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Effects of Rosiglitazone on Metabolic Parameters and Adiponectin Levels in Fructose-Fed Rats

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Abstract

Aim. To investigate the effect of the peroxisome proliferators-activator receptor gamma agonist, rosiglitazone, on metabolic parameters and adiponectin levels in an animal model of the metabolic syndrome.

Material and methods. Metabolic syndrome was induced in 32 male Wistar rats by adding a fructose in drinking water for 12 weeks. During the last 4 weeks, 16 rats were treated with rosiglitazone (5 mg/kg/day), while the remaining 16 did not receive any medication (fructose group). Another control group consumed standard rat chow and water for 12 weeks.

Results. Chronic fructose administration induced a significant increase in systolic blood pressure (SBP), body weight, serum triglycerides (TG), free fatty acids (FFA), insulin, glucose AUC_{0-120} (during oral glucose tolerance test) and decreased serum high density lipoprotein (HDL) cholesterol and adiponectin concentrations compared with the control group. Treatment with rosiglitazone over the final 4 weeks reversed these effects and significantly reduced SBP, TG, FFA, insulin concentrations and glucose AUC_{0-120} compared with the fructose group. In addition, rosiglitazone increased serum levels of adiponectin twofold from 3.44 ± 0.46 to 7.03 ± 1.30 μg/ml.

Conclusion. This study indicates that rosiglitazone treatment improves the components of the metabolic syndrome, which is accompanied with an increase in adiponectin concentrations.

Introduction

The metabolic syndrome is characterized by the following components: impaired glucose tolerance, dyslipidemia, hypertension and/or abdominal (central) obesity. In the literature, it is also found under different names, such as: syndrome X, "deadly quartet" and insulin resistance syndrome, and its importance in the modern world is increasing, because the results from many prospective studies have shown that those who meet the definition of this syndrome are twice as likely to die from, and three times as likely to have, a myocardial infarction than those who do not, and four to five times more likely to develop type 2 diabetes (1).

The pathogenesis of the metabolic syndrome is complex and multifactorial, but it is considered that the insulin resistance and adiposity (especially in the abdominal region) are crucial factors for its development (2, 3). Insulin resistance is a state of decreased

tissue reactivity to circulating insulin levels. It causes impaired glucose uptake in the periphery (skeletal muscles, adipose tissue) and an increased glucose production in the liver, which leads to increased insulin needs for maintaince of blood glucose values within the normal range (4).

Adipose tissue is no longer considered as a passive storage depot for triglycerides and fatty acids, but rather an active metabolic organ that produces various biologically active molecules (referred to as adipocytokines) which act as autocrine, paracrine or endocrine regulators of many physiological and patophysiological processes in the organism. Among them, a special attention and scientific interest has been lately focused on adiponectin, which is thought to be the molecular link between adiposity and the insulin resistance. Adiponectin is a protein of 30 kD and is exclusively produced in the adipose tissue. Many studies have demonstrated that adiponectin possesses antidiabetic, anti-atherogenic and anti-inflammatory characteristics. Plasma concentrations of adiponectin are decreased in the metabolic syndrome; therefore therapeutic strategies that increase adiponectin levels could be potentially useful for the treatment of the metabolic syndrome, as well as prevention and/or delaying the development of manifest type 2 diabetes and atherosclerotic coronary vascular disease (5-7).

Thiazolidinediones, a new class of insulin senzitizing drugs including rosiglitazone and pioglitazone, provide an effective approach for treating type 2 diabetes. They elicit their effects through activating the nuclear peroxisome proliferator-activated receptor gamma (PPAR-gamma). Many in vitro and in vivo studies have shown that treatment with thiazolidinediones affects many factors involved in lipid metabolism, insulin signal pathways, glucose phosphorilation and glucose transport, leading toamelioration of insulin resistance and improvement of the imapired glucose tolerance in type 2 diabetics (8).

