Effect of Telmisartan in Experimentally Induced Diabetetes Mellitus in Rats

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Abstract:

Background: Increased oxidative stress is involved in the pathogenesis of diabetic nephropathy and neuropathy. Angiotensin II is a know factor in the pathogenesis of diabetic complications. The protective effects of ACEIs is known in diabetic nephropathy. Thus, Angiotensin receptor antagonists may have the same role. In this study, possible antidiabetic effect of Telmisartan and its tissues antioxidant effect in (STZ) induced diabetic rats, were studied

Methods : The present study was done on 40 rats. They were divided into 2 main groups. Group I: 10 rats as control group, received distilled water. Group II: 30 rats subdivided into 3 equal subgroups as follow: Subgroup IIA: control diabetic group, received 55 mg/kg STZ intraperitoneally. Sub group IIB: diabetic rats, received 10 mg/kg telmisartan daily intragastrically. Sub group IIC: diabetic rats received 10mg/kg gliclazide daily intragastrically. Diabetes was induced by intraperitoneal injection of 55 mg/kg STZ for 8 weeks evidenced by significant increase in serum glucose, HBA_{1c} and decreased Hb levels.

Results : Diabetic rats showed a significant increase in tissue TBARs and a significant decrease in tissue (GSH) and (SOD) enzymes. Telmisartan or Gliclazide in diabetic rats produced a beneficial effect on serum glucose, Hb, HBA_{1c} and restored tissue GSH and SOD with a fall in tissues TBARS.

Conclusion : Telmisartan might be proved useful in the treatment of diabetes and its complications, as Gliclazide is restricted by its secondary failure rate and side effects.

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Introduction

Studies have suggested that increased oxidative stress is involved in the pathogenesis of diabetic nephropathy and neuropathy. [1] Diabetic cystopathy is a manifestation of peripheral neuropathy.[2] The cause of diabetic neuropathy is multifocal and may include altered glucose metabolism, ischemia and super oxide induced free radical formation. [3]

Hyperglycemic episodes which complicate controlled cases of diabetes are closely associated with increased oxidative and stress which can trigger the development of diabetic complications. [1]

Angiotensin can induce direct pro-oxidative effects on the vascular endothelium, mediated by intra endothelial reactive species formation via a new family of NADPH oxidative subunits. [4] Also, Angiotensin converting enzyme inhibition in vivo reduces the apparent formation of peroxynitrite.

Telmisartan is an angiotensin II blocker used in treatment of hypertension. A structural resemblance between telmisartan and pioglitazone a peroxisome proliferator activated receptor (PPARY) ligand that approved for the treatment of type II diabetes has been observed which supports the possibility that certain molecules might have the capacity not only to regulate blood pressure[6] and oxidative stress production [1] but also activate an intracellular nuclear hormone receptor involved in carbohydrate and lipid metabolism; such bifunctional molecules could treat both the hemodynamic and biochemical features of diabetes mellitus and metabolic syndrome. Moreover given that blockade of the renin- angiotensin system at the same time could also lead to the development of new anti diabetic antioxidant drugs with improved safety profiles. [1,6]

Though sulfonylureas are valuable in treatment of diabetes mellitus, their use is restricted by their secondary failure, adverse effects and or the existence of diabetic complications in spite of their use.[8]

The aim of the present study is to investigate the possible antidiabetic effect of Telmisartan and studying its antioxidant effect in experimentally induced diabetes mellitus in rats.

Methods

Drugs used

Streptozotocin (STZ): powder, 1 gm supplied by *Sigma*

Telmisartan: Micardis tablets, 80 mg supplied by *Boehringer Ingelheim*

Gliclazide: Diamicron tablets, 80 mg supplied by *Servier.*

Animals used

Forty male albino rats were used throughout this study weighing (150-200 gm each) and obtained from animal house (Mansoura Faculty of Medicine, Egypt). They were housed individually in plastic cages. They were put under similar housing condition. Rats were allowed free access to food and water.

Experimental protocol

STZ was dissolved in 0.1M cold sodium citrate buffer, PH 4.5 at a dose 55 mg/kg. [9]

Telmisartan was dissolved in a pathogen free distilled water to make a concentration of 1 mg/ml.

Gliclazide was dissolved in pathogen free distilled water to make concentration of 1mg/ml.

Treatment

Forty male albino rats were randomly divided into 2 main groups

Group I: Consisted of 10 animals which were considered as control group and received distilled water for 8 weeks.

