HEMODYNAMIC AND METABOLIC EVALUATION OF AN ACUTE LEFT VENTRICULAR FAILURE MODEL INDUCED BY CORONARY ARTERY EMBOLIZATION

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There is a need for a stable, reproducible experimental model of acute heart failure. In this study we have evaluated the hemodynamic and metabolic effects of left ventricular failure produced by embolization via left main coronary artery with 50 μm plastic microspheres in 56 dogs. Fifteen dogs had either arrhythmias or unstable hemodynamics and were excluded. In the remaining 41 dogs, the embolization caused a stepwise increase in left ventricular end-diastolic pressure from 5.6 ± 0.3 (Mean ± SEM) to 14.8 ± 0.4 mmHg, a decrease in left ventricular dP/dt max from 2627 ± 81 to 1812 ± 46 mmHg/sec, a decrease in cardiac output (28%), a fall in systemic arterial pressure (9%), and an increase in total peripheral vascular resistance (31%) (all $p < 0.01$, $n = 41$). Myocardial lactate fell from 43.1 ± 5.3 to 8.4 ± 5,3 μmol/1 and myocardial uptake of fatty acids was reduced by 42% ($p < 0.01$, $n = 22$). The amount of left ventricular necrosis was assessed after 24 hours by a technique using triphenyl tetrazolium staining. In this group, 41 ± 3% of the left ventricle was necrotic and there was a good correlation between the extent of necrosis and left ventricular end-diastolic pressure ($r = 0.75$, $n = 12$, $p < 0.01$).

In conclusion, embolization via the left main coronary caused a reproducible and stable model of left ventricular failure in dogs. The left ventricular end-diastolic pressure seems of reflect the extent of myocardial necrosis in this model.

Acute myocardial infarction with left ventricular failure or cardiogenic shock is often a main cause of death in hospitalized patients [1-3]. The extent of myocardial necrosis usually exceeds 40% of the left ventricle in patients dying of cardiogenic shock [2,3]. Clinical and experimental studies have shown that the myocardial infarct size and thus the prognosis can be influenced by interventions in the early phase of the evolving myocardial infarction [4.6]. However, therapeutic interventions in the non-failing left ventricle might not have the same effect as in the left ventricle with impaired pump function. Thus, a suitable animal model for the study of pathophysiological mechanisms involved and for the assessment of therapeutic interventions in acute heart failure is needed.

Many different techniques have been employed for inducing acute left ventricular failure [7-10]. However, the use of these earlier described models has been limited by their hemodynamic instability, high incidence of arrhythmias and lack of reproducibility [11]. Recently, an experimental model based on graded, selective embolization of the left coronary artery in dogs with 50 μm plastic microspheres is described [12]. In the present study of left ventricular failure, we have evaluated the hemodynamic and metabolic characteristics, the incidence of arrhythmias and the relationship between the extent of myocardial necrosis and the resultant hemodynamics.

MATERIALS AND METHODS

Animal Preparation - Experiments were carried out in 56 overnight fasted mongrel dogs of either sex with a body weight of 17 to 33 kg. General anesthesia was induced with sodium pentobarbital, 25 mg/kg intravenously, and small supplementary doses were given as necessary to maintain a constant level of anesthesia. The dogs were placed in the left lateral position and ventilation was maintained through a cuffed endotracheal tube with a volume-controlled respirator (Harvard Apparatus). Polyethylene cannulac were placed into the jugular and femoral veins for intravenous infusions. Electrocardiograms (standard and precordial leads) were monitored continuous-

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ly throughout the experiments. Heparin (5000 I.U.) was administered intravenously to avoid clotting of the catheters.

Hemodynamic Measurements - Arterial pressure (Statham P23 ID transducer, Statham Instruments) was recorded from a polyethylene cannula introduced into the aorta through the left carotid artery. A Millar microtip transducer catheter (Millar Instruments) was introduced into the left ventricular cavity through the femoral artery to measure left ventricular pressures. For adjustment of zero level, left ventricular pressure was also measured through the fluid-filled lumen in the millar catheter (Statham P23 ID transducer). The maximal rate of rise of left ventricular pressure, LV dP/dt max, was recorded by means of a differentiator. All these variables were recorded continuously on a polygraph (Gould 2800 recorder, Gould Instruments). Cardiac output (CO, I/min) was determined by the thermodilution technique, using a thermistor in the pulmonary artery and calculated by a computer (Elecath, Electro-Catheter Corporation). The coefficient of variation for duplicate cardiac output measurements was 5%. In addition to the directly obtained variables, total peripheral vascular resistance (TPR, dyn x sec x cm-^{5}) was calculated as (mean aortic pressure/CO) x 80. Stroke volume (SV, ml/beat) was calculated as CO/heart rate.

