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Does telmisartan prevent hepatic fibrosis in rats with alloxan-induced diabetes?☆

Zekai Halici [a], Habip Bilen [b], Fatih Albayrak [c], Abdullah Uyanik [d], Ramazan Cetinkaya [d], Halis Suleyman [a], Osman Nuri Keles [e], Bunyami Unal [e,*]

[a] Department of Pharmacology, Ataturk University School of Medicine, Erzurum, Turkey
[b] Internal Medicine, Division of Endocrinology and Metabolism, Ataturk University School of Medicine, Erzurum, Turkey
[c] Internal Medicine Division of Gastroenterology, Ataturk University School of Medicine, Erzurum, Turkey
[d] Internal Medicine Division of Nephrology, Ataturk University School of Medicine, Erzurum, Turkey
[e] Departments of Histology and Embryology, Ataturk University School of Medicine, Erzurum, Turkey

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ABSTRACT

Background/aims: This study evaluated the effect of telmisartan on the livers of diabetic rats and also aimed to determine the hepatic distribution and role of transforming growth factor β (TGF-β) in diabetes-related hepatic degeneration while taking into account the possible protective effects of telmisartan. *Methods:* Fifteen adult male rats were used and divided into three groups: the non-diabetic healthy group, alloxan-induced diabetic control group, and the alloxan-induced diabetic telmisartan group. The non-diabetic healthy group and the diabetic control group were exposed to saline for 30 days, while the group treated with diabetic drugs was orally administered telmisartan for 30 days (10 mg/kg/day). At the end of the experiment, the rats were sacrificed and the livers were dissected and transferred into the fixation solution. The livers were then evaluated using stereological and histopathological methods. *Results:* Our study of the numerical density of hepatocytes shows a significant difference between the diabetic control group and diabetic rats treated with telmisartan. Immunohistochemical staining for TGF-β in liver sections of the diabetic rats treated with telmisartan showed no immunoreactivity. The diabetic control group was determined to be strongly immunoreactive to TGF-β. *Conclusion:* Results suggest that telmisartan may reduce type-I diabetes mellitus-induced hepatic injury by suppressing activated hepatic stellate cells through concomitant TGF-β1 down-regulation.

1. Introduction

Many clinical trials have proven that using some antihypertensive drug groups such as angiotensin receptor type I (AT_1 receptor) blockers to reduce blood pressure decreases the complications of hypertension (Bloch and Basile, 2004). Unlike other antihypertensive drugs, angiotensin receptor type I blockers are beneficial for reducing nephropathy, coronary vascular disease, and paralysis in diabetic patients. No longer, angiotensin AT_1 receptor blocker usage in hypertensive patients with heart failure, renal failure, and especially diabetes became an inevitable indication. Nevertheless, though all angiotensin AT_1 receptor blockers are thought to be clinically beneficial, there is increasing evidence that each type does not exert the same benefits, especially in chronic treatment (Miura et al., 2005).

Telmisartan, a common angiotensin AT_1 receptor blocker, shows antidiabetic effects by activating peroxisome proliferator-activated receptor-γ (PPAR-γ) (Benson et al., 2004). The structural similarities between telmisartan and pioglitazon, the PPAR-γ ligand, have been detailed in previous studies. In a study performed according to these findings at physiological concentrations, it was found that of angiotensin AT_1 receptor blockers, only telmisartan caused significant PPAR-γ activation (Benson et al., 2004). Furthermore, telmisartan is known to cause the following in vitro responses characteristic of PPAR-γ activation: adiposity differentiation and selective organization of the genes that play a role in lipid and carbohydrate metabolism (Benson et al., 2004).

Diabetes mellitus is one of the most prevalent chronic diseases in the world. Chronic liver disease and diabetes mellitus can co-exist in a single individual, and the liver is a frequent site of unrecognized injury in diabetes mellitus patients. The mortality rate in diabetes mellitus patients is generally not due to classical diabetes-related complications (micro and/or macro vascular complications), but rather to an increased risk of hepatocellular failure via nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and cirrhosis. Chronic liver injury is typically silent in its progression, and the presence of liver disease in patients with progressively worsening insulin resistance may not be

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* Corresponding author. Tel.: +90 4422316590; fax: +90 4422360968.
E-mail address: bunyamiunal@gmail.com (B. Unal).

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recognized until patients develop manifestations of the metabolic syndrome such as diabetes, hypertension, hyperlipidemia, and vascular disease (Grundy et al., 2005). In patients with chronic liver diseases such as cirrhosis, the renin–angiotensin system is reportedly activated (Rockey and Weisiger, 1996; Arroyo et al., 1981).

