A CANINE MODEL OF DILATED CARDIOMYOPATHY INDUCED BY REPETITIVE INTRACORONARY DOXORUBICIN ADMINISTRATION

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Objective: A simple and reproducible large animal model of dilated cardiomyopathy has yet to be developed. This study was performed to establish a canine model of dilated cardiomyopathy. **Methods:** Six closed-chest purebred beagles weighing 8 to 12 kg (10 ± 1.9 kg) underwent intracoronary infusion of doxorubicin (Adriamycin). Low-dose (0.7 mg/kg) doxorubicin was infused into the left main coronary artery through a 5F Judkins catheter. Infusions were repeated weekly for 5 weeks. We evaluated the effects on cardiac hemodynamics, chamber size, the neuroendocrine system, and cardiac ultrastructure before and 1 and 3 months after five intracoronary infusions of doxorubicin. **Results:** Three months after treatment, fractional shortening (mean $\pm$ standard error of the mean) had decreased from $36.5\% \pm 0.8\%$ to $21.7\% \pm 1.4\%$ ($p = 0.0003$), and left ventricular ejection fraction had decreased from $71.0\% \pm 3.3\%$ to $36.3\% \pm 5.5\%$ ($p = 0.001$). The left ventricular diastolic dimension had increased from 27.8 ± 0.9 to 35.5 ± 0.6 mm ($p = 0.003$), and the left ventricular end-diastolic volume had increased from 27.5 ± 1.8 to 38.3 ± 1.9 ml ($p = 0.015$). Left ventricular end-diastolic pressure had increased from 8.5 ± 0.9 to 14.5 ± 1.1 mm Hg ($p = 0.01$), and the stroke volume had decreased from 16.7 ± 0.9 to 11.5 ± 0.4 ml ($p = 0.001$). During the same period, the plasma norepinephrine concentration also increased from 114 ± 27.4 to 423 ± 88.9 pg/ml ($p = 0.024$), and plasma atrial natriuretic peptide levels increased from 33.8 ± 7.0 to 76.5 ± 14.8 pg/ml ($p = 0.012$). Histologic changes such as myofiber atrophy and cytoplasmic vacuolation, accompanied with interstitial fibrosis, were found predominantly in the left ventricle. **Conclusion:** Repeated intracoronary infusions of doxorubicin represent a simple and reliable technique to produce dilated cardiomyopathy in the dog. This model can be used to evaluate the effects of new therapies, especially surgical treatments such as dynamic cardiomyoplasty and reduction ventriculoplasty, on dilated cardiomyopathy. (J Thorac Cardiovasc Surg 1998; 115:1367-73)

Heart failure is a syndrome of associated abnormal physiologic characteristics that include left ventricular dysfunction and dilation, activation of the neuroendocrine system, and restriction of phys-

ical activity. The progression of these characteristics during the evolution of heart failure has been difficult to determine in human beings because of the uncertainty of when heart failure in fact begins or the effects of age, concomitant diseases, and medications. Because of these problems, animal models of chronic heart failure are important in evaluating the effects of new therapies for heart failure. Many methods to produce a heart failure model have been reported in various animal species.¹⁻⁵ However, it is difficult to measure cardiac hemodynamics accurately in small animals.

In the clinical setting, end-stage left ventricular failure caused by cardiomyopathy can now be treated with heart transplantation, cardiomyoplas-

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Table 1. Serial left ventriculographic and echocardiographic data at baseline, and 1 month and 3 months after treatment