Group II: Consisted of 30 animals. Further divided into 3 equal subgroups (10 rats each) as follow:

Subgroup IIA: served as control diabetic group and received STZ in a dose of 55 mg/kg intraperitoneally

Sub group IIB: diabetic rats received telmisartan in a dose of 10 mg/kg daily[11] intragastrically by gavage for 8 weeks.

Sub group IIC: diabetic rats received gliclazide in a dose of 10mg/kg daily[12] intragastrically by gavage for 8 weeks.

Diabetes mellitus was induced by one dose injection of STZ intraperitoneally. Blood glucose level on the 3rd day following injection of STZ was measured and rats with fasting blood glucose $\geq$ 250 mg % were considered diabetic. [10]

Animals were killed after 8 weeks of the treatment. The fasting rats were sacrificed by cervical decapitation. Blood was obtained for measurement of serum glucose, blood Hb and HBA_{1c}. The liver, kidney, and urinary bladder were dissected out, washed in ice cold saline, patted dried and persevered at-8°C till determination of TBARS, GSH, and SOD.

Biochemical analysis

1. Serum glucose was determined: according to the enzymatic glucose oxidase method of Trinder. [13]
2. Blood Hb was determined: according to the method of Drabkin and Austin.[14]
3. Blood HBA1c was determined: according to the method of Nayak and Patt Abiraman.[15]
4. TBARS was determined in hepatic and renal homogenates: according to the method of Brogan et al., [16] and in urinary bladder homogenate to Kodama et al. [17]
5. Reduced glutathione (GSH) was determined in hepatic, renal, and urinary bladder homogenates: according to the method of Beutler et al.[18]
6. Super oxide dismutase (SOD) was determined in hepatic, renal and urinary bladder homogenates according to the method of Nandi and Chatterjee. [19]

Statistical Analysis

- Analysis was done by SPSS program (statistical package for social science)
- Multiple comparisons were performed by independent t test statistical analysis between 2 groups
- "Scheffe" test was done

All the results were expressed as the mean ± SE for ten animals in each group.

Results

A). Criteria of diabetes

Effect of diabetes on fasting serum glucose, blood hemoglobin (Hb) and glycosylated Hb (HBA_{1c})

As shown in Table 1, diabetes produced a significant increase in fasting blood glucose as compared to control group (285.353±7.5&95.130± 5.2, respectively P < 0.05). Furthermore, diabetes produced a significant decrease in blood hemoglobin (8.93±0.47), but a significant increase in glycosylated Hb(HBA_{1c}) (12.490±0.75) as compared to control group(10.880 ±0.87&6.501±0.31 respectively) .

Effect of gliclazide, on fasting serum glucose, blood hemoglobin (Hb) and glycosylated Hb (HBA_{1c}) in diabetic rats (Table 1).

Gliclazide, as shown in Table (1) produced a significant decrease in fasting serum glucose as compared to diabetic control group; 116.767±4.5 & 285.353±7.5, respectively). Moreover, Gliclazide, produced a significant increase in blood hemoglobin as compared to diabetic control group (11.99 ±0.81 &8.93±0.47, respectively). However, it produced a significant decrease in glycosylated Hb (HBA_{1c}) as compared to diabetic control group (7.656±0.39 &12.490±0.75, respectively) P <0.05.

As compared to non-diabetic control group, there is no significant difference.

Effect of telmisartan on fasting serum glucose, blood hemoglobin (Hb) and glycosylated Hb (HBA_{1c}) in diabetic rats (Table 1):

Telmisartan (Table 1) produced a significant decrease in fasting serum glucose as compared to diabetic control group; (108.093±5.02 and 285.353±7.5, respectively). Moreover, Telmisartan, produced a significant increase in blood hemoglobin as compared to diabetic control group (12.480±0.49 & 8.93±0.47, respectively), however, it produced a significant decrease in glycosylated Hb (HBA_{1c}) as compared to diabetic control group (7.063±0.34 & 12.490±0.75, respectively)

As compared to non-diabetic control group, there is no significant difference.

Comparison between the effect of telmisartan & gliclazide on fasting serum glucose, blood hemoglobin (Hb) and glycosylated Hb (HBA_{1c}) in diabetic rats (Table 1):

There is no significant difference between them. Both telmisartan and gliclazide normalize serum glucose, Hb and HBA_{1c} in diabetic rats as compared to non diabetic control group.

