The Effects of Dobutamine, Propranolol and Nitroglycerin on an Experimental Canine Model of Congestive Heart Failure

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ABSTRACT—A new experimental model of acute congestive heart failure was established in open-chest dogs, and it was employed to examine the effects of dobutamine, propranolol and nitroglycerin. The model was induced by intracoronary administration of saponin, volume loading and intravenous infusion of methoxamine. Left ventricular end-diastolic pressure (LVEDP) increased from 7.9±0.6 to 24.2±1.4 mmHg, and aortic blood flow (AoF) decreased from 0.89±0.06 to 0.53±0.041/min. Systemic vascular resistance (SVR) increased from 9618 ± 585 to 16492 ± 1213 dynes · sec/cm5 and right atrial pressure (RAP) increased from 2.5±0.2 to 4.2±0.4 mmHg. Furthermore, VMax decreased from 71.6±5.1 to 45.8±2.9 1/sec, and the time constant of left ventricular pressure decay (T) increased from 40.0±2.6 to 90.2±7.9 msec. These hemodynamic changes were stable for up to 80 min. Dobutamine improved cardiac function by increasing Vmax and by decreasing T. Consequently, dobutamine increased AoF and decreased LVEDP, while there was no change in SVR. Nitroglycerin reduced LVEDP, SVR and T; increased AoF; and did not change Vmax. Propranolol produced no improvement in the hemodynamics or cardiac function. These results indicate that the present congestive heart failure model is characterized by global left ventricular dysfunction with lowered cardiac output and increased peripheral vascular tone, and it is beneficial for evaluating the pharmacological properties of drugs for acute congestive heart failure.

Keywords: Congestive heart failure (canine model), Dobutamine, Propranolol, Nitroglycerin

To develop therapeutic agents for the treatment of congestive heart failure (CHF), drug evaluation in experimental heart failure models with clinical relevance is very important. In the last decade, many types of experimental animal models for heart failure have been developed (1-6), and many drugs are evaluated by using these models. Hitherto, several models of failing heart have depended on excessive doses of propranolol (4, 5), barbiturates (5) or a surgical method such as coronary ligation, pulmonary artery banding or coronary embolism (1). However, some models have certain weakness, e.g., unstable hemodynamics due to arrhythmia, insufficient changes in left ventricular end-diastolic pressure or cardiac output.

Recently, CHF has been treated mainly with diuretics, vasodilators and cardiotonic agents (7-9). These drugs improve left ventricular filling pressure, increase cardiac output and reduce peripheral resistance (6). In addition to load-reduction and improvement of myocardial contractility, it is important to evaluate the effects of drugs on the diastolic function of the left ventricle, because the abnormalities of left ventricular relaxation have been demonstrated to accompany clinical CHF (7).

In the present study, we developed a new experimental canine model of CHF by intracoronary injections of saponin, which is widely used for the skinning of smooth muscle cell membranes (10), volume-loading and methoxamine infusion. Moreover, we investigated the effects of conventional therapeutic drugs that are already used for the treatment of CHF.

MATERIALS AND METHODS

Experimental preparations

Twenty-seven mongrel dogs of either sex, weighing 8.0-14.5 kg, were anesthetized with sodium pentobar-
bital (30 mg/kg, i.v.) and artificially ventilated using a
volume limited respirator (Takashima-Shoten, Tokyo)
with room air (a tidal volume of 20 ml/kg at a rate of 20
beats/min). The heart was exposed by removing the
3rd - 6th ribs, and the heart was cradled with the pericar
dium. An electrical magnetic flow probe (Nihon Kohden,
Tokyo) was placed around the ascending aorta to mea
sure aortic blood flow (AoF). A monopolar electrode was
attached to the free wall of the left ventricle to monitor
the local electrocardiogram. Heart rate (HR) was deter
mined from the R-R interval of the electrocardiogram.
Polyethylene catheters were inserted into the right atrium
from the subclavicular vein to measure right atrial pres
sure (RAP) and into the abdominal aorta from the left
femoral artery to measure arterial pressure. A catheter-tip
pressure transducer (Millar, Houston, TX, USA) was in
serted into the left ventricle through the right femoral ar
tery to measure left ventricular pressure (LVP). The max
imal rate of the first derivative of LVP, LVmaxdP/dt, was
obtained by a differential amplifier (NEC San-ei, Tokyo).
These parameters were continuously recorded on an 8-
channel pen recorder (NEC San-ei).