Myocardial blood flow (MBF, ml/min) was measured as coronary sinus blood flow by the continuous infusion thermodilution technique [13], using a = 7 F two thermistor catheter (Wilton Webster Laboratory) introduced from a jugular vein to the coronary sinus. The blood flow was calculated from the formula:

$$F_1 \times 1.19 \times \frac{TB\text{-}TI}{TB\text{-}TM} - 1$$

where TB, TI and TM represent the temperature of blood, injection and mixture of blood and injectate, respectively. F, is the volume of injectate per minute and 1. 19 is a constant derived from the density and specific heat of saline solution and blood. The coefficient of variation for duplicate MBF measurements was 5%.

Metabolic Measurements - Blood samples were obtained simultaneously from the aorta and coronary sinus directly before each measurement of MBF. Oxygen content (ml 0,/ml blood), was analyzed in duplicate using a LEX 02 CON-K (Lexington Instrument), Myocardial oxygen consumption was calculated as the product of (arterial - coronary sinus) oxygen content and MBF. Plasma concentrations of free fatty acids (FFA) were measured by a spectrophotometric method14, and myocardial uptake of FFA was calculated from myocardial plasma flow and anterio-coronary sinus differences in plasma FFA concentrations. Blood lactate concentrations were measured by a lactate dehydrogenase method using a commercial kit (Sigma Chemical Company).

Induction of Left Ventricular Faillure - To induce left ventricular failure, initially the left main coronary artery was catheterized with a Judkins coronary artery catheter (= 7 F, USCI Cardiology and Radiology Products) under fluoroscopic control. It was introduced through a femoral artery, and polystyrene microspheres OM Company) with a diameter of 52 ± 4.0 μm (Mean Fig.1 - Effect of coronary embolization by 50 um microspheres injected into the left coronary artery. ECG = Eletrocardiogram; LV + dP/dt and LV -dP/dt = Positive and negative maximal rate or rise in left ventricular systolic pressure; AP = Arterial pressure; LVDE = Left ventricular end-diastolic pressure.

SD) were injected into the coronary artery. The microspheres were diluted with macrodex and saline (dextran 70, 60 mg/ml to a suspension of 1 mg microspheres per ml (i.e., 12,000 microspheres per ml). The first dose of microsphere suspension was 0.5 ml/kg body weight, and all subsequent doses were 0.25 ml/kg, and administered every 5 minutes. Injection of microspheres was discontinued when LV dP/dt max had been reduced by 30% or left ventricular end-diastolic pressure (LVEDP) increased to 13 mmHg, when measured 5 minutes after the last dose.

Protocol for Hemodynamic and Metabolic Studies - Hemodynamic and metabolic measurements were done before the start of coronary artery embolization, 5 minutes following every microsphere injection, and every 15 minutes after end of embolization for 105 minutes. Forty-five minutes were allowed to elapse after the last dose of microspheres to verify the stability of the model. If LV dP/dt max, LVEDP or cardiac output had changed by 20% or more between 30 and 45 minutes after embolization, or there was evidence of shock with mean arterial blood pressure < 70 mmHg, the animals were excluded from the study. Ventricular arrhythmias, as defined by premature ventricular beats exceeding 10 beats per minute or atrial fibrillation, were also reason for exclusion from the study.

Quantitation of Myocardial Necrosis - The extent of myocardial necrosis was studied in 12 dogs sacrificed 24 hours after induction of heart failure. The hearts were excised, the left ventricle dissected free from adjacent tissue and frozen at -201C. The left ventricle was then sliced parallel to the atrioventricular grove into 3 mm thick slices using an electric meat slicer. Assessment of necrotic myocardium was done after staining the slices in phosphate- buffered 1% triphenyl tetrazolium chloride (TTC) (Sigma Chemical Company) for 10 minutes at 37*C and subsequently immersing the slices in a 10% solution of formaldehyde. TTC stains viable, dehydrogenase-containing myocardium red, whereas necrotic tissue which is depleted of dehydrogenase is unstained and appears gray 15,16. Tracings of each slice were drawn on transparent plastic sheets, and the quantitation of necrosis was made by cross-point counting of the slices.

Histology - Transmural 0.5 cm thick pieces were taken from both the free wall and interventricular septum from basal, mid and apical regions of the left ventricle. The pieces were fixed in 4% buffered formalin, dehydrated in ethanol and embedded in JB-4 plastic (Polysciences) 3 μm sections were cut

with glass Ralph knives and stained with toluidine blue.

Statistical Analysis - All data are presented as mean ± SEM. The effects of coronary artery embolization on hemodynamic and metabolic variables were analyzed statistically by the use of analysis of variance [17]. Significant results found by analysis of variance were evaluated by Wilcoxon's two-tailed test paired samples [18]. Standard linear regression analysis was employed to determine the nature of the linear relationship between two variables. It was regarded as statistically significant when the p value was less than 0. 05.

