

Effect of renin inhibition on adipokines in diabetic rats

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Abstract: Insulin resistance predicts development of type 2 diabetes mellitus (DM). Adipocytes release tumor Necrosis factor-alpha (TNF- α), and adiponectin. They modulate whole-body insulin sensitivity. The disturbance in the relationship between good and bad adipokines may cause insulin resistance. The renin-angiotensin aldosterone system (RAAS) plays a role in DM and the consequence of cardiovascular complications development. It is considered as a target for therapy. The present objective examined the relationship between renin angiotensin system and DM. There were, Group (1): Normal non obese rats, Group (2): Obese diabetic rats, Group (3) : Obese diabetic rats with telmisartan, Group (4): Obese diabetic rats with enalapril, Group (5): Obese diabetic rats with aliskiren. There was a significant increase in serum glucose, lipid profile [triglycerides (TGs), low-density lipoprotein cholesterol (LDL), total serum cholesterol (TC)], tumor Necrosis factor-alpha (TNF- α), malondialdehyde (MDA) and a significant decrease in adiponectin associated with minor changes in superoxide dismutase (SOD) activity in the obese diabetic rats. Administration of telmisartan, enalapril and or aliskiren caused a significant improvement in serum lipid profile and adiponectin, a minor improvement in SOD activity, a decrease in TNF- α and or MDA. Conclusion: Renin angiotensin blockers significantly improve the metabolism and oxidative dysfunctions in Type 2 DM and aliskiren may show a promising powerful therapy.

Keywords: Renin -angiotensin system, adipokines, type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a widespread chronic disease (Chaturvedi, 2007). It is composed of insulin resistance, a decrease in insulin secretion and, it is a predisposing factor for cardiovascular disease (CVD) (American Diabetes Association, 2006; Chaturvedi, 2007). Good Blood glucose control may decrease vascular complications, but cardiovascular disorders still complicate many diabetic patients (Copper, 2004; Chaturvedi, 2007). Insulin resistance is a defect response in the tissue to insulin effect (Xu *et al.*, 2003), and it is followed by hyperglycemia (Saad *et al.*, 1989). Adipocytes secrete adipokines as leptin, TNF- α , and adiponectin. They regulate energy expenditure and modulate whole-body insulin sensitivity (Saad *et al.*, 1989; Xu *et al.*, 2003). Disturbance between different adipokines may result in metabolic defect and insulin resistance in obesity and type2 DM (Wellen *et al.*, 2003; Lobner and Fuchtenbusch, 2004; Dyck *et al.*, 2006). Adiponectin, is an anti atherosclerotic anti-inflammatory hormone and is released by white adipocytes, to promote insulin sensitivity (Bouskila *et al.*, 2005; Kadowaki *et al.*, 2006; Guerre-Millo, 2008). Low serum adiponectin in obesity leads to metabolic disorders, type 2 DM and cardiac lesion (Gallagher *et al.*, 2008; Cornier *et al.*, 2008). Adiponectin receptors are in the liver and skeletal muscle, they are AMP-activated protein kinase and enhance fatty acid utilization (Kadowaki *et al.*, 2006). Also, TNF- α is a pro-inflammatory atherosclerotic adipokine that is released

from macrophages, lymphocytes and adipocytes. has involved in the development of insulin resistance through abnormal phosphorylation of insulin receptor substrate (IRS)- 1 (Hotamisligil *et al.*, 1993).

Different studies showed the beneficial effects of RAAS blockade on the onset and progression of type 2DM (Copper, 2004; Scheen, 2004; Leiter and Lewanczuk, 2005). Their blockade improves insulin response, stimulates insulin release and affects islet cell directly. RAS antagonist-induced vasodilation, may facilitate muscle blood flow and pancreatic blood flow (Scheen, 2004). RAS antagonist-mediated inhibition of sympathetic system may also increase insulin sensitivity.