Systemic vascular resistance (SVR) was calculated as
([mean arterial pressure (MAP) - RAP]/AoF). The time
constant of isovolumic left ventricular pressure decay,
"T", was calculated from a plot of a negative LVmaxdP/dt
versus LVP according to the best fit method (11, 12). Vmax,
which is an index of the contractility of the left ventricle,
was calculated from the pressure-velocity curve by
manual linear extrapolation. Signals from 10 - 15 con
secutive beats were overlapped with an X-Y plotter (NEC
San-ei) at each point for analysis of T and Vmax.

Production of acute CHF

The CHF model was established as illustrated in Fig. 1.
Branches of the left anterior descending artery (LAD) and
left circumflex artery (LCX) were cannulated for drug ad
ministration. Threads were placed on the artery of each
catheter proximally to allow occlusion of the coronary
arteries. During total occlusion of the LCX for 30 sec, the
peripheral area of LCX was filled with 1 to 1.5 ml of sapo
nin solution (1 mg/ml), which was injected from the
catheter to produce global impairment of the myocar
dium. The same procedure was repeated in the LAD to
injure the peripheral area of LAD.

Saline (30 ml/kg) was injected intravenously for 10 to
20 min for volume-loading and subsequently methox
amine, an α-adrenergic stimulant, was titrated intravenously
to maintain MAP at the level before saponin injections
throughout the experiment. When left ventricular end
diastolic pressure (LVEDP) elevated over 18 mmHg, the
animal was determined to have CHF.

Preparation of heart failure

Six of 27 CHF dogs were used to examine changes in
systemic hemodynamics and cardiac function during each
procedure. These animals were also used in experiments
with nitroglycerin. Before intracoronary injection of sapo
nin into the LCX, saline was injected to examine the
influence of 30-sec total occlusion on cardiac function and
systemic hemodynamics. Subsequently the same
procedure was performed to produce the CHF model.

Stability of the heart failure model

The stability of the CHF model was evaluated in 6
animals. Time-dependent changes in all parameters were
monitored for 80 min just following the establishment of
the model.

Effects of dobutamine, propranolol and nitroglycerin

Dobutamine was administered intravenously as a bolus
at doses of 0.1 - 10 μg/kg to 6 CHF dogs. Propranolol
was administered intravenously as a bolus at doses of
1 - 300 μg/kg to 7 CHF dogs. Nitroglycerin was admin
istered intravenously by continuous infusion at doses of
0.1 - 40 μg/kg/min to 8 CHF dogs. Each dosing level of
nitroglycerin was administered for at least 3 min.

Pathological analysis

After the experiments, the animals were sacrificed with a
lethal dose of sodium pentobarbital. Samples of cardiac
tissue were taken from the free wall of the left ventricle and
fixed in neutral buffered formalin. Sections of the
tissue were stained by hematoxylin-eosin to observe any
pathological changes.
Drugs
Saponin (Wako Pure Chemical, Osaka), methoxamine (Mexane®; Nippon Shinyaku, Kyoto), nitroglycerin (Millisrol®; Nippon Kayaku, Tokyo), propranolol (Inderal®; ICI Pharma, Osaka) and dobutamine (Dobutrex®; Shionogi, Osaka) were used in this study. These drugs were dissolved in or diluted with saline.

Statistical analyses
Data are presented as the mean±S.E. Changes in the data were analyzed by Student’s t-test, which compared the initial and experimental values. Time-dependent changes in the data were evaluated with analysis of variance for repeated measures. Statistical significance was accepted if the P values were less than 5%.

RESULTS
Hemodynamic characteristics of the CHF model
Intracoronary injection of saline (1-1.5 ml) to the LCX during 30-sec total occlusion caused no significant changes in hemodynamics and cardiac function. In contrast, a single injection of saponin to the LCX caused a slight decrease in MAP, AoF, left ventricular systolic pressure (LVSP) and negative LVmaxdP/dt and an increase in SVR and T. Additional injection of saponin to the LAD produced further changes in these parameters, as well as significant decreases in positive LVmaxdP/dt and Vmax. Intravenous administration of saline (30 ml/kg) decreased SVR, LVmaxdP/dt and Vmax, and it increased RAP, Vmax and T.