RESULTS

Embolization Procedure - The left main stem of the left coronary artery was successfully catheterized in all dogs. Each dose of microspheres injected into the coronary artery resulted in a rapid decrease in the positive and negative LV dP/dt max and increase in LVEDP with only small changes in arterial pressure and with gradual normalization within minutes (fig. 1). Repeated injections effected a gradual and predictable reduction in LV dP/dt max and increase in LVEDP. The average number of doses used for induction of acute heart failure was 8.0 ± 0.3 doses (Mean h SEM) (range 5.14 doses), which corresponds to an average of 6.5 x 105 microspheres injection for each experimental animal.

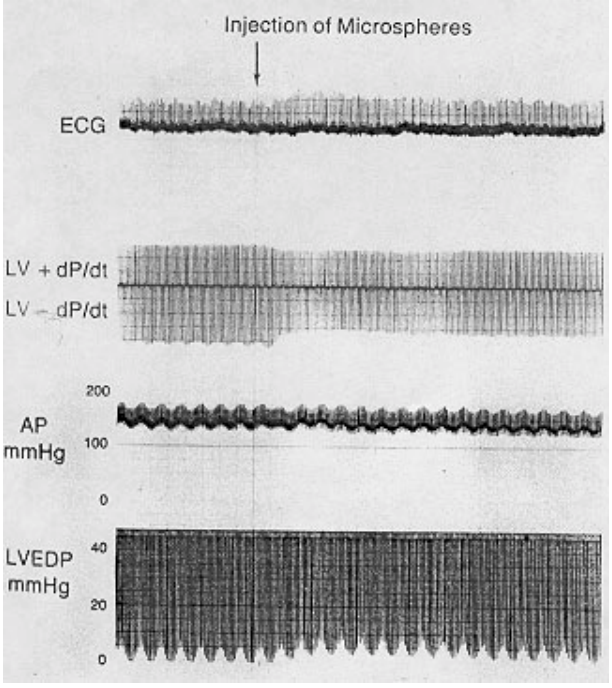

Fig.1 - Effect of coronary embolization by 50 um microspheres injected into the left coronary artery. ECG = Eletrocardiogram; LV + dP/dt and LV -dP/dt = Positive and negative maximal rate or rise in left ventricular systolic pressure; AP = Arterial pressure; LVDE = Left ventricular end-diastolic pressure.

Table I describes the success with the coronary embolization technique in 56 dogs. Unstable hemodynamics and atrial fibrillation were the most frequent exclusions.

TABLE I - Success rate and complications in the acute heart failure modem induced by coronary artery embolization.

	Number of Experiments	Percentage
Total number of experiments	56	100
Successful experiments	41	73
Complications	15	27
Unstable hemodynamics	9	
Atrial fibrillation	5	
Frequent premature ventricular beats	1	
Ventricular tachycardia or fibrillation	0	

Hemodynamic Effects - By the end of coronary embolization, the left ventricular function was severely depressed with a decrease in LV dP/dt max by 30%, increase in LVEDP by 164% and decrease in cardiac output by 28% (tab. ID. LV dP/dt max and LVEDP remained unchanged throughout the following 105 minutes while cardiac output tended to decrease slightly. Mean arterial blood pressure was moderately decreased (9%), and this was accompanied by an increase in total peripheral vascular resistance (31%). The mean arterial pressure tended to decrease and total peripheral resistance to increase slightly throughout the observation period. However, when tested for the whole period after end of embolization, none of them changed significantly. Myocardial blood flow was moderately reduced after embolization, but the slightly further decrease during the observation period was not significant.

Metabolic Effects - Myocardial oxygen consumption markedly decreased after embolization (36%), but did not change significantly in the follow-up period (tab. ID. The arterial lactate concentration Increased (38%), whereas the myocardial uptake of lactate was reduced after embolization (81%). In 5 of 19 experiments the uptake of lactate was reversed to release. The arterial concentration of F7A and myocardial uptake of F7A fell by 22% and 42%, respectively. Arterial concentrations and myocardial uptake of lactate and FFA were not significantly changed in the 105 minute follow-up period.

Electrocardiographic Changes - The ECG revealed elevation of the ST-segment suggestive of acute myocardial ischemia. This change was seen in most animals during and after the embolization procedure both in standard (fig. 2) and precordial leads. During the following 24 hours development of Q waves, negative T waves and lowering of R waves was seen (fig. 2). Premature ventricular beats were occasionally seen closely related to the microsphere injection, but in the majority of dogs no ventricular arrhythmia was recorded.

TABLE II - Hemodynamic and metabolic effects of coronary embolization. Means ± SEM of 41 experiments are given.

