Metformin HCl has curative effect on rebuilding of ventricular diastolic functions in high-fat-diet fed rats

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Abstract: Myocardial lipid accumulation due to diabetes and/or obesity plays a role in the progression of left ventricular diastolic dysfunction. Our aims were to exhibit the correlation between histopathologic stage of the liver and cardiac functions, and to evaluate the effects of metformin HCl and rosiglitazone on myocardial functions. Thirty-two male Sprague-Dawley rats were divided into four groups to exhibit the correlation between histopathologic stage of the liver and cardiac functions and to determine whether metformin HCl and rosiglitazone have effects on cardiac functions. For 20 weeks, one group was fed standard rat basic diet, whereas the other groups were on high-fat-diet. During the last 4 weeks, metformin HCl was given to the third group, rosiglitazone to the fourth group. Histological evaluation of rat livers yielded significantly higher steatosis grade in high-fat-diet group and different fibrosis stages among groups. Also, there was significant correlation between diastolic functions and steatosis grade/fibrosis stage of rat liver. Electrophysiological study of hearts via Langendorff technique showed better coronary perfusion pressures and diastolic functions in standard-diet and metformin HCl groups compared to other groups. Metformin HCl improves LV diastolic dysfunction and coronary perfusion pressures.

Keywords: Myocardial steatosis, metformin HCl, rosiglitazone, diastolic dysfunction, coronary perfusion.

INTRODUCTION

The recent increase in obesity and diabetes mellitus (DM) all around the world give rise to obesity and/or DM-related heart diseases such as heart failure with worse prognosis compared with those without DM. Although heart failure is generally attributed to coexisting disorders such as coronary artery disease and hypertension, metabolic derangements in the myocardium may play a pivotal role, too. (Andersson *et al*., 2010 a).

Under conditions of excess fatty acid production, such as in obesity or DM, the balance between lipolysis and lipogenesis is in favor of excess intracellular triglyceride (TG) accumulation in the heart [myocardial steatosis], leading to lipotoxic injury and then to the development of cardiomyopathy that can be reversed by weight lose (Lindsey and Marso, 2008; Zhou *et al.*, 2000). Myocardial steatosis adversely effects left ventricular (LV) diastolic function rather than systolic function (Zhou *et al.*, 2000) .

For many years, many investigations have been performed on laboratory animals and finally on patients. Metformin HCl and rosiglitazone are two of the pharmaceutical agents which have been demonstrated to cause improvement in hepatic steatosis. Metformin HCl is a biguanide drug, reverses hyperglycemia, improves metabolic control and insulin deficiency disappears. Rosiglitazone is a thiazolidinedione drug (peroxisome proliferator-activated receptor gamma agonist) decreases plasma glucose levels by improving pancreatic islet b-cell function and insulin sensitivity. Treatment with metformin HCl and/or rosiglitazone activates peripheral lipogenic pathways by the aid of reversal of insulin deficiency and the fat in ectopic sites (liver, heart) migrates to peripheral adipose tissues (Lingvay *et al.*, 2007). So they decrease hepatic steatosis primarily by the way of insulin, their favorable efficiencies on myocardial steatosis have also been studied recently.

The aims of the present study were to exhibit the correlation between histopathologic stage of the liver and cardiac functions and to determine whether metformin HCl and rosiglitazone have effects on myocardial functions and liver steatosis in rats.

MATERIALS AND METHODS

Study group

Thirty-two male Sprague-Dawley rats, obtained from University Animal Research Laboratory, weighing 300± 50g (8 weeks of age) were studied. Cages for rats were based in an animal room at 22±2°C, with a 12⁄12 hour light:dark cycle. At first rats were weighed, then food and water were followed up daily in the course of the study. Rats were given standard rat basic diet (SD) including 15% protein, 2.5% fat, 15% cellulose, 14% fuller's earth, 13% water (pellets; Bait Industry Incorporated Company, Elazığ, Turkey). Rats could drink tap water ad libitum

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until 8 weeks of age. All the regulations for the use and care of experimental animals were obeyed, and also experiment was approved by the Local Animal Research Laboratory Ethical Committee (No: B.30.2.DİC.1H.00.00/26.

