Pharmacological Reports
2011, 63, 956–966
ISSN 1734-1140

Beneficial role of telmisartan on cardiovascular complications associated with STZ-induced type 2 diabetes in rats

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Abstract:
We studied the effect of an eight-week treatment with telmisartan (5 mg $kg^{-1}day^{-1}$) on cardiovascular complications that are associated with type 2 diabetes in a neonatal rat model. Type 2 diabetes was induced by the administration of 90 mg/kg streptozotocin (STZ), *ip*, in two-day-old rats. The development of diabetes was checked 12 weeks after STZ administration, and the animals were divided into different groups. Telmisartan treatment was given for eight weeks. At the end of the eight-week treatment, various biochemical and cardiac parameters were measured. Diabetic rats exhibited hyperglycemia, hyperinsulinemia, hyperlipidemia, increased blood pressure and heart rate, increased creatinine, cardiac enzyme and C-reactive protein (CRP) levels, a reduction in the rate of pressure development and decay, cardiac hypertrophy and oxidative stress. Chronic treatment with telmisartan significantly prevented STZ-induced hypertension and tachycardia and elevated fasting glucose and insulin levels. It significantly prevented the dyslipidemia and significantly reduced the elevated creatinine and CRP levels and the levels of other cardiac enzyme markers, like lactate dehydrogenase and creatinine kinase, in diabetic rats. There was an increase in rate of blood pressure development and decay with telmisartan treatment. Telmisartan also produced beneficial effects by preventing cardiac hypertrophy, which was evident from left ventricular collagen levels, the cardiac hypertrophy index and the left ventricular hypertrophy index in diabetic rats. Telmisartan successfully prevented oxidative stress, which was evidenced by a decrease in malondialdehyde and an increase in glutathione, catalase, superoxide dismutase levels. In conclusion, our data suggest that telmisartan prevented STZ-induced metabolic abnormalities and cardiovascular complications in type 2 diabetes.

Key words:
cardiovascular complications, cardiac hypertrophy, telmisartan, type 2 diabetes mellitus

Introduction

Diabetes mellitus and hypertension are two of the most common diseases, and the frequency of both diseases increases with advancing age [57]. A prospective cohort study of 12,550 adults reported that the development of type 2 diabetes was almost 2.5 times more likely in persons with hypertension than in their normotensive counterparts [24]. Hypertension and type 2 diabetes are interrelated metabolic disorders that strongly predispose an individual to atherosclerotic cardiovascular disease (CVD) and renal failure [67].

More-aggressive treatment, such as reducing blood pressure to < 130/85 mmHg, is recommended when

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diabetes and hypertension coexist [57]. In the epidemiological UK Prospective Diabetes Study (UKPDS), each 10 mmHg reduction in mean systolic blood pressure was associated with reductions in risk of 12% for any complication related to diabetes, 11% for myocardial infarction and 13% for microvascular complications [63]. In light of these facts, it is clear that the control of blood pressure in diabetics is positively more beneficial for the progression of diabetic complications. The choice of anti-hypertensive therapy in the diabetic population needs to be considered in the context of not only reducing the blood pressure, microvascular and macrovascular complications, but also in providing cardioprotection and the prevention of interstitial fibrosis. Various groups of anti-hypertensives have been evaluated experimentally and clinically regarding the co-existence of diabetes mellitus and hypertension [23].

Interrupting the effects of the renin-angiotensin system (RAS) may improve the cellular actions of insulin by several mechanisms. Local activation of angiotensin-II (Ang-II) in a hypertrophic left ventricle triggers matrix metalloproteinase-mediated extracellular matrix degradation (i.e., left ventricular remodeling) at least in part through an Nicotinamide adenine dinucleotide phophate (NADPH) oxidase-induced oxidative stress and the subsequent activation of nuclear factor kappa B (NF-κB) [61]. Ang-II may directly contribute to oxidative stress by increasing the vascular production of superoxide radicals that, by reacting with nitric oxide to form peroxynitrite, reduces the levels of free nitric oxide [7]. Ongoing oxidative stress can further add to myocardial damage in diabetes mellitus [64].

