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Original Article

Possible Hepatoprotective Effect of Telmisartan in a Rat Model of Metabolic Syndrome

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ABSTRACT

Metabolic syndrome (MetS), a complex of highly debilitating disorders including hypertension, diabetes mellitus, and dyslipidemia, is associated with the development of visceral obesity. Telmisartan, an angiotensin II receptor blockers and partial agonist on peroxisome proliferative activated receptor-gamma (PPAR-γ), showed a promising protective effect on MetS. The present study investigated the possible hepatic protective effect of telmisartan in an albino rat model of MetS with a focus on some proinflammatory cytokines: MCP-1 and TNF-α protein in hepatic tissue homogenates. Adult albino rats were divided into three groups and treated for 8 weeks as follow: group 1 fed standard rat's diet and served as normal control group; group 2 fed high carbohydrate-high Fat Diet (HCHF); group 3 fed high carbohydrate-high Fat Diet (HCHF); plus telmisartan at a dose of 5 mg/kg/day by oral gavage. A pilot study was done on the effect of temisartan alone on these markers under the regular chow diet condition and no changes were reported on their levels compared to group (1). Administration of telmisartan in group (3) showed a significant decrease in liver index, a decrease in hepatic triglycerides, a decrease in serum ALT enzyme and a significant reduction in hepatic MCP-1 and TNF-α protein with a significant reduction in the elevated non-invasive mean blood pressure. The results indicate that telmisartan could be considered as a potential adjuvant therapy of MetS.

Keywords: metabolic syndrome, Telmisartan, liver, albino rats.

INTRODUCTION

The metabolic syndrome (MetS) is characterized by obesity concomitant with other metabolic abnormalities such as hypertriglyceridemia, reduced high-density lipoprotein level (HDL), elevated blood pressure and raised fasting glucose level. It is imperative to develop animal models of MetS to help determining the possible pathophysiology and the different mechanisms of suggested drugs to be used in the treatment of this syndrome (Kawamoto *et al*., 2005).

MetS is associated with disturbances in the lipid metabolism together with affection of many pathways by which inflammation is activated with its negative impact on many vital organs. The World Health Organization (WHO)

and National Cholesterol Education Program–Adult Treatment Panel III (NCEP–ATP III) guidelines determine the criteria of MetS to be an association of (abdominal obesity, low HDL, hypertriglyceridemia, hypertension and dysglycemia) (Roberts and Barnard, 2005).

A Clinical evidence of an evolving role of inflammatory mediators in patients with the metabolic syndrome that help in the development of a pathological damage such as atherosclerosis, oxidative stress, obesity (particularly visceral adiposity), insulin resistance, and disturbances in the immune system are important targets for drugs used in treatment of MetS. In light of recent developments, interventions to decrease oxidative stress, inflammation and insulin resistance are the aim of the work of many clinical trials done on patients suffering from MetS (Kintscher *et al*., 2007).

Pretreatment by telmisartan (as an angiotensin II receptor blocker type 1(AT1)) to isolated neuronal PC12 cells, exposed to neurotoxicity by high glucose oxidative damage, was proved to elicit an anti-oxidant effect via increasing activity of reduced glutathione, superoxide dismutase enzyme and catalase enzyme as well as an inhibition of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase according to Eslami *et al*., (2014).

Several therapies of MetS, including diet and antioxidants have been tried to treat patients, However, at present there are no approved drugs that can reliably reduce all of the metabolic risk factors over the long term especially on the liver, and so there is growing interest in therapeutic strategies that might target multiple risk factors more effectively, thereby minimizing problems with polypharmacy (Grundy, 2006).

A pilot study, that was conducted in our laboratory, revealed that telmisartan might serve as a potential therapy for non-alcoholic fatty liver disease (NAFLD) when it was administered by oral gavage for 8 weeks to wister rats on cholesterol diet. The aim of the present study to investigate the possible hepatic protective effect of telmisartan in an albino rat model of MetS with a focus on some proinflammatory cytokines: MCP-1 and TNF-α protein in hepatic tissue homogenates.