	Before Embolization	End of Embolization	After Embolization 15 Min	15 Min	75 Min	105 Min
LV dP/dtmax, mmHg/sec	2627 ± 81	1812 ± 46 ***	1781 ± 49 ***	1670 ± 43 ***	1608 ± 49 ***	1630 ± 4l ***
LVEDP, mmHg	5.6 ± 0.3	14.8 ± 0.4 ***	15.6 ± 0.5 ***	17.3 ± 0.6 ***	18.6 ± 1.0 ***	17.2 ± 0.8 ***
CO, l/min	3.35 ± 0.08	2.40 ± 0.08 ***	2.19 ± 0.07 ***	1.93 ± 0.06 ***	1.85 ± 0.09 ***	1.82 ± 1.82 **
HR, beats/min	152 ± 4	139 ± 4 **	139 ± 4 **	136 ± 3 **	139 ± 4 **	138 ± 4 **
AP, mmHg	128 ± 3	117 ± 3 **	117 ± 3 **	116 ± 3 **	110 ± 6 ***	108 ± 5 ***
TPR, dyn.sec.cm	3147 ± 123	4127 ± 210 ***	4557 ± 205 ***	4735 ± 201 ***	4761 ± 249 ***	4737 ± 237 ***
MBF, ml/min (n=40)	lM6 ± 4	106 ± 5 **	102 ± 5 **	93 ± 4 ***	96 ± 4 ***	92 ± 4 ***
MVO, ml/min (n=40)	M5.5 ± 0.7	9.9 ± 0.7 **	-	10.1 ± 0.5 * *	11.4 ± 0.7 *	11.1 ± 0.7 **
Lactate arterial, mmol/l (n=19)	1.49 ± O.16	2.05 ± 0.16 *	-	2.45 ± 0.17 **	2.22 ± 10 *	2 .35 ± 0.07 **
Lactate uptake, umol/min (n=19)	43.1 ± 5.3	8.4 ± 5.3 **	-	17.0 ± 5.0 *	15.0 ± 2.2 *	14.5 ± 2.5 **
FFA arterial umol/l (n=22)	452 ± 33	354 ± 28 **	-	367 ± 34 **	414 ± 65 *	423 ± 57 *
FFA uptake, umol/min (n=22)	12.0 ± 1.0	7.0 ± 1.1 ***	-	6.5 ± 0.9 *	7.5 ± 1.4 ***	7.4 ± 0.9 ***

* p < 0.05, ** p < 0.01 and *** p < 0.001 versus values before coronary embolization.

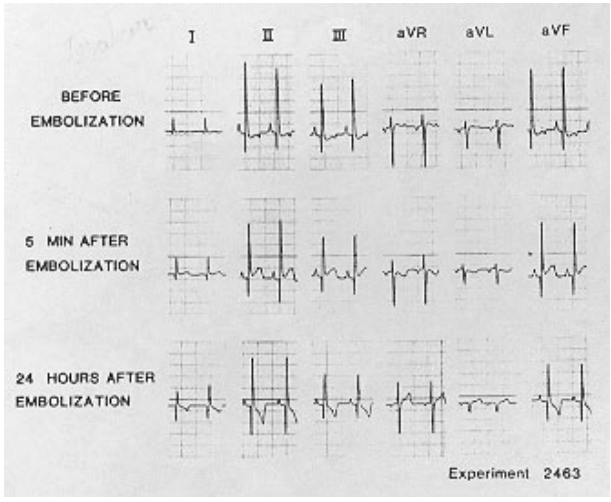

Fig. 2 - Eletrocardiogram from an experiment showing ST-segment elevation in lead I, II, III and a VF after 5 minutes and development or negative T waves in the same leads during 24 hours.

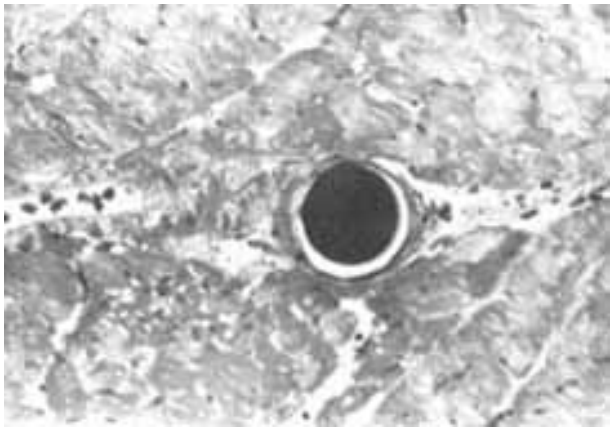

Fig. 3 - Left ventricular wall 24 hours after coronary artery embolization showing a carbonized microesphere impacted in a small arteriole.