Ligands for peroxisome proliferator activated receptor γ (PPARγ) improve insulin sensitivity, reduce triglyceride levels, and decrease the risk for atherosclerosis [6]. Telmisartan, which is an AT_1 receptor antagonist, has unique agonist activity at the PPARγ *in vitro* [5]. Telmisartan has the strongest binding affinity for the AT_1 receptor, compared with other angiotensin receptor blockers [30]. Previous studies from our laboratory have shown that telmisartan prevents STZ-induced metabolic abnormalities and the cardiovascular complications of type 1 diabetes in rat [21]. Various clinical trials have demonstrated that telmisartan induces a regression of left ventricular hypertrophy and vascular remodeling [39, 47]. Telmisartan efficacy is equivalent to ramipril in patients with vascular disease or high-risk diabetes and is associated with less angioedema [45]. Telmisartan is well tolerated in patients who are unable to tolerate ACE inhibitors and modestly reduces the risk of the composite outcome of cardiovascular death, myocardial infarction, or stroke [62]. A meta-analysis of randomized controlled trials indicated that telmisartan provides superior blood pressure control over ACE inhibitors (e.g., enalapril, ramipril and perindopril) and has fewer drug-related adverse events and better tolerability in hypertensive patients [74]. Therefore, in the light of above-mentioned facts, the present investigation was performed to study the effect of chronic telmisartan treatment on cardiovascular complications associated with type 2 diabetes in rats.

Materials and Methods

The protocol of this experiment was approved by our institutional animal ethical committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), the Ministry of Social Justice and Empowerment, Government of India (Protocol No. LMCP/07/07).

Animals

Sprague Dawley rats from an inbred colony were bred under well-controlled conditions of temperature, humidity and a 12 h/12 h light-dark cycle with diet and water provided *ad libitum*. Two-day-old neonates of either sex were injected intraperitoneally (*ip*) with 90 mg/kg STZ (Sigma, USA) in a 0.9% NaCl solution. Control neonates received an equivalent amount of isotonic saline alone. Twelve weeks after the injection of STZ, animals were checked for fasting glucose levels using a commercially available kit (Span Diagnostics Ltd., India). The animals with fasting glucose levels > 140 mg/dl were considered diabetic. The rats were then randomly divided into four groups of 6 animals: control, control treated with telmisartan, diabetic control and diabetic treated with telmisartan. Telmisartan was dissolved in distilled water and was administered orally (*po*) at a dose of 5 mg $kg^{-1}day^{-1}$ for eight weeks with food and water *ad libitum*.

Blood collection and serum analysis

At the end of eight weeks of telmisartan treatment, body weight, food intake and water intake were determined. Thereafter, animals were fasted for 12 h, and blood samples (2.5 to 3 ml from each rat) were collected from the retroorbital plexuses of each rat under light ether anesthesia. The serum was separated and analyzed spectrophotometrically for glucose, cholesterol, triglycerides, creatinine, C-reactive protein (CRP), lactate dehydrogenase (LDH) and creatinine kinase (CK) (Shimadzu UV-1601, Japan) using commercially available biochemical diagnostic kits (Bayer Diagnostics Ltd., India). Serum insulin was estimated by radioimmunoassay using kits from the Board of Radiation and Isotope Technology, Mumbai, India and a γ-counter (Packard, USA).

Hemodynamic parameter measurements

At the end of eight weeks, blood pressure, heart rate and the rate of pressure development and decay were recorded using carotid artery cannulation. Briefly, the animals were anesthetized with ketamine (100 mg/kg, *ip*) + xylazine (7 mg/kg, *im*). The body temperature was maintained at 37 ± 1°C during the experiment. The carotid artery behind the trachea was exposed and cannulated for the measurement of hemodynamic parameters using a transducer (SS 13L) and Biopac MP35 Systems (Biopac Systems Inc., USA). The hemodynamic parameters that were observed included systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MAP), the rate of pressure development (dp/dt_{max}) and the rate of pressure decay (dp/dt_{min}). All of the data were analyzed using Biopac Student Lab Pro software (Version 3.7.2).

Measurement of cardiovascular parameters

After the hemodynamic recording, the animals were sacrificed, the hearts were excised, and the extraneous tissues were separated. The wet weight of the entire heart and left ventricle was noted to calculate the index of cardiac hypertrophy as the wet heart weight to body weight ratio and the left ventricular hypertrophy index as the left ventricular weight to heart weight ratio. Quantification of left ventricular myocardial hydroxyproline concentrations was performed according to the method of Prockop and Udenfriend [50]. Antioxidant levels were measured from the left ventricular tissue. The left ventricular malondialdehyde (MDA) levels [43], reduced glutathione (GSH) levels [8], superoxide dismutase (SOD) levels [40], and catalase levels [1] were measured.

Statistical analysis

Results are presented as the mean ± SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at a p value < 0.05.

