Original Article

Effect of Telmisartan, Angiotensin II Receptor Antagonist, on Metabolic Profile in Fructose-Induced Hypertensive, Hyperinsulinemic, Hyperlipidemic Rats

Yehuda KAMARI^{1,2)}, Ayelet HARARI²⁾, Aviv SHAISH²⁾, Edna PELEG¹⁾,
Yehonatan SHARABI¹⁾, Dror HARATS²⁾, and Ehud GROSSMAN¹⁾

The metabolic syndrome (MS) is a common risk factor for cardiovascular disease and type-2 diabetes. Recently, telmisartan, an angiotensin II receptor antagonist that has an antihypertensive effect, has been reported to be a partial peroxisome proliferator-activated receptor γ (PPAR γ) agonist. The anti-diabetic hormone adiponectin has been recognized as a marker of *in vivo* PPAR γ activation. Therefore, we studied telmisartan's effect on the metabolic profile and adiponectin levels in a fructose-induced hypertensive, hyperinsulinemic, hyperlipidemic rat model. Twenty-four male Sprague-Dawley rats were divided into three groups (eight in each). One group of control rats was fed standard chow for 5 weeks while a second was fed a fructose-enriched diet. A third group was fed a fructose-enriched diet for 5 weeks and treated with telmisartan 5 mg/kg/day during the last 2 weeks. Fructose feeding increased systolic blood pressure (mean $\pm$ SEM), from 130 $\pm$ 1 to 148 $\pm$ 2 mmHg, insulin from 0.26 $\pm$ 0.03 to 0.68 $\pm$ 0.08 ng/mL, and triglycerides from 102 $\pm$ 6 to 285 $\pm$ 23 mg/dL (p <0.05 for all variables). Telmisartan treatment reversed these effects and reduced blood pressure to 125 $\pm$ 2 mmHg, insulin levels to 0.41 $\pm$ 0.07 ng/mL, and triglycerides to 146 $\pm$ 18 mg/dL (p <0.05 for all variables), while attenuating the increase in body weight during weeks 3 to 5. In contrast, telmisartan did not affect plasma adiponectin levels. In conclusion, although telmisartan is considered a partial PPAR γ agonist, its beneficial effect in the fructose-induced hypertension, hypertriglyceridemia, and hyperinsulinemia rat model is apparently not mediated by adiponectin elevation but rather by direct inhibition of AT1 receptor. (*Hypertens Res* 2008; 31: 135–140)

Key Words: metabolic syndrome, telmisartan, adiponectin, peroxisome proliferator-activated receptor γ

Introduction

The metabolic syndrome (MS), characterized by the clustering of hypertension, insulin resistance, and dyslipidemia, is a common risk factor for cardiovascular disease and type-2 diabetes (1, 2). To achieve optimal prevention of cardiovascular outcomes in hypertensive patients, a multi-factorial approach

is required, combining lifestyle modification, blood pressure lowering, and control of dyslipidemia. In addition, prevention of type-2 diabetes has become recognized as an important component of cardiovascular risk reduction in patients with essential hypertension (3, 4). Since insulin resistance is the core malfunction that leads eventually to the development of type-2 diabetes, improving insulin sensitivity can be beneficial and contribute to lowering the risk for developing diabe-

From the ¹⁾Hypertension Unit and ²⁾Institute of Lipid and Atherosclerosis Research, Sheba Medical Center, Tel Hashomer and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Address for Reprints: Ehud Grossman, M.D., Internal Medicine D and Hypertension Unit, The Chaim Sheba Medical Center, Tel Hashomer 52621, Israel. E-mail: grosse@post.tau.ac.il

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tes. Activation of the renin-angiotensin system (RAS) contributes to the development of insulin resistance. β -Cell dysfunction due to angiotensin-mediated increases in oxidative stress, inflammation, and free fatty acids concentrations, as well as glucotoxicity, lipotoxicity, and advanced glycation, potentially contribute to insulin resistance related to RAS activation (5). Although a number of clinical and experimental studies suggested that angiotensin II receptor antagonists can improve insulin sensitivity and decrease the incidence of new-onset type-2 diabetes in patients with hypertension, the exact mechanisms underlying these beneficial effects are not fully understood (6–8).