Yokohama et al. (2004) have demonstrated that the renin–angiotensin system plays a role in hepatic fibrosis and that losartan treatment significantly reduces the blood markers of hepatic fibrosis and aminotransferase levels. In vitro experiments and studies in animals and humans have suggested a possible relationship between the renin–angiotensin system and the pathogenesis of insulin resistance. Moreover, angiotensin AT_1 receptor antagonists may thus become inevitable in diabetes mellitus characterized by liver diseases. For example, recent studies have suggested that angiotensin-II may impair glucose metabolism through its effects on insulin signaling pathways, tissue blood flow, oxidative stress, sympathetic activity, and adipogenesis (Ogihara et al., 2002; Velloso et al., 1996; Folli et al., 1997; Paolisso et al., 1997). Angiotensin-II, which is a physiologically active product of the renin–angiotensin system and also a vasoconstrictor substance, has many physiological effects such as vascular hormonal secretion and tissue growth. These effects in turn stimulate the proliferation of mesangial cells, cardiac fibroblasts, and hepatic stellate cells while increasing the synthesis of extracellular matrix proteins through in vivo and in vitro induction of transforming growth factor β (TGF-β) expression (Dzau et al., 1991; Bataller et al., 2000). TGF-β has various effects on cell proliferation, differentiation, migration, and survival. Furthermore, TGF-β plays a role in multiple biological processes, including development, carcinogenesis, fibrosis, wound healing, and immune responses (Roth et al., 1998). TGF-β has also been reported to be stored but not synthesized in substantial amounts in hepatocytes (Roth et al., 1998). The suggestion that angiotensin-II may be a mediator of fibrosis in diabetic rat livers raises the question of whether the angiotensin-receptor inhibitor telmisartan could be effective in preventing hepatic fibrosis.

The goal of this study was to evaluate the effect of telmisartan, an angiotensin-II receptor blocker, on the livers of alloxan-induced diabetic rats using three different methods: conventional light microscopy, electron microscopy, and stereology. We also aimed to determine the hepatic distribution and role of TGF-β in diabetes-related hepatic degeneration while examining the possible protective effects of telmisartan through immunohistochemical methods.

2. Materials and methods

2.1. Animals

Adult male Wistar albino rats weighing 200–210 g were obtained from the Ataturk University Experimental Animal Laboratory in the Medicinal and Experimental Application and Research Center. The rats were kept in our laboratory under controlled environmental conditions. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and approved by the local animal care committee of Ataturk University.

2.2. Alloxan-induced diabetes

Diabetes was induced in male Wistar albino rats by intraperitoneal administration of aqueous alloxan monohydrate (Sigma-Aldrich Co.) at a single dose of 150 mg/kg body weight according to previously described methods (Gupta, 1994). It was dissolved in 0.9% NaCl solution, freshly prepared, and injected intraperitoneally to rats that were fasted for one night. In the non-diabetic group, 0.9% NaCl solution at the same volume was injected intraperitoneally. After alloxan application, the pancreas secretes insulin at high levels. As a consequence, fatal hypoglycemia can occur. To prevent this adverse effect, 5 ml 20% glucose solution were injected intraperitoneally 4–6 h after alloxan treatment. We then added a 5% glucose solution to the rats' drinking water for 24 h to prevent possible hypoglycemia. After 72 h, fasting blood samples were collected via the tail vein. The samples were then used to measure blood glucose levels using the Accu-Check Active blood glucose monitor (Souza et al., 2007). At the end of the third day, animals with serum glucose levels higher than 200 mg/dl were considered diabetic and were included in the study.

2.3. Experimental groups

The rats were separated into three groups: the non-diabetic healthy group, the diabetic control group, and the diabetic telmisartan group.

2.4. Drug administration

In our study, there were five rats in each group. The non-diabetic healthy group and the diabetic control group were exposed to 0.9% saline for 30 days (1 ml/day), while the group treated with diabetic drugs was orally administered telmisartan (Micardis 80 mg tablet, obtained from Boehringer Ingelheim Ilaç Tic., Istanbul) for 30 days (10 mg/kg/day) (Schäfer et al., 2007). All rats were sacrificed on the 31st day of the experiment by an overdose of a general anesthetic (Thiopental sodium, 50 mg/kg). The livers were dissected immediately and transferred into a 10% formaldehyde solution for stereological and histopathological evaluation.

2.5. Histological procedures

2.5.1. Light microscopy

Livers were fixed in 10% formaldehyde, dehydrated in a graded-alcohol series, embedded in paraffin wax, and sectioned using a Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany). In our study, we obtained two section thicknesses: 5 μm for histopathological examinations and 40 μm for stereological evaluations. Sections were stained with Hematoxylin-Eosin (H-E).

2.5.2. Electron microscopy

The liver samples for electron microscopic examination were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, post-fixed in 1% phosphate-buffered osmium tetroxide, dehydrated in a graded-alcohol series, and immersed in propylene oxide. The specimens were then embedded in Araldite CY 212 (Agar, Cambridge, UK). Sections were cut into thicknesses of between 70 and 80 nm using an ultramicrotome (Nova LKB Bromma, Sweden) for histological evaluation at the ultrastructural level. They were finally observed with a JEOL JEM-1200 electron microscope at an accelerating voltage of 80 kV after staining with uranyl acetate and lead citrate.

