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Telmisartan ameliorates hyperglycemia and metabolic profile in nonobese Cohen-Rosenthal diabetic hypertensive rats via peroxisome proliferator activator receptor−γ activation

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Abstract

The importance of hypertension treatment has expanded beyond blood pressure management to include additional risk factors, mainly diabetes. It was considered of interest to test the effect of telmisartan, an angiotensin receptor 1 antagonist and peroxisome proliferator activator receptor−γ partial agonist, on Cohen-Rosenthal diabetic hypertensive nonobese (CRDH) rats, a unique model combining both pathologies. Its effect was examined on fat-derived and inflammatory agents in CRDH. To determine the extent of the drug's peroxisome proliferator activator receptor−γ modulating beneficial metabolic actions, results were compared with those obtained with valsartan and rosiglitazone in CRDH and Cohen diabetic rat (CDR). Telmisartan and valsartan were given in drinking water at 3 and 12 mg/kg/d, whereas rosiglitazone (3 mg/kg/d) was given as food admixture for a period of 5 months. Blood pressure, glucose, insulin, adiponectin, leptin, and tumor necrosis factor α were examined. Telmisartan and valsartan significantly ($P < .01$) reduced blood pressure, whereas telmisartan and rosiglitazone considerably reduced blood glucose levels to normoglycemic levels ($P < .01$) in these 2 strains. Insulin levels were not affected by telmisartan and valsartan but were slightly reduced by rosiglitazone in CDR. In contrast to valsartan, adiponectin was significantly (60%, $P < .01$) increased by telmisartan in both CDR and CRDH, whereas rosiglitazone induced a 60% and 180% increase in CRDH and CDR animals, respectively, on day 30 of treatment. Co-treatment with GW9662 averted telmisartan-induced rise of adiponectin. Tumor necrosis factor α declined in telmisartan-treated rats, less so with rosiglitazone, but not valsartan. Telmisartan also induced downsizing of epididymal adipocytes compared with valsartan. Leptin levels were significantly increased by valsartan ($P < .05$) but reduced by telmisartan and rosiglitazone. The telmisartan-induced increase in adiponectin was most probably associated with a decrease in glucose and tumor necrosis factor α levels. Therefore, in addition to its hypotensive effect, telmisartan demonstrated beneficial thiazolidinedione-like effects.

1. Introduction

Numerous studies have shown that interruption of the renin-angiotensin system by angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ARBs) can correct, reverse, or improve endothelial function in hypertension, diabetes, and hypercholesterolemia [1]. Recent evidence indicates that the ARB telmisartan structurally resembles the insulin sensitizer pioglitazone, a thiazolidinedione ligand of peroxisome proliferator activator receptor−γ (PPAR-γ) used for the treatment of type 2 diabetes mellitus. Peroxisome proliferator activator receptor−γ is an established therapeutic target in the treatment of insulin resistance, diabetes, and metabolic syndrome. Activation of PPAR-γ leads to the expression of key target genes that mediate

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beneficial effects on glucose and lipid metabolism [2]. Developed and marketed primarily as an ARB, telmisartan can bind to and activate PPAR-γ, leading to improved insulin sensitivity both in vitro [3] and in vivo [4]. In line with this evolving understanding of telmisartan's action as a dual vascular/metabolic modulator, we further investigated the effect of telmisartan on key molecules in the metabolic disorder. We recently provided preliminary evidence of telmisartan's ability to lower both arterial pressure and blood glucose in the Cohen-Rosenthal diabetic hypertensive (CRDH) rat [5]. This unique rat model combines hereditary hypertension conferred by a genetic spontaneous hypertensive rat (SHR) background with hereditary susceptibility to diabetes that depends on particular nutritional cues [6,7].

The present study was undertaken to expand these preliminary reports and examine the involvement of fat-derived and inflammatory agents in the beneficial effect of telmisartan in CRDH rats. To further elucidate telmisartan mode of action, its effects were compared with valsartan as well as with the PPAR-γ agonist rosiglitazone in CRDH and Cohen diabetic rat (CDR) animals.

2. Method

2.1. Experiments

2.1.1. Effect of telmisartan in CDR, CRDH, and SHR animals

The SHR; CDR, a type 2 diabetic animal model; and CRDH diabetic-hypertensive rats (n = 10 each), of similar age (5 ± 0.4 months) and weight range (300-335 g), were administered telmisartan at 3 mg/kg/d in their drinking water for a period of 5 months, whereas control groups (n = 6 each) received tap water. Both CRDH and CDR rats were fed a sugar-rich, copper-free diet. Blood pressure (BP); blood glucose level (BGL); and serum insulin, adiponectin, leptin, and TNFα were evaluated throughout.

2.1.2. Comparative study: effects of telmisartan, valsartan, and rosiglitazone on CRDH and CDR

Both strains, CRDH and CDR, were divided into 3 treatment groups (n = 9-10), each receiving telmisartan (3 mg/kg/d), valsartan (12 mg/kg/d), and rosiglitazone (3 mg/kg/d) for 90 days. Telmisartan and valsartan were given in drinking water, whereas rosiglitazone was given as food admixture. Rosiglitazone dosage was adjusted according to animals' weight and their water/food consumption; animals were weighed twice a week, and their food consumption was examined once a week. Rats were maintained on a 14-hour–light/10-hour–dark cycle in a room with constant temperature of 23°C and humidity of approximately 50%, in accordance with animal studies guidelines approved by the Institutional Animal Care Committee at Tel Aviv University.

