Congestive Heart Failure Model in Rabbits: Effects of Digoxin and a Drug Containing Toad Venom

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ABSTRACT—A low-output-type heart failure model was established in rabbits by protease treatment of the surface of the left ventricular anterior wall. Heart rate, aortic blood flow (AoF), left ventricular pressure (LVP) and maximal rate of rise of LVP (max dP/dt) in this model were maintained at lower levels than in normal rabbits, while left ventricular end-diastolic pressure (LVEDP) and systemic vascular resistance (SVR) were maintained at higher levels, and mean blood pressure (MBP) remained at a normal level. Intraduodenal administration of digoxin and a drug containing toad venom (Kyushin®: KY) improved the hemodynamic parameters by increasing the AoF, LVP and max dP/dt and by decreasing the LVEDP and SVR without a significant change in MBP. These results suggest that the beneficial effects of digoxin and KY on this heart failure model originate from their cardiotoxic activity.

The most critical prerequisite for developing useful cardiotoxic drugs is to establish their efficacy in experimental models of heart failure which possess high clinical relevance. A variety of experimental models of heart failure have been documented so far (see a review by Smith and Nuttall (1)). In these models, except for genetically determined heart failure models, heart failure was induced by surgical procedures such as pulmonary artery binding, aortic constriction, aortic valve incision or coronary embolism or induced by pharmacological means such as administration of cardiotoxic doses of barbiturates, adriamycin or isoproterenol. The required fine surgical procedures and instrumentation for estimating altered cardiac function often demand the use of dogs or larger animals.

Recently, Sonoki et al. (2–4) have reported a new canine heart failure model that was produced by injecting protease into the left ventricular free wall. This model possesses the hemodynamic alterations characteristic to the clinically observed low-output-type of congestive heart failure with peripheral vasoconstriction. It has been shown that clinically used pharmacological interventions such as dobutamine, isoproterenol and prazosin improved the altered hemodynamic parameters in this model by their expected mechanism of action (4). Furthermore, this model has advantages of minimum inter-animal variation in the established heart failure and of not requiring complicated surgical technique.

In an attempt to establish a heart failure model in smaller experimental animals, we considered that this method might be appropriate for rabbits, which are often used...
in pharmacological laboratories because they are easy to handle. In this paper, we describe the successful application of their methods to rabbits and the characteristics of altered cardiac functions in this model.

MATERIALS AND METHODS

Male albino rabbits (Japan domestic strain) purchased from Sankyo Lab. Service (Tokyo) were used after an acclimatization period of at least 6 days. The rabbits, weighing 2.0–2.5 kg, were anesthetized by intraperitoneal administration of urethane (1.0 g/kg), fixed in a supine position, and artificially ventilated with a respirator (Shinano, SN-480-6). The chest was opened at the second intercostal space. Left ventricular systolic pressure (LVP) and end-diastolic pressure (LVEDP) were measured by a pressure transducer (Bentley Trantec, model 800) connected to a polyethylene catheter introduced into the left ventricle. The maximal rate of rise of LVP (max dP/dt) was obtained by differentiating the LVP signal using a differentiator (Nihon Kohden, ED-601G). Mean blood pressure (MBP) was measured via a polyethylene catheter inserted into the left femoral artery. Heart rate (HR) was monitored by a heart rate counter (Nihon Kohden, AT-601G) triggered by the femoral artery pressure pulse. A flowmeter probe (Nihon Kohden, FB-060T) was placed around the ascending aorta, and the aortic blood flow (AoF) was measured by an electromagnetic flowmeter (Nihon Kohden, MFV-1200, linear in the range of 0.2–19.99 ml/min). Systemic vascular resistance (SVR) was calculated by dividing MBP by AoF, and thus SVR in this study did not include the coronary vascular resistance. All parameters were recorded on a multichannel polygraph (Nihon Kohden, RM-6300).