The results from several recent studies indicate that PPAR-gamma agonists affect a much broader spectrum of processes in the organism (inflammation, endothelial function, atherosclerosis...) and that some of these effects could be a result of an altered adipocytokines secretion. Therefore, beside their current official indication (manifest diabetes mellitus- type 2), PPAR-gamma agonists could have a potential role in the treatment of other metabolic and vascular diseases (9-11).

In the present study, we evaluated the effect of rosiglitazone, a PPAR-gamma agonist, on metabolic profile and adiponectin levels in fructose-fed rats that represent a nutritive, animal model of the metabolic syndrome.

Material and methods

Groups

Male Wistar rats (200 $\pm$ 25 g) were kept at the experimental stable of the Institute of Preclinical and Clinical Pharmacology and Toxicology. The animals were housed in standard cages (four rats/cage) and maintained under controlled room temperature and humidity with 12/12-hour light-dark cycle. Rats were fed a standard commercial chow and had a free access to drinking water. All performed procedures were in accordance to the principles for care and use of laboratory animals (12).

The rats were divided into 3 groups: group 1 (n=16): represents a control group, and consumed standard rat chow and drinking water in a period of 12 weeks; group 2 (n=16): consumed fructose as a 10% solution in the drinking water for a period of 12 weeks; and group 3 (n=16): consumed fructose as a 10% solution in the drinking water for a period of 12 weeks + rosiglitazone (ALKALOID AD, Macedonia) in a dose of 5 mg/kg/day by intragastric tube in the last 4 weeks.

Fructose solution was prepared fresh daily during the 12 weeks, by dissolving fructose (ADM Corn Processing) in the drinking water.

Study parameters

Systolic blood pressure (SBP), body weight, serum triglycerides (TG), free fatty acids (FFA), HDL (high density lipoprotein) cholesterol, insulin, adiponectin and glucose $AUC_{0\text{-}120}$ (Area Under Curve) were determined at the beginning of the study (week 0), after 8 weeks of fructose diet and at the end of the study after 4 additional weeks of fructose diet and a pharmacological treatment with rosiglitazone (week 12).

Pletismographic method for measurment of systolic blood pressure

Seven days before the beginning of the experiment, the animals were trained for measument of the systolic blood pressure on the tail by pletismographic method (IITC, Life Science, California, USA). After each measurment, the rats were warmed on temperature of approximately 37^{o}C (30 minutes), and afterwards each rat was placed in an immobilization cham-

ber. Five consecutive measurments were made for each animal (minimal and maximal values were excluded, and from the remaining three an averige value was calculated.

Oral glucose tolerance test (OGTT)

Twelve hours before the beginning of the test, food was removed from the cages and the bottles with 10% fructose solution were replaced with water. After 12 hour-fast, each rat was adimistered 2 g glucose/kg bw (as a 30% solution) by intragastric tube. The last dose of the investigated drug administered approximately 24 hours before the glucose load. Glucose levels were measured in the following time points: 0, 30, 60 i 120 minutes after glucose administration. Blood was taken from the tail vein of each rat. Glucose concentration were determined by the glucose-oxidase method. The obtained glucose values in all time points were used for calculation of AUC_{0-120} (Area Under Curve) of glucose.

Methods for determination of serum concentrations of TG, HDL cholesterol, insulin, adiponectin and FFA

Blood samples were withdrawn by venepunction from the retroorbital sinus of the rats (under light ether anesthesia). Five hours before, food was removed from the cages and the bottles with 10% fructose solution were replaced with water.

Serum triglycerides and HDL holesterol concentrations were measured by standard enzymatic colorimetric methods on Integra 400+ (Roche Diagnostics GmbH, Manheim, Germany). For determination of serum insulin and adiponectin concentrations a commmercial ELISA kit were used (Mercodia, Uppsala, Sweden and B-Bridge International Inc, California, USA, respectively). For determination of serum free fatty acids an enzymatic colorimetric kit was applied (Roche Diagnostics GmbH, Pennzberg, Germany).