Study protocol

Four groups were randomly maintained, first one (control group) was given SD for 20 weeks, whereas the others were on high-fat-diet (HFD) for 20 weeks. Fresh HFD in pellet form was prepared everyday by mixing 25 g butter with 100 g standard diet, thus 65% of caloric supply in HFD was maintained by fat.

After 20 weeks, all groups were nourished with SD for 4 weeks. First two groups were without any pharmacological agent (group 1: SD group, group 2: HFD group), whereas metformin HCl was administered to the third group (HFD+MET group) at 250mg/kg/day by oral gavage and rosiglitazone to the fourth group (HFD+ROS group) at 4 mg/kg/day by oral gavage.

At the end of the study periods, animals were weighed (Sartorius BP 1215) and anesthetized by intramuscular injection of 75mg/kg ketamine HCl and 15 mg/kg xylazine. After exploration, whole blood was collected by cardiac puncture, then heart and liver were excised immediately.

Langendorff perfusion system examination

The heart was placed into an ice-cold modified Krebs-Henseleit solution at 4^{o}C to cease contractions. Then it was cleaned of surrounding tissues and was perfused retrogradely by noncirculating Langendorff technique (MAY 0702, Commat, Ankara, Turkey) through the aorta tied to a stainless steel cannula of the perfusion system. The pulmonary artery was incised to facilitate a complete coronary drainage in the ventricles. Daily prepared modified Krebs-Henseleit solution with the following composition (mM): NaCl 118, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25 and glucose 11 was used as the perfusion solution. Continuous oxygenation of solution with 95% O2 and 5% CO2 was maintained at pH of 7.4 and at temperature of 37^{o}C (MAY WBC 3044 Water Bath and Circulator, Commat, Ankara, Turkey). Perfusion of heart was kept under constant flow condition (12ml/min) by the way of a peristaltic pump (MAY PRS 9508, Commat, Ankara, Turkey). Our preliminary studies represented that cardiac and coronary variables were not changed due to infusions during the study.

Side arm of the aortic cannula connected to a pressure transducer (FDT-10A, Commat, Ankara, Turkey) provided measurement of coronary perfusion pressure. A liquid-filled latex balloon attached to another pressure transducer was inserted into the left ventricle via the mitral valve to measure peak systolic and end diastolic pressures with preserved diastolic balloon pressure at 8 mmHg. The difference between the systolic and diastolic pressures yielded left ventricular developed pressure (LVDP) which is a sign of contractile force. The signals of the left ventricular pressure allowed calculation of heart rate. Additionally, the maximum and minimum rates of increase of left ventricular pressure (dp/dt max. and dp/dt min.) were determined to evaluate the systolic and diastolic functions of LV. A data acquisition and analysis system (MP 30 B Amplifier Modules, Biopac System. Inc. Santa Barbara, CA, USA) was used to analyze hemodynamic parameters. The hearts were equilibrated for 30 minutes (min) to establish a stable baseline.

Histological examination

Liver tissues taken for histological evaluation were stained with haematoxylin-eosin to evaluate necro-inflammatory grading (steatosis grade) and with masson trichrome to evaluate non-alcoholic steatohepatitis (fibrosis stage). A scoring system was used to specify histological changes.