2.1.3. PPAR-γ activation by telmisartan

Using CRDH rats expressing both hypertension and diabetes, we intended to investigate whether the induced effects achieved by telmisartan are associated with the activation of PPAR-γ. Four groups of animals were subjected to 2-week treatment with (*a*) telmisartan at 3 mg/(kg d) (n = 8); (*b*) telmisartan + GW9662 (GW), a potent selective PPAR-γ antagonist (catalog no. M6191; Sigma-Aldrich, St Louis, MO), at 3 and 1 mg/kg/d, respectively (n = 10); (*c*) GW9962 alone at 1 mg/kg/d (n = 7); and (*d*) control CRDH fed sugar diet (n = 7). Telmisartan was given in drinking water (as previously), whereas GW was given by gavage. Blood pressure, BGL, and adiponectin levels were examined prior and at the end of experiment. Animals' weight and food and water consumption were monitored twice a week.

2.2. Blood pressure and glucose

Systolic and diastolic BPs were measured before treatment and periodically throughout the study using a noninvasive tail-cuff instrument (BP-2000 series II; Visitech Systems, Apex, NC). Postprandial BGL was determined using a standard glucometer (Bayer, Leverkusen, Germany), 2 hours postfeeding after 16-hour (overnight) fasting period, bimonthly. Morning feeding habituation was established by previous 3 consecutive overnight fasting and morning feedings.

2.3. Humoral parameters

Blood samples collected from the retroorbital sinus, under light anesthesia, were centrifuged at 3500 rpm for 25 minutes; and serum was stored at −80°C. Serum was analyzed for adiponectin, leptin, and insulin levels using commercially available rat radioimmunoassay kits (Linco Research, St Charles, MO) and, for TNFα levels, using enzyme-linked immunosorbent assay kit (Invitrogen-Biosource, Nivelles, Belgium), all according to manufacturer's instructions. The effect of the drugs on the insulin resistance was evaluated by comparing the homeostasis model assessment (HOMA) index obtained according to the following formula: [fasting insulin (in microunits per milliliter) × fasting glucose (in milligrams per deciliter)]/405.

2.4. Histology of epididymal fat

At the end of the study, animals were anesthetized with pentobarbital, 50 mg/kg; and epididymal fat tissue was collected, fixed in 10% paraformaldehyde solution at 4°C, dehydrated, embedded in paraffin, and cut into 4-μm sections. Paraffin-embedded sections were stained with hematoxylin and eosin; and using light microscopy, adipocytes numbers in telmisartan- and valsartan-treated CRDH groups were compared.

2.5. Statistical analyses

Variables for each of the strains were compared with their baseline levels (pretreatment values) using paired *t* test. Differences between treatment and vehicle groups within each strain and between strains were analyzed using mixed

effects models. The differences relative to corresponding baselines in treated groups were analyzed using 2-way analysis of variance to assess telmisartan absolute effect on each of the parameters tested. Correlation between 2 parameters and the significant levels of correlation were analyzed by Pearson correlation analysis. Analysis of data was done using statistics software SPSS version15 (SPSS, Chicago, IL). Data are presented as mean ± SEM, and $P < .05$ was considered significant.

3. Results

3.1. Blood pressure and BGL

Telmisartan significantly reduced BP (systolic/diastolic) from 194/154 ± 2/4 to 122.1/89.7 ± 2/4 mm Hg in SHR, from 162/134 ± 2/3 to 105/78 ± 1/2 mm Hg in CRDH, and from 146/109 ± 0.8/2 to 110/92 ± 2/2 mm Hg in CDR ($P < .01$). This reduction was further confirmed by telmisartan in the comparative study, reaching 110/79 ± 5/7 mm Hg ($P < .01$) on day 90 of treatment of CRDH rats. Valsartan induced significant but delayed reduction of BP, reaching 124/93 ± 3/7 mm Hg ($P < .02$). Rosiglitazone reduced systolic BP in CRDH by approximately 11 mm Hg and diastolic BP by approximately 14 mm Hg, reaching 154/115 ± 1/3 mm Hg on day 90 of treatment. Blood pressure in the normotensive CDR group was also lowered by both drugs: by telmisartan from 141/121 ± 2/4 to 118/91 ± 9/8 mm Hg ($P < .02$) and, slightly but not significantly, by valsartan from 138/114 ± 6/3 to 128/98 ± 3/8 mm Hg on day 90 of treatment. Telmisartan-induced reduction in BP was not inhibited by a concomitant treatment with GW in CRDH rats: 154/119 ± 2/3 declined to 130/100 ± 3/4 ($P < .01$) in group A (telmisartan alone), 153/119 ± 3/4 to 126/104 ± 3/4 ($P < .01$) in group B (telmisartan + GW), 154/121 ± 5/8 to 156/131 ± 6/7 in group C (GW alone), and 153/116 ± 4/5 to 154/120 ± 2/4 mm Hg in group D (control).w

Telmisartan induced a significant reduction in BGL from 246 ± 12 to 113 ± 4 mg/dL on day 30 in CRDH animals and to 94 ± 3 mg/dL on day 115 in CDR, which had a similar initial BGL. Blood glucose level in control groups of CDR and CRDH animals remained around 200 mg/dL throughout the study. In SHR, initial BGLs of approximately 100 mg/dL were not affected by either treatment.