MATERIALS AND METHODS

Materials

Telmisartan was obtained as a powder from Boehringer Ingelheim, International (GmbH, Germany); lard fat was obtained from special Egyptian Commercial Factory, Cairo, Egypt. Fructose and cholic acid were obtained from Sigma chemical (St Louis, MO, USA); Triglyceride, and ALT kits were obtained from Biodiagnostic (Cairo, Egypt). All other chemicals were obtained from Sigma chemical (St Louis, MO, USA).

Animals

Adult Wister Albino rats weighing 150-200 g were obtained from animal house (Faculty of Medicine, Missiry Medical Research Center, Ain Shams University, Cairo, Egypt). They were housed in pharmacology laboratory, Faculty of Medicine, Ain Shams University, Cairo, Egypt under controlled standard conditions of temperature and light. Group (1) was allowed a free access to standard laboratory chow (El-Gomhoryaa Company, Cairo, Egypt) and water. While groups (2&3) had a special diet regimen that is mentioned below to induce a model of metabolic syndrome (MetS).

The effect of telmisartan alone on these parameters in animals on regular chow diet were examined in a pilot study before the whole experimental study was initiated. No changes were reported on their levels compared to control normal fed diet group (1).

Experimental protocol and rats'grouping

After laboratory acclimatization for 1 week, the rats were divided into three experimental groups (each group n=7 rats). The duration of the study was 8 weeks as follow:

Group (1): rats on standard diet, received an equivalent volume of saline for 8 weeks (as the solvent of telmisartan) and served as control group.

Group (2): received high carbohydrate-high Fat Diet (HCHF) diet, as a model of MetS, and they were administered by equivalent volume of saline for 8 weeks, being the solvent of telmisartan

Group (3): telmisartan- treated (5 mg/kg/day) for 8 weeks by oral gavage according to Wienen *et al*., (2001), the drug was dissolved in saline.

This group will be fed on HCHF diet as group (2).

Ethics

All procedures will be conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals, as well as the guidelines of the Animal Welfare Act.

Induction of a rat model of metabolic syndrome (MetS) in group (3)

HCHF consists of a mixture of both fructose and lard in the following concentrations: fructose (52%) + lard (24%) mixed + cholic acid together with 1% dried broken bread in the form of small pastes placed in cages of rats. This will be accompanied by supplementation of 25% fructose in drinking water. This type of food and sweetened water will be supplied daily for 8 weeks to rats of group (3) (Poudya *et al*., 2010; Panchal and Brown, 2011).

Measurements

At the end of the 8th week, all rats were anesthetized by intraperitoneal injection of urethane (1g/kg ip for each rat) and blood samples were collected from abdominal aorta and processed for biochemical measurements. Then, rats were sacrificed and their livers were rapidly collected and homogenized to measure MCP-1 and TNF-α in tissue homogenates.

1- Measurement of liver index:
   Liver index was calculated from the equation: (liver weight/body weight) ×100 (Inaba *et al*., 2012).
2- Serum levels of alanine amine transferasenase (ALT):
   Serum level of ALT was measured using ALT biochemical kit on biochemistry automatic analyzer (Hitachi7600).
3- liver tissue content of triglycerides:
   Triglyceride was assayed in hepatic tissue using commercially available kits after lipid extraction as described by (Folch *et al*., 1957).
4- MCP-1 and TNF-α protein in hepatic tissue homogenate
   MCP-1 level was determined using ELISA kit for rat MCP-1 (Camarillo, CA, USA). TNF-α protein was determined using commercially available rat TNF-α ELISA kit (Raybiotechmique®, USA) according to the company's instructions.
5- Protein Determination
   The protein content of the liver homogenates was measured spectrophotometrically by Bradford method. TG hepatic tissue level was expressed in mg/g tissue protein while both levels of MCP-1 and TNF-α were expressed in pg/mg tissue protein.
6- Measurement of non-invasive mean blood pressure (mean BP) in all rats of tested groups as indicated by manual of the AD instrument (PowerLab) as follows (Shams Eldine, 2015):

The Blood Pressure Analysis View displays the average cycles and the mean BP was calculated by the module. In the Analysis pane of the Blood Press ure Settings dialog you set the number of Cycles to average. The values of mean non-invasive blood Pressure of all tested rats were recorded on a window of the monitor of Power Lab AD Instrument.