2.5.3. Stereological method

2.5.3.1. Tissue sampling procedures for stereology. On the basis of a pilot study, 8–10 sections were obtained. All 40-μm thick sections were evaluated without carrying out any sampling procedure.

2.5.3.2. Number estimation using the optical dissector method. The hepatocytes in the stained sections were counted using the optical dissector counting method (Howard and Reed, 1998; Kaplan et al., 2001) at a stereology workstation composed of a CCD digital camera (Optronics MicroFire), a personal computer, a computer-controlled motorized specimen stage (BioPrecision MAC 5000 controller system), and a light microscope (Leica DM4000 B). Hepatocytes were counted using a 20× Leica Plan Apo objective (NA = 1.40) and the total magnification was *M*, which allowed for accurate recognition.

Table 1
A sampling strategy used for stereological analysis for non-diabetic healthy group.

Non-diabetic healthy group	
Group 1 – Animal 1	
Number of sampling sites for microscopic	31
Counting frame area (*XY*) (μm^2)	4900 (70 μm × 70 μm)
Disector height (*Z*) (μm)	16
Disector volume (*XYZ*) (μm^3)	78400 (70 μm × 70 μm × 16 μm)
Sampling grid area (*XY*) (μm^2)	3062500 (1750 μm × 1750 μm)
Section thickness (μm)	28
Section periodicity	1
The number of dissector particles	721
Hepatocytes' numerical density formulas	The number of dissector particles / counting frame area × disector height × number of sampling sites
Hepatocytes' numerical density value	721/4900 μm^2 × 16 μm × 31 = 0.0002967 N/μm^3)

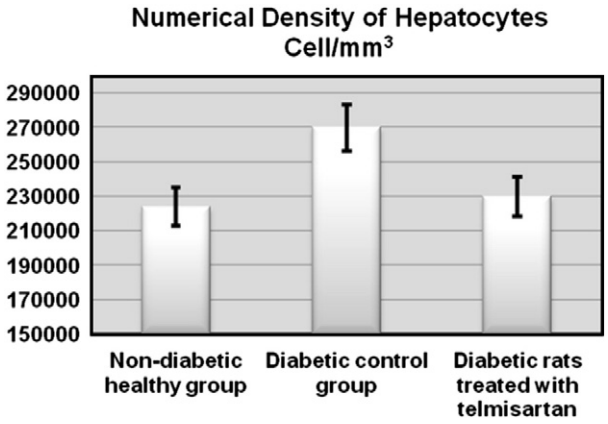

Fig. 1. Numerical density of hepatocytes in all groups was shown (S.E.M ±).

2.5.3.3. Microscopy stages for stereology. All details of the microscopy stages for samples belonging to our study are summarized in Table 1.

For our number estimations, we used an approach based on methods described by Gundersen et al. (1999) to obtain coefficient of error (C.E) values (Gundersen and Jensen, 1987). The generally accepted highest limit of C.E in stereological studies is 5% (Gundersen and Jensen, 1987). In our study, the C.E was 0.05 (variance due to noise: 911; variance of systematic random sampling: 659.33, $m=1$; total variance: 1570.33, $m=1$; coefficient of error, Gundersen: 0.04, $m=1$).

2.5.3.4. Estimation of hepatocytes and hepatocyte nuclei volume. All 40-μm-thick sections obtained to estimate the number of hepatocytes using the optical dissector method was utilized without carrying out any sampling procedure. Although some requirements for obtaining full randomness in the section-sampling stage while estimating the volume of the small objects have been mentioned in the literature (Mattfeldt et al., 1990; Nyengaard and Gundersen, 1992), we did not meet these requirements completely. Sections were obtained in the coronal plane without randomness in orientation, and volume estimations were applied as described by Dorph-Petersen et al. (2004).

2.5.4. Immunocytochemistry

2.5.4.1. Immunohistochemical staining of TGF-β1. It was performed using a streptavidin biotin-staining method (Vector Laboratories, Burlingame, CA). Following the removal of araldite from the semi-thin sections to eliminate endogenous peroxidase activity, the tissue was exposed to 0.3% H_2O_2 in absolute methanol for 30 min at room temperature. The slides were placed in 10 mmol/L citrate buffer (pH 6.0) for 10 min to retrieve interesting tissue antigens. After the slides were placed in phosphate-buffered saline (PBS) for 15 min, avidin/biotin block (Avidin/biotin blocking, Invitrogen, Turkey) serum blocker was applied for 10–15 min. The samples were then rinsed in PBS. Sections were incubated in primary antibody (TGF β RI (V-22) Antibody, Santa Cruz Biotechnology, Santa Cruz, CA) at a 1:200 dilution in primary antibody dilution buffer (primary antibody diluent, GeneTex, USA) for 1 h at room temperature. In the following step, after sections were washed in PBS for three 5-minute intervals, a secondary biotinylated anti-rabbit antibody (Vector Laboratories) was applied to each section surface for 30 min at room temperature. The color reaction was developed with the diaminobenzidine detection kit (Vector Laboratories) and counterstained with hematoxylin. Finally, slices were rinsed in tap water for 3 min, dehydrated through 95% ethanol for 1 min and 100% ethanol for 2–3 min, cleared in xylene for 2–5 min, covered with mounting medium, and examined with a light microscope (Olympus BH-40, Japan) (Takahashi et al., 2007).