In the comparative study, BGL declined significantly in the CRDH groups on both telmisartan and rosiglitazone: from 211 ± 22 and 208 ± 9 mg/dL to 115 ± 6 and 123 ± 3 mg/dL, respectively ($P < .01$). A minor reduction was induced by valsartan from 224 ± 25 to 184 ± 8 mg/dL on day 90 ($P < .05$). Likewise, BGL was reduced in CDR by telmisartan and rosiglitazone by nearly 100 mg/dL (276 ± 17 to 180 ± 13 mg/dL and 232 ± 18 to 132 ± 8 mg/dL, respectively), whereas valsartan elicited an insignificant reduction of 35 mg/dL, observed on day 90.

Telmisartan-induced decline in BGL (298 ± 40 to 182 ± 19 mg/mL, $P < .02$) was attenuated by concomitant treatment with GW (310 ± 48 to 270 ± 23 mg/mL), whereas GW alone did not affect glucose levels, which remained at higher values in parallel to control group at a range of 312 ± 43 mg/mL.

3.2. Biochemical parameters

3.2.1. Insulin

In the first experiment, serum insulin levels were not affected by telmisartan in CRDH, whereas it was slightly increased in CDR on day 150 of treatment (1.4 ± 0.27 to 1.89 ± 0.29 ng/mL, $P = .055$) (Fig. 1A and B). The initial values of insulin in CRDH (3.06 ± 0.65 ng/mL) (including the vehicle group) were approximately 2 fold higher than those obtained in CDR (1.4 ± 0.27 ng/mL) and SHR (0.72 ± 0.13 ng/mL) animals, yet comparison of the absolute value of insulin change induced by telmisartan showed no strain or time dependency. In the comparative study (Table 1), insulin levels were significantly increased ($P < .05$) in CRDH rats

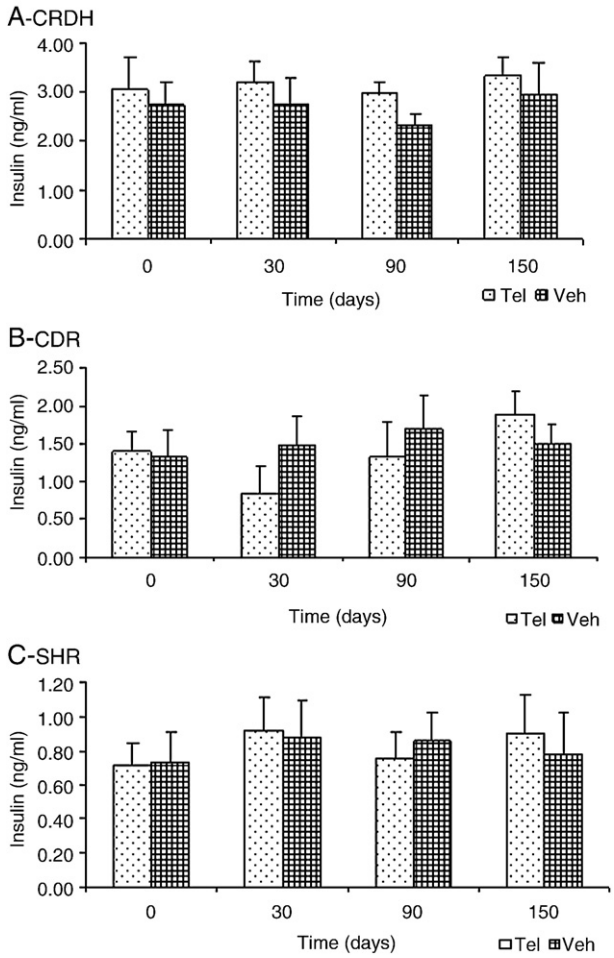

Fig. 1. Insulin levels in telmisartan- and vehicle-treated rats: (A) CRDH, (B) CDR, and (C) SHR. Results are presented as mean ± SEM. Student paired *t* test was performed for the assessment of significance (*) in comparison to baseline. Differences between vehicle and treated groups were evaluated by mixed analysis (†). *P* value < .05 was considered significant. Tel indicates telmisartan; Veh, vehicle.

Table 1
Adiponectin, leptin, insulin, and TNFα in CRDH and CDR animals treated with telmisartan, valsartan, and rosiglitazone

	Time (d)	CRDH			CDR		
		Telmisartan (n = 9)	Valsartan (n = 11)	Rosiglitazone (n = 9)	Telmisartan (n = 9)	Valsartan (n = 11)	Rosiglitazone (n = 9)
Adiponectin	0	7.04 ± 0.45	7.25 ± 0.33	7.69 ± 0.55	5.07 ± 0.50	5.57 ± 0.20	6.21 ± 0.34
(μg/mL)	30	11.08 ± 0.95†,§	8.07 ± 0.96	11.79 ± 0.56†,§	8.21 ± 0.27†,§	3.04 ± 0.24*	14.56 ± 1.68†,§
	90	7.78 ± 0.43	7.13 ± 0.56	10.09 ± 0.70†,§	7.54 ± 0.54†,§	4.74 ± 0.29	7.48 ± 0.45†,‡
Leptin (ng/mL)	0	2.43 ± 0.26	2.98 ± 0.22	2.79 ± 0.23	2.79 ± 0.29	3.31 ± 0.45	3.40 ± 0.22
	30	3.04 ± 0.45	2.50 ± 0.22	2.33 ± 0.10*,§	3.16 ± 0.50	2.83 ± 0.35	4.12 ± 0.46
	90	1.78 ± 0.24*,‡	3.85 ± 0.41*	2.03 ± 0.27†,‡	3.05 ± 0.53	3.09 ± 0.24	3.39 ± 0.27
Insulin (ng/mL)	0	1.31 ± 0.3	1.02 ± 0.12	0.98 ± 0.11	1.19 ± 0.16	1.62 ± 0.27	1.83 ± 0.34
	30	1.48 ± 0.23	1.19 ± 0.16	1.20 ± 0.28	0.73 ± 0.19	1.16 ± 0.22	1.34 ± 0.18
	90	1.46 ± 0.21	1.41 ± 0.17*	1.13 ± 0.32	1.26 ± 0.13	1.22 ± 0.10	1.27 ± 0.21*
HOMA index	0	12.90 ± 1.54	10.07 ± 1.37	14.39 ± 3.21	12.42 ± 1.54	11.73 ± 4.10	19.28 ± 4.77
	30	11.64 ± 1.85	13.40 ± 2.74	9.27 ± 1.20*	9.44 ± 1.67	18.03 ± 4.39	14.60 ± 3.13
	90	8.48 ± 2.12*,§	21.71 ± 4.05*	10.07 ± 2.88*,§	8.56 ± 1.41*	17.95 ± 2.23*	7.81 ± 1.15*,‡
TNFα (pg/mL)	0	27.13 ± 2.63	26.31 ± 4.1	23.89 ± 2.32	23.35 ± 3.37	25.13 ± 3.68	28.32 ± 4.26
	30	21.13 ± 3.57*	23.45 ± 3.27	21.12 ± 3.51	21.91 ± 1.95	24.41 ± 3.50	24.45 ± 3.98
	90	12.32 ± 4.23†,§	24.96 ± 4.69	17.02 ± 2.19*,‡	18.16 ± 1.34†	21.93 ± 3.38	19.89 ± 4.23†