Statistical evaluation

The data are evaluated with the statistical programs Statistica for Windows Windows 8.0 and KINETICA™ 4.2 (Innaphase corporation, USA).

The differences between the determined timepoints, as well as the differences between the groups were analyzed with the Student "t test" for dependent and independent samples, respectively. Values for $p < 0.05$ were considered as statistically significant.

Results

The metabolic and haemodynamic parameters of different experimental groups during the study are summarized in Table 1.

The chronic fructose administration in drinking water (10% solution) in a period of 8 weeks induced a metabolic syndrome in the experimental animals. The values of systolic blood pressure, serum triglycerides and free fatty acids were significantly increased, whereas the serum HDL holesterol and adiponectin concentrations were significantly decreased, compared to the basal values (week 0) in the same group ($p<0.001$ for all parameters), as well as compared to the measured values at week 8 in the control group of animals that consumed ordinary drinking water ($p<0.001$ for all parameters). Fructose administration induced a devel-

Table 1: Metabolic and haemodynamic parameters of different experimental groups during the study.

Treatment	Week	SBP (mmHg)	TG (mmol/L)	HDL (mmol/L)	FFA (mM)	Adiponectin (μg/ml)	insulin (pmol/L)	BW (g)
Control (H_2O)	0	114 ± 8	0.61 ± 0.10	0.90 ± 0.11	0.30 ± 0.10	4.21 ± 0.42	131 ± 14	20 ± 13
	8	118 ± 8	0.65 ± 0.11	0.93 ± 0.10	0.33 ± 0.08	4.26 ± 0.40	135 ± 11	236 ± 16
	12	122 ± 5	0.70 ± 0.12	0.91 ± 0.09	0.34 ± 0.06	4.19 ± 0.36	134 ± 11	258 ± 16
Fructose	0	117 ± 5	0.63 ± 0.13	0.91 ± 0.08	0.31 ± 0.09	4.28 ± 0.43	136 ± 15	202 ± 10
	8	141 ± 5	2.01 ± 0.29	0.71 ± 0.07	0.61 ± 0.15	3.48 ± 0.65	241 ± 22	248 ± 17
	12	146 ± 4	2.09 ± 0.21	0.70 ± 0.07	0.62 ± 0.12	3.28 ± 0.53	260 ± 22	269 ± 15
Fructose+ ROSI	0	115 ± 8	0.59 ± 0.18	0.89 ± 0.10	0.32 ± 0.12	4.05 ± 0.67	134 ± 14	200 ± 13
	8	140 ± 7	2.03 ± 0.26	0.73 ± 0.09	0.62 ± 0.17	3.44 ± 0.46	244 ± 27	244 ± 18
	12	130 ± 6	1.31 ± 0.17	0.75 ± 0.08	0.45 ± 0.14	7.03 ± 1.30	152 ± 18	275 ± 15

ROSI- rosiglitazone; SBP- systolic blood pressure; TG- triglycerides; FFA- free fatty acids; HDL- high density lipoproteins cholesterol; BW- body weight.

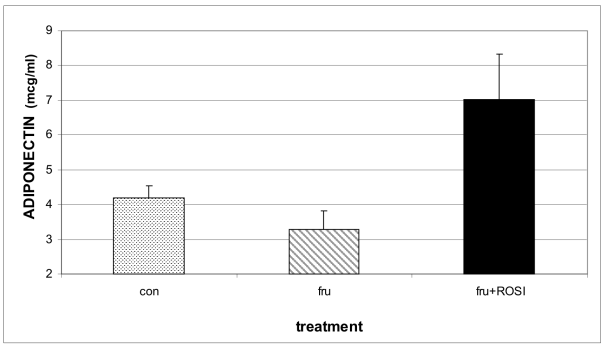

Figure 1: Serum adiponectin concentrations in the control group (con), fructose group (fru) and fructose+rosiglitazone treated group (fru+ROSI) at the end of the study (week 12).

opment of insulin resistance, which is evident from the glucose AUC_{0-120} (753 mmol/L*min) calculated from the OGTT in comparison to glucose AUC_{0-120} (540 mmol/L*min) of the control group (p<0.001). These values were accompanied with higher serum insulin concentrations (control group: 135 pmol/L; fructose: 241 pmol/L; p<0.001).