Results

General features of experimental rats

The neonatal type 2 diabetic rats exhibited significantly higher levels of serum glucose. No glucose was detectable in the urine of control animals. Diabetic rats showed a loss of body weight, polyphagia, and polydipsia. Chronic treatment with telmisartan failed to prevent the loss of body weight but significantly reduced the elevated food and water intake in STZ-diabetic rats (Tab. 1).

Biochemical parameters

Neonatal STZ-diabetic rats exhibited significant ($p < 0.05$) hyperglycemia and hyperinsulinemia compared to control rats. Treatment with telmisartan produced a significant ($p < 0.05$) decrease in elevated serum glucose levels and a significant ($p < 0.05$) decrease in insulin levels (Tab. 1).

There was a significant ($p < 0.05$) increase in total cholesterol, LDL, VLDL, and triglyceride levels and a significant ($p < 0.05$) decrease in HDL-cholesterol levels in neonatal STZ-diabetic rats, compared with control rats. Treatment with telmisartan significantly ($p < 0.05$) reduced the elevated total cholesterol, LDL, VLDL and triglyceride levels in diabetic rats and increased the lowered HDL-cholesterol levels (Tab. 1).

Neonatal STZ-diabetes also produced a significant ($p < 0.05$) increase in serum creatinine levels compared to control rats. Chronic treatment with telmisartan significantly ($p < 0.05$) reduced the elevated creatinine levels of diabetic rats (Tab. 1).

Tab. 1. Effect of telmisartan treatment on general features and biochemical parameters

Parameter	CON (n = 6)	COT (n = 6)	DIC (n = 6)	DIT (n = 6)
Body weight After treatment (g)	291.36 ± 1.07	231.24 ± 0.39*	234.73 ± 2.86*	237.1 ± 2.61
Food intake (g/animal/day)	13.3 ± 0.5	12.3 ± 0.2	15.9 ± 0.3*	13.6 ± 0.3#
Water intake (ml/animal/day)	22.2 ± 0.4	23.7 ± 0.6	27.9 ± 0.4*	22.3 ± 0.7#
Serum glucose (mg/dl)	113.89 ± 5.67	129.37 ± 7.94	265 ± 46.84*	158.97 ± 5.65#
Serum insulin (μU/ml)	43.68 ±12.14	45.57 ±7.37	113.63 ± 19.46*	74.83 ± 2.02#
Serum total cholesterol (mg/dl)	47.38 ± 4.27	48.71 ± 4.83	71.05 ± 7.33*	49.78 ± 3.52#
Serum LDL cholesterol (mg/dl)	14.24 ± 1.21	13.65 ± 2.11	24.75 ± 2.18*	14.91 ± 1.98#
Serum VLDL (mg/dl)	7.87 ± 1.05	8.75 ± 1.35	20.49 ± 2.66*	9.55 ± 1.01#
Serum triglyceride (mg/dl)	39.37 ± 5.27	43.74 ± 6.73	102.43 ±13.31*	47.77 ± 5.05#
HDL-cholesterol (mg/dl)	21.64 ± 2.58	20.56 ± 0.83	11.86 ± 1.66*	18.73 ± 1.28#
Serum creatinine (mg/dl)	0.14 ± 0.009	0.14 ± 0.007	0.265 ± 0.009*	0.16 ± 0.01#

* Significantly different from control ($p < 0.05$). # Significantly different from diabetic control ($p < 0.05$). Values are expressed as means ± SEM, n = 6 in each group. CON – non-diabetic control animals. COT – control animals treated with telmisartan (5 mg $kg^{-1}day^{-1}$). DIC – diabetic animals. DIT – diabetic animals treated with telmisartan (5 mg $kg^{-1}day^{-1}$)

Cardiovascular parameters

Biochemical markers

Neonatal STZ-diabetes produced a significant ($p < 0.05$) increase in serum LDH and CK levels compared to control rats. Chronic treatment with telmisartan significantly ($p < 0.05$) reduced the elevated serum LDH levels and CK levels of diabetic rats (Tab. 2).

Neonatal STZ-diabetes also produced a significant ($p < 0.05$) increase in serum CRP levels, compared with levels in control rats. Chronic treatment with telmisartan significantly ($p < 0.05$) reduced the elevated CRP levels of diabetic rats (Tab. 2).