2.6. Statistical analysis

The mean numerical densities (Nv) and mean volume values of hepatocytes and their nuclei were compared using an independent samples T test (SPSS for Windows version 13.0). In this study, $P<0.05$ was accepted as significant.

3. Results

3.1. Stereological results

a. *Numerical density of hepatocytes*: The numerical density of hepatocytes in all groups is summarized in Table 2 and Fig. 1.

When these values were statistically evaluated, a significant difference was noted between the diabetic control group (Group 2) and the diabetic rats treated with telmisartan (Group 3) ($P<0.05$).

b. *Geometric volume of hepatocytes*: The geometric volume of hepatocytes in all groups is summarized in Table 3 and Fig. 2.

In terms of the geometric volume of the hepatocytes, there were no significant differences between groups ($P>0.05$).

c. *Geometric volume of hepatocyte nuclei*: The geometric volume of hepatocyte nuclei in all groups is summarized in Table 4 and Fig. 3.

When results were evaluated with respect to the geometric volume of hepatocyte nuclei, we did not observe any significant differences between groups ($P>0.05$).

Table 2
Numerical density of hepatocytes in all groups.

Groups	Numerical density of hepatocytes (N/μm^3)	Standard deviation	Standard error mean
Group 1 ($N=5$)	0.000240993	0.00004	0.00002
Group 2 ($N=5$)	0.000271573	0.00002	0.00001
Group 3 ($N=5$)	0.000225880	0.00003	0.00002

Table 3
Geometric volumes of hepatocytes in all groups.

Groups	Geometric volumes (μm^3)	Standard deviation	Standard error mean
Group 1 ($N=5$)	3794.276	301.33	134.76
Group 2 ($N=5$)	3934.42	700.15	313.11
Group 3 ($N=5$)	4129.342	409.93	183.33

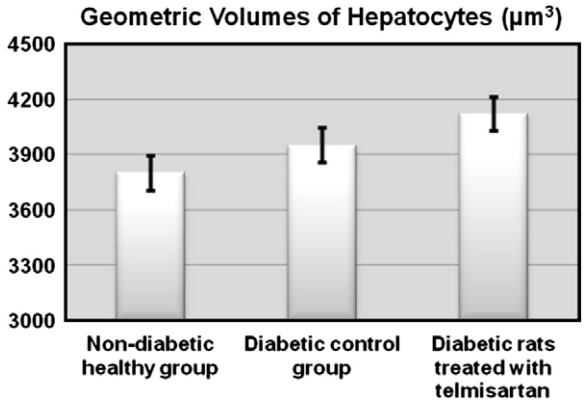

Fig. 2. Geometric volumes of hepatocytes in all groups were shown (S.E.M ±).

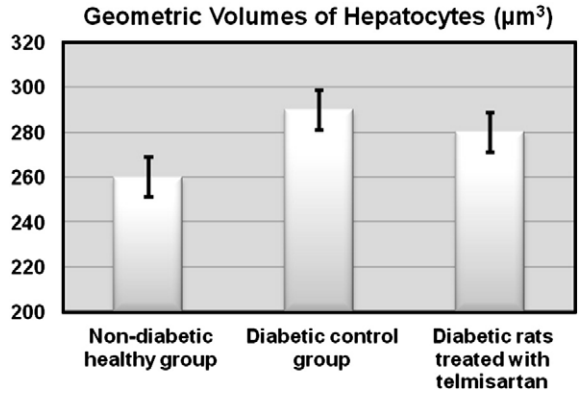

Fig. 3. Geometric volumes of hepatocyte nuclei in all groups were shown (S.E.M ±).

3.2. Histopathological results

3.2.1. Conventional light microscopic examination

For the conventional histopathological examination under a light microscope, all slides were stained with H-E to define routine histological structures. In the non-diabetic group (Group 1), known healthy liver histology was seen (Fig. 4A and B). The hepatocytes of the diabetic group contained more acidophilic cytoplasm than those of the control group. In the hepatocytes of the diabetic group, hydropic degeneration was noted; the perinuclear cytoplasm of these hepatocytes was dense or granular and the nuclei were abnormally shaped and basophilic dyed. In some hepatocytes, the nuclei were peripherally localized and contained distinct nucleoli. Moreover, in the light microscopical sections of the diabetic group, there were abundant kupffer cells with acidophilic cytoplasm and dense, basophilic nuclei. Significant mononuclear cell infiltration was observed both between the cell plates and around the vessels in the diabetic control group (Group 2; Figs. 4 and 5B). Most notably in the portal area, there was cytoplasmic shrinkage and both dark and small acidophilic cell nuclei (Fig. 5C, D, and E). Moreover, many necrotic foci were found in the sections of the diabetic control group (Group 2; Fig. 5E). Histological views of the telmisartan-treated diabetic rats (Group 3; Fig. 6) were similar to those of the non-diabetic healthy group (Fig. 4).