RAAS antagonism could reduce angiotensin II induced oxidative stress and decrease free fatty acids from adipocytes resulting in better cellular insulin signaling and insulin action (Scheen, 2004; Leiter and Lewanczuk, 2005).

This work investigated the action of renin angiotensin aldosterone system (RAAS) antagonists on adipokines in diabetic obese rats.

MATERIALS AND METHODS

Experiments were done according to the Guide for Care and Use of Laboratory Animals (1985), NIH, Bethesda and were approved by our local committee of Animal Care and Use.

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The experimental diabetic rat model: The sixty five male sprague dawley rats (150-180g), were undergone into two different types of diet regimen groups for 6 weeks. Ten rats were fed a normal fat diet (NFD) (3150 kcal/g; fat (5%) protein (21%), starch (60%), fibers (3%), vitamins and minerals (1%), while 55 rats were fed a high fat diet (HFD) (5300kcal /g; fat (15%) composed of 10% as lard and 1% cholesterol powder) protein (21%), starch (60%), fibers (3%), vitamins & minerals (1%). After two weeks, hyperglycemia was induced by an intraperitoneal injection of a single dose (35mg/ kg in citrate buffer) of freshly prepared Streptozotocin (STZ)(Sigma ,USA) (0.08M, pH 4.8) after an overnight fast (Reaven and Ho, 1991; Reed *et al.*, 2000).

Drugs investigated

Telmisartan (Micardis tablets, 80 mg supplied by Boehringer Ingelheim company), orally in a dose of 10 mg/kg (Wienen, 2001).

Enalapril (Ezapril tablets, 10mg supplied by the Apex Pharmaceutical company) orally in a dose of 5 mg/kg (Itohet *et al.*, 2002).

Aliskiren (Rasilez tablets, 150mg supplied by Novartis Europharm limited company), orally in a dose of 10 mg/kg (Wood *et al.*, 2005).

Experimental design

Two days after STZ injection and after establishment of hyperglycemia. The rats were divided:

Group 1 (10 rats): Normal non obese rats.

Group 2 (10 rats): Obese diabetic rats without treatment and received 1ml saline (vehicle) orally.

Group 3 (15 rats): Obese diabetic rats received telmisartan (angiotensin receptor blocker* AT1 blocker*) for 12 weeks.

Group 4 (15 rats): Obese diabetic rats received enalapril (angiotensin converting enzyme inhibitor *ACE inhibitor*) for 12 weeks.

Group 5 (15 rats): Obese diabetic rats received aliskiren (Renin inhibitor) for 12 weeks.

Collection of Blood Samples

Blood Samples were obtained from the ophthalmic venous plexus after overnight fasting. Each blood sample was then centrifuged at 1000 rpm and their sera were preserved at -20°C till biochemical analysis of the following: a) Glucose. b) Lipid profile: Triglycerides (TGs), LDL, total cholesterol. c) Oxidants (MDA), TNF- α & Antioxidant (SOD). d) Adiponectin.

Biochemical measurements

Serum glucose determination

Serum glucose was determined using a Randox reagent colorimetric assay kit (Sigma, USA), according to Barham and Trinder (1972).

Serum lipid profile determination

Lipid profile was performed using Spinreact colorimetric assay Kits, total cholesterol, triglycerides according to (Richmond, 1973; Allain *et al.*, 1974; Fassati and Prencipe, 1982) and LDL-cholesterol (Friedewalde *et al.*, 1972).

Serum malondialdehyde (MDA) determination

MDA was determined according to (Ohkawa, 1979; Draper and Hadley 1990). Precipitation of serum by trichloroacetic acid and then reaction of thiobarbiturate with MDA, leading to the formation of a thiobarbiturate reactive product that was colorimetric measured.

Serum TNF- α determination

Serum TNF- α was determined by enzyme-linked immunosorbent assay (ELISA) (Immunotech Beckman Coulter kit), according to the manufacturer's instructions.