B). Oxidative stress parameters

(i) TBARS level

Effect of diabetes on TBARS level in liver, kidney and urinary bladder in diabetic rat (Table 2):

There was a significant increase in tissue TBARS in liver, kidney and urinary bladder (1.712±0.048, 2.237±0.04 and 11.576±0.5, respectively) in diabetic group compared to control group (0.839±0.035, 1.246±0.049 and 3.311±0.3, respevtively)

Effect of gliclazide, on TBARS level in liver, kidney and urinary bladder in diabetic rat (Table 2):

Gliclazide produced a significant decrease tissue TBARS in liver, kidney and urinary bladder (1.410±0.026,1.783±0.03 &5.529±0.4,respectively), as compared to diabetic control group.

Effect of telmisartan on TBARS level in liver, kidney and urinary bladder in diabetic rat (Table 2):

Telmisartan (Table 2) produced a significant decrease tissue TBARS in liver, kidney &urinary bladder in diabetic control group (0.978±0.049, 1.380±0.07 &5.241±0.4, respectively) as compared to control group (0.839±0.035, 1.246±0.049&3.311±0.3, respectively)

Table (1). Effect of gliclazide and telmisartan on Serum glucose(mg/dl) blood Hb (gm/dl) and HBA_{1c} % in control and diabetic rats. Mean ±SE.

P1: Test of significance between control normal and diabetic rats. P2: Test of significance between non treated diabetic rats and diabetic treated with telmisartan .P3: Test of significance between non treated diabetic rats and diabetic treated with gliclazide. P4: Test of significance between control normal rats and diabetic treated with telmisartan . P5: Test of significance between control normal rats and diabetic treated with gliclazide ,NS= non significant ,S .E.= standard Error.

Groups	Serum glucose mg/dl	Blood Hb gm/dl	blood HBA_{1c} %
Normal Control	95.130± 5.2	10.880 ±0.87	6.501±0.31
Diabetic control	285.353±7.5 $P_1 < 0.05$	8.93±0.47 $P_1 < 0.05$	12.490±0.75 $P_1 < 0.05$
Diabetic +telmisartan	108.093 ±5.02 $P_2 < 0.05$ P_4 NS	12.480±0.49 $P_2 < 0.05$ P_4 NS	7.063±0.34 $P_2 < 0.05$ P_4 NS
Diabetic +gliclazide	116.767±4.5 $P_3 < 0.05$ P_5 NS	11.99 ±0.81 $P_3 < 0.05$ P_5NS	7.656±0.39 $P_3 < 0.05$ P_5 NS

Table (2). Effect of gliclazide and telmisartan on tissue lipid peroxides(TBARS, mM/100g of tissue) in liver, kidney ,and urinary bladder of control and diabetic rats. Mean ±SE.

P1 : Test of significance between control normal and diabetic rats. ,P2 : Test of significance between non treated diabetic rats and diabetic treated with telmisartan,P3: Test of significance between non treated diabetic rats and diabetic treated with gliclazide,P4: Test of significance between control normal rats and diabetic treated with telmisartan,P5: Test of significance between control normal rats and diabetic treated with gliclazide,NS= non significant , SE.= standard Error.

Groups	lipid peroxides mM/100g of tissue		
	liver	kidney	urinary bladder
Normal control	0.839±0.035	1.246±0.049	3.311±0.3
Diabetic control	1.712±0.048 $P_1 < 0.05$	2.237±0.04 $P_1 < 0.05$	11.576±0.5 $P_1 < 0.05$
Diabetic + telmisartan	0.978±0.049 $P_2 < 0.05$ P_4 NS	1.380±0.07 $P_2 < 0.05$ P_4 NS	5.241±0.4 $P_2 < 0.05$ P_4 NS
Diabetic gliclazide	1.410 ± 0.026 $P_3 < 0.05$ P_5 NS	1.783±0.03 $P_3 < 0.05$ P_5 NS	5.529±0.4 $P_3 < 0.05$ P_5 NS

Comparison between the effect of telmisartan and gliclazide on TBARS level in liver, kidney and urinary bladder in diabetic rat:

There is no significant difference between them. Both telmisartan and gliclazide normalize TBARS level in liver, kidney and urinary bladder in diabetic rats as compared to non diabetic control group.

2. Reduced glutathione (GSH) level and activity of superoxide dismutase:

Effect of diabetes on reduced glutathione (GSH) level and activity of superoxide dismutase (SOD) in liver, kidney and urinary bladder in diabetic rats (Table.3).

As shown in Table 3, diabetes produced a significant decrease in tissue GSH in liver, kidney and urinary bladder (22.264±1.1, 21.183±1.4 &15.611±1.1, respectively) as compared to control group (51.964±2.4 , 35.813±1.3 and 31.752±1.4 respectively).