Histology - Specimens for histologic examination were taken 24 hours after coronary embolization. Scattered carbonized microspheres were seen on light microscopy in small arterioles (fig. 3). There were widespread patchy areas of contraction band. ing necrosis throughout the left ventricular wall, with moderate infiltration by leukocytes in some places (fig. 4).

Extent of Left Ventricular Necrosis - The amount of necrosis of the left ventricular wall using a TTC staining technique was on the average 41A ± 3.4% when determined in dogs sacrificed after 24 hours. The necrotic areas were distributed transmurally and were found in all parts of the left ventricle. No significant correlation was found between the extent of necrosis and the number of microspheres per gram of the ventricle which were used to induce heart failure (r=0.56, n=12, N. S.). There was a close linear correlation between the extent of myocardial necrosis and the change in LVEDP induced by the embolization (r=0.75, n=12, p<0.01) (fig. 5). However, no significant correlation was found between the extent of myocardial necrosis and LV dP/dt max (r=0.48, n=12, N. S.) or cardiac output (r=0.35, n=12, N. S.).

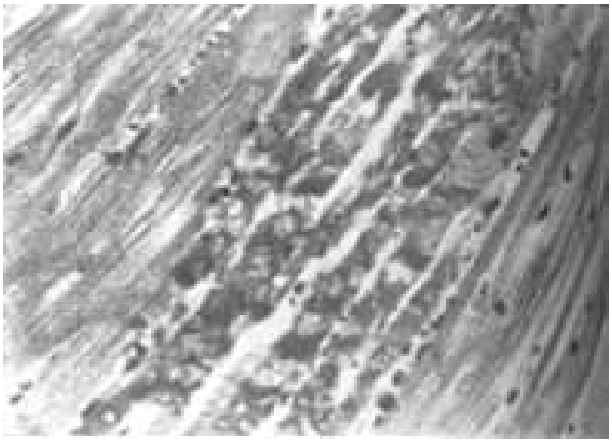

Fig. 4 - Left ventricular myocardium showing contraction banding necrosis and infiltration by neutrophilis 24 hours after coronary embolization.

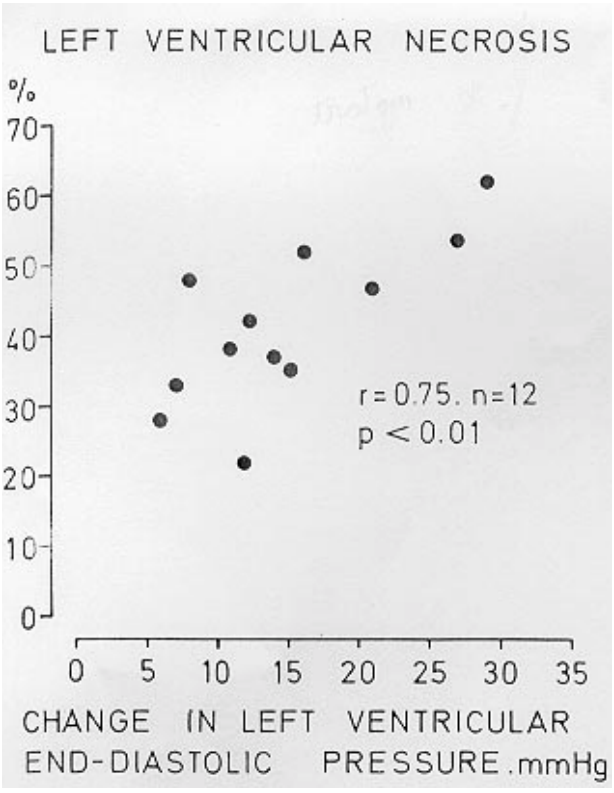

Fig. 5 - Relation between the extent of left ventricular necrosis 24 hours after embolization and the increase in left ventricular end-diastolic pressure by the end of embolization.

DISCUSSION

A number of cardiovascular diseases may result in congestive heart failure with the myocardium unable to pump sufficient blood to the peripheral tissues. Congestive heart failure is manifested hemodynamically by a decrease in myocardial contractility and cardiac output, accompanied by a compensatory increase in left ventricular end-diastolic pressure [19]. However, congestive heart failure can occur with normal systolic function [2O]. The most common cause of congestive heart failure is chronic myocardial ischemia due to coronary atherosclerosis. Other causes such as microvascular hyperreactivity and spasm may be the major components of congestive cardiomyopathies [21].