Adiponectin has been shown to have insulin-sensitizing properties in murine models (9–13). Several studies have shown that some of the beneficial effects of peroxisome proliferator-activated receptor γ (PPAR γ) activation are mediated by the increase in adiponectin levels (14, 15). A few recent studies found that telmisartan, an angiotensin II type 1 (AT1) receptor antagonist, is a partial PPAR γ nuclear receptor agonist (16–19). However, there are conflicting data regarding telmisartan's *in vivo* effects on metabolic parameters in animal models of the metabolic syndrome (16, 20). Therefore, we assessed telmisartan's effects on adiponectin and metabolic indices in fructose-induced hypertensive, hyperinsulinemic, hyperlipidemic rats.

Methods

Twenty-four male Sprague-Dawley rats weighing 220 to 250 g were used in the study. A control group ($n=8$) was fed a normal chow diet. Sixteen rats were fed a fructose-enriched diet (Teklad-Harlan, Madison, USA) consisting of 21% protein, 5% fat, 60% carbohydrate, 0.49% sodium, and 0.49% potassium, for 5 weeks. The rats fed the fructose-enriched diet (FED) were further subdivided into two groups: one group of 8 rats was given FED for 5 weeks (nontreated group) while the other group of 8 rats was given FED for 5 weeks, with telmisartan 5 mg/kg/day administered in the last 2 weeks of the experiment (treated group).

The experiments were conducted according to the Guidelines for Animal Care and Treatment of the hospital's Animal Ethics Committee. The rats were maintained in a temperature-controlled room (22°C) and kept on a 14/10 light/dark cycle. Telmisartan was administered by dissolving the commercially available drug in the drinking water at an initial concentration of 40 mg/L. Because fluid intake was not scaled linearly with body weight, drug intake based on body weight tended to decline over time. Therefore, the drug concentration in the drinking water was increased during the course of the study every week to maintain the scheduled dosing. The fluid intake was similar in all the groups. Body weight was measured before the start of the experiment and then weekly. Systolic blood pressure (SBP) was measured weekly in conscious rats by the indirect tail-cuff method using an electrophymo-

manometer and a pneumatic pulse transducer (Narco Biosystems, Houston, USA). The mean of five consecutive readings was recorded.

Blood samples from a retro-orbital sinus puncture were taken, under light anesthesia with isoflurane after 5 h of fasting, at the beginning of the experiment and after 3 and 5 weeks. Plasma adiponectin concentrations were determined using an RIA kit (Linco Research, Billerica, USA). Insulin levels were assessed using a rat insulin ELISA kit (Mercodia, Uppsala, Sweden), and triglyceride levels were assayed with an automated analyzer of an enzymatic colorimetric reaction (Olympus AU 270, Hamburg, Germany).

Data Analysis

Results are presented as mean $\pm$ SEM. Differences between series of data were assessed by two-way paired and unpaired Student's *t*-tests for group comparison. A *p* value of less than 0.05 was considered significant.

Results

Effects of Fructose-Enriched Diet on Parameters of the Metabolic Syndrome

Baseline parameters were the same for all three groups. At 3 weeks, the chow and fructose-fed groups showed similar weight gains. Three weeks of the FED induced the MS as manifested by increases in blood pressure (from 130 $\pm$ 1 to 145 $\pm$ 1 mmHg, $p<0.05$), plasma insulin (from 0.26 $\pm$ 0.02 to 0.38 $\pm$ 0.03 ng/mL, $p<0.05$), and triglycerides (from 102 $\pm$ 6 to 184 $\pm$ 13 mg/dL, $p<0.05$) (Fig. 1A–C). Plasma adiponectin did not change in chow-fed rats. However, similar to a finding in our recent report (21), 3 weeks of fructose feeding increased plasma adiponectin levels, from 3.1 $\pm$ 0.3 to 4.8 $\pm$ 0.6 μ g/mL ($p<0.05$) (Fig. 1D).