Table 1. Changes of hemodynamics and left ventricular function in response to maneuvers to produce congestive heart failure in open-chest dogs

| Parameters          | Control | LCX (i.c.a) | Total saponin | Saline (i.v.) | Methoxamine (i.v.) |
|---------------------|---------|-------------|---------------|--------------|-------------------|
|                     |         | Saline      | Saponin       |              |                   |
| MAP (mmHg)          | 98 ± 7  | 97 ± 8      | 89 ± 7**      | 81 ± 9*      | 77 ± 7*           | 102 ± 7          |
| HR (beats/min)      | 138 ± 9 | 139 ± 9     | 137 ± 9       | 143 ± 12     | 123 ± 9**         | 117 ± 8*         |
| AoF (l/min)         | 0.75 ± 0.06 | 0.75 ± 0.05 | 0.71 ± 0.06** | 0.66 ± 0.06* | 0.87 ± 0.1        | 0.49 ± 0.07**    |
| SVR (dyne·sec/cm²)  | 10491 ± 1122 | 10444 ± 1073 | 10128 ± 1108* | 9989 ± 1470  | 6995 ± 825**      | 17753 ± 2275**   |
| RAP (mmHg)          | 2.0 ± 0.4 | 1.9 ± 0.4   | 1.9 ± 0.4     | 1.9 ± 0.3    | 3.8 ± 0.5**       | 2.8 ± 0.3**      |
| LVSP (mmHg)         | 120 ± 10 | 118 ± 9     | 112 ± 10**    | 103 ± 11*    | 101 ± 9*          | 123 ± 10         |
| LVEDP (mmHg)        | 10.5 ± 1.5 | 10.5 ± 1.5  | 10.5 ± 1.5    | 12.8 ± 2.3   | 19.9 ± 1.7**      | 26.4 ± 0.8**     |
| (+)LVmaxdP/dt (mmHg/sec) | 1448 ± 156 | 1465 ± 156  | 1384 ± 148    | 1108 ± 183*  | 988 ± 160**       | 943 ± 140**      |
| (−)LVmaxdP/dt (mmHg/sec) | -1255 ± 109 | -1180 ± 93  | -1056 ± 110** | -852 ± 147** | -732 ± 109**      | -803 ± 81**      |
| Vmax (1/sec)        | 68.6 ± 3.7 | 68.8 ± 4.1  | 67.8 ± 4.8    | 56.5 ± 5.3*  | 50.6 ± 4.4**      | 43.4 ± 2.5**     |
| T (msec)            | 36.6 ± 8  | 39.2 ± 7.8  | 42.1 ± 9.7*   | 55.5 ± 15.3* | 74.2 ± 17.3*      | 97.4 ± 14.3**    |

Fig. 2. Changes in hemodynamics and left ventricular function in acute congestive heart failure (CHF). Each point and vertical bar represents the mean±S.E. of 27 animals. **P<0.01, significantly different from the control value. AoF: aortic blood flow, SVR: systemic vascular resistance, RAP: right atrial pressure, LVEDP: left ventricular end-diastolic pressure.
LVEDP and T. Subsequent continuous titration of methoxamine induced further decreases in AoF, LVm, dP/dt and \( V_{\max} \) and induced increases in SVR, LVEDP and T (Table 1).

The changes in the systemic hemodynamics and cardiac function of 27 CHF animals are summarized in Fig. 2 and Table 2. Saponin-induced heart failure caused significant decreases in AoF and \( V_{\max} \) and caused significant increases in SVR, RAP, LVEDP and T. In addition, HR, positive \( LV_{\max} dP/dt \) and negative \( LV_{\max} dP/dt \) decreased significantly. There was no significant changes in MAP and LVSP because these parameters were kept constant by infusion of methoxamine.

The stability of the CHF model
The time-dependent changes in systemic hemodynamics and cardiac function are shown in Fig. 3. There were no significant changes in any parameters during 80 min of observation after the establishment of the CHF model.

