Diabetes mellitus type 1 and cardiac failure were experimentally induced in 55 Wistar adult rats. The animals were divided in four groups and treated as follows: group 1 (streptozotocin and adriamycin), group 2 (streptozotocin), group 3 (adriamycin), group 4 (healthy control rats). 70 days after administration, the rats were euthanized. Body weight, cardiac weight and liver weight were assessed. Cardiac gravimetric values were completed with the assessment of longitudinal diameter and transversal diameter of the heart, interventricular septum and free walls of the left and right ventricle. All values were used for calculation of longitudinal cardiac diameter/transversal cardiac diameter ratio, heart weight/body weight ratio, ventricular ratio, liver weight/body weight ratio. Rats from group 1 and group 2 presented the highest degree of hypertrophy of the left ventricle, ventricular ratio being 8.33 ± 2.53 and 6.25 ± 1.85, respectively (P<0.05). Increased values of heart weight/body weight ratio and liver weight/body weight were also recorded in group 1 and group 2, with 0.37(10^2) ± 0.06 and 4.19(10^2) ± 0.79 respectively in group 1 (P<0.05), 0.40(10^2) ± 0.04 and 3.73(10^2) ± 0.74 respectively in group 2 (P<0.05). Transversal diameter/longitudinal diameter ratio recorded no significant differences between all groups, but significant differences occurred between group 3 and control.

Key words: adriamycin, cardiac failure, diabetes mellitus, streptozotocin

INTRODUCTION

Rats with streptozotocin induced diabetes mellitus represent the most used experimental model in cardiovascular research of our days (Rees DA et al. 2005). These models followed clinical and morphological investigations, focused on cardiac activity and morphology, integrity of peripheral microvascular architecture and function and response to various therapy protocols of therapy. Clinical and experimental research of the last years proved the associated diabetes mellitus type 1 or 2 and cardiac failure in the same patient (Factor et al., 1981; Abaci et al., 1999; Dahm-Jandeleit et al., 2000; Sowers et al. 2001; Asbun et al., 2006). The
diagnosis of diabetic cardiomyopathy can be made with accuracy only when coronary artery disease, hypertension, alcoholism etc. are excluded (Fischer et al., 1984; Gregor P et al., 1984; Ishikawa et al., 1999). Clinically, these patients exhibit all signs of a congestive cardiac failure. Morphological investigations of the heart revealed hypertrophy of myocardium (Fein et al., 1980; Factor et al., 1984), apoptosis and necrosis of myocardocytes (Nunoda et al., 1985; Fiordaliso et al., 2000; Fustaci et al., 2000; Shiomi et al., 2003), myocardial fibrosis (Fein et al., 1985; Shimizu et al., 1993), important lesions of coronary arteries and their branches as acellular capillaries, microaneurisms, poor formation of collateral vessels in the myocardium, hypertrophy of arteriolar media together with a reduced lumen of arterioles (Gherasim et al., 1985; Lorenzi et al., 1991; Vranes et al., 1999; Rizzoni et al., 2003). Microvascular lesions are widely spread, including cerebral cortical arterioles, expressed as branched or stellate vascular smooth muscle and endothelial cell degeneration (Moore et al., 1985). The lesions of experimental diabetes are not confined to the small vessels, as the aorta presented a histologically disrupted architecture (Karasu et al., 1997).

Gross assessment of heart in experimental animals represents one of the important issues, lesions as cardiac hypertrophy or cardiac dilation being objectively determined. Controversial results are recorded concerning diabetes mellitus cardiomyopathy, left ventricular hypertrophy or dilated cardiomyopathy being equally mentioned among other results (Braslasu et al., 2005; Asbun et al., 2006). Adriamycin induced cardiac failure exhibits almost always ventricular dilation, cardiac hypertrophy, and overall enlargement of the heart revealed by absolute heart weight and heart weight/body weight ratio (Sun et al., 2001).

Our research is based on an experimental model which induces streptozotocin diabetes mellitus type 1 cardiomyopathy and adriamycin cardiac failure. Previous researches do not specify cardiac remodelling considering other parameters, but heart weight/body weight ratio and liver weight/body weight ratio. This study aims to assess and correlate some parameters as ventricular ratio, heart weight/body weight ratio, longitudinal cardid diameter/transversal cardiac diameter ratio, liver weight/body weight ratio, for an objective characterization of diabetic and adriamycin induced cardiomyopathy and its consequences.