Grading and staging

Steatosis was graded 0-3, according to the percent of hepatocytes involved. (Involved hepatocytes are <5% in Grade 0,5%-33% in Grade I, 33%-66% in Grade II, 66% in Grade III) Staging was based on the severity of fibrosis. (Stage 0: No fibrosis, Stage 1: Perisinusoidal fibrosis, Stage 2: Portal fibrosis, Stage 3: Bridginig fibrosis, Stage 4: Cirrhosis)

STATISTICAL ANALYSIS

The statistical packages for SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for analysis. Mean and standard deviation were calculated for continuous variables. Because of the small group sizes, Mann Whitney U test was preferred to analyze the medians of independent groups. The normality of the continuous data was controlled by Shapiro-Wilk test and the differences of the variables between different groups were assessed by one way ANOVA test. Two tailed p values less than 0.05 were accepted statistically significant.

RESULTS

Weight Gain

At the end of 20 weeks, HFD fed rats were heavier than SD fed rats (p .001). After 24 weeks, all groups differed from each other on the basis of body weight, however weight gaining was significantly less in rats supplemented with metformin HCl or rosiglitazone (p .001) (table 1).

Histological features of liver

The steatosis grade was significantly higher in HFD group than other groups (Grade 2 vs 0, p .001) (table 1). Also,

Table 1: Data regarding body weights, histopathologic examination and Langendorff System (countable values as mean and standard deviations).

Variables	SD	HFD	HFD+MET	HFD+ROS	p value
Weight at 20th week (gram)	326.8±4.4	340.6±3.2	340.3±3.3	335±4	□ .001
Weight at 24th week (gram)	333.8±1.3	376.5±2.3	348.2±2.3	348.3±2.1	□ .001
Steatosis Grade	0	2	0	0	□ .001
Fibrosis Stage	0	3	1	1	□ .001
Pressure (mmHg)	45.2±6.8	37.1±7.7	47.4±3.8	36.9±3.2	.001
Rate (/minute)	229.3±25.7	242.3±26.4	259.4±15.9	242±19.5	.083
LVDP (mmHg)	67.6±8.9	56.6±15	75.1±10.5	70.3±9.9	.021
dp/dt max. (mmHg/second)	1105.3±259.2	948±51.8	982.1±71.4	1027.4±169.4	.256
dp/dt min. (mmHg/second)	2037.8±86.3	1531.3±130.3	1979.6±125.9	1600.4±168.2	□ .001

Pressure: Coronary perfusion pressure, LVDP: left ventricular developed pressure, dp/dt max.: the maximum rate of increase of left ventricular pressure, dp/dt min.: the minimum rate of increase of left ventricular pressure. All P values in Table 1 correspond to the comparisons of 4 groups.

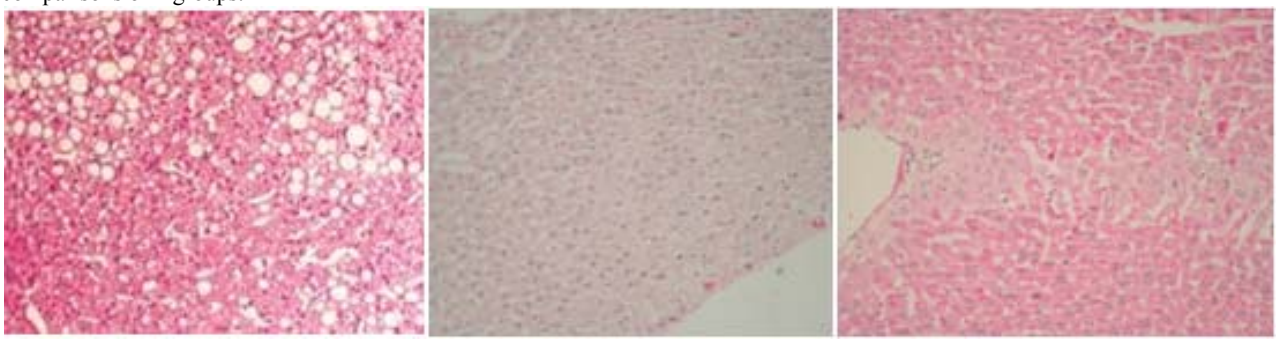

Fig. 1: Haematoxylin and eosin stained sections of rat livers (left: HFD group, middle: metformin HCl group, right: rosiglitazone group)