Histopathological changes in the CHF model
The typical pathological changes in the present CHF model are shown in Fig. 4. Remarkable myocardial vacuolization, coagulation and lysis were observed. These changes were observed in mosaic fashion. Coronary embolism or stenosis were not observed in the coronary arteries.

Table 2. Changes of hemodynamics in canine experimental congestive heart failure (CHF) model

| Parameters          | Control     | CHF         |
|---------------------|-------------|-------------|
| MAP (mmHg)          | 100±2       | 100±2       |
| HR (beats/min)      | 136±5       | 120±5**     |
| LVSP (mmHg)         | 116±3       | 116±3       |
| \(+\)LV_{\max} dP/dt (mmHg/sec) | 1703±92     | 1152±71**   |
| \(-\)LV_{\max} dP/dt (mmHg/sec) | −1781±133   | −1018±73**  |

Each value represents the mean±S.E. of 27 animals. **P<0.01, significantly different from the value measured before CHF-preparation. MAP: mean arterial pressure, HR: heart rate, LVSP: left ventricular systolic pressure.

Fig. 3. Time-dependent changes of hemodynamics and left ventricular function. Each point and vertical bar represents the mean±S.E. of 6 animals. MAP: mean arterial pressure, HR: heart rate, AoF: aortic blood flow, SVR: systemic vascular resistance, RAP: right atrial pressure, LVSP: left ventricular systolic pressure, LVEDP: left ventricular end-diastolic pressure, LV: left ventricle.
Effects of dobutamine, propranolol and nitroglycerin

Figures 5–7 show the dose-dependent changes in cardiovascular parameters following intravenous administration of dobutamine, propranolol or nitroglycerin. Administration of dobutamine significantly increased positive $\text{LV}_{\text{max}}\text{dP/dt}$ and decreased T at doses of 0.3–10 $\mu$g/kg. Furthermore, MAP, HR, AoF, LVSP, negative $\text{LV}_{\text{max}}\text{dP/dt}$ and $V_{\text{max}}$ increased significantly at 1–10 $\mu$g/kg. RAP and LVEDP decreased significantly at 3 and 10 $\mu$g/kg. SVR was not changed significantly (Fig. 5).

Intravenous administration of propranolol decreased $V_{\text{max}}$ at doses of 10–300 $\mu$g/kg, while no significant change in T was observed. HR and AoF were decreased significantly at doses of 3–300 $\mu$g/kg. Positive and negative $\text{LV}_{\text{max}}\text{dP/dt}$ were decreased significantly at doses of 30–300 and 3–300 $\mu$g/kg, respectively. MAP and LVSP decreased at doses of 10–300 $\mu$g/kg. SVR increased at doses of 100 and 300 $\mu$g/kg. There were no significant changes in LVEDP or RAP (Fig. 6).

Intravenous administration of nitroglycerin produced a significant decrease in LVSP, LVEDP and T at doses of 1–40 $\mu$g/kg/min. MAP and SVR decreased and AoF increased significantly at doses of 4–40 $\mu$g/kg/min. Furthermore, RAP decreased significantly at 10 and 40 $\mu$g/kg/min. However, there were no remarkable changes in HR, positive and negative $\text{LV}_{\text{max}}\text{dP/dt}$ or $V_{\text{max}}$ (Fig. 7).

The relationship between AoF and LVEDP after intravenous administration of dobutamine, propranolol or nitroglycerin are shown in Fig. 8. AoF is plotted on the ordinate and LVEDP on the abscissa. As compared with the initial state before saponin treatment, the establishment of the CHF model produced remarkable changes in AoF and LVEDP, as described above. The intravenous administration of dobutamine caused a dose-dependent increase in AoF and decrease in LVEDP, i.e., it shifted these parameters towards the control values. However, propranolol decreased AoF with little or no change in LVEDP. Nitroglycerin changed these parameters in a similar manner to dobutamine.
Fig. 5. Effects of intravenous administration of dobutamine on hemodynamics and left ventricular function. Each point and vertical bar represents the mean ± S.E. of 6 animals. *P < 0.05, **P < 0.01, significantly different from the control value. Abbreviations are defined in Fig. 3.