Hemodynamics	Baseline		1 mo		<i>p</i> Value vs baseline	3 mo		<i>p</i> Value vs baseline	<i>p</i> Value vs 1 mo
	(mean $\pm$ SE)	95% CI	(mean $\pm$ SE)	95% CI		(mean $\pm$ SE)	95% CI		
LVEDV (ml)	27.5 $\pm$ 1.8	22.8-32.2	38.5 $\pm$ 1.9	33.7-43.3	0.018	38.3 $\pm$ 1.9	33.5-43.2	0.015	0.96
LVESV (ml)	7.8 $\pm$ 1.0	5.3-10.4	23.0 $\pm$ 2.9	15.6-30.4	0.0009	24.3 $\pm$ 2.1	19.0-29.6	0.002	0.73
LVEF (%)	71 $\pm$ 3.3	62.3-79.4	41.0 $\pm$ 5.0	28.1-53.9	0.0001	36.3 $\pm$ 5.5	22.2-50.4	0.001	0.48
LVDd (mm)	27.8 $\pm$ 0.9	25.4-30.3	34.2 $\pm$ 0.9	31.7-36.6	0.018	35.5 $\pm$ 0.6	34.1-36.9	0.003	0.08
LVDs (mm)	17.8 $\pm$ 0.8	15.8-19.9	26.5 $\pm$ 1.0	24.0-29.0	0.003	27.8 $\pm$ 0.8	25.7-30.0	0.0009	0.01
%FS (%)	36.5 $\pm$ 0.8	34.4-38.6	22.7 $\pm$ 1.1	20.0-25.4	0.0001	21.7 $\pm$ 1.4	18.1-25.3	0.0003	0.14
IVSd (mm)	9.3 $\pm$ 0.4	8.2-10.4	7.8 $\pm$ 0.7	6.2-9.5	0.19	7.5 $\pm$ 0.3	6.6-8.4	0.06	0.47
PWd (mm)	7.8 $\pm$ 0.6	6.3-9.4	7.0 $\pm$ 0.7	5.2-8.8	0.50	6.5 $\pm$ 0.2	5.9-7.1	0.12	0.36
IVSs (mm)	11.0 $\pm$ 0.6	9.4-12.6	9.1 $\pm$ 0.2	8.6-9.6	0.01	8.5 $\pm$ 0.3	7.8-9.2	0.02	0.19
PWs (mm)	10.1 $\pm$ 0.3	9.4-10.8	8.9 $\pm$ 0.3	8.2-9.6	0.0006	8.5 $\pm$ 0.2	7.9-9.1	0.002	0.06

The values are expressed as mean $\pm$ the standard error (SE) of the mean. The *p* values are calculated by repeated-measures analysis of variance with the Scheffe F test. CI, Confidence interval; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; LVDd, left ventricular diastolic dimension; LVDs, left ventricular systolic dimension; %FS, fractional shortening; IVSd and PWd, end-diastolic wall thickness of interventricular septum and posterior wall; IVSs and PWS, end-systolic wall thickness of interventricular septum and posterior wall.

ty,⁶ or reduction ventriculoplasty.⁷ Research thus should be performed in an animal model of cardiomyopathy. However, the experimental evaluation of these new therapies has been hampered because a stable and reproducible large animal model of chronic heart failure is still lacking. Especially, a model of dilated cardiomyopathy has never been established. Previous efforts to create chronic heart failure in large animals required complex surgical procedures.⁸⁻¹⁰ If we want to assess the effects of surgical treatments like transplantation, cardiomyoplasty, or ventriculoplasty, a large animal model of nonreversible heart failure not requiring complex thoracic procedures such as thoracotomy or pericardiotomy would be the most appropriate. The purpose of this study was to establish a simple and reproducible model of dilated cardiomyopathy in closed-chest beagles.

Materials and methods

Nine purebred adult beagles weighing 8 to 11 kg were used for the study. All animals were kept in clean cages and were provided with regular food and sterile water. They received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the Institute of Laboratory Animal Resources and the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). The protocol was approved by the Kobe University School of Medicine Experimental Animals Committee.

Hemodynamic evaluation. For each hemodynamic measurement, the beagles were anesthetized with sodium pentobarbital (25 mg/kg, intravenously) and permitted to breathe spontaneously. Echocardiography, cardiac catheterization, and left ventriculography were performed before the treatment and 1 and 3 months after the last

intracoronary injection of doxorubicin (Adriamycin). All echocardiograms were performed with the dog lying in the left decubitus position. Left ventricular echocardiograms were obtained at the chordal level just below the tips of the mitral leaflets with an echocardiograph (SSH-140A, Tohshiba Medical Inc., Tokyo, Japan). All cardiac catheterizations were performed under sterile conditions. A 7.5F Swan-Ganz catheter (Baxter Healthcare Corp., Irvine, Calif.) was advanced from the femoral vein to the pulmonary artery and a 4F pigtail catheter from the femoral artery to the left ventricle for pressure measurement. Arterial and intracardiac pressures were measured with a polygraph (363, NEC San-ei Instruments, Ltd.) and recorded (8M14, NEC San-ei Instruments Ltd.). Cardiac output was measured by the thermodilution technique and was based on the mean of at least five measurements recorded by a cardiac output computer. Left ventriculograms were obtained during each catheterization immediately after completion of the hemodynamic measurements with the dog placed on its right side. Left ventriculograms were recorded on videotape and analyzed with a Cardio 500 device (Kontron Elektronik GmbH). Correction for image magnification was made with a calibrated grid placed at the level of the left ventricle. Left ventricular volumes were measured by the area-length method.¹¹