After completing the instrumentation for estimating cardiac functions, we proceeded to the protease treatment of the left ventricular free walls. Instead of the original method of injecting a protease solution into the myocardium, we invented a protease-soaked filter paper method. A stack of three filter paper disks (Toyo Filter Paper, 5B, 5 mm in diameter) was placed at the center of the left ventricular anterior wall and then soaked with the protease solution (37.5 U/10 µl, 10 µl/kg body weight). Five minutes after the treatment with the protease solution, the stack of disks was removed; and 5 min later, the same procedure was repeated. After removing the paper disks and a bolus injection of saline (5 ml/kg), infusion of a 6% dextran solution containing methoxamine (20.7 µg of methoxamine in 0.3 ml of 6% dextran in saline) was commenced through a polyethylene tube placed in the femoral vein and continued until the end of the experiment. The infusion rate (0.06–0.68 ml/min) was adjusted so that the MBP was maintained at the level prior to the treatment with protease. These experimental conditions have been confirmed by preliminary experiments to be adequate to establish the heart failure and to keep the rabbits alive for one hour at least. Then the rabbits were allowed to stand for about 30 min until the individual parameters stabilized. After having confirmed the establishment of heart failure, hemodynamic effects of intraduodenally administered digoxin (8 mg/kg) and KY (20 mg/kg) were evaluated and compared with those of the vehicle.

Digoxin (Sigma), KY (Kyushin), urethane (Sigma), protease (Sigma, Type I), methoxamine hydrochloride (Sigma), heparin sodium (Mochida) and dextran 70 injection (JP XI) were used. Six pills (90 mg) of KY contain 5 mg of toad venom, 3 mg of oriental bezoar, 5 mg of velvet horn, 6 mg of antelope horn, 6 mg of dried bile, 23 mg of ginseng, 7.5 mg of pearl and 2.7 mg of borneol. The specifications of this product were regulated and confirmed by quantitative measurement of cinobufagin and glycohyodeoxycholic acid and by qualitative tests on 6 components. KY and digoxin were suspended in distilled water and administered intraduodenally. In the vehicle group, only distilled water was administered instead of the drug suspension.

Statistical significance of data was estimated
using Student’s or Welch’s (5) t-test (two-tailed) after the F-test. When the population variance was unequal (Fcal > F distribution (a = 0.025)), the latter procedure was employed. Significance was established when a P value was less than 0.05.

RESULTS

Repeated application of the protease-soaked filter paper to the left ventricular free wall resulted in significant decreases in MBP, HR, AoF, LVP and max dP/dt and significant increases in LVEDP and SVR (Table 1). With the saline injection followed by methoxamine infusion, MBP was restored to almost the same level as that before the protease treatment; thus SVR increased further. LVP and max dP/dt also showed a tendency to increase, but were not restored to the pre-protease levels. AoF remained low, and LVEDP and SVR remained at high levels (Table 1). These hemodynamic changes are very similar to the low-output-type congestive heart failure observed in a clinical setting.

With the elapse of time, HR, AoF, LVP and max dP/dt were gradually decreased in this low-output-type congestive heart failure model, whereas LVEDP and SVR gradually increased as shown in Figs. 1 and 2. Four out of 12 rabbits that received the vehicle alone died within an observation period of 2 hour (11−75 min).

By intraduodenal administration of 8 mg/kg digoxin to the rabbit with heart failure, AoF and max dP/dt were significantly increased in a period of 15−120 min after administration (Fig. 2). LVEDP was decreased at 90 min, and SVR was decreased during 15 to 90 min (Fig. 2). Significant inhibition of the decrease in HR was observed at 90 min after administration (Fig. 2).

Intraduodenal administration of KY also produced cardiotonic effects similar to those observed after administration of digoxin. As shown in Fig. 2, administration of 20 mg/kg KY produced increases in AoF, LVP and max dP/dt and decreases in LVEDP and SVR.

MBP was not significantly influenced by these drugs (Fig. 2).

None of the animals given 8 mg/kg digoxin or 20 mg/kg KY died.