MATERIAL AND METHODS

Our study considered 55 adult Wistar rats, divided in 4 unequal groups as follows:

- group 1 represented by 25 rats with diabetes mellitus type I induced by streptozotocin (STZ) administration and cardiac failure induced by adriamycin (ADR) administration;
- group 2 represented by 16 rats with diabetes mellitus type I induced by streptozotocin administration;
- group 3 represented by 9 rats with cardiac failure induced by adriamycin administration;
- group 4 represented by 5 healthy rats.
Diabetes mellitus was induced by single or double administration, via intraperitoneal injection with streptozotocin (Sigma-Aldrich), 60 mg/kg. The application was repeated 21 days after the first administration, because of normal or insignificantly increased glycemia. Cardiac failure was obtained with adriamycin, 2.5 mg/kg, 6 administrations, with 3 days between each application. The experiment lasted for 70 days, the rats being investigated along the entire period: clinical examination and complementary methods of evaluation of cardiac activity. The value of glycemia in rats with diabetes was assessed using biochemical methods (Reflotron-Glucose-Roche) or with rapid tests (glucose meter Accu-Chek Go Roche). Diabetic rats were treated with insulin according to the protocol.

At the final of the experimental period, the rats were euthanized, blood being sampled for final biochemical investigations. Body weight, cardiac weight and liver weight were assessed. Cardiac measurements were completed with the assessment of longitudinal diameter (distance between apex and base of the heart) and transversal diameter (distance between left side and right side of the heart at the point of the atrioventricular sulcus). The heart was fixed 24 hours in 10% solution of formaldehyde. After fixation, a transversal section in atrioventricular sulcus was made, followed by measurements of thickness of the interventricular sept (IS) and free walls of the left ventricle (LV) and right ventricle (RV). All values were used for calculation of longitudinal cardiac diameter/transversal cardiac diameter ratio, heart weight/body weight ratio, ventricular ratio, liver weight/body weight ratio. Ventricular ratio was calculated using the formula: \((LV + IS)/RV\) (Robinson and Grant Maxie, 1993).

All data were expressed as mean ± standard error of mean (SEM). Significant differences between mean values of multiple groups were evaluated using one way analysis of variance ANOVA. Significant differences between mean values of two groups were evaluated by Student's t test. In both tests, values of \(P<0.05\) were considered statistically significant.

RESULTS

The values of longitudinal diameter/transversal diameter ratio in Group 1 (STZ and ADR), Group 2 (STZ), Group 3 (ADR) and Group 4 (CON) were 1.231±0.279, 1.243±0.128, 1.341±0.120 and 1.182±0.134, respectively (Figure 1). There were no statistically significant differences between all groups, but significant differences occurred among Group 3 (ADR) and control (\(P<0.05\)). The difference between the means was 0.16 ± 0.06.

Ventricular ratio exhibited conclusive results, significant differences between all groups being recorded (\(P<0.05\)). The values of ventricular ratio were 8.33±2.53 in Group 1 (STZ and ADR), 6.25±1.85 in Group 2 (STZ), 5.11±1.27 in Group 3 (ADR) and 4.90±0.38 in Group 4 (CON) (Figure 2). Data proved that ventricular ratio presented significant differences between Group 1 (STZ and ADR) and Group 2 (STZ), the difference between the means being 2.08±0.73. Same significant differences were recorded between Group 1 (STZ and ADR) and
Group 3 (ADR), the difference between the means being 3.22±0.88. There were no significant differences between Group 2 (STZ) and Group 3 (ADR).

The values of heart weight/body weight ratio exhibited statistically significant differences between all studied groups (P<0.05) (Figure 3). The values of this parameter were 0.37±0.06 in Group 1 (STZ and ADR), 0.40±0.04 in Group 2 (STZ), 0.44±0.07 in Group 3 (ADR) and 0.34±0.01 in Group 4 (CON). Student's t test proved that there was a significant difference between Group 1 (STZ and ADR) and Group 3 (ADR), the difference between the means being -0.07±0.02. Same significant differences were recorded between Group 3 (ADR) and Group 4...
(CON), the difference between the means being 0.10±0.03. There were no significant differences between Group 1 (STZ and ADR) and Group 2 (STZ); Group 2 (STZ) and Group 3 (ADR).