Data Analysis

Results are expressed as mean ±SD (Standard Deviation). Statistical analysis was performed by analysis of variance followed by Tukey's *post hoc* using GraphPad Prism version 3.00 for Windows 97 (Graph Pad Software, San Diego, CA, U.S.A.). Differences with $p< 0.05$ were considered to be statistically significant

RESULTS

Effect of telmisartan on liver index in HCHF model of MetS in albino rats

Figure 1 illustrates that liver index was significantly ($p<0.05$) increased in HCHF non-treated group (2) compared with control group (1). It was significantly ($p<0.05$) decreased in telmisartan-treated group compared with HCHF non-treated group (2)

Effect of single dose oral gavage of telmisartan for 8 weeks on serum levels of alanine amine transferase (ALT) in U/L

Table 1 shows a significant ($p<0.05$) reduction in serum ALT (IU/L) and triglycerides (TGs) in hepatic homogenates of HCHF telmisartan-treated rats group (3) compared to HCHF-non-treated group (2). Both parameters

were decreased to values in telmisartan-treated group that were comparable to control group (1).

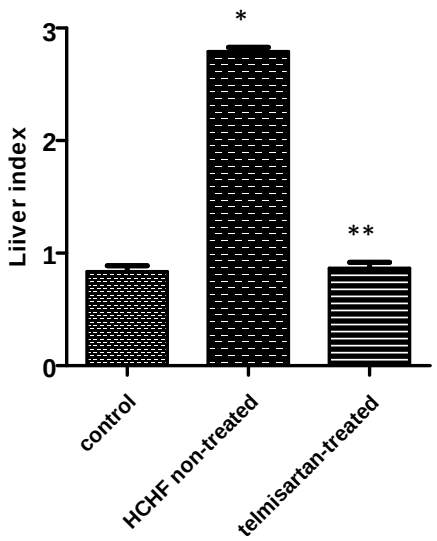

Figure (1): Changes in liver index after 8 weeks of single daily oral gavage of telmisartan in HCHF-fed rat's model of MetS (group 3) compared to control group (1) and HCHF non-treated group (2).

* $p<0.05$= significant increase in liver index in HCHF non-treated group (2) compared to the control albino rats group (1).

** $p<0.05$ = significant reduction in liver index in HCHF telmisartan-treated group (3) compared to the HCHF –non treated albino rats group (2).

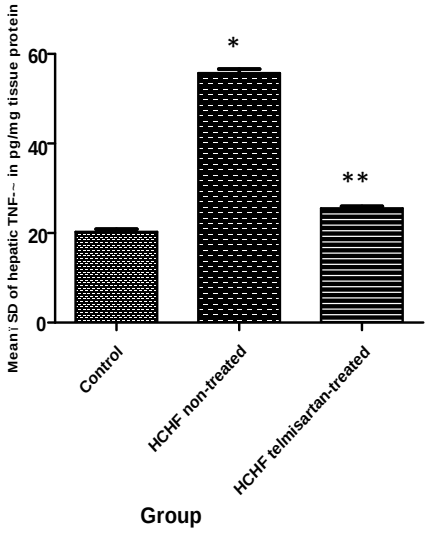

Figure 2: Changes in levels of TNF-α (pg/mg hepatic tissue protein) after 8 weeks of single daily oral gavage of telmisartan in HCHF-fed rats model of MetS (group 3) compared to control group (1) and HCHF non-treated group (2).

* $p<0.05$= significant increase in levels of both TNF-α in HCHF non-treated group (2) compared to the control albino rats group (1).

** $p<0.05$ = significant reduction in levels of both TNF-α in HCHF telmisartan-treated group (3) compared to the HCHF –non treated albino rats group (2).