In this way, a useful experimental model for investigation of the metabolic syndrome and the effects of rosiglitazone on its components was obtained.

Treatment with rosiglitazone over the final 4 weeks improved the insulin sensitivity, as assessed by a decrease of serum insulin concentrations (260 ± 22 vs. 152 ± 18 pmol/L; p<0.001) and glucose AUC_{0-120} (623 ± 40 vs. 810 ± 48 mmol/L*min; p<0.001) compared with the fructose group. Rosiglitazone significantly reduced serum levels of triglycerides (1.31±0.17 vs. 2.09 ± 0.21 mmol/L; p<0.001) and free fatty acids (0.45 ± 0.14 vs. 0.62 ± 0.12 mmol/L; p<0.01), but induced only a minor increase of serum HDL holesterol concentrations (0.75 ± 0.08 vs. 0.70 ± 0.07 mmol/L; p=0.09). The four-week treatment with this PPAR-

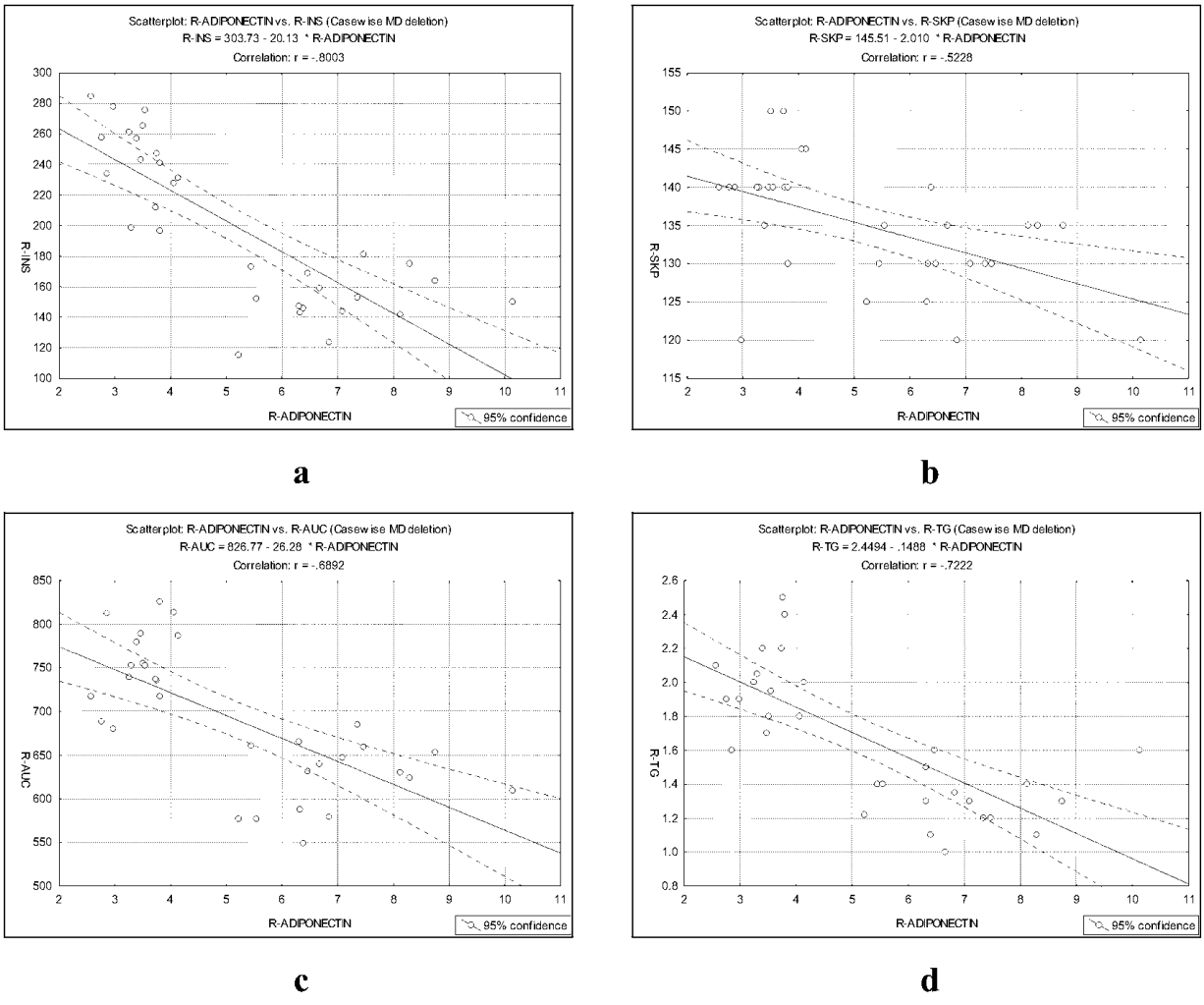

Figure 2: Correlations between adiponectin levels and serum insulin concentrations (a), systolic blood pressure (b), glucose AUC (c) and serum triglycerides (d) after treatment with rosiglitazone.

gamma agonist increased the body weight of the experimental animals (244 ± 18 g at week 8 compared to 275 ± 18 g at week 12; p<0.01), but this weight gain was not statistically significant from the animals that consumed only fructose (p=0.27). Treatment with rosiglitazone significantly reduced the levels of the systolic blood pressure (130 ± 6 vs. 146 ± 4 mmHg; p<0.01).

In addition, rosiglitazone increased serum levels of adiponectin twofold from 3.44 ± 0.46 μg/ml at the beginning of the treatment (week 8) to 7.03 ± 1.30 μg/ml at week 12 (p<0.001) (Figure 1). The detected adiponectin levels negatively correlated with serum insulin concentrations (r= -0.80; p<0.001), glucose AUC_{0-120} (r= -0.69; p<0.001), triglycerides (r= -0.72; p<0.001) and systolic blood pressure (r= -0.52; p<0.01) (Figure 2). No statistically significant correlation was established between adiponectin and HDL cholesterol (r= 0.05; p=0.978) after rosiglitazone treatment.

Discussion