![Figure 3. Values of heart weight/body weight ratio in the studied groups. Each column represents the mean ± SEM for each experimental group. P<0.05 vs. CON](image)

The values of liver weight/body weight ratios were 4.19±0.79 in Group 1 (STZ and ADR), 3.73±0.74 in Group 2 (STZ), 4.52±0.94 in Group 3 (ADR) and 3.40±0.36 in Group 4 (CON), statistically significant differences being recorded between all groups (Figure 4). Only groups treated with adriamycin recorded significant differences comparing with the control group (Group 1 and Group 4, difference between means 0.79±0.36; Group 3 and Group 4, difference between means 1.12±0.36).

![Figure 4. Values of liver weight/body weight ratio in the studied groups. Each column represents the mean ± SEM for each experimental group. P<0.05 vs. CON](image)
For a better image of the data, the results of this experiment are concentrated in table 1.

Table 1. Mean values of studied parameters in control and experimental groups of rats

<table>
<thead>
<tr>
<th></th>
<th>Longitudinal diameter / transversal diameter ratio</th>
<th>Ventricular ratio</th>
<th>Heart weight/body weight ratio (x10²)</th>
<th>Liver weight/body weight ratio (x10²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 STZ and ADR</td>
<td>1.231±0.279</td>
<td>8.33 ± 2.53* †</td>
<td>0.37±0.06*</td>
<td>4.19±0.79*</td>
</tr>
<tr>
<td>Group 2 STZ</td>
<td>1.243±0.128</td>
<td>6.25±1.85*</td>
<td>0.40±0.04</td>
<td>3.73±0.74</td>
</tr>
<tr>
<td>Group 3 ADR</td>
<td>1.341±0.120*</td>
<td>5.11±1.27*†</td>
<td>0.44±0.07†</td>
<td>4.52±0.94†</td>
</tr>
<tr>
<td>Group 4 CON</td>
<td>1.182±0.134*</td>
<td>4.90±0.38</td>
<td>0.34±0.01†</td>
<td>3.40±0.36†</td>
</tr>
</tbody>
</table>

Variance analysis

- Insignificant differences between groups (P = 0.5019)
- Significant differences between group 3 and group 4 (P<0.05)
- Significant differences between all groups (P=0.0001)
- Significant difference between group 1 and group 2, group 1 and group 3 (P<0.05)
- Significant differences between group 1 and group 3 (P<0.05)
- Significant differences between group 1 and group 3 (P<0.05)
- Significant differences between group 3 and group 4 (P<0.05)
- Significant differences between group 3 and group 4 (P<0.05)
- Significant differences between group 3 and group 4 (P<0.05)

**DISCUSSION**

Comparing the values of longitudinal transversal cardiac diameter ratio we have seen that there were no significant differences between experimental groups. The biggest values were obtained in Group 3 (animals treated with adriamycin) and were also observed in adriamycin induced cardiomyopathy in hamsters (Okumura et al., 2002).

Previous researches can explain these findings as a decreased ejection fraction of the heart, especially in the left ventricle, inducing a subsequent dilation of the chamber (Shan et al., 1996; Singal et al., 1997). This dilation is not always correlated with the increase of cardiac diameters and a significant increase of longitudinal diameter/transversal diameter ratio implicitly. It is possible that a
cardiac remodelling process occurred in Group 3, the heart being spherically shaped, without a significant increase of its dimensions.

The values of ventricular ratio presented significant differences between all experimental groups. Animals from Group 1 recorded the biggest value, being double compared with controls. Thus, double administration of streptozotocin and adriamycin were grossly expressed as left ventricle hypertrophy. These results are previously explained as a possible interstitial fibrosis, thickening of arteriolar media, endothelial cells and basement membrane changes (Vranes et al., 1999). Progressive narrowing of arteriolar lumen induces myocardial ischemia and subsequent replacement of apoptotic myocardocytes with collagen (Lorenzi et al., 1991; Kumar et al., 2001). On the other hand, experimental models associate diabetes with hypertension. Ischemia induced by hypertensive vasoconstriction generates the same cardiac fibrosis, revealed as hypertrophy of the left ventricle (Dahm-Jandeleit et al., 2000). Group 2 (single streptozotocin administration) recorded higher values compared with controls and lower compared with Group 1 (double administration of streptozotocin and adriamycin), exhibiting hypertrophy of the left ventricle. One question we have to answer to, is how can be explained the significant differences between Group 1 and Group 2. It is possible that the vascular resistance induced by vascular sclerosis or hypertension is bigger in Group 1, producing a supplementary cardiac activity and a bigger ventricular ratio.