Tab. 2. Effect of telmisartan treatment on cardiovascular markers and hemodynamic parameters

Parameter	CON (n = 6)	COT (n = 6)	DIC (n = 6)	DIT (n = 6)
Serum LDH (U/L)	40.48 ± 7.31	53.98 ± 8.95	221.59 ± 21.66*	144.07 ± 10.97#
Serum CK (U/L)	12.38 ± 3.22	15.75 ± 4.21	41.86 ± 7.39*	18.57 ± 1.38#
Serum CRP (mg/l)	2.21 ± 0.86	3.54 ± 0.61	11.91 ± 0.92*	4.28 ± 0.52#
Blood pressure (mmHg)	78 ± 1	81 ± 8	105 ± 1*	72 ± 1#
Heart rate (beats/min)	303 ± 21	317 ± 21	360 ± 25	355 ± 25

* Significantly different from control ($p < 0.05$).# Significantly different from diabetic control ($p < 0.05$). Values are expressed as the means ± SEM, n = 6 in each group. CON – non-diabetic control animals. COT – control animals treated with telmisartan (5 mg $kg^{-1}day^{-1}$). DIC – diabetic animals. DIT – diabetic animals treated with telmisartan (5 mg $kg^{-1}day^{-1}$)

Hemodynamic parameters

The mean blood pressure was significantly ($p < 0.05$) increased after eight weeks in neonatal STZ-diabetic rats, compared with control rats. Telmisartan treatment prevented the STZ-induced increase in blood pressure in diabetic animals (Tab. 2). Heart rate was significantly ($p < 0.05$) higher in neonatal STZ-diabetic rats than in controls. Chronic treatment with telmisartan in diabetic rats exhibited decrease in heart rate, compared to that in diabetic control animals (Tab. 2). Neonatal STZ-diabetic rats exhibited a significantly decreased rate of pressure development and decay, compared with that in control rats. Chronic telmisartan treatment significantly elevated the decreased rate of pressure development and decay of the diabetic rats (Fig. 1).

Cardiac hypertrophy and left ventricular hypertrophy index

Left ventricular collagen levels were also significantly ($p < 0.05$) greater in neonatal STZ-diabetic rats than in control rats. Chronic treatment with telmisartan significantly ($p < 0.05$) reduced the elevated left ventricular collagen levels of diabetic rats (Tab. 3).

Neonatal STZ-diabetic rats exhibited a reduced wet heart weight, compared with that for non-diabetic

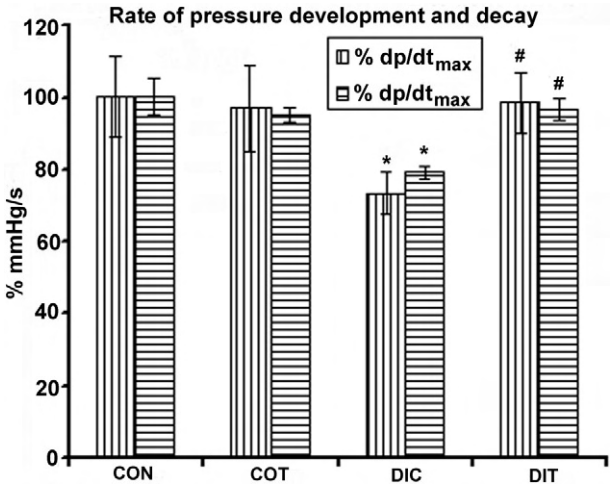

Fig. 1. Effect of telmisartan on the rate of pressure development and decay. Each bar represents the mean ± SEM of 6 experiments. * Significantly different from control ($p < 0.05$). # Significantly different from diabetic control ($p < 0.05$). CON – non-diabetic control animals. COT – control animals treated with telmisartan (5 mg $kg^{-1}day^{-1}$). DIC – diabetic animals. DIT – diabetic animals treated with telmisartan (5 mg $kg^{-1}day^{-1}$)

Tab. 3. Effect of telmisartan treatment on hypertrophic parameters and anti-oxidant levels

Parameter	CON (n = 6)	COT (n = 6)	DIC (n = 6)	DIT (n = 6)
Cardiac hypertrophic index (mg/g)	2.64 ± 0.13	2.83 ± 0.03	3.79 ± 0.08*	2.71 ± 0.05#
LV Hypertrophic index (mg/mg)	0.66 ± 0.01	0.66 ± 0.01	0.76 ± 0.02*	0.67 ± 0.02#
LV Collagen levels (mg/g LV tissue)	1.70 ± 0.14	1.75 ± 0.04	3.08 ± 0.25*	1.84 ± 0.14#
SOD (units/min/mg protein)	2.37 ± 0.25	2.07 ± 0.25	0.63 ± 0.14*	1.82 ± 0.22#
Catalase (units/min/mg protein)	6.286 ± 0.625	6.059 ± 0.77	2.10 ± 0.31*	5.52 ± 0.63#
MDA (nmoles/mg protein)	3.69 ± 0.54	3.17 ± 0.79	7.93 ± 1.01*	3.92 ± 0.95#
GSH (µg/mg protein)	8.52 ± 1.65	7.18 ± 1.49	1.73 ± 0.38*	6.34 ± 0.40#