Data presented as the mean ± SEM.
* $P < .05$ vs corresponding baseline (day 0).
† $P < .01$ vs corresponding baseline (day 0).
‡ $P < .05$ vs valsartan group of same strain at corresponding day of treatment.
§ $P < .01$ vs valsartan group of same strain at corresponding day of treatment.

treated with valsartan and were slightly decreased in CDR on day 90 of treatment. Telmisartan did not induce any notable change in insulin levels in either strain, whereas rosiglitazone caused a moderate ($P = .054$) decrease on day 30 of treatment in CDR.

3.2.2. HOMA index

Evaluation of the HOMA index indicated an impressive decline in the rosiglitazone- and telmisartan-treated CRDH and CDR rats. Treatment with valsartan on the contrary rather induced an increase in insulin resistance indicated by higher HOMA index (Table 1).

3.2.3. Adiponectin

There was a significant increase in adiponectin level in CRDH from baseline to days 30 and 90 of treatment: from 5.77 ± 0.45 to 8.46 ± 0.43 μg/mL ($P < .001$) and 6.98 ± 0.2 μg/mL ($P < .05$), respectively (Fig. 2A). In addition, adiponectin levels were significantly higher in telmisartan than control group on days 30 and 90: 5.5 ± 1.02 and 5.56 ± 0.42 μg/mL, respectively ($P < .01$). In CDR, adiponectin reached a peak value of 5.83 ± 0.24 on day 150 ($P < .01$) compared with baseline and control group ($P < .001$) (Fig. 2B). Serum adiponectin in SHR increased significantly on days 90 and 150 vs baseline control group ($P < .001$ and $P < .01$, respectively) (Fig. 2C). Notably, adiponectin levels in CRDH animals were approximately 2- and 3-fold higher at baseline than in CDR and SHR, respectively.

When telmisartan was compared with other drugs (Table 1), telmisartan induced a significant ($P < .01$) rise (60%, relative to baseline) in adiponectin in both CRDH and CDR on day 30 of treatment (Fig. 3). Rosiglitazone induced a similar rise in adiponectin levels in CRDH animals (Fig. 3A) and a 3-fold increase ($P < .01$) in CDR animals as compared with baseline (Fig. 3B). A sustained significant rise ($P < .03$) in adiponectin was observed on day 90 in CDR animals treated with rosiglitazone. Valsartan, on the other hand, induced no marked increase in adiponectin levels in CRDH, but rather decreased adiponectin levels in CDR. Telmisartan and rosiglitazone induced a significantly higher rise in adiponectin in comparison to valsartan in CRDH ($P < .01$) on day 30 and in CDR on days 30 and 90 ($P < .01$).

Telmisartan-induced rise in adiponectin levels within the 2-week treatment (5.05 ± 0.55 to 6.55 ± 0.65, $P < .05$) was averted by co-treatment with GW (5.41 ± 0.66 to 5.81 ± 0.63) (Fig. 4). Treatment with GW alone did not affect the levels of adiponectin, which remained at a basal level similar to control group.

3.2.4. Weight

In the first experiment, animals in all 3 strains had small weight gain from baseline to 150 days of treatment: CRDH from 333 ± 1.67 to 360 ± 1.75 g, CDR from 322 ± 5.44 to 342 ± 4.67 g, and SHR from 306 ± 9.27 to 362 ± 10.94 g. Weight gain from baseline to day 150 in the vehicle group was as follows: CRDH from 341 ± 2.34 to 364 ± 11.78 g, CDR from 312 ± 4.21 to 365 ± 3.92 g, and SHR from 317 ± 6.23 to 382 ± 8.56 g.