Intracoronary doxorubicin. Intracoronary infusions were performed with a catheter. Under pentobarbital anesthesia (25 mg/kg, intravenously), a 5F Judkins catheter was inserted into the left main coronary artery with the aid of a fluoroscope through a 5F sheath placed in the femoral artery. Doxorubicin was infused at a rate of 0.7 mg/kg in six beagles. The doxorubicin was dissolved in 20 ml of 0.9% sodium chloride and delivered by bolus injection into the coronary artery. Infusions were repeated each week for a total of 5 weeks. This protocol was established during preliminary studies in six beagles in which the dose of doxorubicin was adjusted to prevent immediate lethal myocardial injury. At preliminary study beagles with a dose of 1.0 mg/kg or 1.5 mg/kg died before

Table II. Serial cardiac catheterization data at baseline, and 1 month and 3 months after treatment

Hemodynamics	Baseline (mean ± SE)	95% CI	1 mo (mean ± SE)	95% CI	p Value vs baseline	3 mo (mean ± SE)	95% CI	p Value vs baseline	p Value vs 1 mo
HR (beats/min)	120 ± 5.5	106-134	139 ± 3.1	131-147	0.018	145 ± 5.3	131-158	0.009	0.14
RAP (mm Hg)	2.7 ± 0.7	0.8-4.5	2.8 ± 0.4	1.8-3.9	0.82	2.2 ± 0.2	1.7-2.6	0.54	0.17
RVP (mm Hg)	36 ± 2.9	28.6-43.4	38 ± 1.8	33.5-42.9	0.31	36 ± 1.9	31.4-40.9	0.94	0.14
RVEDP (mm Hg)	2.3 ± 0.4	1.2-3.4	2.8 ± 0.3	2.0-3.6	0.46	2.5 ± 0.2	1.9-3.1	0.74	0.53
PAP (mm Hg)	34 ± 2.8	27.1-41.2	35 ± 1.8	30.2-39.5	0.83	36 ± 1.9	31.0-41.0	0.54	0.32
PCWP (mm Hg)	5.2 ± 0.5	3.9-6.4	11.5 ± 0.8	9.5-13.5	0.001	12.5 ± 1.3	9.1-15.9	0.005	0.20
LVP (mm Hg)	128 ± 5.3	115-142	130 ± 7.3	111-149	0.83	135 ± 8.1	114-156	0.51	0.09
LVEDP (mm Hg)	8.5 ± 0.9	6.0-11.0	13.7 ± 1.5	9.9-17.5	0.001	14.5 ± 1.1	11.6-17.4	0.0001	0.09
AoP (mm Hg)	127 ± 5.2	114-141	120 ± 7.2	102-139	0.28	123 ± 8.1	102-143	0.49	0.18
CO (L/min)	1.99 ± 0.07	1.74-2.24	1.71 ± 0.10	1.52-1.90	0.008	1.66 ± 0.05	1.53-1.79	0.005	0.14
SV (ml)	16.7 ± 0.9	14.4-18.9	12.3 ± 0.6	10.9-13.8	0.003	11.5 ± 0.4	10.4-12.6	0.001	0.14

The values are expressed as mean ± the standard error (SE) of the mean. The *p* values are calculated by repeated-measures analysis of variance with the Scheffe F test. *CI*, Confidence interval; *HR*, heart rate; *RAP*, right atrial pressure; *RVP*, right ventricular pressure; *RVEDP*, right ventricular end-diastolic pressure; *PAP*, pulmonary arterial pressure; *PCWP*, pulmonary artery capillary wedge pressure; *LVP*, left ventricular pressure; *LVEDP*, left ventricular end-diastolic pressure; *AoP*, aortic pressure; *CO*, cardiac output; *SV*, stroke volume.

Table III. Plasma neurohormones at baseline and 3 months after treatment

Neurohormones	Baseline (mean ± SE)	95% CI	3 mo (mean ± SE)	95% CI	p Value vs baseline
ANP (pg/ml)	33.8 ± 7.0	15.9-51.8	76.5 ± 14.8	38.5-114.5	0.012
NA (pg/ml)	114 ± 27.4	43.6-184.7	423 ± 88.9	193.9-651.1	0.024
DOA (pg/ml)	33.7 ± 6.1	18.0-49.3	68.5 ± 17.1	24.5-112.5	0.081
PRA (ng/ml/hr)	1.32 ± 0.13	0.98-1.65	2.58 ± 0.50	1.29-3.87	0.077

The values are expressed as mean ± the standard error (SE) of the mean. The *p* values are calculated by repeated-measures analysis of variance with the Scheffe F test. *CI*, Confidence interval; *ANP*, atrial natriuretic peptide; *NA*, noradrenaline; *DOA*, dopamine; *PRA*, plasma renin activity.

completion of 5 weeks. These results suggest that such doses may cause acute anthracycline cardiotoxicity characterized by pericarditis, myocarditis, acute left ventricular failure, and arrhythmias.¹²

Neurohormonal evaluation. Venous blood samples were obtained from conscious dogs before catheterization at baseline and 3 months after the last infusion. Plasma norepinephrine and dopamine concentrations were measured by high-performance liquid chromatography. Plasma renin activity and atrial natriuretic peptide levels were measured by radioimmunoassay.