* Significantly different from control ($p < 0.05$). # Significantly different from diabetic control ($p < 0.05$). Values are expressed as the means ± SEM, n = 6 in each group. CON – non-diabetic control animals. COT – control animals treated with telmisartan (5 mg $kg^{-1}day^{-1}$). DIC – diabetic animals. DIT – diabetic animals treated with telmisartan (5 mg $kg^{-1}day^{-1}$)

rats. However, the ratio of heart weight to body weight, which is a measure of cardiac hypertrophy, was significantly ($p < 0.05$) higher in diabetic rats than in control rats. Furthermore, the left ventricular weight to heart weight ratio, which is a measure of left ventricular hypertrophy, was also significantly ($p < 0.05$) higher in diabetic control animals than in non-diabetic control animals. Chronic treatment with telmisartan significantly ($p < 0.05$) reduced the elevated cardiac hypertrophy index and left ventricular hypertrophy index of diabetic rats (Tab. 3).

Anti-oxidant levels

Neonatal STZ-diabetic rats exhibited significantly decreased SOD, catalase and glutathione levels in the left ventricle, compared with control rats. Chronic treatment with telmisartan significantly increased the left ventricular SOD, catalase and glutathione levels of diabetic rats. Neonatal STZ-diabetic rats also exhibited significantly increased MDA levels in the left ventricle, compared with control rats. Chronic treatment with telmisartan significantly increased the left ventricular MDA levels of diabetic rats (Tab. 3).

Discussion

In present study, neonatal STZ-diabetic rats exhibited the cardinal signs and characteristics of diabetes, i.e., significant weight loss and increase in food and water intake, compared with that for control animals. Treatment with telmisartan significantly reduced the elevated food and water intake in diabetic rats, but it did not prevent body weight loss. Because there was a significant difference in the food and water intake in the telmisartan-treated rats, there may be further difference in the glucose, insulin, lipid and cardiac enzyme levels. However, previous studies have reported that a decrease in food and water intake does not necessarily control biochemical parameters, but there may be several proposed mechanisms for these effects. Anti-hypertensives do not differ in the lowering of blood pressure under diabetic conditions, but they may produce differential effects on the metabolic and cardiovascular alterations that are observed [22].

Neonatal diabetic STZ rats show significant glucose intolerance when 1.5 g/kg glucose is orally ad-