3.2.2. Immunohistochemical and electron microscopic examinations

According to histological investigations in immunohistochemically TGF-β stained liver sections of telmisartan-treated diabetic rats (Group 3; Fig. 7A and C), we observed no immunoreactivity in either the cytoplasm or the nuclei of the hepatocytes (Fig. 7C). However, we did note weak reactivity in the cytoplasm of a few interstitial and endothelial cells (Fig. 7A and C). In the diabetic control group, we detected strong immunoreactivity for TGF-β in not only the connective tissue of the liver but also the cytoplasm of the hepatocytes (Fig. 7B and D). Furthermore, there was dense immunoreactivity in most of the kupffer cells (Fig. 7B and D).

In the electron microscope examination of all three groups (Fig. 8), we observed normal ultrastructures in liver sections from Group 1 (Fig. 8A). In the diabetic group, we defined more fat droplets and free ribosomes, enlarged smooth endoplasmic reticuli, many intracytoplasmic vacuoles, swollen mitochondria with irregular membranes, and perinuclear edema (Fig. 8B). In this group, we also observed flattened microvilli on the sinusoidal side of the hepatocytes, hepatocyte nuclei with irregular contours, and activated Kupffer cells with large and dense nuclei containing an increased number of lysosomes and cytoplasmic vacuoles (Fig. 8B). In some sections from Group 3, normal liver ultrastructures similar to those seen in the sections from Group 1 were noted (Fig. 8C).

Table 4
Geometric volumes of hepatocyte nuclei in all groups.

Groups	Geometric volumes of hepatocytes' nuclei (µm³)	Standard deviation	Standard error mean
Group 1 ($N=5$)	263.2666	17.019	7.611
Group 2 ($N=5$)	292.1984	41.061	18.363
Group 3 ($N=5$)	285.3416	87.088	38.947

4. Discussion

In the current study, we evaluated the effects of telmisartan on liver cells in alloxan-induced diabetic rats using three distinct methods. We estimated the number of hepatocytes and determined whether or not this number changed in rats depending on telmisartan treatment and diabetes mellitus. We also determined that the livers of diabetic rats that were and were not treated with telmisartan produced TGF-β1. We further determined the localization of TGF-β1 in hepatocytes after it was produced.

The circulating renin–angiotensin system has been known as a hormonal system that plays a crucial role in hemodynamic regulation by virtue of its vasoconstriction and sodium retention (Campbelli, 1987; Leung et al., 2003). This system produces a physiologically active peptide, angiotensin-II, which functions on target organs by binding to its type I and type II receptors (De Gasparo et al., 2000). It has been considered that activated angiotensin AT_1 receptors play an important role in the regulation of intracellular Ca2+ homeostasis, cell contraction, proliferation, and induction of proinflammatory cytokines and pro-fibrogenic actions (Leung et al., 2003; De Gasparo, 2002). For this reason, it has been thought that inhibiting the renin–angiotensin system by blocking its receptors will provide a beneficial pathway for treating cardiovascular, renal, and hormonal diseases and metabolic syndrome (Van Guilder et al., 2008; Adler et al., 2007; Waeber and Feihl, 2007). Some recent studies have shown that various tissues or organs such as the carotid body, epididymis, and pancreas express renin–angiotensin system components to regulate physiological and pathophysiological conditions (Campbell, 1986; Leung et al., 2000, 2001; Leung and Carlsson, 2001). It has been asserted in experimental studies that the renin–angiotensin system plays a major role in the pathogenesis of liver cirrhosis due to increasing intrahepatic pressure and because angiotensin AT_1 receptor blockers may counteract the effects of angiotensin-II (Rockey and Weisiger, 1996; Leung et al., 2003; Schneider et al., 1999).

Our stereological results demonstrate that administration of telmisartan resulted in significant differences with regard to the numerical density of hepatocytes in rat livers when compared to the diabetic control group. Furthermore, the difference between the diabetic control group and the non-diabetic healthy group was significant. A 12% increase in the numerical density values of hepatocytes was found in the diabetic control group when compared to the non-diabetic healthy