Group 3 (single administration of adriamycin) presented an inferior value compared with Group 1 and Group 2. Despite minor differences between Group 3 and control, we evaluate that this finding reveals the debut of a dilated cardiomyopathy.

The values of heart weight/body weight ratio recorded significant differences between all groups. This ratio was bigger than controls in Group 1 and 2, probably induced by interstitial fibrosis (Lorenzi et al., 1991).

Reference data concerning this parameter are very few. Medium body weighted rats (238 g) recorded a heart weight/body weight ratio with a value of 0.30 \((10^2)\) (Ayers and Jones, 1978). The references show that previous experimental models which have used adriamycin as the inducing agent for congestive cardiac failure, heart weight/body weight ratio varied in very large limits. These values ranged between 2.38 \((10^3)\pm0.08\) (Siveski-Iliskovic et al., 1994) and 5.78 \((10^3)\pm0.2\) (Mukherjee et al., 2003). The value of heart weight/body weight ratio was smaller than in the control group, inderferently of experimental model. In our experiment, rats of Group 3 recorded an intermediary value (comparing to those metioned as references), but was bigger than in the control group. All references reviewed in this study presented decreased values of heart weight/body weight ratio in adriamicyn treated groups (comparing with control groups) (Saltiel and McGuire, 1983; Kumar et al., 2001; Sowers et al., 2001; Okumura et al., 2002; Shad et al., 2007).

The values of liver weight/body weight ratio exhibited significant differences between all studied groups. Analyzing the data, a constant increase of the values of this ratio is noticed in treated groups. Group 1 (double administration of streptozotocin and adriamycin) and Group 2 (single administration of
streptozotocin) presented close values. Group 3 (single admistration of adriamycin) recorded the biggest value of the experiment. We consider that the increase of liver weight/body weight ratio is the effect of congestive heart failure, expressed as a possible hyperplasia of arteriolar leiomyocytes and perivasular fibrosis (Militaru et al., 2006).

In conclusion, the rats with streptozotocin induced diabetes type 1 included in Groups 1 and 2 presented hypertrophy of the left ventricle, revealed by increased values of ventricular ratio, compared with the control group. Same groups exhibited a significant increase of heart weight/body weight ratio and liver weight/body weight ratio, compared with the control group. These figures were smaller than the mean value of adriamycin treated rats. Longitudinal cardiac diameter/transversal cardiac diameter ratio did not record significant differences between all experimental groups, except Group 3 treated with adriamycin, when compared with control rats.

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REFERENCES


GRAVIMETRIJSKA I MORFOMETRIJSKA ISPITIVANJA KOD WISTAR PACOVA SA EKSPERIMENTALNIM DIJABETESOM TIPA 1 I SRČANOM SLABOŠĆU

CIOBOTARU E, MILITARU M, BRASLASU CM, BRASLASU ED, SOARE T, JOITA S i DINESCU G

SADRŽAJ

U ovim ispitivanjima je korišćeno 55 odraslih pacova soja Wistar podeljenih u četiri nejednake grupe. Pacovima oglednih grupa su eksperimentalno indukovani *Diabetes mellitus* tip I i srčana slabost dok je četvrta gupa služila kao kontrolna (zdrave jedinke). Pacovima prve ogledne grupe aplikovani su streptozotocin i adriamicin, druge streptozotocin a treće adriamicin. Pacovi su autanazirani nakon perioda od 70 dana i merene su njihova telesna masa, masa srca i jetre. Srčane gravimetrijske vrednosti su izračunavane nakon merenja longitudinalnog i transverzalnog dijmetara srca, kao i dijmetara septuma i zida leve i desne komore. Ove vrednosti su korišćene za izračunavanje: odnosa longitudinalni/ transverzalni dijmetar, odnosa masa srca/masa tela, komornog indeksa i odnosa masa jetre/masa tela. Pacovi prve i druge grupe imali su najveću hipertrofiju zida leve komore i kod njih je komorni indeks bio 8,33±2,53, odnosno 6,25±1,85 (p<0,05). U ovim grupama su bili povećani i odnosi: masa srca/masa tela kao i masa jetre/masa tela. Odnos longitudinalni/transverzalni dijmetar srca je bio sličan u svim grupama a značajne razlike su utvrđene samo između treće i kontrolne grupe.