In the comparative study (Fig. 5A, B), after rosiglitazone treatment, rats of both strains showed higher weight gain over time compared with baseline and with telmisartan-treated groups ($P < .05$). In contrast, there was no significant weight gain in the telmisartan-treated CRDH or CDR. Valsartan-treated CRDH had a detectable weight

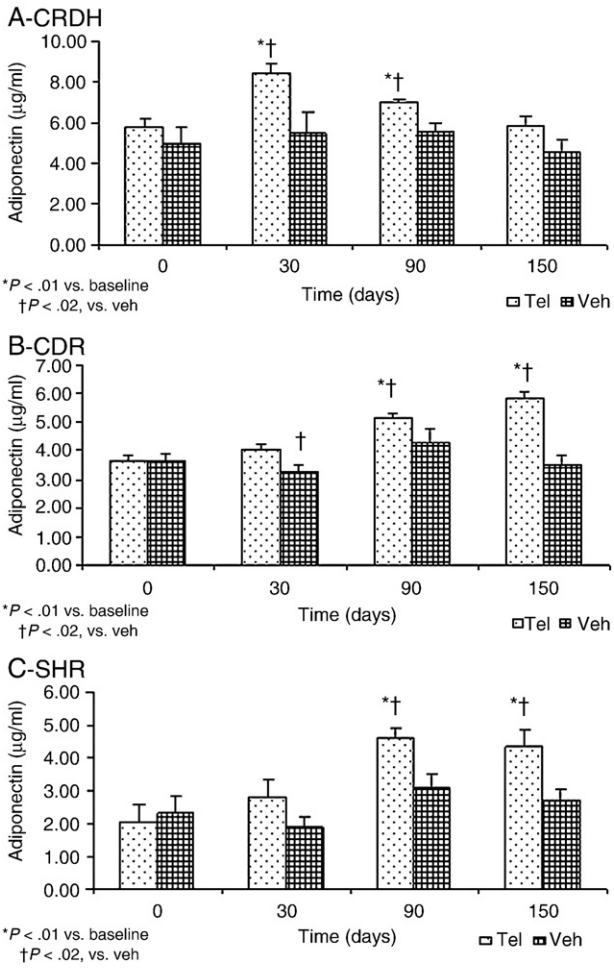

Fig. 2. Adiponectin levels in telmisartan-treated animal strains and animals receiving tap water only: (A) CRDH, (B) CDR, and (C) SHR. Results are presented as mean ± SEM. Student paired *t* test was performed for the assessment of significance (*) in comparison to baseline. Differences between vehicle and treated groups were evaluated by mixed analysis (†). $P < .05$ was considered significant.

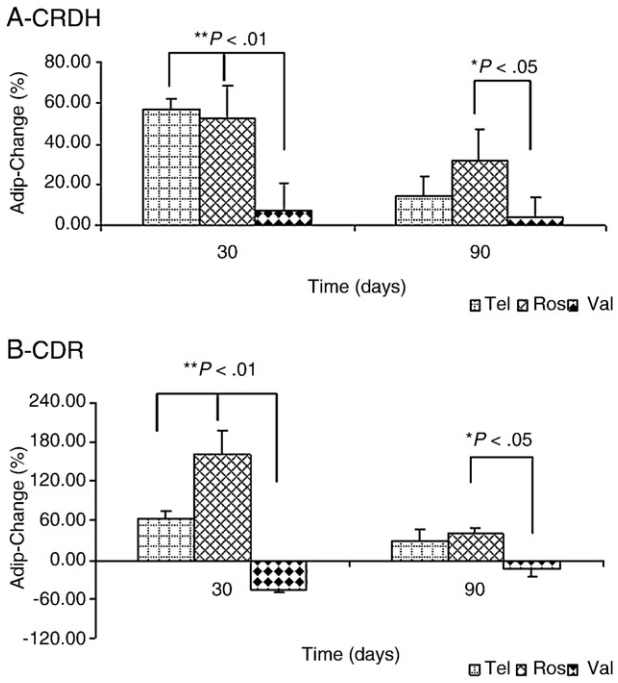

Fig. 3. Changes in adiponectin (expressed as percentage change of mean relative to corresponding baseline) at 30 and 90 days posttreatment in CRDH (A) and CDR (B) animals treated with telmisartan, valsartan, and rosiglitazone, relative to corresponding baseline.

gain only in the first 10 days of treatment, which remained unchanged thereafter, whereas weight increased gradually with time in CDR, reaching levels obtained by the rosiglitazone group and exceeding those of the telmisartan group ($P < .05$). Food intake measured once a week, which ranged between 19 and 28 g per animal, was not affected by any of the treatments or by the corresponding change in body weight in each treatment. Co-treatment with GW during the 2 weeks of treatment by telmisartan did not affect CRDH weight, which remained within the normal weight range as the control animals.

3.2.5. Leptin

Leptin level demonstrated a monotonic significant reduction over time, reaching 2.41 ± 0.2 ng/mL in CRDH and 1.52 ± 0.19 ng/mL in CDR on day 90. On day 150 of treatment, leptin levels were significantly reduced ($P < .01$) to 2.17 ± 0.24 ng/mL in CRDH (Fig. 6A) and 1.54 ± 0.14 ng/mL in CDR (Fig. 6B) as compared with baseline. In SHR, leptin declined significantly on day 150 (1.35 ± 0.15 ng/mL, $P < .05$) compared with baseline (Fig. 6C). Comparing telmisartan- and vehicle-treated groups in the different strains showed a significant reduction in leptin on days 90 and 150 in CRDH and CDR ($P < .05$). Telmisartan-induced leptin reduction is strain dependent ($P < .02$); reduction in CRDH was 2-fold and 4-fold higher than in CDR and SHR animals, respectively.

