METHODS

Development of an Experimental Model of Cardiac Failure Combined with Type I Diabetes Mellitus

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The doses and mode of streptozotocin injection for modeling heart failure combined with type 1 diabetes mellitus have been determined. Combined disease was induced in animals by injecting the selected streptozotocin dose (60 mg/kg intraperitoneally) at the stage of heart failure formation (2 weeks after coronary occlusion). This protocol of experiment led to development of hyperglycemia, body weight loss, and formation of myocardial cicatrix and hypertrophy corresponding to signs of heart failure paralleled by diabetes mellitus.

Key Words: *heart failure; type 1 diabetes mellitus; streptozotocin; coronary occlusion*

Combination of diabetes mellitus (DM) with chronic coronary disease (CD) significantly deteriorates the disease prognosis and promotes the development of heart failure [6,7]. The development of pathogenetically valid therapeutic approaches implies deep understanding of the metabolic and electrophysiological processes in cardiomyocytes under conditions of DM combined with ischemic lesions. Several experimental models are used in studies of DM and postinfarction heart remodeling [1,2,4,7]. However, combined modeling of these diseases is virtually not carried out for many reasons, for example, because each of these models alone is a strong exposure for animals.

We developed an experimental model of heart failure, induced by myocardial infarction, combined with DM1.

MATERIALS AND METHODS

Experiments were carried out on adult male Wistar rats (180-200 g). Initiation of postinfarction remodeling and the resultant development of heart failure of ischemic origin were induced by coronary artery occlusion. Occlusion of the left descending coronary artery was carried out by the standard protocol [1,2]. After the intervention the animals were kept under standard vivarium conditions. Cardiomyocyte remodeling was morphologically verified 6 weeks after the operation. The development of heart and left ventricle hypertrophy was evaluated by the proportions left+right ventricles/body weights and left ventricle/heart weights [7].

Diabetic disorders were induced using the experimental model based on streptozotocin injection [3-5,7]. Streptozotocin (Sigma) was dissolved *ex tempore* in 0.01 M citrate buffer (pH 4.5) and injected to animals in a single dose. The doses of 40, 50, and 65 mg/kg intravenously and 60 mg/kg intraperitoneally were compared. Intact animals and animals injected with equivalent volumes of citrate buffers intravenously or intraperitoneally served as controls. The animals were observed for 4 weeks after streptozotocin injection. The development of diabetic disorders was verified by changes in blood glucose concentration and body weight. Blood glucose concentrations were measured by the enzymatic colorimetric method (Biocon Diagnostic)

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every other day during week 1 after the drug injection and then weekly. Body weights were measured weekly throughout the entire period of observation.

The combined disease was modeled by inducing DM 2 weeks after coronary artery occlusion. The animals were then kept under standard vivarium conditions for 6 weeks. The data were statistically processed (*M*±*SEM*), the significance of differences was evaluated by the nonparametric Mann–Whitney *U* test.

RESULTS

Comparative study of the effects of single intravenous streptozotocin doses showed that the dose of 40 mg/kg virtually did not change blood glucose concentration, while 50 and 65 mg/kg initiated a pronounced stable increase of this parameter (Table 1). In addition, just 3 weeks after streptozotocin injections in doses of 50 and 65 mg/kg a significant body weight loss was recorded (Table 2). Poor survival was also characteristic of these groups of animals. By the end of week 4 postinjection, the survival was 60 and 25%, respectively (Table 2). These results did not recommend the above streptozotocin doses and intravenous route of its administration for combined disease induction. Streptozotocin in a single dose of 60 mg/kg, injected intraperitoneally, was the optimal method. This protocol led to a pronounced stable increase of blood glucose levels (Table 1). Body weight changed just negligibly, while survival by the end of observation was 95% (Table 2).

The results obtained after intraperitoneal streptozotocin dose of 60 mg/kg following coronary occlusion are presented in Table 3. A significant elevation of blood glucose level was attained, in fact comparable to that in the group of DM monodisease. Importantly that combined disease led to body weight loss more significant than in monodisease.