Histologic evaluation. At the completion of the study, dogs were killed and the hearts were fixed in 10% formalin. Transmural tissue samples from the left ventricular and right ventricular free walls were embedded in paraffin blocks. Tissue samples 5 μm thick were stained with hematoxylin and eosin and Masson's trichrome stain and then analyzed.

Control protocol. Three beagles served as control animals. Control dogs were anesthetized in an identical manner as dogs subjected to heart failure and had five intracoronary infusions of normal saline solution.

Statistical analysis. All data are presented as the mean value ± standard error. Variables were compared by means of a one-way repeated-measures analysis of variance to determine the effects of doxorubicin. When the differences were determined by the one-way repeated-measures analysis of variance to be significant, the differences were further analyzed by the Scheffe F test.

Results

All six beagles receiving doxorubicin in a dose of 0.7 mg/kg survived and were available for analysis. The beagles' weights did not change significantly from the baseline values. A pleural effusion developed in one dog, and pericardial effusions developed in three dogs.

Hemodynamic findings. Serial echocardiographic and angiographic data are shown in Table I and cardiac catheterization data are summarized in Table II. All beagles had normal baseline cardiac function. During the course of evolving heart failure, a significant decline in left ventricular contractility and significant left ventricular dilation were observed (Fig. 1). The left ventricular diastolic and systolic dimension and volume increased significantly at 1 and 3 months after treatment. The fractional shortening and left ventricular ejection fraction decreased significantly. Catheterization revealed that the heart rate, pulmonary capillary wedge pressure, and left ventricular end-diastolic pressure increased significantly. Cardiac output and stroke volume decreased significantly. No significant changes were observed in hemodynamics, chamber

Fig. 1. Echocardiograms demonstrating a dilated left ventricle and depressed myocardial contractility. **A,** Before treatment. **B,** Three months after treatment.

size, and contractility between 1 month and 3 months after the last intracoronary infusion of doxorubicin, except for the left ventricular systolic dimension, which increased significantly from 26.5 ± 1.0 at 1 month to 27.8 ± 0.8 mm at 3 months ($p = 0.01$). End-systolic thickness of interventricular septum and left ventricular posterior wall decreased significantly. A near significant decrease in end-diastolic wall thickness was also found.

Neurohormonal findings. The changes of plasma catecholamines, plasma renin activity, and atrial

natriuretic peptide are shown in Table III. The plasma norepinephrine concentration and plasma atrial natriuretic peptide level increased significantly. Plasma renin activity and dopamine levels also increased, but the increase was not significantly different from baseline.

Histologic findings. Degenerative changes with loss of myofibrils and cytoplasmic vacuolation were found predominantly in the left ventricular myocardium (Fig. 2). The amount of interstitium increased, and interstitial fibrosis was noted. The myocytes

Fig. 2. Microscopic appearance of representative myocardial specimens showing loss of myofibrils with scattered cytoplasmic vacuolation in the left ventricular myocardium. The interstitial space is increased and interstitial fibrosis is demonstrated by Masson's trichrome staining (original magnification $\times 200$).

were regularly arranged without signs of disarray, and no inflammatory cells were seen.

Control animals. No significant hemodynamic and neurohormonal effects occurred in the control animals, and no degenerative changes were observed in the myocardium with intracoronary infusion of saline solution.

