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A Novel, Innovative Ovine Model of Chronic Ischemic Cardiomyopathy Induced by Multiple Coronary Ligations

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Abstract

Heart failure is one of the fastest-growing epidemics worldwide in health care today. Although a wide variety of animal models exist to create chronic heart failure, there are few truly successful, reproducible models with ischemic dilation *and* mitral regurgitation. Six healthy sheep (36 ± 5 kg) underwent multiple, strategic coronary artery ligations on the left ventricle (LV). Six to eight ligations were performed transmurally on three of four segments of the LV: anterior, lateral, and posterior. Side branches of the left anterior descending and circumflex arteries were ligated to create multiple, patchy areas of myocardial infarction. Cardiac global and regional systolic function was assessed by echocardiography and cardiac magnetic resonance imaging (MRI). The extent, the characteristics, and the location of the myocardial infarction were qualitatively and quantitatively assessed by late gadolinium enhancement imaging. The overall mortality rate was 16.7% (1/6 animals). Animals who survived showed a significantly reduced ejection fraction (mean $60 \pm 5\%$ to $28 \pm 7\%$; $P < 0.05$); additionally, two out of the remaining five (40%) animals developed mild to moderate mitral regurgitation quantified by cardiac MRI. Furthermore, each animal developed clinical signs of heart failure (tachycardia, dyspnea, and tachypnea) consistent with global, dilated cardiomyopathy noted on MRI. Creating and reproducing a model of global, ischemic cardiomyopathy with functional mitral regurgitation is an arduous task. We have developed a promising model of ischemic heart failure using multiple ligations, which mimics the sequelae of human cardiomyopathy. Our proposed model is highly effective, reproducible, and may be used for experimental research on heart failure (cardiac assist devices, heart transplant, etc.).

Keywords

Coronary ligation; Chronic heart failure; Animal model; Sheep

INTRODUCTION

Heart failure is one of the fastest-growing epidemics worldwide in health care today. Epidemiological studies have revealed an annual incidence of 0.3–1.0% in the USA and Europe (1). Various animal models have already been developed to create chronic heart failure (2–12); however, there are only few truly successful, reproducible models with ischemic dilation *and* mitral regurgitation.

Beside the option to create an animal model of chronic ischemic heart failure by coronary microembolizations (3–6), one other option is to ligate the coronary arteries. The earliest studies to ligate coronary arteries with simple suture techniques to develop a large animal model of chronic ischemic cardiomyopathy were described by Harris et al. in the 1940s (2,13,14). The authors ligated the left anterior descending coronary artery in dogs. Moainie et al. modified this model by ligating the first and second branches of the left anterior descending coronary artery, which reliably creates a moderate-sized myocardial infarction (2,7).

In our working group at the Division of Cardiac Surgery of the Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA, we had previously used Moainie's model for the last years to create chronic heart failure in sheep. Although this model replicates a safe and reproducible model of chronic ischemic cardiomyopathy, it fails to create functional mitral regurgitation. Additionally, a further limitation is the fact that the animals develop a region of complete akinesia on the anterior and lateral wall of the left ventricle (LV), which leads to a compensatory hyperkinesis of the posterior regions of the heart. We thus decided to perform multiple, strategic coronary artery ligations distributed over the LV to induce global LV failure and therefore avoid hyperkinetic areas of the LV.

We describe our new, innovative model of multiple, strategic coronary artery ligations to create a robust, replicable model of chronic ischemic cardiomyopathy in sheep.

MATERIALS AND METHODS

All animals were handled according to the guidelines of the American Physiological Society. The experimental protocol was approved by the Harvard Standing Bioethical Committee on Animals of the Harvard Medical School, Boston, MA, USA.

Heart failure animal models

Six healthy sheep (36 ± 5 kg) underwent multiple, strategic coronary artery ligations on the LV. A left lateral thoracotomy was performed through the fourth intercostal space, followed by a pericardiotomy. Six to eight ligations were performed transmurally on three of four segments of the LV: anterior, lateral, and posterior, each 2 cm apart from each other. Side branches of the left anterior descending and circumflex arteries were occluded by suture ligations to create multiple, patchy areas of myocardial infarction. A chest tube was placed before the thoracotomy was closed in layers, and the animal recovered. Cardiac magnetic resonance imaging (MRI) was performed 8 weeks after infarction. By this time point all animals developed heart failure, defined as an LV ejection fraction (EF) <35% and a 100% increase in LV volume at end-diastole (EDV).

Anesthesia and postoperative care

Animals were sedated with Telazol (6 mg/kg) and endotracheally intubated. Anesthesia was maintained with 1–2% isoflurane. Animals received magnesium (2 mg intravenously), amiodarone (1.5 mg/kg intravenously), and lidocaine (3 mg/kg intravenously) before inducing the infarction and an infusion of amiodarone (0.01 mg/kg/min) and lidocaine (2

mg/min) for 60 min afterwards. Animals received buprenorphine (5 ug/kg intramuscularly every 12 h for the first two postoperative days) for pain control and cefazolin (4 mg/kg intramuscularly every 12 h for the first two postoperative days) for antibiotic prophylaxis.