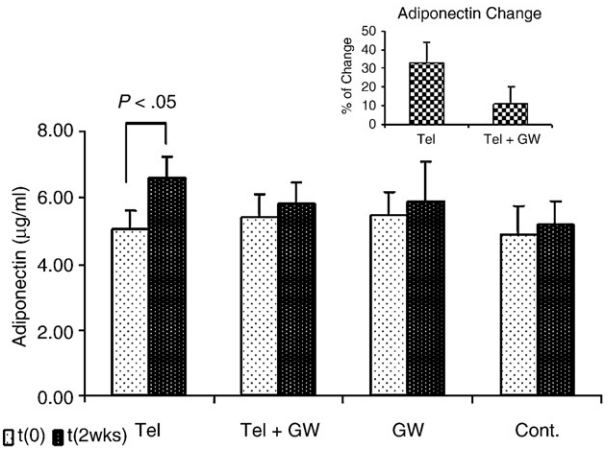

Fig. 4. Effect of telmisartan (3 mg/kg/d) and cotreatment with GW (1 mg/[kg d]) or GW alone on serum adiponectin before and after treatment. Data are presented as mean ± SEM, and $P < .05$ vs baseline is considered significant.

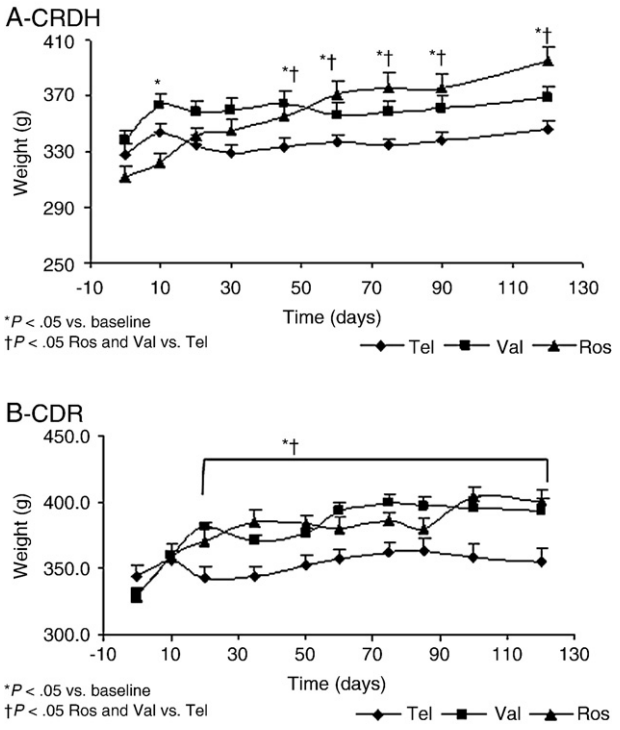

Fig. 5. Weight of animals treated with telmisartan, valsartan, and rosiglitazone in CRDH (A) and CDR (B). Weight is presented as mean ± SEM. Telmisartan-treated group differed significantly from both valsartan- and rosiglitazone-treated groups in both strains. *$P < .05$ vs baseline, and †$P < .05$ vs other treatment groups. Val indicates valsartan; Ros, rosiglitazone.

In the comparative study, leptin levels exhibited an opposite pattern of change to that of adiponectin in CRDH animals, but not in CDR; there was a significant leptin decline in the rosiglitazone groups on days 30 and 90, but only on day 90 in the telmisartan group ($P < .02$) (Table 1). The decrease in leptin levels in these groups paralleled the impressive increase in leptin in the valsartan-treated group, resulting in a significant ($P < .05$) difference between the treatments.

3.2.6. TNFα

Before treatment, TNFα was higher in CRDH (27.7 ± 2.91 pg/mL) and CDR (23.35 ± 3.37 pg/mL) than in SHR (15.40 ± 1.7 pg/mL) ($P < .01$) (Fig. 7). A strain-dependent reduction pattern of TNFα was induced by telmisartan ($P < .05$): Tumor necrosis factor α declined until day 90 in all strains and only on day 150 in CRDH ($P < .05$). Unfortunately, TNFα levels in the vehicle groups were not evaluated because of technical mishap. In the comparative study, telmisartan induced a significant decrease in TNFα in the CRDH and CDR groups ($P < .01$) relative to baseline (Table 1). In addition, TNFα levels were lower in the telmisartan- than in the valsartan–treated group ($P < .05$). Rosiglitazone reduced TNFα levels on day 90 of treatment in both strains.

3.2.7. Adipocyte size

After 3 months of treatment, epididymal adipocytes in the telmisartan group were markedly smaller than those in the valsartan-treated CRDH rats (Fig. 8). This was also evidenced by the significantly higher number of cells per field in the former: 138 ± 7 vs 83 ± 3 ($P < .02$).

3.2.8. Interparameter correlation

A negative correlation was observed between adiponectin rise and the lowering of both BGL and TNFα in telmisartan-treated CRDH ($R = −0.509$, $P < .001$ and $R = −0.511$, $P < .05$, respectively) and in telmisartan-treated CDR ($R = −0.412$, $P < .05$ and $R = −0.487$, $P < .05$, respectively), whereas a negative correlation was seen only in BGL vs adiponectin in rosiglitazone-treated CRDH ($R = −0.645$, $P < .03$). The rise in adiponectin and the decline in leptin levels were most prominent in the CDR groups treated with telmisartan ($R = −0.595$, $P < .01$) and