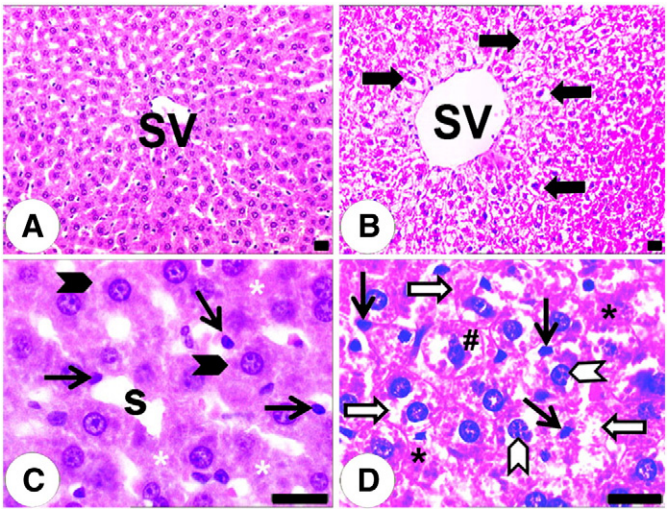

Fig. 4. Light micrographs of non-diabetic healthy group (A, C) and diabetic group (B, D). **SV,** central vein; **s,** sinusoid; thick arrow, hepatocytes with dense peri-nuclear cytoplasm and hydropic degeneration; black arrowhead, hepatocytes with healthy nuclei and cytoplasm; thin arrow, Kupffer cells (these cells were healthy appearance in C and they had asidophilic cytoplasm and dense basophilic nuclei in D); transparent arrow, hepatocytes with hydropic degeneration; transparent arrowhead, nuclei with distinct nucleolus; **#,** hepatocytes with abnormal shaped nuclei and hydropic degeneration; asterisks sign healthy (white) and asidophilic cytoplasm (black). Dye: H-E; Bars: 30 μm.

group. Finally, we determined that there was a significant difference between the diabetic control group and the diabetic rats treated with telmisartan.

In insulin-resistant states such as type II diabetes mellitus, it has been claimed that the number of hepatocytes increases in a condition that is known as hepatic hyperplasia. Certainly, this probably occurs to regulate energy imbalance (Yang et al., 2001). We suggest that the number of increased cells not only decreases the trophic factor, which may be loaded apoptosis, but also causes an increase in the number of mitochondria, which are major sources of oxidants. Indeed, diabetes has been previously shown to increase mitochondrial oxidative stress (Prow et al., 2008).

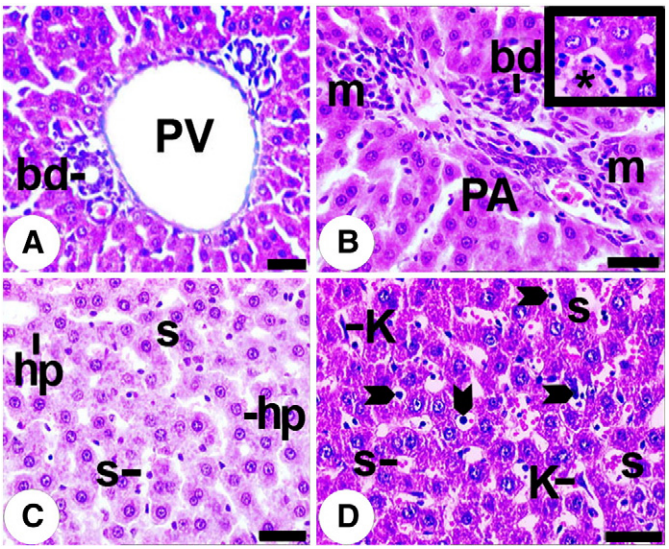

Fig. 5. Light micrographs of non-diabetic healthy group (A, C) and diabetic group (B, D). PV, portal vein; bd, bile duct; **s,** sinusoid; PA, portal area; m, mononuclear cell infiltration; hp, hepatocytes plates; K, Kupffer cell; asterisk, necrotic foci with mononuclear cell infiltration; black arrowhead sign mononuclear cells in sinusoids. Dye: H-E; Bars: 60 μm.

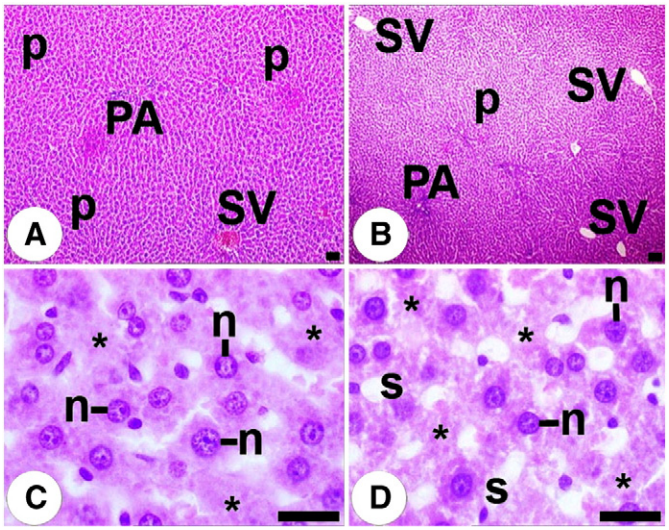

Fig. 6. Light micrographs of non-diabetic healthy group (A, C) and diabetic group treated with telmisartan (B, D). SV, central vein; p, parenchyma; s, sinusoid; PA, portal area; n, nucleus of hepatocytes, asterisks sign cytoplasm of hepatocytes but more metacromatic ergastoplasm were seen D than that of C. Dye: H-E; Bars: 40 μm.