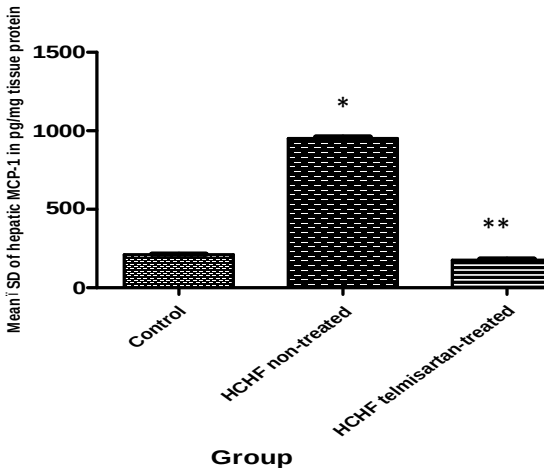

Figure 3: Changes in levels of MCP-1 (pg/mg hepatic tissue protein) after 8 weeks of single daily oral gavage of telmisartan in HCHF-fed rat's model of MetS (group 3) compared to control group (1) and HCHF non-treated group (2).

* $p<0.05$= significant increase in levels of MCP-1 (pg/mg hepatic tissue protein) in HCHF non-treated group (2) compared to the control albino rats group (1).

** $p<0.05$ = significant reduction in levels of MCP-1 (pg/mg hepatic tissue protein) in HCHF telmisartan-treated group (3) compared to the HCHF –non treated albino rats group (2).

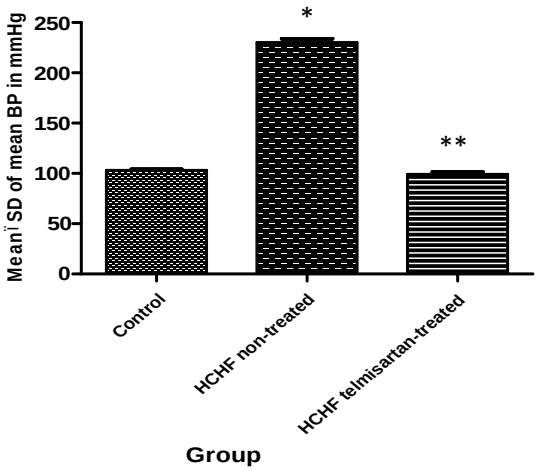

Figure 4: Changes in non-invasive mean BP (mmHg) after 8 weeks of single daily oral gavage of telmisartan in HCHF-fed rat's model of MetS (group 3) compared to control group (1) and HCHF non-treated group (2).

*$p<0.05$= significant increase in non-invasive mean BP (mmHg) in HCHF non-treated group (2) compared to the control albino rats group (1).

** $p<0.05$ = significant reduction in non-invasive mean BP (mmHg) in HCHF telmisartan-treated group (3) compared to the HCHF –non treated albino rats group (2).

Table (1): Serum levels of alanine amine transferase (ALT) in U/L and hepatic tissue triglycerides in all tested rats'groups

Parameters	Mean ±SD		
	Control	HCHF non-treated	HCHF telmisartan-treated
Serum ALT (IU/L)	41.75±2.4	265.9±12.1*	32.5±2.0**
Liver TG (mg/g tissue)	11.9±0.19	45.5±5.9*	7.5±0.7**

* p<0.05= significant increase in both serum ALT enzyme and in hepatic Triglycerides (TG) in HCHF-fed non-treated group compared to the control non-treated albino rats group.

** p<0.05 = significant reduction in both serum ALT enzyme and in hepatic Triglycerides (TG) in HCHF-fed non-treated group compared to the HCHF non-treated albino rats group.

Effect of telmisartan on hepatic proinflammatory cytokines: MCP-1 and TNF-α levels (pg/mg hepatic tissue protein) in HCHF-rat model of metabolic syndrome

HCHF non-treated rats (group 2) had a significant (P<0.05) increase in hepatic TNF-α and MCP-1 levels as compared with control group (1). HCHF Telmisartan-treated group (3) had significantly reduced MPC-1 and TNF-α levels (P<0.05) in comparison to HCHF non-treated group (2) (Figures 2&3).