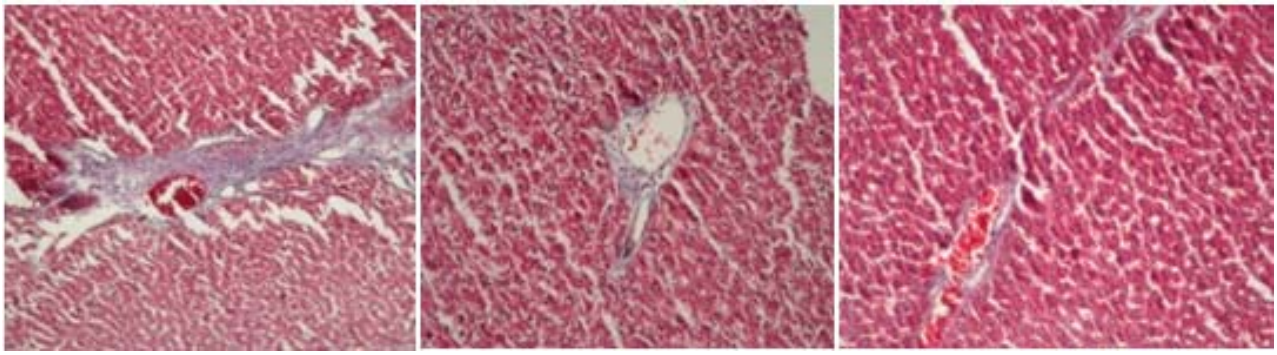

Fig. 2: Masson trichrome stained sections of rat livers (left: HFD group, middle: metformin HCl group, right: rosiglitazone group)

there were significant differences between fibrosis stages (SD of fibrosis stage 0, HFD of stage 3, other groups of stage 1, p□.001). Metformin HCl and rosiglitazone supplement added to HFD feeding protected rat liver from steatosis completely and from fibrosis to some extent (fig. 1, 2).

Data derived from langendorff perfusion system

Mean heart rate was highest in HFD+MET group, lowest in SD group with no statistical significance; mean LVDP value was lowest in HFD group, and highest in HFD+MET group (p=.014) (table 1).

Mean coronary perfusion pressures of SD and HFD+MET groups were better compared to HFD and HFD+ROS groups (fig. 3). Also, HFD+MET group had slightly higher coronary perfusion pressures compared to SD group, as well as HFD group compared to HFD+ROS group. (SD vs HFD, p=.039; SD vs HFD+ROS, p=.032; HFD+MET vs HFD, p=.006; HFD+MET vs HFD+ROS, p=.005).

Mean dp/dt max. value was lowest in HFD group and highest in SD group, but the difference was not significant (p=.229) (fig. 4). On the other hand, mean dp/dt min. values in SD and HFD+MET groups were significantly higher compared to HFD and HFD+ROS groups (p□ .001).

There was correlation between dp/dt min. and steatosis grade/fibrosis stage of rat liver (respectively, p=.003 and p=.001). Statistical analysis did not show any relationship between other Langendorff System data and steatosis grade/fibrosis stage.

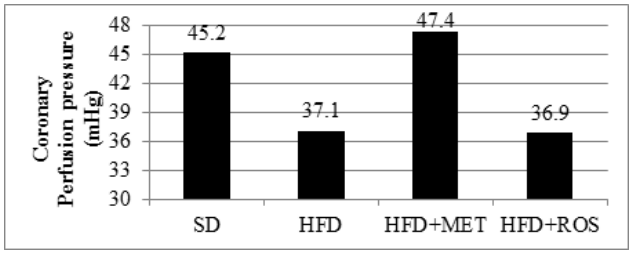

Fig. 3: Mean coronary perfusion pressures (p = .001)

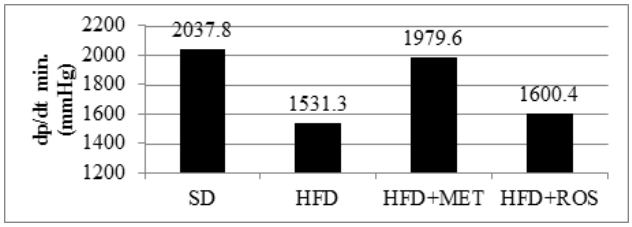

Fig. 4: Mean dp/dt min. values (p .001).