Whichever mechanisms mentioned in the literature are responsible for these changes, a lot of findings related to proliferations, apoptosis, and degeneration overlap with our stereological and histopathological findings in the diabetic control group.

Our other stereological finding was related to the geometric volume, or cell-body volume, of hepatocytes. We did not determine any differences between the non-diabetic healthy group and the diabetic control group, between the non-diabetic healthy group and the diabetic rats treated with telmisartan, nor between the diabetic control group and the diabetic rats treated with telmisartan.

When we review our findings, we discover that in alloxan-induced diabetic rats (type I diabetes mellitus), the numerical density of hepatocytes increased compared to both the non-diabetic healthy group and the diabetic rats treated with telmisartan.

We can say that although type I diabetes mellitus causes an increase in both the number and volume of hepatocytes, telmisartan

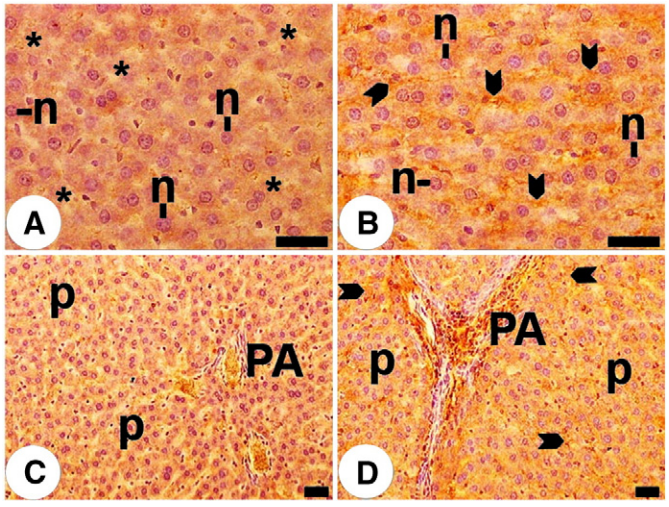

Fig. 7. Light micrographs of diabetic group treated with telmisartan (A, C) and diabetic group (B, D) obtained from immunohistochemically stained for TGF-β. PA, portal area; p, parenchyma; n, nucleus of hepatocytes, asterisks sign cytoplasm of hepatocytes wasn't observed immune positive; black arrowhead, cytoplasm of hepatocytes have immune positive cytoplasm for TGF-β; Dye: H-E; Bars: 50 μm.

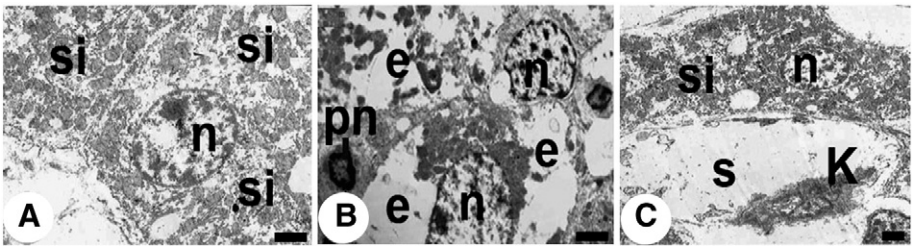

Fig. 8. Electron micrographs of non-diabetic healthy group (A), diabetic group (B) and diabetic group treated with telmisartan (C). n, nucleus, si, cytoplasm; s, sinusoid; pn, picnotic nucleus; e, cytoplasmic edema; K sign multinuclear Kupffer cell. Dye: Uranyl acetate-Lead citrate; Bars: 2 μm.

exhibits a slight extension tendency in hepatocyte volume ($P>0.05$) instead of increasing the number of hepatocytes.

Hepatocytes are structures that are vital to metabolic, endocrine, and exocrine functions. Impairment of any of these functions may cause a serious insufficiency in the regulation of lipid metabolism or glucose homeostasis (Brock and Dorman, 2007).

At this stage, the following question must be addressed: what is necessary for the proper functioning of the above processes and what happens if those necessities are not met? For example, insulin resistance can be described as a state in which a given concentration of insulin fails to produce the expected biological response (Brock and Dorman, 2007). The expected effects of insulin occur mainly via insulin receptors or the insulin-like growth factor 1 receptor; binding to those receptors initiates tyrosine kinase signaling and activates effectors within various target cells, mediating glucose metabolism and a host of other responses (Michael and Ruderman, 2001). Of course, in a state of insulin resistance, insulin doesn't produce those effects (Brock and Dorman, 2007). Insulin resistance may result in a lot of undesirable conditions relating to metabolism, such as increased storage of fatty acids via impairment of the insulin signaling pathway, inhibition of mitochondrial β-oxidation, and changes in hepatic glucose metabolism via elevations in postprandial glucose production (Gujral et al., 2004; Saleh et al., 1999; Arner, 2002). When glucose homeostasis and lipid metabolism fail in hepatocytes because of insulin resistance or some other cause, this situation may result in increased oxidant production, which has been proven to have many side effects that cause cell dysfunction (Brock and Dorman, 2007).