Unlike many other cardiovascular disorders, congestive heart failure is difficult to produce in animal models so that it exactly mimics the causes and hemodynamic conditions in patients with heart failure. Many different techniques have been employed for inducing left ventricular failure in animals including coronary artery ligation [7], induction of thrombosis [8], embolization with metallic mercury [9,22,23], rapid pacing [24], DC counter-shock [25], aorto-caval fistula [26], mitral insufficiency [27], supraventricular aortic stenosis [28], or plastic microspheres with a diameter Of 190 450 Am 10. The other approach in experimental models is to take advantage of animals that spontaneously develop cardiomyopathy, but these are models with severe limitations [29]. Although used for specific purposes, all these models have some disadvantages due to the method of producing the failure, lack of reproducibility, or the hemodynamic instability of the animal model [11].

Since the great majority of patients with heart failure have as the cause myocardial ischemia, a model to closely represent the clinical setting should also be produced by ischemia. Indeed, embolization via the left main coronary artery with 50 μm plastic microspheres has been tested successfully recently 12 and has been shown to clearly reproduce the hemodynamic findings encountered in patients with acute myocardial infarction and congestive heart failure [31]. This type of induction is very easily accomplished and has the advantage of being done in closed chest animals. The degree of failure (mild, moderate or severe) can be chosen beforehand and be accomplished by the number of microspheres injected. The decrease in contractility measured in this study by LV dP/dt max, and the increase in left ventricular end-diastolic pressure are graded and predictable. The hemodynamic changes are stable for a long enough time period allowing for the evaluation of drug intervention [32-33]. The yield is high, with three of four dogs used having desirable CHP without arrhythmias. In this study, nine percent of the animals developed atrial fibrillation and were excluded. It is possible that the atrial fibrillation was caused by the coronary sinus catheter and, in its absence, the incidence could be much lower.

In the present model the lowering of the contractility index, LV dP/dt, was invariably associated with other signs of severe left ventricular failure. The left ventricular filling pressure was markedly elevated while cardiac output and stroke volume were severely depressed. The aortic blood pressure was only moderately depressed due to the marked increase in systemic vascular resistance. This model is thus not a model of cardiogenic shock like most earlier models, but the hemodynamic profile is consistent with the findings in congestive heart failure. The tachycardia usually present in man with LV failure was not found in this model. On the contrary, some reduction in heart rate was observed after coronary embolization. This was probably due to the barbiturate anesthesia used in the present study which inhibits cardiovascular reflexes [34].

This model represents a modification of that introduced by Agress et al. [10] who injected successive doses of microspheres with a diameter of 190-450 μm in the aortic root of closed chest dogs while obstructing the distal aortic flow. As an endpoint for the embolization procedures, they used a fall in aortic blood pressure of 30%. Unlike the present study which used smaller microspheres and selective coronary injections, the hemodynamic effects in their model were quite unpredictable and ventricular fibrillation occurred frequently.

The carbonized microspheres were distributed throughout the left ventricular wall obliterating arterioles and associated with multiple small infarcts indicating a global, but patchy left ventricular ischemia. The electrocardiographic alterations and the decrease of myocardial lactate extraction, shifting to lactate release in five dogs, is clear evidence of the acute ischemia caused by the coronary embolization. The extensive degree of global myocardial ischemia was documented by the finding that about 40% of the left ventricular wall was necrotic after 24 hours. There was close correlation between the elevation in LVEDP at the end of embolization and the percentage of LV necrosis twenty-four hours later. This supports the fact that the severity of heart failure is related to the degree of necrosis as shown before in patients with acute myocardial infarction dying in cardiogenic shock[2].

The decrease in arterial concentration of free-fatty acids probably is related to the lower perfusion of adipose tissue found in situations with low cardiac output and high peripheral vascular resistance [35]. Thus, patients with acute myocardial infarction commonly have elevated arterial concentrations of free fatty acids [36]. However, when these patients develop cardiac failure with compromised peripheral circulation they frequently have low fatty acid concentrations [37]. The lowering of myocardial fatty acid uptake is a combination of lower fatty acid supply and lower myocardial blood flow.

Further investigation is necessary to evaluate the chronic evolution of the model. Nevertheless, this acute phase can be of great utility to evaluate interventions that could improve left ventricular performance. Indeed it has been done using the angiotensin converting enzyme inhibitor enalapril [32] and synthetic atrial natriuretic factor [33].

In conclusion, coronary, embolization in closed-chest, anesthetized dogs was associated with a large and sustained decrease in cardiac output, cardiac contractility (LV dP/dt) and a rise in LVEDP. The model is stable for several hours and mimics the hemodynamic findings encountered in patients with heart failure. However, the extrapolation of these results to the clinical setting should be done only with the utmost caution because animal models mimic only certain characteristics of the human pathologic condition.