Serum superoxide dismutase determination

SOD was colorimetric measured according to Nikishimi *et al.* (1972) and Winterbourn *et al.* (1975). The assay relies on the ability of the enzyme to inhibit the fanzinee methosulphate mediated reduction of nitroblue tetrazolium dye.

Serum adiponectin determination

Serum Adiponectin was determined by rat ELISA assay Kit (Biovendor, diagnostic research) according to the manufacturer's instructions

STATISTICAL ANALYSIS

The biochemical data were expressed as mean $\pm$ standard deviation (SD). Analysis were performed using ANOVA followed by post-hoc multiple comparison (Scheffe text). P value ≤ 0.05 was considered as statistically significant. The statistical analysis of results was done by using SPSS (Statistical Package for Social Science) program, version 13, 2004, for windows XP professional.

RESULTS

Effect of renin- angiotensin system blockers on serum glucose and lipid profile

Compared to the normal non diabetic group, diabetic rats showed that the sera of glucose, TGs, LDL and total cholesterol were significantly increased ($p < 0.05$) (table 1). These parameters were improved significantly in the tested groups on comparing to the diabetic rats (table 1).

Effect of renin-angiotensin system blockers on serum MDA, TNF- α

Compared to the normal non diabetic group, there was a significant increase in serum MDA and or TNF- α in the diabetic rats ($p < 0.05$) (table). While, on comparing to the diabetic group there was a significant reduction in serum MDA and TNF- α of the tested groups (table 2).

Effect of renin-angiotensin system blockers on serum SOD and adiponectin

Compared to the normal non diabetic group, the diabetic rats had a non significant decrease in serum SOD activity ($p>0.05$) and a significant decrease in adiponectin level ($p<0.05$), while treatment with telmisartan, enalapril and or aliskerin also caused a non significant improvement in SOD activity and a significant increase in adiponectin level compared to the diabetic group ($p>0.05$) (table 3).

DISCUSSION

Diabetes Mellitus, is a disease of metabolic dysregulation where there is an impairment of glucose uptake and utilization, lipid metabolism alteration and accumulated various lipid species in the circulation and tissues. Also, there is a disruption of metabolic signaling pathways that regulate insulin secretion from pancreatic islet β -cells (Deborah *et al.*, 2008).

The basic pathophysiological mechanisms responsible for the metabolic derangements occurred in diabetes mellitus has remained elusive, although it has been investigated, Type 2 DM, is the predominant form and an independent risk factor for cardiovascular disease. This type is best known by insulin resistance and impaired insulin secretion (Chaturvedi, 2007). Insulin resistance has often preceded hyperglycemia onset, anticipating the development of type 2DM (Saad *et al.*, 1989). The imbalance between beneficial (adiponectin) and harmful (TNF- α) adipokines released from adipocytes may result in insulin resistance that is present in obesity and type 2 DM (Wellen *et al.*, 2003; Lobner and Fuchtenbusch, 2004).

Plasma concentrations of adiponectin (an adipose-specific) are decreased in obese subjects and type 2 diabetic patients, which contribute to the pathogenesis of the metabolic syndrome, risk factors of type 2DM and

Table 1: Effect of RAAS blockers on serum glucose and lipid profile

Group	Glucose (mg/dl)	LDL cholesterol (mg/dl)	TG (mg/dl)	Total cholesterol (mg/dl)
Controlgroup (10rats)	76.6 $\pm$ 12.40	38.67 $\pm$ 5.69	68.9 $\pm$ 12.60	80.08 $\pm$ 8.47
Diabetesgroup (10rats)	355.8 $\pm$ 34.50 ^a	117.5 $\pm$ 9.17 ^a	114.9 $\pm$ 27.17 ^a	127.6 $\pm$ 13.12 ^a
Diabetes+telmisartan (15rats)	139.4 $\pm$ 29.32 ^b	46.7 $\pm$ 10.37 ^b	74.45 $\pm$ 8.30 ^b	82.5 $\pm$ 8.48 ^b
Diabetes+lisinopril (15rats)	133.23 $\pm$ 20.08 ^b	49.13 $\pm$ 7.08 ^b	72.42 $\pm$ 10.60 ^b	85.67 $\pm$ 6.42 ^b
Diabetes+aliskerin (15rats)	124.05 $\pm$ 23.40 ^b	52.3 $\pm$ 11.90 ^b	76.1 $\pm$ 7.87 ^b	88.34 $\pm$ 5.27 ^b