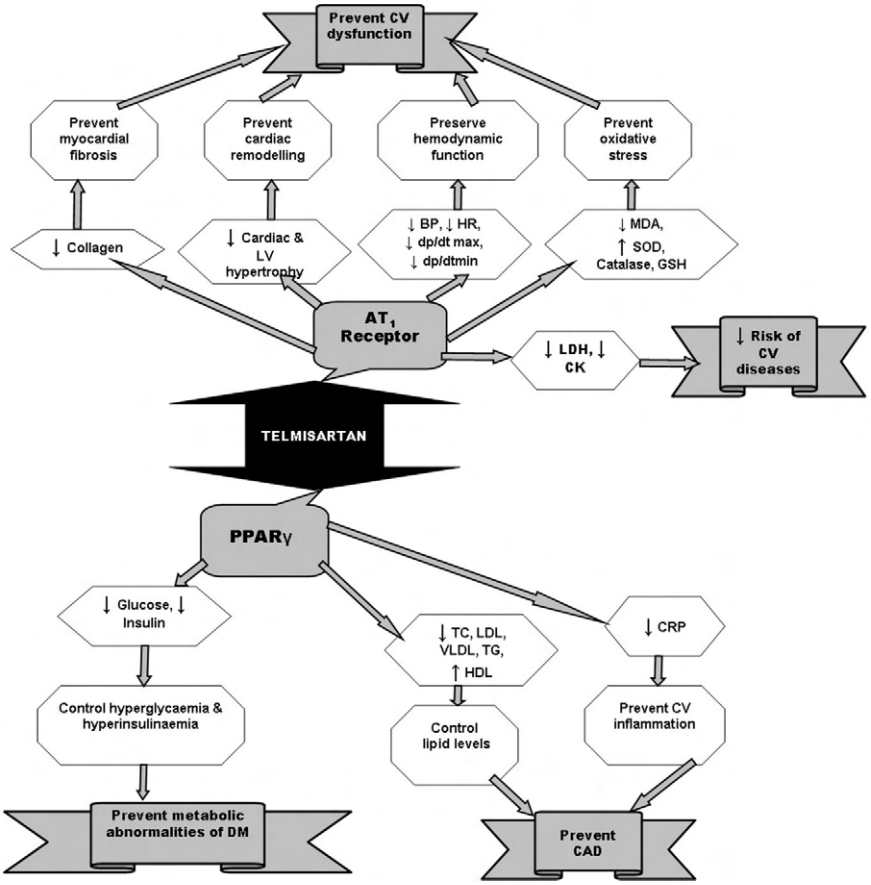

Fig. 2. Summarized effect and beneficial role of telmisartan *via* AT_1 receptor and PPARγ. PPARγ – peroxisome proliferator activated receptor γ, CV – cardiovascular, LC – left ventricular, BP – blood pressure, HR – heart rate, dp/dt_{max} – rate of pressure development, dp/dt_{mim} – rate of pressure decay, MDA – malondialdehye, SOD – super oxide dismutase, GSH – reduced glutathione, LDH – lactate dehydrogenase, CK – creatinine kinase, TC – total cholesterol, TG – triglyceride, CRP – C-reactive protein, DM – diabetes mellitus, CAD – coronary artery diseases, ↑ - decrease, ↓ – increase

ministered, and they also have high levels of insulin [48]. Hyperinsulinemia with low hepatic excretion and the hypersecretion of β cells are also reported in mildly glucose intolerant obese subjects [10]. In the present study, STZ produced a significant increase in glucose levels that was associated with a significant increase in insulin levels in diabetic rats. Treatment with telmisartan significantly reduced the serum glucose and insulin levels of the diabetic rats. In the early components of the insulin signaling cascade, Ang-II negatively affects insulin signaling. In diabetic rat hearts, the inhibition of the AT_1 receptor prevents the decline of glucose transporter-4 (GLUT-4). Angiotensin receptor blockers may also increase the protein expression of GLUT-4 in the skeletal muscle and myocardium [14, 55]. Furthermore, telmisartan acts as a partial agonist of PPARγ and influences the expression of PPARγ target genes [5]. Telmisartan structurally resembles pioglitazone [46]. Therefore, the glucose lowering effect might be due to an improvement in insulin sensitivity, and the reduction in the serum glucose levels may be attributed to various mechanisms. Further studies are required to determine the precise mechanism of the improvement in insulin sensitivity. Numerous hyperglycemia-related mechanisms, like protein kinase C activation, the generation of reactive oxidant stress, poly(ADP ribose) polymerase (PARP) activation, and the accumulation of advanced glycoxidation, are hypothesized to mediate micro- and macro-vascular complications [4]. The actions of insulin on the myocardium during chronic systemic hyperinsulinemia bear directly on the commonly observed finding of cardiac hypertrophy in diabetic cardiomyopathy [26]. Because telmisartan controls hyperglycemia and hyperinsulinemia, it might be beneficial for the prevention of diabetic cardiomyopathy as depicted in Figure 2.

Circulating levels of free fatty acids are elevated in diabetes [18]. Free fatty acids may impair endothelial function through several mechanisms, including the increased production of oxygen-derived free radicals, the activation of PKC, and the exacerbation of dyslipidemia [27]. In the present study, diabetic animals showed elevated triglyceride, cholesterol, LDL,

and VLDL levels and decreased HDL levels, compared with control animals. Serum cholesterol, triglyceride, LDL, and VLDL levels were significantly decreased by treatment with telmisartan in diabetic rats. Although PPARγ regulates lipid metabolism and telmisartan exerts partial agonistic activity on PPARγ [68], the control of dyslipidemia might be due to a direct AT_1 inhibition because the action of telmisartan is not mediated by adiponectin [31]. Benson et al. [5] have also reported that telmisartan administration causes a significant reduction in triglyceride levels in rats that are fed a high-fat, high-carbohydrate diet compared to treatment with losartan. Ang-II modulates the effects of oxidized LDL on endothelial cell function [35]. The positive relationship between plasma triglyceride concentrations and coronary events has been reported previously, and both hypertriglyceridemia and low HDL are associated with endothelial dysfunction [33]. The reduction in cholesterol and triglyceride levels by telmisartan suggests a beneficial effect on coronary heart diseases.