DISCUSSION

In this study, we described a new model for low-output type congestive heart failure in rabbits, which was produced by applying the methods of Sonoki et al. with modifications introduced by us (2). In their original methods, Sonoki et al. (2) used dogs and injected a protease solution into the left ven-

| Parameters | Before protease | After protease | Before drug (−0 min) |
|------------|----------------|---------------|---------------------|
| MBP (mmHg) | 62.2 ± 2.2     | 50.3 ± 2.5**  | 66.0 ± 2.2          |
| HR (beats/min) | 258.7 ± 4.5 | 241.4 ± 5.3*  | 250.7 ± 5.3         |
| AoF (ml/min) | 268.2 ± 11.5  | 183.6 ± 10.2** | 179.9 ± 8.8**       |
| LVP (mmHg) | 106.4 ± 2.5    | 89.8 ± 3.0**  | 102.3 ± 2.6         |
| LVEDP (mmHg) | 2.74 ± 0.62   | 7.00 ± 0.93** | 7.28 ± 0.76**       |
| Max dP/dt (mmHg/sec) | 5402 ± 243 | 3503 ± 196**  | 4009 ± 192**        |
| SVR (mmHg-min/ml) | 0.25 ± 0.01 | 0.30 ± 0.02*  | 0.41 ± 0.03**       |

The data are expressed as the mean ± S.E. (n = 45). *, P < 0.05, **, P < 0.01, as compared with the value measured before protease treatment using the t-test. MBP: mean blood pressure, HR: heart rate, AoF: aortic blood flow, LVP: left ventricular systolic pressure, LVEDP: left ventricular end-diastolic pressure, Max dP/dt: maximal rate of rise in left ventricular pressure, SVR: systemic vascular resistance.
tricular free wall. In preliminary experiments, however, we found that it was very difficult to adjust the dose of protease in rabbits. Rabbits died after the injection of protease if the dose exceeded a critical level or no detectable changes in hemodynamic parameters were observed if the dose was lower than that level. Therefore, we invented a novel way of protease application described in this study: an application of protease solution dropwise to disks of filter paper placed on the left ventricular anterior wall. After the treatment with protease, MBP, HR, AoF, LVP and max dP/dt all decreased, and SVR and LVEDP increased significantly. It is likely that max dP/dt and LVP decreased because of the abrupt waning of ventricular muscle contraction due to damage of the myocardial cells by protease, and that this deteriorated myocardial contractility induced the decreases in AoF and MBP and the increase in LVEDP. In order to add a further congestive factor and to increase further the peripheral vascular resistance, the humoral volume was increased by the intravenous injection of saline, and the peripheral vasculature was constricted by methoxamine infusion. By these treatments, MBP was increased to almost the same level as that before protease treatment. However, AoF still remained lower than the pre-protease level, and LVEDP and SVR remained at higher levels. Thus, our cardiac failure model was accompanied by low AoF and peripheral vasoconstriction. This method could easily produce a constant degree of heart failure state with good reproducibility.

Intraduodenal administration of digoxin increased AoF, max dP/dt and LVP without any significant change in MBP, and it decreased LVEDP and SVR in this congestive heart failure model. The fact that digoxin decreased LVEDP, which is an important index of cardiac function (6), suggests that this model is useful for evaluating the effects of these drugs on heart failure.

Intraduodenal administration of KY also increased AoF, max dP/dt and LVP without any significant change in MBP; and it de-
creased LVEDP and SVR, as digoxin did. This suggests that the mode of action of this drug on the heart failure model is apparently similar to that of digoxin. It is well-known that toad venom and/or bufosteroids such as bufalin and cinobufagin, which are constituents of KY, have a cardiotonic action resembling that of cardiac glycosides (7-10), although the metabolism of cinobufagin is reportedly much different from that of digitalis (11).

In conclusion, it was shown in the present study that by protease treatment, a congestive heart failure model could be produced experimentally in rabbits, and we also found that digoxin and KY improved the state of cardiac failure through their cardiotonic action.

Fig. 2. Effects of intraduodenal administration of digoxin and KY on hemodynamics in anesthetized rabbits with the experimental congestive heart failure induced by protease. The data are expressed as the mean ± S.E. *: P < 0.05, **: P < 0.01, as compared with the vehicle group using the t-test. AoF: aortic blood flow, LVP: left ventricular systolic pressure, LVEDP: left ventricular end-diastolic pressure, Max dp/dt: maximal rate of rise in left ventricular pressure, SVR: systemic vascular resistance, MBP: mean blood pressure. ○: Vehicle (n = 8); • : KY, 20 mg/kg, i.d. (n = 5); ■: Digoxin, 8 mg/kg, i.d. (n = 5).

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