All results are expressed as mean $\pm$ standard deviation (SD).

a=significance between control and diabetic groups.

b=significance between diabetic and tested groups.

Table 2: Effect of RAAS blockers on serum MDA and TNF- α

Group	MDA (nmol/l)	TNF- α (pg/ml)
Controlgroup (10 rats)	5.02 $\pm$ 0.60	0.9 $\pm$ 0.25
Diabetic group (10 rats)	9.81 $\pm$ 2.10 ^a	5.56 $\pm$ 1.15 ^a
Diabetes+telmisartan (15 rats)	5.6 $\pm$ 0.70 ^b	1.5 $\pm$ 0.27 ^b
Diabetes+lisinopril (15 rats)	5.9 $\pm$ 0.50 ^b	1.2 $\pm$ 0.30 ^b
Diabetes+aliskrin (15 rats)	5.19 $\pm$ 0.77 ^b	1.33 $\pm$ 0.29 ^b

All results are expressed as mean $\pm$ standard deviation (SD).

a=significance between control and diabetic groups.

b=significance between diabetic and tested groups.

Table 3: Effect of RAAS blockers on serum SOD and adiponectin in diabetic rats

Group	SOD (%inhibition)	Adiponectin (μ g/ml)
Control group (10 rats)	58.26 $\pm$ 12.92	4.1 $\pm$ 0.64
Diabetic group (10 rats)	45.5 $\pm$ 7.90	2.5 $\pm$ 0.49 ^a
Diabetes+telmisartan (15 rats)	52.8 $\pm$ 13.02	3.6 $\pm$ 0.50 ^b
Diabetes+lisinopril (15 rats)	50.2 $\pm$ 5.90	4.01 $\pm$ 0.75 ^b
Diabetes+ aliskerin (15 rats)	49.5 $\pm$ 9.20	3.74 $\pm$ 0.70 ^b

All results are expressed as mean $\pm$ standard deviation (SD).

a=significance between control and diabetic groups.

b=significance between diabetic and tested groups.

cardiovascular disease with abdominal obesity and insulin resistance (Cornier *et al.*, 2008; Gallagher *et al.*, 2008).

The role of the rennin – angiotensin system (RAS) in the pathogenesis of DM and its associated cardiovascular complications is very important. Thus, it can be targeted for pharmacological interference (Cooper, 2004).

ACE inhibitors and Ang II type 1 (ATI) receptor blockers can decrease proteinuria and the progression of diabetic glomerulosclerosis (Carey and Siragy 2003). Stiefel *et al.*, (2011), also showed the involvement of angiotensin II and aldosterone in promoting cardiometabolic syndrome.

So, in this work the effect of renin angiotensin system inhibitor (aliskiren) on serum adipokines in Type 2 diabetic rats had been investigated and compared with the other two ang II antagonists.

Diabetic rats showed increased serum glucose, TGs, LDL and or total cholesterol. The treatment with telmisartan, enalapril and or aliskiren caused a significant improvement in all these parameters Stiefel *et al.* (2011). and others concluded the relation between visceralobesity and carbohydrate & lipid metabolism disorders. Scheen (2004), Leiter and Lewanczuk (2005) proved different benefits of RAS blockade in these conditions for example preserving cellular potassium and magnesium, that leads to better insulin action. This is related to the reduction of sympathetic over activity or even due to the direct effect of RAS blockade on insulin signaling, glucose transporters as well as promotion of adipocytes differentiation.