Effects of High fat diet on Mean non-invasive blood pressure (mean BP) in the presence and absence of telmisartan

Figure 4 depicts that HCHF telmisartan-treated group (3) for 8 weeks at a dose of 5 mg/kg /day by oral gavage significantly (p< 0.05) lowered mean BP compared to HCHF non- treated group (2).

DISCUSSION

There is a strong evidence that there is an excessive accumulation of triglycerides in hepatocytes that is accompanied by lipid peroxidation and over-production of inflammatory cytokines in metabolic syndrome. These changes lead to a harmful liver damage in this syndrome (Poudyal *et al*, 2010).

The present study investigated the effect of telmisartan on hepatic index, serum ALT enzyme level, hepatic tissue triglycerides and some pro-inflammatory cytokines as TNFα and MCP-1 in high-carbohydrate High fat (HCHF) diet to induce a rat model of metabolic syndrome. The study demonstrated a significant increase in liver index, with a significant reduction in both serum ALT enzyme and hepatic tissue contents of triglycerides, in addition to a significant reduction in some pro-inflammatory cytokines, which are TNF-α and MCP-1 in the telmisartan-treated group (3) with HCHF diet with a significant reduction in the elevated non-invasive mean blood pressure being one of the angiotensin receptor inhibitor. Telmisartan was found, in different studies, to induce a modulating and hepatoprotective effect in non-alcoholic fatty liver disease (NAFLD) (Fan *et al*., 2003; Benson *et al*., 2004; Fujita *et al*., 2007).

The hepatocyte is injured and leaks ALT enzyme in metabolic syndrome. The laboratory measurement of this enzyme is a useful test to follow-up the status of liver injury (Hennes *et al*., 1990). George *et al*., (2003) reported that telmisartan showed almost normalization of serum ALT in a rat model of chronic steato-hepatitis which is similar to the result of serum ALT measurement in the present study of HCHF-model of metabolic syndrome in rats.

Administration of telmisartan to rats on high cholesterol diet showed a significant decrease in liver triglycerides (Adaramoye *et al*., 2005). Fujita *et al*., (2007) explained this finding to be due to reduction in both the biosynthesis and redistribution of cholesterol among the lipoprotein molecules which suggest that telmisartan possesses a triglyceride lowering effect in hepatic injury due to high fat diet or metabolic conditions similar to the metabolic syndrome.

Additionally, it can be noticed from the present results that the hepatic levels of pro-inflammatory cytokines, MCP-1 and TNF-α, were significantly increased in HCHF non-treated group (2). Oral gavage of telmisartan for 8 weeks reduced levels of MCP-1 and TNF-α which might provide an evidence of its possible anti-inflammatory effect in this rat model of metabolic syndrome.

Tagaki *et al.*, (2013) revealed via an interesting study that Telmisartan, by its unique peroxisome proliferator-activated receptor-gamma-inducing property, improves metabolic parameters in metabolic syndrome. The findings were reported from the first meta-analysis of

randomized controlled trials (RCTs) collected from MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials using PubMed and OVID. Ten reports of RCTs, enrolling a total of 546 patients with metabolic syndrome, were identified and included. Pooled analysis suggested significant reductions in the following: % changes of fasting glucose, glycosylated hemoglobin and homeostasis model assessment index with a significant increase in % changes of adiponectin among patients with metabolic syndrome randomized to telmisartan versus control therapy. This study concludes that telmisartan therapy would be a powerful drug to significantly improve metabolic disorders in patients with metabolic syndrome.

CONCLUSION

In addition to its significant reducing effect on hepatic tissue triglyceride and non-invasive mean BP; telmisartan would provide a hepatic protective effect in HCHF model of metabolic syndrome by restoring serum level of ALT enzyme and liver index. It also could reduce the inflammatory process that accompany this syndrome by reducing the levels of pro-inflammatory cytokines as TNF-α and MCP-1.

DISCLOSURE

The author reports no conflicts of interest in this work.

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