DISCUSSION

Diabetic cardiomyopathy means the presence of myocardial abnormalities which is not due to a significant etiology such as coronary artery disease or hypertension. Bauters et al. detected some constant changes in diabetic cardiomyopathy such as microangiopathy, metabolic disturbances and myocardial fibrosis (Bauters *et al.*, 2003).

Many arguments have established that lipid oversupply to cardiomyocytes leads to the development of diabetic cardiomyopathy (Unger, 2002). In the normal heart, lipolysis and lipogenesis are balanced. In patients with DM, insulin resistance causes excessive FA delivery from adipose tissue ending with uptake in the heart. Excess FAs exceeding the oxidizing requirements of the organ are sequestered as TGs to provide cardiomyocytes a reserve of FAs, producing fatty acyl-CoA esters, diacylglycerol, and ceramide (Unger, 2002). Collection of these FA intermediates induce mitochondrial dysfunction and reactive oxygen species, which damage cells directly leading to apoptosis and then myocardial dysfunction. Myocardial dysfunction is sometimes induced indirectly by inflammatory cascades as well (Zhou *et al.*, 2000; Ouwens *et al.*, 2005; Young *et al.*, 2002).

Animal studies have shown that excess accumulation of TGs in cardiomyocytes impairs LV function and promotes cardiac fibrosis and apoptosis in obese rats and in high-fat-fed rabbits (Zhou *et al.*, 2000; Ouwens *et al.*, 2005; Carroll and Tyagi, 2005). But, in humans, there are emerging yet limited data for myocardial lipid accumulation. Histopathology of explanted hearts among diabetic transplant recipients has shown myocardial lipid accumulation (Sharma and Chetti, 2005). Also, use of magnetic resonance spectroscopy (MRS) has provided that myocardial lipid accumulation occurs in vivo in humans, specifically among individuals with DM and in those with insulin resistance (McGavock *et al.*, 2007).

LV and RV functions are impaired in patients with high myocardial TG content. Septal thickness and left ventricular mass correlates strongly with myocardial lipid content, suggesting that myocardial lipids contribute to the formation of ventricular hypertrophy (Szczepaniac *et al.*, 2002). By MRS, it has been shown that excess myocardial TG deposition impairs left ventricular longitudinal systolic and diastolic functions, while preserving circumferential and radial functions (Ng *et al.*, 2009). This finding supports the hypotheses claiming that increased myocardial stiffness, increased resting myocyte tension and deposition of glycated end products are related with diabetic cardiomyopathy (van Heerebeek *et al.*, 2008).

Although steatosis has been suggested to be able to precede the onset of asymptomatic LV systolic dysfunction (McGavock *et al.*, 2007), most of the studies argued that myocardial lipid accumulation is independently associated with impaired LV diastolic function (Andersson *et al.*, 2010 a; Lindsey and Marso, 2008; Chiu *et al.*, 2005), perhaps due to coronary microangiopathy or small vessel disease (Nagueh *et al.*, 2004), with no correlation between myocardial TG content and left ventricular ejection fraction (Rijzewijk *et al.*, 2010). Our study supported the literature in this regard. In HFD and HFD+ROS groups, LV diastolic functions of rats were impaired, whereas LV systolic funtions were preserved in all groups. SD and HFD+MET groups had better coronary perfusion as well as diastolic function, suggesting that TG deposition in coronary vessels consistent with vascular stiffness was one of the causes of diastolic dysfunction. However, more prominent impairment of diastolic function comparing to coronary perfusion in HFD and HFD+ROS groups supported that there were other causes, as well.