RESUMO

Existe uma necessidade de um modelo experimental reproduzível e estável da insuficiência cardíaca aguda. Neste estudo, realizado em 56 cães, avaliamos os efeitos hemodinâmicos e metabólicos da insuficiência ventricular esquerda, produzidos por embolia via artéria coronária principal esquerda com microsferas plásticas de 50 μm. Foram excluídos 15 cães que apresentaram tanto arritmias como hemodinâmica instável. Nos 41 cães restantes, a embolia causou aumento gradativo da pressão endodiastólica final do ventrículo esquerdo de 5,6 ± 0,3 média ± EPM) para 14,8 ± 0,4 mmHg, diminuição da dP/dt max ventricular esquerda de 2627 ± 81 para 1812 ± 46 mmHg/s, redução do débito cardíaco (28%), queda da pressão sistêmica arterial (9%) e aumento da resistência vascular periférica total (31%) (todos $p < 0,01$, $n = 41$). O consumo de oxigênio do miocárdio foi reduzido em 36% ($p < 0,01$, $n = 41$), o lactato do miocárdio declinou de 43,1 ± 5,3 para 8,4 ± 5,3 /μmol/l e a absorção dos ácidos graxos no miocárdio foi reduzida em 42% $p < 0,01$, $n = 22$). O total da necrose ventricular esquerda foi estimado após 24 horas, através de uma técnica que emprega o corante trifenil terazólio. Nesse grupo, 41% ± 3 do ventrículo esquerdo estavam necrosados, existindo boa correlação entre a extensão da necrose e a pressão endodiastólica final do ventrículo esquerdo ($r = 0,75$, $n = 12$, $p < 0,01$).

Concluindo, a embolia via artéria coronária principal esquerda provocou um modelo reproduzível e estável da insuficiência ventricular esquerda em cães. A pressão endodiastólica final do ventrículo esquerdo parece refletir a extensão da necrose miocárdica nesse modelo.

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REFERENCES

1. Norris, R. M.: Sammel, N. L. - Predictors of late hospital death in acute myocardial infarction. Progr. Cardiovasc. Dis. 22: 129, 1980.
2. Page, D. L.; Caufield, J. B.; Kastor, J. A.; Desanctis, R. W.; Sanders, C. A. - Myocardial changes associated with cardiogenic shock. N. Engl. J. Med. 285: 133, 1971.
3. Mathey, D.; Bleibed, W.; Hanrath, P.; Effert, S. - Attempt to quantitate relation between cardiac function and infract size in acute myocardial infarction. Br. Heart. J.36: 271, 1974.
4. Rude, R. E.; Muller, J. E.; Braunwald, E. - Efforts to limit the size of myocardial infarct. Ann. Intern. Med. 95: 736, 1981.
5. Herlitz, J.; Hjalmarson, A.; Holmberg, S. et al. - Limitation of infarct size in acute myocardial infarction with metoprolol . Am. J. Cardiol. 51: 1282, 1983.
6. The International Collaborative Study Group: Reduction of infarct size with the early use of timolol in acute myocardial infarction. N. Engl. J. Med. 310: 9, 1984.
7. Ellis, P. R. Jr.; Bailas, N. J.; Viskos; J. D., Wong, S. H.; Hyland, J. W. -Experimental heart failure in dogs. Arch. Surg. 89: 299, 1964.
8. Salazar, A. E. - Experimental myocardial infarction. Induction of coronary thrombosis in intact closed-chest dogs. Circ. Res. 9: 1351, 1961.
9. Much, S.; Moguilevsky, H. C.; Pietra, G.; Shaffer, A. B.; Hirsh, L. J.; Fishman, A. P. - A reproducible model of cardiogenic shock in the dog. Circulation, 49: 205, 1969.
10. Agress, C. M.; Rosenberg, M. J.; Jacobs, H. I.; Binder, M. J.; Schneiderman, A.; Clark, W. G. - Protracted shock in the closed-chest dog following coronary embolization with graded microspheres. Am. J. Physiol. 170: 536, 1952.