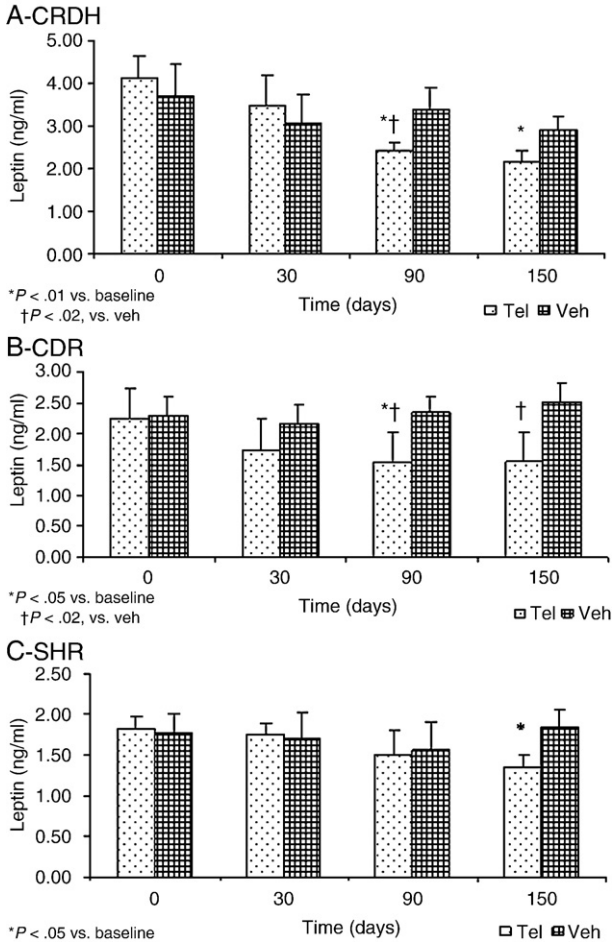

Fig. 6. Leptin levels in telmisartan-treated animal strains and animals receiving tap water only: (A) CRDH, (B) CDR, and (C) SHR. Results are presented as mean ± SEM. Student paired t test was performed for the assessment of significance (*) in comparison to baseline. Differences between vehicle and treated groups were evaluated by mixed analysis (†). $P < .05$ was considered significant.

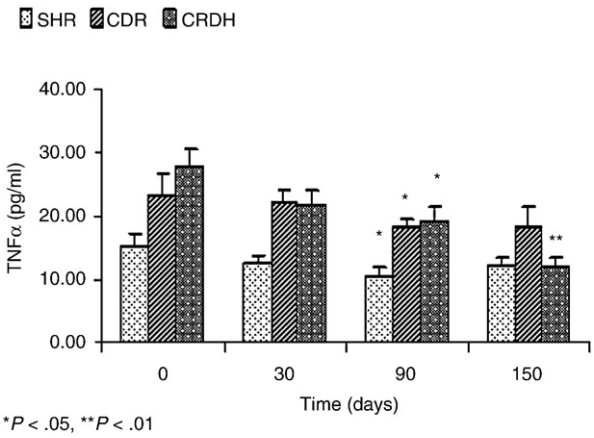

Fig. 7. Levels of TNFα in the 3 telmisartan-treated groups—SHR, CDR, and CRDH animals—at different time points in the study. The TNFα levels are presented as mean ± SEM. Significance was evaluated vs corresponding baseline.

rosiglitazone ($R = -0.453$, $P < .05$), whereas there was an opposite negative correlation (decline in adiponectin and rise in leptin) in CRDH ($R = -0.602$, $P < .02$) on day 90 of valsartan treatment.

4. Discussion

We have demonstrated that telmisartan, an ARB combining both pharmacologic actions as angiotensin receptor 1 antagonist and partial PPAR-γ agonist, proves to be effective in the treatment of both hypertension and hyperglycemia. In CRDH, a unique model of combined genetically transmitted hypertension and diabetes [6,7], and in SHR rats, telmisartan effectively reduced hypertension to normotensive values faster than another ARB, valsartan, whereas rosiglitazone, a PPAR-γ agonist, had mild reduction of BP in CRDH rats. The dosing of telmisartan (3 mg/kg/d) and valsartan (12 mg/kg/d) chosen for this study was at acceptable proportion considering the clinical and experimental use [8]. Whether this superior efficacy achieved by telmisartan is due to the activation of PPAR-γ or a mere outcome of the improving glucose homeostasis in such animals is not clear. Experimental evidences demonstrated that PPAR-γ activation has beneficial effects on BP control in spontaneously hypertensive rats [9], in salt-sensitive hypertensive rats [10], and in renovascular hypertension model [11]. Inhibition of telmisartan-induced increased nitric oxide bioavailabity in vivo by GW9662 [12] and nitric oxide secretion in senescence endothelial cells in vitro by PPAR-γ