Convenient to our results, metformin HCl has been asserted to improve cardiac metabolism and functions in DM and even to attenuate the development of heart failure (Gundewar *et al.*, 2009; Sasaki *et al.*, 2009). Andersson et al. demonstrated that 'metformin HCl shortens isovolumic relaxation time and improves early diastolic longitudinal tissue velocity, independent of the blood glucose lowering effect' (Andersson *et al.*, 2010 b).

Exact mechanism of metformin HCl in improvement and/or prevention of diastolic dysfunction has not been fully understood. Metformin HCl stimulates AMP kinase

(AMPK), which has a pivotal role in regulation of cardiac metabolism under stressed conditions (Hardie, 2004). By the aid of AMPK, metformin HCl has been demonstrated to attenuate pacing-induced heart failure development in dogs, and to decrease the subsequent degradations of cardiomyocyte ATP synthesis in mice exposed to occlusion of the left coronary artery (Gundewar *et al.*, 2009; Sasaki *et al.*, 2009). Metformin HCl diminishes Activin A which has been linked to contractile dysfunction in cardiomyocytes and also reduces cross-linkage of advanced glycemic end products, thus inhibits ventricular stiffness and cardiac fibrosis (Chen *et al.*, 2013). Besides the effects on myocardium, it increases myocardial microcirculation by improvements in endothelial function and arterial stiffness, which explains preserved coronary perfusion of metformin HCl taking rats in this study (Agarwal *et al.*, 2010). This study did not prove the mechanism of metformin HCl; but at least, exhibited that improvement of diastolic dysfunction can not be achieved only with insulin regulation. If so, rosiglitazone could not be ineffective. To identify the complex mechanism, research at molecular and cellular level is needed.

Additionally there was a significant correlation between the myocardial diastolic dysfunction and the steatosis grade/fibrosis stage of liver. This is important because it means that patients with hepatic steatosis must be alerted for the possible impairment of cardiac functions at the present time or in the near future. The fact, that rosiglitazone supplementation did not improve diastolic function although preserved liver steatosis and fibrosis, surprised us. According to the literature, rosiglitazone reverses hepatic steatosis, even improves myocardial steatosis (Ackerman *et al.*, 2010; McGuire *et al.*, 2009). We think that impaired LV diastolic function in HFD+ROS group was not due to myocardial steatosis, probably due to coronary microangiopathy or fluid retention which is a well known side effect causing abnormalities in LV diastolic function and LV volumes, without LV systolic function impairment, suggesting no adverse effects on LV contractility as in our study (Sharaf *et al.*, 2008). In somuch that rosiglitazone has been withdrawn from markets in many countries, because of high heart failure and death rates. However, in 2013 June, FDA advisory committee considered data from three long-term outcome studies (RECORD, ADOPT, DREAM), suggesting no sufficient correlation between cardiac diseases/death and rosiglitazone treatment, and regarded that rosiglitazone should continue to be marketed in the United States. Even if rosiglitazone use does not cause heart failure, present study demonstrated that it does not have beneficial effect to improve LV diastolic dysfunction and/or impaired coronary perfusion due to HFD feeding.

CONCLUSION

HFD feeding causes diastolic dysfunction of left ventricle while systolic functions are stil preserved. Metformin HCl has curative effect on rebuilding of ventricular diastolic functions due to high-fat-diet, also it improves coronary perfusion, but rosiglitazone does not have favourable effect on diastolic function or coronary perfusion. So, in obese diabetic patients especially with hepatic steatosis, high probability of diastolic dysfunction should be kept in mind and metformin may be preferred as antidiabetic agent in those patients, even in the absence of diastolic dysfunction.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institutional ethical committee. This article does not contain any studies with human participants performed by any of the authors.

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