C-reactive protein (CRP) is an acute phase reactant that is elevated in type 1 and type 2 diabetes [13, 49]. After acute myocardial infarction, the circulating concentration of human CRP is increased [15]. CRP is also an important predictor of MI and CHD death in patients with unstable angina [52]. CRP selectively binds non-oxidized low-density lipoprotein found within atheromatous plaques and enhances complement activation [2, 9]. In the present study, a significant rise in CRP level in STZ-induced type 2 diabetic rats was found. Treatment with telmisartan significantly reduced CRP levels. Telmisartan dose-dependently inhibits AGE-induced ROS generation and the subsequent CRP gene and protein induction [69]. Therefore, telmisartan may work as an anti-inflammatory agent by suppressing the expression of the AGE receptor *via* PPARγ activation in the liver [69]. This pathway is one of the possible mechanisms for the decrease in CRP levels and the improvement in cardiovascular dysfunction that was observed in the present study.

Abnormally high activities of serum CKMB are claimed to be a specific and extremely sensitive index of myocardial necrosis or ischemia [53]. LDH levels are also reported to be increased in type 2 diabetic patients and may serve as a cardiovascular risk-related marker [25]. In our study, we found a significant increase in serum LDH and CK levels in diabetic rats. Treatment with telmisartan significantly reduced LDH and CK levels, which further substantiates its beneficial effect for the reduction of cardiovascular risk in diabetes mellitus, similar to the reports of Goyal et al. [21].

Cardiac fibrosis is one of the main modulators of diastolic cardiac stiffness [73]. The heart is densely populated with angiotensin II receptors, and stimulation of the Ang-II signal transduction pathways promotes various important actions in the myocardium, including myocyte hypertrophy with left ventricular hypertrophy (LVH), apoptosis in myofibroblasts, collagen deposition within muscle fibers, and fibrosis of myocardial tissue [37]. Accumulation of cardiac fibrosis can result from the excessive production of collagen by fibroblasts and from a decrease in the degradation of collagen by metalloproteinases. In addition to non-enzymatic glycosylation of collagens, the dysregulation of collagen-degrading matrix metalloproteinases (MMPs) and their tissue inhibitors is a hallmark for myocardial fibrosis in diabetes. MMP-2 activity is downregulated by high glucose levels and by Ang-II [3]. Low proteolytic activity of MMPs contributes to diabetes-induced renal fibrosis [29]. Consequently, an increase in collagen content in the STZ group leads to diastolic dysfunction. Alterations in the diastolic filling of the left ventricle (LV) that are associated with reciprocal changes in the LV collagen gene suggest that an increase in interstitial cardiac collagen causes cardiac fibrosis. This fibrosis results in greater LV stiffness and a decrease in LV wall compliance, which leads to diastolic dysfunction and eventual heart failure in diabetes [41]. In our study, we found a significant increase in left ventricular collagen deposition in diabetic rats, and telmisartan treatment significantly reduced elevated left ventricular collagen levels. An enhanced expression of matrix metalloproteinase and an augmented oxidative stress represent the pathological consequences of Ang-II [34, 42]. The AT_1 receptor is involved in the development of cardiac hypertrophy by increasing ECM accumulation [11, 66]. Olmesartan treatment is beneficial for sympatho-excitatory cardiac hypertrophy [70]. Furthermore, in hypertensive Dahl salt-sensitive rats, the local activation of Ang-II in hypertrophic LV triggers ECM degradation, which results in LV remodeling. Treatment with telmisartan preserves LV shape and function and ECM density and decreases oxidative stress-mediated protein degeneration [61]. Ang-II stimulates cardiac collagen production by promoting transforming growth factor-β_1 (TGF-β_1) synthesis *via*

AT_1 receptor activation [28]. Therefore, it is possible that a decrease in cardiac collagen levels by telmisartan may be associated with AT_1 receptor antagonism and an improvement in cardiac function, as shown in Figure 2.