Also, diabetes produced a significant decrease in tissue superoxide dismutase (SOD) in liver, kidney and urinary bladder (3.050 ± 0.22, 7.850 ± 0.20 and 6.520 ± 0.42, respectively) as compared to control group (9.230±0.37, 14.940±0.60 and 11.250 ± 0.40, respectively).

Effect of gliclazide on reduced glutathione (GSH) level and activity of superoxide dismutase in liver, kidney and urinary bladder in diabetic rats (Table 3)

As shown in Table 3, gliclazide produced a significant increase in activity of superoxide dismutase in liver, kidney and urinary bladder (6.568 ± 0.31, 14.300 ± 0.62 &12.394 ± 0.5, respectively) as compared to control diabetic group (3.050 ± 0.22, 7.850 ± 0.20 &6.520 ± 0.42, respectively).

Effect of telmisartan on reduced glutathione (GSH) level and activity of superoxide dismutase in liver, kidney and urinary bladder in diabetic rats (Tab.3)

Table (3). Effect of gliclazide and telmisartan on GSH(mg/100g tissue) Level and SOD(ug protein) activity in liver, kidney and urinary bladder of control and diabetic rats. Mean ±SE. P1: Test of significance between control normal and diabetic rats.
P2: Test of significance between non treated diabetic rats and diabetic treated with telmisartan .
P3: Test of significance between non treated diabetic rats and diabetic treated with gliclazide.
P4: Test of significance between control normal rats and diabetic treated with telmisartan .
P5: Test of significance between control normal rats and diabetic treated with gliclazide
NS= non significant SE.= standard Error.

group	GSH mg/100g tissue			SOD ug protein		
	liver	kidney	urinary bladder	liver	kidney	urinary bladder
Normal control	51.964±2.4	35.813±1.3	31.752±1.4	9.230±0.37	14.940±0.60	11.250±0.40
Diabetic control	22.264±1.1 P_1<0.05	21.183±1.4 P_1< 0.05	15.611±1.1 P_1 < 0.05	3.050±0.22 P_1 < 0.05	7.850±0.20 P_1 < 0.05	6.520±0.42 P_1 < 0.05
diabetic + telmisartan	39.817±1.4 P_2< 0.05 P4 NS	28.506±1.3 P_2<0.05 P4 NS	26.570± 1.3 P_2 < 0.05 P4 NS	6.810±0.4 P_2 < 0.05 P4 NS	13.793±0.5 P_2 < 0.05 P4 NS	10.450±0.7 P_2 < 0.05 P4 NS
Diabetic + gliclazide	39.838±1.6 P_3 <0.05 P_5 NS	28.256±1.2 P_3< 0.05 P_5 NS	25.938±1.05 P_3 < 0.05 P_5 NS	6.568±0.31 P_3 < 0.05 P_5 NS	14.300±0.62 P_3 < 0.05 P_5 NS	12.394±0.5 P_3 < 0.05 P_5 NS

Telmisartan produced a significant increase in activity of superoxide dismutase in liver, kidney and urinary bladder (6.810±0.4, 13.793±0.5, and 10.450±0.7, respectively) as compared to control diabetic group (3.050±0.22, 7.850±0.20 &6.520±0.42, respectively) (Table 3).

Comparison between the effect of telmisartan and gliclazide on reduced glutathione (GSH) level and activity of superoxide dismutase in liver, kidney and urinary bladder in diabetic rats (Table 3)

There is no significant difference between them. Both telmisartan and gliclazide normalize (GSH) level and activity of superoxide dismutase in liver, kidney and urinary bladder in diabetic rats as compared to non diabetic control group.

Discussion

In this study the antioxidant effect of either telmisartan or gliclazide was studied. The STZ induced diabetic rats is a well established model for diabetes studies. STZ has been shown to have special specific cytotoxic effects on islet B cells.[16]

Effect of STZ induced diabetes in rats:

STZ diabetic animals showed a significant increase in serum glucose, and or blood glycosylated Hb. This is in consistant with study of Rakieten et al.[9] Furthermore, rats showed a significant increase in malonaldialdehyde level in liver, kidney and urinary bladder which is similar to the study of Levy et al. [27] On the other hand, they showed significant decrease in reduced GSH and SOD activity in liver, kidney and urinary bladder. [1,9,32] This finding supports study of Sozmen et al., [32] who showed that oxidative stress in diabetes coexists with a reduction in anti oxidant capacity(reduced GSH and SOD) and this can increase the deletrious effects of free radicles.

