explain the beneficial effects of this drug in heart failure in man.
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