The development of LVH is a considerable risk for morbidity and mortality in hypertension [12]. Diabetes mellitus is independently associated with abnormal LV relaxation, which is similar to the well-known impaired relaxation that is associated with hypertension [36]. LV remodeling in type 2 diabetes includes an increase in LV mass, increased LV wall thicknesses and a reduction in LV systolic chamber and myocardial function [16]. Poorly controlled hypertension, obesity, type 2 diabetes, and insulin resistance with hyperinsulinemia promote LVH [5]. Excessive levels of insulin, in combination with Ang-II and/or aldosterone, enhance the proliferative effects of these substances on myocardial cells [32]. In the present study, cardiac hypertrophy and left ventricular hypertrophy index were increased in diabetic hearts, and treatment with telmisartan significantly reduced the cardiac hypertrophy and LVH index of diabetic animals. The inhibition of RAAS promotes the regression of pathological LVH more effectively than other anti-hypertensive agents [38]. Ang-II stimulates fibrous tissue formation by promoting TGF-β_1 synthesis *via* AT_1 receptors and is a major determinant in cardiac remodeling [60]. AT_1 receptor activation plays a role in the molecular changes that are associated with coronary matrix remodeling in diabetes [28]. Furthermore, AT_1 receptor blockade reduces myocardial hypertrophy, decreases myocardial fibrosis, and attenuates cardiac remodeling [56]. From the ongoing discussion, it can be said that telmisartan produces a beneficial effect on cardiac and LVH.

An increase in blood pressure after treatment with streptozotocin has been reported previously [19]. A number of factors are involved in the pathogenesis of hypertension in diabetes mellitus. In our study, the blood pressure of diabetic animals was higher than that of control animals. Telmisartan successfully prevented the increase in blood pressure in diabetic animals. Mean blood pressure remained significantly lower in telmisartan-treated diabetic rats than in diabetic control rats. Oral administration of telmisartan has been demonstrated to be an effective blood pressure-lowering agent in adult patients with mild to moderate hypertension in numerous randomized clinical trials [20]. In the present study, the heart rate of STZ-diabetic animals was significantly higher than that of non-diabetic animals. Telmisartan treatment exhibited a significant decrease in heart rate. Ang-II exerts an inhibitory influence on the baroreceptor reflex control of heart rate, which is mediated by the AT_1 receptor [44]. Because telmisartan is an AT_1 receptor antagonist, it is possible that it may attenuate the Ang-II-mediated inhibition of the baroreceptor reflex. Left ventricular dysfunction has been associated with a decrease in L-developed pressure, dp/dt_{max} and dp/dt_{min} [65, 71]. In the present study, diabetic rats exhibited a significantly decreased rate of pressure development and decay, compared with control rats. Chronic treatment with telmisartan significantly elevated the decreased rate of pressure development and decay in diabetic rats. Therefore, the results of the hemodynamic study indicate the beneficial role of telmisartan in left cardiovascular dysfunction.

Diabetes is associated with nephropathy. In present study, serum creatinine levels were elevated in diabetic rats, and treatment with telmisartan significantly reduced elevated creatinine levels. However, the mechanism of action of this effect remains unknown. Because telmisartan decreased elevated glucose and insulin levels, it might control the complications associated with hyperglycemia and hypoinsulinemia. This control may be the reason for the decrease in creatinine levels in diabetic rats.

The accumulation of ROS is a characteristic feature of oxidative stress, and a relationship between oxidative stress and the vascular complications in diabetes was suggested previously [59]. Oxidative stress is associated with complications of diabetes [51] and has been linked to insulin resistance *in vitro* and *in vivo* [17]. When glucose and free fatty acids increase, they cause oxidative stress and the activation of stress-sensitive signaling pathways [17]. The activation of these pathways, in turn, worsens both insulin action and secretion, which leads to overt type 2 diabetes. In our study, there was a high level of serum MDA and a low level of SOD, catalase, glutathione in the diabetic group, compared with levels in normal animals. Chronic telmisartan treatment reduced the elevated level of MDA and increased the levels of SOD, catalase, glutathione, which indicates a decrease in oxidative stress. Zhao et al. [72] reported that angiotensin II leads to cardiac oxidative stress through an enhancement of reactive oxygen species production at sites of cardiac injury, and aldosterone partially mediates the Ang-II-induced cardiac proinflammatory/profibro-

genic phenotype. Therefore, because telmisartan is an AT_1 receptor antagonist, it might inhibit Ang-II-induced oxidative stress.

Conclusions

In conclusion (Fig. 2), our data suggest that telmisartan prevented the metabolic abnormalities of diabetes by controlling hyperglycemia and hyperinsulinemia in STZ-induced diabetic rats. Additionally, an improvement in the lipid profile and hemodynamic functions, a reduction in LDH, CK, CRP, collagen and hypertrophic indices and the prevention of oxidative stress suggest that telmisartan is beneficial in the prevention of cardiovascular complications and dysfunctions that are associated with type 2 diabetes mellitus.

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Received: August 7, 2010; **in the revised form:** February 7, 2011; **accepted:** February 18, 2011.