Fig. 6. Effects of intravenous administration of propranolol on hemodynamics and left ventricular function. Each point and vertical bar represents the mean ± S.E. of 7 animals. *P < 0.05, **P < 0.01, significantly different from the control value. Abbreviations are defined in Fig. 3.
DISCUSSION

In the present study, we report a new experimental model of acute CHF in anesthetized dogs that was produced by means of intracoronary injections of saponin, volume loading and infusion of methoxamine. In these manipulations, both contraction and relaxation of the left ventricle were impaired. LVEDP was elevated up to 18 mmHg, SVR and RAP were increased significantly, and cardiac output was decreased. In addition, these hemodynamic changes were sustained for at least 80 min after the establishment of CHF, which is probably sufficiently long for the evaluation of drug effects. Intravenous administration of dobutamine and nitroglyc-
In the present acute model of CHF, intravenous administration of methoxamine produced an increase in AoF and a decrease in LVEDP. Dobutamine predominantly improved cardiac function and resulted in a preload reduction and increase in cardiac output. In contrast, nitroglycerin predominantly decreased preload and afterload, which was followed by the improvement of left ventricular relaxation. Propranolol did not improve the parameters of cardiac function at all in this model. These results demonstrate that the present model has potential for differentiating or evaluating the effects of drugs on acute CHF.

Intracoronary injection of saline with total coronary occlusion for 30 sec did not affect any parameters. In contrast, a single administration of saponin to the LCX caused a slight but significant decrease in MAP and AoF and an increase in T. Furthermore, subsequent saponin treatment to the LAD caused a decrease in positive $LV_{max}$ dP/dt and $V_{max}$. Thus, saponin depressed the contractility of the left ventricle. In addition, abrupt dyskinesis of the myocardium due to saponin induced a reduction in blood pressure and LVSP, while SVR and RAP were not altered, and LVEDP remained in the normal range.

Recently, saponin has been widely used for the chemical skinning of smooth muscle cell membranes for the purpose of increasing the permeability of the membrane to substrates (10). In the present experiments, saponin produced a global myocardial necrosis. Histologically, diffuse necrotic lesions were observed with the existence of intact or non-necrotic myocardial cells in a mosaic fashion.

To worsen the heart failure condition, intravenous administration of saline and continuous titration of methoxamine were performed. With these procedures, the present model showed a recovery in MAP to the pre-saponin level, while there were further decreases in myocardial pumping activity and cardiac output, and the increases in both preload (LVEDP) and afterload (SVR). Therefore, the present CHF model is characterized by a lowered cardiac output and increased peripheral vascular tone.

Dobutamine improved cardiac contractility and relaxation, decreased left ventricular filling pressure and increased cardiac output, while no remarkable change in peripheral resistance was observed. These results agree well with previous reports (6, 13). The alteration of cardiac function appeared predominantly at lower doses; and subsequently, LVEDP and RAP decreased at higher doses, indicating that dobutamine improved the hemodynamics via direct inotropic action on the myocardium.

The efficacy of propranolol for the therapy of chronic CHF has been investigated because this drug induces an up-regulation of myocardial $\beta$-adrenergic receptors (14). In the present acute model of CHF, intravenous administration of propranolol suppressed cardiac function and increased both peripheral resistance and left ventricular filling pressure. Previous studies indicated that the hemodynamics and cardiac output were further impaired with propranolol (4, 5).

Intravenous infusion of nitroglycerin, which has both venous and arterial dilating effects, caused a reduction of preload and afterload, as has been previously reported in experimental and clinical heart failure (15, 16). Furthermore, nitroglycerin decreased the time constant of left ventricular relaxation (T), without remarkable changes in $V_{max}$. Preload reduction has been demonstrated to alter the left ventricular filling pattern and improve left ventricular relaxation (17). Thus, the increase in AoF with nitroglycerin could be explained by the improvement of the diastolic function of the left ventricle associated with changes of systemic hemodynamics.

In conclusion, we have established a new acute congestive heart failure model with global dysfunction of the left ventricle caused by intracoronary injection of saponin, volume loading and intravenous infusion of methoxamine. This model is characterized by lowered cardiac output and increased peripheral vascular tone, with these changes being stable for at least 80 min. Dobutamine and nitroglycerin produced load-reduction and increased cardiac output consistent with the expected actions of these drugs. These results indicate that this CHF model with global myocardial dysfunction may be useful for the evaluation of drugs in acute CHF.

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