Cardiac MRI

Serial cardiac MRI was performed in all surviving animals at 8 weeks after multiple coronary ligation to confirm the development of chronic heart failure (Fig. 1). All surviving animals were studied in a 3.0-T CMR system (Signa CV/i, GE Healthcare, Waukesha, WI, USA) ($n = 5$) in lateral decubitus position under general anesthesia and proper ventilation with an eight-element phased-array surface coil. Typical cardiac MRI study consisted of cine steady-state free precession imaging (repetition time, 3.4 min; echo time, 1.2 min; in-plane spatial resolution, 1.6×2 mm) of LV function and late gadolinium enhancement (LGE) imaging (repetition time, 4.8 min; echo time, 1.3 min; inversion time, 200–350 min) for myocardial scar. Peripheral pulse gating and breath holding were used as much as possible to minimize cardiac and respiratory motion, respectively. When breath holding was not successfully achieved, a free-breathing technique was applied using the multiple excitations per cycle approach associated with reduced views per segment. Cine imaging and LGE imaging were obtained in 8–14 matching short-axis (8-mm thick with 0-mm spacing) and 3 radial long-axis planes. A previously described segmented inversion-recovery pulse sequence for LGE was used starting 10 min after 0.2 mmol/kg dose of gadolinium DTPA.

Quantitative analysis of LGE and LV function parameters

Cine function analysis was performed offline with validated software (QMASS 7.1, Medis Medical Imaging Systems, Inc., Leiden, the Netherlands). The cardiac phase that demonstrated the largest LV cavity size was defined as end-diastole and the smallest LV cavity size as end-systole. A single experienced observer manually traced the endocardial contours of the right ventricle (RV) and the LV to determine the ventricular chamber EDV and volume at end-systole (ESV). The papillary muscles were included in the ventricular cavity volume. If the basal slice contained both ventricular and atrial myocardium, the contours were drawn up to the junction of the atrium and the ventricle and joined by a straight line through the blood pool. For the right ventricle (RV) basal slice in both end-diastole and end-systole, if the pulmonary valve was visible, only the portion of the volume surrounded by trabeculated myocardium below the level of the pulmonary valve was considered ventricle and included. For the inflow portion of the RV, the blood volume was excluded from the RV volume if the surrounding wall was thin and not trabeculated, as this was considered to be part of the right atrium. RV EF and LV EF were computed using Simpson's rule—EF was calculated as the difference between EDV and ESV as a percentage of EDV.

We interpreted LGE as present or absent by the consensus of two cardiologists. LGE was considered present only if myocardial enhancement was confirmed on both short-axis and matching long-axis locations. The myocardial mass of LGE (grams) was then quantified by a semiautomatic detection method using a signal intensity threshold of >3 SD above a remote reference region as previously reported (Fig. 1).

Hemodynamic evaluation—Hemodynamic data were analyzed at baseline and 2 months after the operation (Table 1). Cardiac function was evaluated using echocardiography and MRI. All hemodynamic measurements were obtained in sedated animals. Electrocardiography was performed at baseline, continuously during the intervention (pre-, intra-, and postoperatively), and at the end of the experiment 2 months after coronary ligation.

Statistical analysis—Clinical and hemodynamic data were documented at baseline and 2 months after the coronary ligations. All data are reported as means $\pm$ SD. Calculations were performed with STATISTICA software package (Statistica 6.0, StatSoft Inc., Tulsa, OK, USA) on a personal computer; *t*-test for matched pairs was used and *P* values of 0.05 were considered statistically significant.

RESULTS

Six healthy sheep (36 ± 5 kg) underwent multiple, strategic coronary artery ligations on the LV. Six to eight ligations were performed transmurally on three of four segments of the LV: anterior, lateral, and posterior, each 2 cm apart from each other. Side branches of the left anterior descending and circumflex arteries were ligated to create multiple, patchy areas of myocardial infarction. The overall mortality rate was 16.7% (1/6 animals). One sheep died intraoperatively due to ventricular fibrillation; all other sheep (5/6) survived the complete time period of the study (2 months), and all surviving animals developed clinical signs of heart failure as indicated by increased heart rate at rest (62 ± 5 beats per minute [preoperatively] to 102 ± 4 beats per minute [8 weeks] [$P < 0.05$]) and increased respiratory rate at rest (25 ± 3 [preoperatively] to 42 ± 6 [8 weeks] [$P < 0.05$]) (all heart-failure sheep developed bilateral pulmonary rales and pleural effusion). The average EF of the remaining animals had significantly decreased from $60 \pm 5\%$ to $28 \pm 7\%$. The hemodynamic baseline data are presented in Table 1 and are compared with the postoperative data (2 months after coronary ligation). LV EDV increased from 61.1 ± 6.6 mL at baseline to 185 ± 15 mL ($P < 0.05$), while LV ESV increased from 24.4 ± 4.9 mL at baseline to 124 ± 12 mL ($P < 0.05$). Additionally, cardiac output decreased from 6.0 ± 0.2 L at baseline to 4.5 ± 0.6 L ($P < 0.05$). Two of the remaining five (40%) animals developed mild to moderate mitral regurgitation revealed by echocardiography and cardiac MRI. Measuring the resulting regurgitant volume, we revealed an increase to 10 ± 2 mL regurgitant volume, calculating an average regurgitant fraction of 0.16 from the LV to the left atrium.