Diabetes-related mitochondrial oxidative stress is known (Prow et al., 2008) and may be one of the factors responsible for changes in the number and volume of hepatocytes. The antioxidative effects of many angiotensin AT_1 receptor blockers such as telmisartan have been studied (Cianchetti et al., 2007), and we think that the beneficial effects of telmisartan on hepatocytes can be partially based on the drug's antioxidative properties.

By taking all of the above results into consideration, we tried to explain the positive effects of telmisartan on diabetic rats in terms of the fact that the drug is an angiotensin AT_1 receptor blocker that has antioxidative effects. However, an important point that should not be overlooked is telmisartan's activation of PPAR-γ. Indeed, PPAR-γ is important in that it has been connected to increased insulin sensitivity and HDL levels and decreased inflammation, oxidative stress, cell proliferation, migration, and fatty acid and triglyceride levels (Kurtz and Pravenec, 2004). Amelioration of our stereological findings on diabetic rat livers by telmisartan could be due to an improvement in the diabetes-related fatty acid metabolism disorder caused by PPAR-γ. Furthermore, its antiinflammatory and antioxidative properties undoubtedly also produce beneficial effects.

In this study, the majority of our histopathological findings were confirmed by the stereological results. Histopathological evaluations under both light and electron microscopy of the non-diabetic healthy group and diabetic group treated with telmisartan were normal. However, the diabetic control group had abnormalities (hepatocytes containing unstained cytoplasm, hypertrophied cell bodies, irregularities in the boundaries of central veins, a lot of apoptotic cell nuclei, many large vacuoles in the cytoplasm, and dense inflammatory cells in the portal areas) that indicated cellular degeneration.

Our results, obtained by different examination methods, exhibit a major confirmation of our ultrastructural findings. Although the ultrastructural appearance of the non-diabetic healthy group was similar to that of diabetic rats treated with telmisartan, the diabetic control group clearly showed many pathological changes that indicate cellular damage such as enlarged smooth endoplasmic reticuli, intracytoplasmic vacuoles, and swollen mitochondria in the hepatocytes.

In this study, our most interesting findings were not related to the production of TGF-β1, but rather to the localization of TGF-β1 in liver parenchyma and other support structures called stroma, depending on the experimental group. Although neither the diabetic group treated with telmisartan nor the non-diabetic healthy group demonstrated immunoreactivity in the cytoplasm or nuclei of hepatocytes, the diabetic control group showed strong immunoreactivity in both the cytoplasm of hepatocytes and the Kupffer cells, which are considered components of hepatic stellate cells (Gressner et al., 2007; Marcos et al., 2003). The role of hepatic stellate cells, the principal fibrogenic cell type in the liver, as valid targets for antifibrotic therapy is beginning to be studied (Friedman, 1999). In the literature, it is suggested that other components of hepatic stellate cells, the non-parenchymal structures, are ITO and endothelial cells (Gressner et al., 2007). Indeed, it has been shown that the accumulation of extracellular matrix such as collagen, glycoproteins, and proteoglycans in the liver plays a major role in hepatic fibrosis. The most prominent producers of extracellular matrix are hepatic stellate cells, and especially Kupffer cells, following liver injury due to an autoimmune attack, Hepatitis C or B viral infections, drug toxins, cholestasis, or metabolic disease (Gressner et al., 2007; Wang et al., 1996; Schuppan et al., 2001; Gressner and Weiskirchen, 2006). Activated human stellate cells have been found to express angiotensin AT_1 receptor, and the binding of angiotensin-II to angiotensin AT_1 receptor induces the contraction and proliferation of those cells (Bataller et al., 2000). It has been shown that angiotensin-II stimulates oxidative stress through endothelin-1. Thus, lipid peroxidation has been induced in hepatocytes and hepatic steatosis has in turn been induced in an animal model (Ohishi et al., 2001). These in vitro findings suggest that angiotensin-II plays a role in mediating and exacerbating liver injury, and that it can be controlled with a converting enzyme. During the process of fibrosis, the role of TGF-β1 is considered to be the most important. Angiotensin-II increases TGF-β1 production, which in turn stimulates extracellular matrix production, up-regulates cell-matrix cell adhesion molecules, inhibits degradation, and induces the chemo attraction of macrophages and fibroblasts (Paizis et al., 2001).

In summary, the liver is altered morphologically and functionally during diabetes. Because diabetes is considered a major risk factor for hepatic fibrosis, we have endeavored to show that telmisartan may reduce hepatic injuries resulting from type I diabetes mellitus. At clinically realistic doses, telmisartan strongly inhibits lesion development and liver fibrogenesis by suppressing activated hepatic stellate

cells with concomitant TGF-β1 down-regulation. Because telmisartan is widely used in clinical practice without serious side effects, it may provide an effective new strategy for antifibrosis therapy during hypertension with diabetes. From the translational research point of view, treatment with telmisartan, even at doses that did not lower blood pressure, improved the remodeling of the liver, which is an important target for the pathological consequences of hypertensive patients with or without diabetes mellitus.

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