The metabolic syndrome, which probably develops as a consequence of the insulin resistance, is characterized with impaired glucose tolerance, hyperinsulinemia, dyslipidemia and hypertension. These metabolic disturbances are often accompanied with abdominal (central, visceral) obesity. The cluster of multiple cardiometabolic risk factors that are present in the metabolic syndrome results in an increased risk for development od manifest diabetes mellitus- type 2 and atherosclerotic cardiovascular disease, that are still among the most common causes for morbidity and mortality in the human population. Thus, the pharmacologic treatment of the syndrome should be focused on amelioration of the insulin resistance and reduction of the present cardiovascular risk factors.

In the present study, an experimental model of the metabolic syndrome was used, that was induced by chronic fructose overload of the experimental animals. The fructose diet over a period od 8 weeks caused: hyperinsulinemia, impaired glucose tolerance, hypertrigliceridemia, elevation of serum free fatty acids concentration, hypertension and a decrease of serum HDL cholesterol and adiponectin concentrations. In this way, the effect of the investigated pharmacological treatment with rosiglitazone was evaluated in a nutritive (and not a transgenetic) experimental model that very much resembles the metabolic syndrome which is commonly found in the human population.

The obtained results from this study suggest that rosiglitazone (PPAR-gamma agonist) manifests a beneficial effect in terms of improving the components of the metabolic syndrome. The four-week rosiglitazone treatment induced a significant decrease of the serum insulin concentrations and improved the impaired glucose tolerance (assessed through reduced glucose AUC values during OGTT). Many other experimental and clinical studies are in line with the results from this study and confirm the benefitial effects of the PPAR-gamma agonists (rosiglitazone, pioglitazone, troglitazone) on metabolic and haemodynamic parameters (13-18).