In all surviving animals, no atrial fibrillation occurred, but several incidences of supraventricular arrhythmia occurred postoperatively. Every sheep showed acute ischemic signs (reversible ST-segment elevations) in electrocardiographic evaluation after each coronary ligation. A third heart sound developed in three of surviving five sheep (60%) late in the course of coronary ligation and persisted throughout the period of the study.

DISCUSSION

Experimental animal models of chronic ischemic heart failure were established in several large animals (2) using different approaches, for example, single coronary ligations (7) and coronary microembolizations (3-6). While coronary microembolization models are well established and useful (3-6), due to the fact that the chest remains closed, these animal models require great experience and technical equipment (left heart catheter, fluoroscopy, etc.). The earliest studies using suture ligation of the coronary arteries were described by Harris et al. in the 1940s (13,14). The authors used dogs to ligate the left anterior descending artery in two stages: first by partially occluding the vessel, followed by complete occlusion several minutes later, and they felt that a two-stage technique would reduce the high incidence of ventricular fibrillation associated with coronary ligation (13,14). Moainie et al. modified this model by ligating the first and second branches of the left anterior descending coronary artery (7). This was shown to reliably create a moderate-sized myocardial infarction of approximately 20% LV mass (7). Furthermore, the Moainie model had resulted in postinfarction dilation; EF decreased from $41.2\% \pm 6.7\%$ (baseline) to $29.1\% \pm 5.7\%$ (8 weeks). In addition, at 8 weeks the left ventricular end-diastolic volume increased over 73.2% (7). The advantage of using sutures to ligate the coronary arteries is that complete

cessation of blood flow is achieved in the ligated vessel, via a minimally invasive surgical technique. However, Moainie's model shows several limitations and disadvantages: First, the ligations are performed via an open-chest surgery, which leads to postoperative adhesions. Second, the ligations tend to result in regional infarction areas and thus compensatory hypertrophic regions of the noninfarcted areas. Furthermore, the ligations increase the risk of arrhythmias despite prophylactically administering antiarrhythmic agents. Finally, the stunned myocardium seen in the clinical setting cannot be achieved with the coronary artery ligation model of heart failure. Stunned myocardium, as described by Braunwald et al., occurs when variable degrees of coronary occlusion lead to repeated ischemic myocardial insults and possibly contribute to postischemic chronic left ventricular dysfunction (7,15). Previous studies (7,13,14) have suggested that a single myocardial infarction, sufficient to produce lasting LV dysfunction, is likely to induce hypertrophic compensatory regions. Thus, further enlargement of the LV does not occur and therefore will not lead to functional mitral regurgitation.

The approach used in the present study differs from previous attempts (7,13,14). Our goal was to avoid compensatory areas of hypercontractility; therefore, we performed transmural ligations distributed over the LV to produce a robust degree of myocardial infarction and focus on global changes of LV function. By using a minimally invasive left lateral thoracotomy, we avoid opening of the chest seen with a conventional sternotomy and yet can directly manipulate as needed. This has the added benefit of reducing pro-inflammatory changes.

The present study represents a clinically relevant model of ischemic-induced myocardial infarction. Two months after multiple coronary ligations, we observed clinical signs of chronic heart failure as indicated by tachycardia, tachypnea, and weight gain. Moreover, hemodynamic signs of congestive heart failure were also present. An important feature of the present model of heart failure is the lack of LV function recovery—cardiac changes were irreversible.

In summary, multiple, transmural coronary ligations of the LV creates a robust, replicable model of chronic ischemic heart failure in sheep.

CONCLUSION

We have developed a novel, promising model of chronic ischemic heart failure using multiple ligations, mimicking the sequelae of human cardiomyopathy. Our proposed model is both highly effective and reproducible, with the potential for use in future experimental heart failure research (cardiac assist devices, heart transplant).

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FIG. 1. Cardiac MRI of the chronic heart failure sheep. LGE images in short-axis views showing a transmural large-sized myocardium infarct (MI) in the mid- to distal antero-lateral wall. The black arrows represent the MI in different short-axis slices.

TABLE 1

Hemodynamic baseline data compared with the postoperative data (8 weeks after coronary ligation)

Variable	Preoperative (baseline)	Postoperative (8 weeks)
Heart rate (bpm)	62 ± 5	42 ± 6
EF (%)	60 ± 5	28 ± 7
LV EDV (mL)	61.1 ± 6.6	185 ± 15
LV ESV (mL)	24.4 ± 4.9	124 ± 12
Cardiac output (L)	6.0 ± 0.2	4.5 ± 0.6
Mortality (<i>n</i>)	0	1/6 (16.7%)
Mitral regurgitation (<i>n</i>)	0	2/5 (40%)