11. Feola, M.; Glick, G. - Experimental models of cardiogenic shock. In: Gunnar, R. S. - Shock in Myocardial Infarction. New York, Grime and Stratton, 1974. p. 24.
12. Smiseth, O. A.; Mjos, O. D. - A reproducible and stable model of acute ischemic left ventricular failure in dogs. Clin. Physiol. 2: 225, 1982.
13. Ganz, W.; Tamura, X.; Marcus, H. S.; Donoso, R.; Yoshida, S.; Swan, H. J. C. - Measurement of coronary sinus blood flow by continuous thermodilution in man. Circulation, 44: ,81, 1971.
14. Bergman, S. R.; Carlson, E.; Dannen, E.; Sobel, B. E. -An improved assay 4-(2-thiazolylazo)-resorcinol for non-esterified fatty acids in biological fluids. Clin. Chem. Acts. 104: 53, 1980.
15. Nachlas, M. M.; Shnitka, T. H. - Macroscopic identification of early myocardial infarction by alterations in dehydrogenase activity. Am. J. Path. 42: 379, 1963.
16. Lie, J. T.; Pairolero, P. C.; Holley, X. E.; Titus, J. L. -Macroscopic enzyme mapping of large homogeneous experimental myocardial infarcts of predictable size and location in dogs. J. Thorac. Cardiovasc. Surg. 69: 599, 9175.
17. Winer, B. J. - Statistical Principles in Experimental Design. 2nd ed. New York, McGraw-Hill Book CO., 1971. p. 201.
18. Snedecor, G. W.; Cochran, W. G. - Statistical Methods. 6th ed, Iowa State University Press, Ames, .1967. p. 128.
19. Chatterjee, X.; Parmley, W. W. - Vasodilator therapy for acute myocardial infarction and chronic congestive heart failure. J. Am. Coll. Cardiol. 1: 133, 1983.
20. Dougherty, A.; Naccarelli, G. V.; Gray, E. L.; Hicks, C. H.; Goldestein, R. A. - Congestive heart failure with normal systolic function. Am. J. Cardiol. 54: 778, 1984.
21. Sonnemblick, E. H.; Factor, S.; Strobeck, J. E.; Capasso, J. M.; Fein, F. - The pathophysiology of heart failure; the primary role of microvascular hyperreactivity and spasm in the development of congestive cardiomyopathies. In: Congestive Heart Failure Grime & Stratton, Inc, 1982. p.87.
22. Franciosa, J. A.; Notargiacomo, A. V.: Cohn, J. N. - Comparative haemodynamic and metabolic effects of vasodilator and inotropic agents in experimental myocardial infarction. Cardiovasc. Res. 12: 294, 1978.
23. Leddy, C. L.; Wilen, M.; Franciosa, J. A. - Effects of a new angiotensin converting enzyme inhibitor, enalapril, in acute and chronic left ventricular failure in dogs. J. Clin Pharmacol, 23: 189, 1983.
24. Reigger, A. J. G.; Liebau, G. - The renin-angiotensin-aldosterone system, natriuretic hormone and sympathetic nerve activity in an experimental model of congestive heart failure in the dog. Clin. Sol. 62: 465, 1982.
25. Carlyle, P. F.; Cohn, J. N. - A nonsurgical canine model of chronic left ventricular myocardial dysfunction. Am. Physiol. Soc. H769, 1983.
26. Taylor, R. R.; Covel, J. W.; Ross, J. Jr. - Left ventricular function in experimental aorto-caval fistula with circulatory congestion and fluid retention. J. Clin. Invest. 47: 1333, 1968.
27. Morais, D. J.; Richart, T. S.; Fritz, A. J.; Agree, P. W.; Davila, J. C.; Glover, R. P. - The production of chronic experimental mitral insufficiency. Ann. of Surg. 145: 5DO, 1957.
28. Rogers, W. A.; Bishop, S. P.; Hamlin, R. L. - Experimental production of supravalvular aortic stenosis in the dog. J. Applied. Physiol. 30: 917, 1971.
29. Vargo, T. A.; Wood, J. M.; Schwartz, A. - An effect of ouabain on isolated heart tissue from cardiomyopathic syrian hamsters. J. Mol. Cell. Cardiol. 4: 495, 1972.
30. Detweiler, D. X.; Knight, D. H. - Congestive heart failure in dogs; therapeutic concepts. JAMA, 171: 106, 1977.
31. Hamosh, P.; Cohn, J. N. - Left ventricular function in acute myocardial infarction. J. Clin. Invest. 50: 523, 1971.
32. Sweet, C. S.; Gaul, S.; Reitz, P. M.; Blaine, E. H.; Ribeiro, L G. T. - Mechanism of action of enalapril in experimental hypertension and acute left ventricular failure. J. Hypertension, I (Suppl. D: 53, 1983.
33. Sweet, C. S.; Ribeiro, L. G. T.; Ludden, C. T.; Blaine, E. H.; Slater, E. E.; Nussberger, J.; Nutt, R. F. - Hemodynamic., and renal electrolyte effects of synthetic atrial natriuretic factor in dogs with acute left ventricular failure. Circulation, 70: II-333, 1984.
34. Vatner, S. F. - Effects of anesthesia on cardiovascular control mechanisms. Environmental Health Perspectives, 26: 193, 1978.
35. Rosell, S.: Belfrage, E. - Blood circulation in adipose tissue. Physiol. Rev. 59: 1078, 1979.
36. Vetter, N. J.; Strange, R. C.; Adams, W.; Oliver, M. P. - Initial metabolic and hormonal response to acute myocardial infarction. Lancet, 1: 284, 1974.
37. Rutenberg, H. L.; Pamintuam, J. C.; Soloff, L. A. - Serum-free-fatty-acids and their relation to complications after acute myocardial infraction. Lancet, 2: 559, 1969.