Discussion

Experimental heart failure has been induced by a variety of techniques and in many different species of animals. However, it is hard to evaluate cardiac hemodynamics accurately in small animals. No simple and reliable large animal model of chronic dilated cardiomyopathy has been established. Coleman and associates¹³ first described an experimental model of chronic heart failure in the dog produced by rapid ventricular pacing. However, this model is not ideal because a thoracotomy is required to attach a pacing lead to the left ventricle, and the heart failure induced by rapid pacing may be reversible after the cessation of pacing. Magid and colleagues³ described a model of heart failure caused by chronic experimental aortic regurgitation in rabbits. However, the severity of aortic regurgitation is difficult to regulate and heart failure takes a long time to develop. Millner and coworkers⁹ established a model of chronic left ventricular failure induced by ligating the second diagonal coronary artery in

sheep. This method also requires a thoracotomy and may not induce chronic heart failure in dogs, which have greater collateral vessels than sheep. Sabbah and associates¹⁴ described a simple and reproducible canine model of chronic heart failure owing to myocardial infarction caused by multiple sequential coronary microembolizations. Their technique requires coronary cannulation without thoracotomy, as does our method. However, the Sabbah model represents heart failure caused by myocardial infarction, in other words, ischemic cardiomyopathy, which is different from our model of dilated cardiomyopathy. The mortality rate for their method is higher than that for our method. This increased mortality may be caused by myocardial infarction-induced arrhythmias.

Doxorubicin, anthracycline antibiotics isolated from cultures of *Streptomyces* species, is composed of an anthraquinone chromophore and a 6-carbon amino sugar joined through a glycosidic linkage and displays broad activity against human neoplasms. The clinical value of this compound is limited by the development of degenerative cardiomyopathy.¹⁵ It has been suggested that doxorubicin causes a dose-dependent cardiomyopathy and that cardiotoxicity is related to the peak plasma doxorubicin concentration.¹⁶ Lowering the peak plasma concentration may reduce the amount of doxorubicin entering myocardial cells.¹⁷ Reduction of the peak concentration of

doxorubicin decreases cardiotoxicity but does not diminish myelosuppression, which was related to the total dose.¹⁶ The basis of our method is to deliver higher peak concentrations of the drug to the myocardial cells, while reducing the total systemic dose.

Proposed mechanisms for doxorubicin cardiotoxicity include release of superoxides leading to conversion of membrane unsaturated fatty acids to lipid peroxides,¹⁸ which can inhibit biosynthesis of coenzyme Q₁₀ (ubiquinone) and inhibit sodium-potassium adenosinetriphosphatase. In addition, doxorubicin can cause the release of compounds such as histamine, arachidonic acid metabolites, platelet activating factor, and calcium, which can cause myocyte injury.¹⁹ Pathologic features of doxorubicin cardiotoxicity include characteristic vesiculation of myocardial cells, a loss of subcellular structural elements, mitochondrial degeneration, loss of myofibrils, and progressive atrophy of myofibers. These myocardial changes are cumulative and potentially nonreversible.²⁰

Heart failure is thought to arise directly from the loss of functioning myocardium and from many secondary processes that contribute to the depression of left ventricular function. Chief among these is pathologic remodeling, in which dilation and resultant afterload excess combine to initiate a downward spiral of deterioration in function, with subsequent hemodynamic and neurohormonal adaptations to left ventricular dysfunction. In our model, the depression of left ventricular function is accompanied by activation of the sympathetic nervous system and by increased secretion of atrial natriuretic peptide and plasma renin activity. These findings are consistent with observations made in patients with heart failure.²¹

Our findings indicate that chronic heart failure, characterized by left ventricular dilation, depressed contractility and increased filling pressure, and by myocardial damage with loss of myofibrils and cytoplasmic vacuolation, can be effectively produced in dogs by repetitive intracoronary infusions of doxorubicin. These pathologic changes were observed in the left ventricular free wall and the interventricular septum, but the right ventricle seemed to be affected slightly by doxorubicin. Selective infusion of doxorubicin into the left coronary artery induced toxic effects predominantly in the left ventricle. Previous attempts to induce heart failure with repeated intravenous injections of doxorubicin have been limited by myelosuppression when the drug is given systemically.^{22, 23} In contrast, the method of Magovern and

associates,¹⁰ in which intracoronary doxorubicin was given in small doses, did not produce significant systemic toxicity. However, their technique relies on complex procedures including thoracotomy, pericardiotomy, insertion of a catheter into the diagonal coronary artery of the beating heart, and ligation of the distal coronary artery. Our method requires only cannulation of the coronary artery via the femoral artery and transcatheter infusion of doxorubicin. No significant systemic side effects occurred with our method.

In conclusion, our model closely mimics dilated cardiomyopathy without myocardial ischemia. Five intracoronary injections of a 0.7 mg/kg dose of doxorubicin may be a preferable method of inducing chronic irreversible cardiomyopathy in dogs. This model should be useful for studying the pathophysiology of heart failure and for evaluating the efficacy of pharmacologic therapies, ventricular repair, cardiomyoplasty, or heart transplantation in patients with dilated cardiomyopathy.

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