It must not be left behind that an important role in the insulin-sensibilizing effects of the PPAR-gamma agonist plays their effect on lipid metabolism (19). The mechanisms that are involved in the reduction of hypertriglyceridemia in the experimental animals are still not fully elucidated, but they probably involve a regulation of the enzyme lipoprotein lipase in the adipose tissue (20) and/or decreased synthesis and secretion of HDL cholesterol in the liver (21). The obtained results from our study confirmed the beneficial effect of rosiglitazone in improvement of the lipid profile, by inducing a significant decrease of the serum triglycerides and free fatty acids concentrations, but without statistically significant changes of the serum HDL cholesterol values. The increased serum free fatty acid concentrations are an important inductor for the development of the insulin resistance, because they lead to an increase lipid accumulation in the non-adipose tissues (liver, the skeletal muscle), where the intracelular lipid metabolites interfere with the insulin signal paths, glucose transport, glycogen synthesis and/or gluconeogenesis (22-25). They increase the oxidative stress, which leads to dysregulation of the adipocytokines synthesis (26). Additionaly, an increasing number of evidence suggest that in the early stages of development of diabetes mellitus- type 2, the increased serum FFA concentration induce a dysfunction, and later an apoptosis of the beta cells in the pancreatic islets. Therefore, the reduction of the serum FFA concentrations by using PPAR-gamma agonists could prevent these pathophysiological proccesses (27-29).

Rosiglitazone treatment lowered the systolic blood pressure in the experimental animals. The reduction of the blood pressure during rosiglitazone treatment is probably a consequence of several mechanisms: amelioration of the hyperinsulinemia, increased synthesis of nitric oxide, reduction of endothelin-1 values, reduced expression of angiotensin receptors etc (30-33).

The 4-week treatment with rosiglitazone caused an increased weight gain in the treated rats. The increase of the body weight is well-known and established adverse effect during treatment with the PPAR-gamma agonists, and in the mechanisms of its development several components are implicated: decrease of serum insulin and leptin concentrations (that function as a satiety signals in the central nervous system), enlargment of the adipose depots, increase of the plasma volume etc (15, 34).

The improvement of the parameters of the metabolic syndrome in our study was accompanied with a significant (two-fold) increase of the serum adiponectin concentrations during rosiglitazone treatment. A correlation between adiponectin and the components of the metabolic syndrome (serum insulin, triglycerides, glucose AUC_{0-120}, systolic blood pressure) was also established. Adiponectin plays an important role in the control of the insulin sensitivity of the peripheral organs, as well as in the maintance of the glucose homeostasis (35-37). The obtained results are in agreement with other studies, performed with experimental animals or cell cultures, that indicate an increase of the adiponectin levels as a result of PPAR-gamma agonists treatment (38-41). Sharabi et al (42) detected an increase in adiponectin gene expression in the adipose tissue of fructose-fed rats. Iwaki et al (43) and Combs et al (44) suggest that the mechanism of action of PPAR-gamma agonists is accomplished through activation of the peroxisome-proliferator response element of the adiponectin gene, thus inducing an increase of its expression, and the adipose tissue is stimulated to produce more adiponectin. Still, further studies are needed for a more detailed elucidation of the molecular interactions between PPAR-gamma activation and the adiponectin gene.

This study indicates that rosiglitazone treatment improves the components of the metabolic syndrome. They correlated significantly with the serum adiponectin concentrations, thus suggesting that the regulation of synthesis of this adipocytokine plays an important role in the mechanism of action of the PPAR-gamma agonists. Furthermore, this study points to a way to treat the metabolic syndrome as a whole and not only its components separately, which could prevent or delay the occurence of cardiovascular complications and type 2 diabetes in patients with the metabolic syndrome.

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