Stephen and Benson (2005) reported that telmisartan, is a partial agonist of PPARgamma receptors and affects their expression genes responsible for carbohydrate and lipid metabolism, resulting in reduction of glucose, insulin resistance and lipid levels in obese rats. Imanishi in his study concluded that a direct renin blocker drug that protects the endothelial function and improves atherosclerotic changes, may be due to its Effect on superoxide and vascular peroxynitrite (Imanishi *et al.*, 2008).

Induction of type 2 diabetes produced a significant increase in MDA, TNF- α level and a change in superoxide dismutase activity, compared to the normal control group. This is consistent with several investigators who have evaluated different oxidative stress parameters in different models of diabetes or hyperglycemia. This is because increased oxidative stress is one of the major hypotheses purposed to explain the hyperglycemia-induced onset of diabetic complications. Once formed, reactive oxygen species (ROS) deplete antioxidant system rendering the tissues more liable to oxidative destruction (Niedowicz and Daleke, 2005). Adipose tissue produces

inflammatory adipokines which are shared in oxidative stress, and increased serum insulin (insulin resistance) (Neels and Olefsky, 2006). Aldosterone through mineralo- corticoid receptors in adipose tissue stimulates the expression of TNF- α that influences insulin metabolism .The blockade of the previous receptors indirectly as done in the present work by telmisartan, enalapril and or aliskiren caused a significant decrement in MDA and TNF- α from adipose tissue (Stiefel *et al.*, 2011). Furthermore, administration of telmisartan, enalapril and or aliskiren caused a significant improvement in adiponectin level, the protein that is released from the adipose tissue. It opposes the deleterious actions of TNF- α and improves insulin resistance and inflammation.

It is decreased in diabetic dyslipidemia, insulin resistance over sympathetic stimulation, high blood pressure and in hyperaldosteronism associated with high renin level (Eckel *et al.*, 2005; Falloet *et al.*, 2007; Stiefel *et al.*, 2011).

Hotta *et al.* (2001) revealed the correlation between adiponectin level and insulin resistance. He stated that decreased plasma adiponectin is parallel with the decrease in the insulin sensitivity.

He revealed that plasma adiponectin decreases early in obesity and continues after the progression of diabetes . Therelation between hypertension and insulin resistance is measured in Ran *et al.* study (2006), as rats that were infused withangiotensin II, showed decreased plasma adiponectin level without affection of plasma leptin and TNF α levels.

While, Stiefel *et al.* (2011) explained that angiotensinII and aldosteron are responsible for cardiac complications in metabolic syndrome patients and blockade of mineralo-corticoid receptors is a potential therapeutic strategy to manage these patients.

On the other hand, enalapril, in spite of its therapeutic effect on diabetes as ACE inhibitor, could lead to a reflex increase in plasma renin, which may lead to RAAS 'rebound'. More over, the chymase (an alternative AngII-generating enzyme), is upregulated in diabetes, hypertension and may be a cause of diabetes and vascular lesion in spite of inhibition the ACE (Miyazakiand Takai, 2006).

Lastly, telmisartan and other competitive antagonists that target the renin system, prevent the actions of angiotensin II, but it is not obvious that these drugs are clinically equivalent (Huang *et al.*, 2003). Senchenkova study (2010) reported the thrombotic effect of angiotensin II via angiotensin IV receptors, which is not blocked by telmisartan and other competitive antagonists.

CONCLUSION

RAAS blockade plays a therapeutic effect in type II diabetes mellitus. A direct renin inhibitor (aliskiren) is present nowadays and may provide a greater and different RAAS blockade than ACE inhibitors or ATI antagonists. More studies are needed to explore the more difference between these therapeutic drugs.

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