Telmisartan's Effects on Parameters of the Metabolic Syndrome in Fructose-Fed Rats

Telmisartan administration significantly decreased SBP (148 $\pm$ 2 in nontreated vs. 125 $\pm$ 2 mmHg in telmisartan-treated rats) (Fig. 2). Triglyceride levels were reduced to 146 $\pm$ 18 compared to 285 $\pm$ 23 mg/dL in nontreated rats, and insulin levels to 0.41 $\pm$ 0.07 compared to 0.68 $\pm$ 0.08 ng/mL in nontreated rats ($p<0.05$ for all variables) (Fig. 2). At 5 weeks, body weight was similar in chow and fructose-fed rats, but was slightly, not significantly, lower in telmisartan-treated rats than in nontreated rats. However, telmisartan attenuated the increase in body weight from weeks 3 to 5 significantly (32 $\pm$ 2.0, 26 $\pm$ 2.0, and 12 $\pm$ 3.0 g in control, nontreated, and telmisartan-treated rats, respectively; $p<0.05$ compared to fructose-fed rats).

Fig. 1. Induction of metabolic syndrome: The effects of chow, and fructose-enriched diet, on metabolic parameters after 3 weeks. Systolic blood pressure (SBP) (A), fasting plasma insulin (B), triglyceride (TG) levels (C) and adiponectin (D), at baseline and after 3 weeks (3w) of diet. Data represent mean $\pm$ SEM of 8 animals. * $p < 0.05$ compared to baseline levels.

Telmisartan's Effect on Plasma Adiponectin Levels

At 3 weeks, plasma levels of adiponectin in fructose-fed rats were similar to those in fructose-fed rats designated for telmisartan treatment, and higher than those in chow-fed rats (4.8 ± 0.6 ; 5.5 ± 0.7 and 3.3 ± 0.1 $\mu\text{g/mL}$, respectively, $p < 0.05$). Telmisartan administration during the last 2 weeks of the experiment did not affect plasma adiponectin levels (4.6 ± 0.5 and 4.5 ± 0.3 $\mu\text{g/mL}$ in fructose-fed rats not treated with telmisartan and telmisartan-treated rats, respectively) (Fig. 2D).

Discussion

Accumulating data have shown that PPAR γ activation increases adiponectin levels (14, 15). Recent studies suggested that telmisartan, an angiotensin II receptor antagonist,

is also a partial PPAR γ agonist (16, 17). Therefore, it was expected that telmisartan would increase adiponectin levels. Our results showed that telmisartan can reverse fructose-induced hypertension, hyperinsulinemia, and hypertriglyceridemia. However, these beneficial effects were not coupled with increased plasma adiponectin levels. Previous animal studies examined telmisartan's effect on the metabolic profile in a dietary model of insulin resistance. In male Sprague-Dawley rats fed a high-fat, high carbohydrate diet for 3 months, telmisartan given along with the diet attenuated weight gain as well as glucose, insulin, and triglyceride levels (16, 22). A recently published study questioned whether the reported effects of angiotensin receptor blockers on insulin sensitivity may occur through modulation of the nuclear receptor PPAR γ (20). Telmisartan treatment resulted in significant PPAR γ agonism *in vitro*. However, when tested *in vivo*, telmisartan treatment had no effect on insulin sensitivity (20). The fructose-fed rat model is a commonly used model

Fig. 2. Treatment of the metabolic syndrome with telmisartan: The effect on blood pressure, insulin, triglyceride and adiponectin levels. Effects of chow, fructose enriched diet for 5 weeks (Fruc) and fructose-enriched diet for 5 weeks with telmisartan during the last 2 weeks (Fruc+Telmi), on systolic blood pressure (SBP) (A), fasting plasma insulin (B), triglyceride (TG) levels (C), and adiponectin (D). Data represent mean $\pm$ SEM of 8 animals. * $p < 0.05$ compared to fructose feeding. ** $p < 0.05$ compared to Fruc, and Fruc+Telmi.