Comparison of the metrical characteristics of the hearts in the groups showed some specific features. Simulation of DM alone, in contrast to DM created under conditions of postinfarction cardiosclerosis, caused no heart and left ventricle hypertrophy and hence, no appreciable changes in such parameters as heart to body weight and left ventricle to body weight proportions (Table 3). In combined disease the changes in these parameters were significant in comparison with intact animals. Experimental simulation of combined disease resulted in development of signs of postinfarction cardiosclerosis (formation of myocardial cicatrix and hypertrophy) and of DM (stable elevation of glucose level and body weight loss) (Table 3).

Hence, in combination of coronary stenosis, streptozotocin dose and route of administration selected in our experiments induce in laboratory animals the changes corresponding to heart failure and DM development separately. This model can be used for studies of the specific features of combined disease development and drug therapy.

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TABLE 1. Time Course of Blood Glucose Levels in Animals with Streptozotocin-Induced DM over 4 Weeks (*M*±*SEM*)

Group	Glucose level, mmol/liter						
	initial	day 3	day 5	day 7	week 2	week 3	week 4
Intact (*n*=6)	4.50±0.29	5.10±0.36	3.80±0.21	4.70±0.33	4.70±0.36	4.70±0.35	4.70±0.32
Solvent (*n*=6)	6.10±0.43	6.50±0.51	6.50±0.46	6.90±0.39	7.00±0.48	6.90±0.51	6.90±0.56
Streptozotocin, single dose of							
40 mg/kg intravenously (*n*=10)	6.40±0.63	9.50±0.71	8.70±0.68	7.20±0.56	7.00±0.51	6.9±0.6	7.00±0.55
50 mg/kg intravenously (*n*=15)	5.80±0.58	27.20±2.76*	25.60±1.16	18.00±1.44*	18.20±1.16*	18.00±1.21*	18.30±1.16*
65 mg/kg intravenously (*n*=8)	6.80±0.45	33.20±1.16*	35.30±1.45	33.2±2.1*	28.40±1.87*	25.30±2.22*	26.40±2.31*
60 mg/kg intraperitoneally (*n*=10)	5.50±0.31	19.80±1.07*	20.60±1.16	18.7±1.4*	19.20±1.15*	18.90±1.11*	19.90±1.21*

Note. Here and in Table 2: solvent: group of animals which received a single injection of solvent for streptozotocin dissolution (citrate buffer pH 4.5). *$p<0.01$ in comparison with the group receiving solvent (*U* test).

TABLE 2. Time Course of Body Weight in Streptozotocin DM over 4 Weeks (*M*±*SD*)

Group	Body weight, g				Survival, %
	initial	week 2	week 3	week 4	
Intact (*n*=6)	220.50±17.63	235.0±19.7	253.6±17.0	298.0±23.7	100
Solvent (*n*=6)	209.1±19.7	228.8±18.9	231.5±16.8	241.8±13.09	100
Streptozotocin, single dose of					
40 mg/kg intravenously (*n*=10)	200.6±12.4	194.6±15.7	195.6±14.1	210.4±16.4	100
50 mg/kg intravenously (*n*=15)	196.7±21.3	185.3±20.3	177.6±17.5*	158.2±15.3*	60
65 mg/kg intravenously (*n*=8)	193.3±13.5	185.4±16.9	168.5±13.2*	165.5±14.3*	25
60 mg/kg intraperitoneally (*n*=10)	210.1±12.4	205.9±11.9	201.7±10.3	199.6±11.1	95

TABLE 3. Changes in Glucose Levels and Morphometric Values of Rats under the Effect of Postinfarction Cardiosclerosis and DM in Mono- and Combined Disease

Group	Number of animals	Body weight, g	Glucose, mmol/liter	Heart weight/ body weight, mg/g	Left ventricle weight/body weight, mg/g	Area of cicatrix zone, %
Intact	12	298.0±23.7	6.10±0.37	3.29±0.21	2.13±0.013	-
DM	10	199.6±11.1**	19.90±1.21**	3.19±0.21	2.06±0.21	-
HF+DM	14	177.00±11.83**	16.40±1.35**	4.32±0.45*	2.53±0.15	40.30±2.53
HF	11	242.00±11.17*	7.40±0.13	6.27±0.33*	4.270±0.016*	51.3±8.9

Note. HF: heart failure. Area of the cicatrix zone was calculated in percent of left ventricular area. *p<0.05, **p<0.01 in comparison with intact group.

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