OZturk et al.[20] stated that the STZ induced diabetes may exhibit most of the diabetic complications namely myocardial, vascular, gastro intestinal, nervous, vas deferens, kidney, urinary bladder dysfunction, nerve blood flow and nerve conduction deficits.[20] Oxidative stress caused by angiotensin II lead to the development of diabetic complications pathogenesis. [21] Angiotensin II can induce direct prooxidative effects on vascular endothelium through intra endothelial reactive species formation; it also can induce intra endothelial peroxynitrite formation. [21]

Effect of gliclazide administration in diabetic rats:

It was observed that administration of gliclazide to STZ induced diabetic rats induced improvement in glycemic control that is associated with significant decrease in high serum glucose and blood HBA_{1c} levels. These findings are in accordance with previous study by Jarvinen [33] who reported that gliclazide and or insulin produced a decrease in fasting hyperglycemia at hepatic and peripheral levels resulting in decreased hepatic glucose production and increased glucose uptake by tissues, also gliclazide is a potent insulin secretagogue from pancreas. [34] In addition, gliclazide produced a significant decrease in lipid peroxides and concomitant increase in tissue GSH and SOD activity in diabetic rats. This could be explained by the reduction of hyperglycemia.[33] Hyperglycemia stimulates the production of superoxide anion formation that interact to form a strong oxidant "peroxynitrite" which attacks various biomolecules in the vascular endothelium, vascular smooth muscle, myocardium, and leads to development of diabetic nephropathy, retinopathy and neuropathy as previously stated by Pacher et al.[1]

.Moreover, Ayan et al.,[33] stated that gliclazide administration produced an improveement in diabetic cystopathy through insulin secretagogue effect.

Effect of telmisartan administration in diabetic rats

The study reveals that telmisartan therapy resulted in a significant decrease in serum glucose and HBA_{1c} levels in the diabetic rats that may be considered an improvement in glycemic status. These findings were in accord with many studies [6,22,23,24] which reported that telmisartan has the ability to activate PPARY involved in carbohydrate and lipid metabolism,beside its ability to regulate blood pressure and oxidative stress production. [1]

Telmisartan administration in diabetic rats produced a significant decrease in lipid peroxides that were increased in long duration diabetes in blood, heart, pancreas, urinary system. This is in agree with the study of Pacher et al., & Guzel et al.[1,25] This alteration has been ascribed to increase antioxidant consumption from increased oxidative stress. [26] Lipid peroxidation is one of the characteristic

features of chronic diabetes due to increased free radicals that may react with polyunsaturated fatty acids in cell membranes resulting in lipid peroxidation (and the latter will in turn leads to the elevated production of free radicles)[27] and pancreatic islet cells damage.[28] The significant decrease in lipid peroxides could be due to a decrease in oxidative stress induced by hyperglycemia under telmisartan effect that blocked angiotensin II action at its receptors. [1,6] On the other hand, telmisartan administration produced a significant elevation tissue GSH in diabetic rats. This finding is in agreement with Pacher and Obrosova[1] who stated that blockade angiotensin action could ameloriate oxidative stress induced by angiotensin that generate free radicles resulted in oxidative stress that consume GSH. , further more, increase GSH biosynthesis may be a direct effect of telmisartan (angiotensin blocker).

Oxidative stress in diabetes mellitus coexists with a reduction in the antioxidant capacity that is considered a cooperative defense system protecting the body from free radical damage.[29,32] Glutathione protects the cellular system against toxic effect of lipid peroxidation. [30] Decreased GSH in the liver, kidney and urinary bladder in diabetes is owing to its utilization by oxidative stress as previous mentioned by Anuradha and Selvam.[31]

Furthermore, diabetic rats treated with telmisartan showed a significant increase in SOD activity. SOD scavenges the superoxide radical by converting it to H_2O_2 and molecular oxgen. SOD is decreased in diabetes due to inactivation of the enzyme by oxidative stress [33] and or by its glycation due to diabetes. [32] Increasing SOD activity proves the antioxidant potency of telmisartan resulting in reduced blood glucose leading to inhibition inactivation or glycation of SOD. [32]

Conclusions

This study provides an additional evidence of the antioxidant effect of telmisartan on liver, kidney and urinary bladder tissues. Furthermore it produced an improvement in carbohydrate and lipid metabolism, which could be due to stimulant action on PPARY receptors. The results are in agreement with others who proved that interruption of harmful action of Angiotensin II may go beyond the blood pressure lowering effect. Thus, telmisartan can be considered as a drug for treatment of diabetes and its complications.

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