that mimics human MS in many aspects, including hypertension, hypertriglyceridemia, insulin resistance, and compensatory hyperinsulinemia, but it is not associated with obesity. In the present study, adiponectin levels increased following 3 weeks of fructose feeding. In fact, we recently demonstrated that metabolic stress with a high-carbohydrate diet increased adiponectin levels in two rat models of hypertension and insulin resistance (21). This may be a transitory compensatory mechanism or it may reflect a decrease in adiponectin's ability to interact with its receptors as a sign of target organ resistance to adiponectin. The present study is the first to look at telmisartan's effect on established hypertension and insulin resistance using an environmental and physiologic induction in genetically susceptible rats. In this study, telmisartan effectively treated fructose-induced hypertension, hypertriglyceridemia, and hyperinsulinemia. However, telmisartan did not affect plasma adiponectin, a protein whose levels are known

to increase with existing PPAR γ agonists and to correlate with insulin sensitivity (23–26). We recently showed in fructose fed rats that the PPAR γ agonist rosiglitazone improved the metabolic profile to a degree similar to telmisartan, but with increases in plasma adiponectin levels and gene expression (15). Therefore, it is unlikely that adiponectin mediates telmisartan's beneficial effect on metabolic parameters.

Telmisartan may induce its beneficial metabolic effects by directly inhibiting the AT1 receptor. Indeed, several studies have established the notion that AT1 receptor stimulation by angiotensin II contributes to insulin resistance and its associated deleterious metabolic profile (5, 6). Therefore, the AT1 receptor blockade ameliorates these effects and partially explains the beneficial effects of telmisartan on fructose-induced insulin resistance. Similar to our findings with telmisartan, a few other studies have shown favorable metabolic effects of other ARBs in fructose-fed rats (27–29). Collec-

tively, these data indicate that the beneficial metabolic effect is not telmisartan-specific but rather is associated with AT1 receptor antagonism as a class effect of ARBs. The RAS blockade's beneficial effect on insulin resistance has been explained partially by haemodynamic effects, with improved delivery of insulin and glucose to peripheral tissues, as well as by direct effects on glucose transport and insulin signaling pathways (6). Therefore, normalization of blood pressure by telmisartan in fructose-fed rats could contribute to improved insulin action and a better metabolic profile. However, previous work in our lab has shown that lowering blood pressure in fructose-fed rats by the use of other antihypertensive drugs, such as calcium channel blockers (30), hydrochlorothiazide, or β -blockers (unpublished data), has not had a metabolic impact, suggesting that telmisartan improved insulin resistance *via* blood pressure-independent mechanisms. In addition to the AT1 receptor, the angiotensin II type 2 (AT2) receptor may play a role in telmisartan's beneficial effect in fructose-fed rats. Indeed, it was reported that mice lacking Ang II type 1a receptor exhibited an attenuation of diet-induced weight gain and adiposity through increased energy expenditure (31). Mori *et al.* recently suggested that the AT2 receptor is important for the regulation of energy homeostasis and may be responsible for the attenuation of adiposity and insulin resistance by telmisartan (32). The AT2 receptor's potential contribution to telmisartan's beneficial effect in fructose-fed rats is beyond the scope of this report, and future studies may examine the possible effect of an AT2 receptor antagonist with or without telmisartan on variables of metabolic syndrome in fructose-fed rats. Another possible mechanism by which ARBs may exert their beneficial effects on insulin resistance is their potential to alter adipocyte size. Ang II inhibits the differentiation of adipocytes and increases the number of large adipocytes (33). Therefore, AT1 receptor antagonism can counteract the effect of Ang II, promoting the differentiation and mobilization of preadipocytes to increase the number of small, insulin-sensitive adipocytes. Indeed, treatment with various ARBs has been reported to downsize adipocytes and improve insulin resistance (32, 34). We did not measure adipocyte size, which is a study limitation. In addition, it is possible that telmisartan improved the impairment of hepatic transduction of the leptin signal, which was recently reported to play a role in fructose-induced insulin resistance in rats (35). Further studies need to be conducted in order to address these issues.

In summary, we showed that telmisartan effectively treated fructose-induced hypertension, hyperinsulinemia, and hypertriglyceridemia without increasing adiponectin, a marker of *in vivo* PPAR γ activation. Further studies will elucidate the molecular mechanism by which telmisartan reversed the deleterious metabolic affects in this model.

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