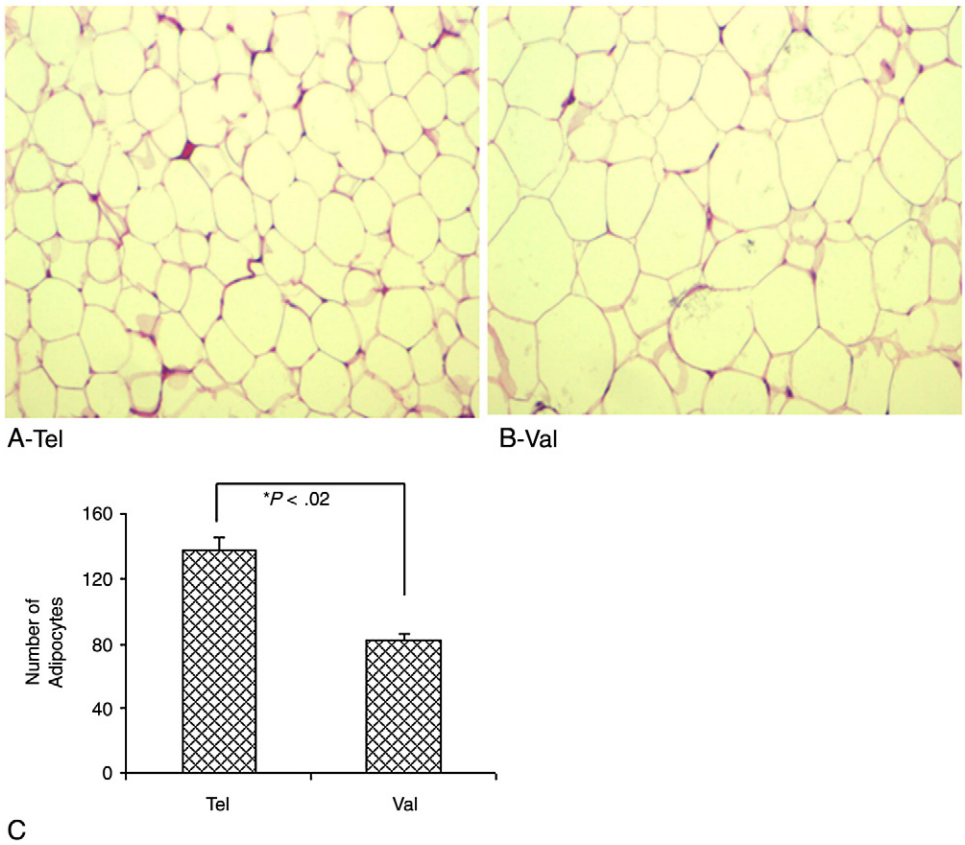

Fig. 8. Representative histology of epididymal fat pads in experimental animals. Hematoxylin and eosin–stained adipocyte at magnification ×200 of CRDH animals treated with telmisartan (A) and valsartan (B). Mean ± SEM of number of adipocytes (C) counted in 3 animals from each treatment group.

small interfering RNA [13] further demonstrates the involvement of PPAR-γ in the regulation of vascular responses. Telmisartan was further shown to down-regulate angiotensin receptor 1 at both the messenger RNA and protein levels through the activation of PPAR-γ in vascular smooth muscle cells [14], a notion of great importance when considering the intricate balance of telmisartan mechanism of action.

The results indicate that telmisartan has a major effect on glucose homeostasis in CDR, a type 2 diabetes mellitus model not accompanied by obesity, and in CRDH rats. We observed a robust, early, and sustained decrease in glucose throughout the treatment in CRDH animals, and a delayed but similarly impressive decline in BGL in CDR.

Rosiglitazone induced unequivocal BGL reduction in both CDR and CRDH animals, with minor reduction in insulin levels in CDR, confirming a role for PPAR-γ–related effects on glucose homeostasis in this model. Because PPAR-γ activation enhances insulin sensitivity, telmisartan could improve peripheral response to insulin sensitivity through its action as a PPAR-γ agonist, a feature lacking in valsartan. Although several angiotensin receptor antagonists were shown to serve as PPAR-γ ligands in binding assays [15], only telmisartan—and, to a lesser extent, candesartan and irbesartan—induced PPAR-γ agonist-like biological effects [16-18]. Replacement of valsartan and candesartan by telmisartan in hypertensive patients with type 2 diabetes mellitus resulted in a significant decrease in fasting insulin [19]. In patients with type 2 diabetes mellitus and metabolic syndrome, telmisartan offered more effective glycemic and lipid control than irbesartan [20]. We now report that valsartan had only a minor BGL-lowering effect, leaving glucose levels at 180 mg/dL, compared with 115 mg/dL with telmisartan treatment. Similarly, 4-week losartan treatment lowered BP but not BGL in CRDH [21]. Of note is the finding in our study that telmisartan nearly normalized BGL but induced no change in insulin levels in either CDR or CRDH rats, whereas valsartan induced an increase in insulin levels on day 90 of treatment.

Activation of PPAR-γ per se leads to increased glucose uptake, even in the absence of a change of insulin blood concentration. In the context of these multiple potential interactions of telmisartan with PPAR-γ, several findings in our study suggest that much of telmisartan's hypoglycemic effect in CDR and CRDH rats is mediated through insulin-sensitizing mechanisms. Activating PPAR-γ can directly increase adiponectin transcription rate [22]. Adiponectin, which is a known insulin sensitizer and enhancer of glucose uptake and metabolism, significantly increased in telmisartan- and rosiglitazone-treated strains. We observed an impressive increase in adiponectin with rosiglitazone, ranging up to 60% in CRDH animals and 180% in CDR on day 30 of treatment. In agreement with our findings [19,23], the rise in serum adiponectin levels was also seen with telmisartan but not valsartan treatment in hypertensive-diabetic patients. Makita et al [24] showed that treatment with telmisartan reduced body weight. The change in body weight correlated inversely to a rise in adiponectin levels in these patients.

Insulin sensitivity in rosiglitazone- and telmisartan-treated animals was improved as revealed by the decline in the means of HOMA. Despite the unchanged insulin levels, this finding indicates an improvement in the fasting glucose levels treated with rosiglitazone and telmisartan but not valsartan. The HOMA has proven to be robust and widely used method for assessing insulin resistance. Furthermore, HOMA was found to correlate well with estimates from euglycemic clamp [25].

Activation of PPAR-γ is associated with potentially beneficial effects on expression and secretion of a range of factors [26] and can also set in motion several anti-inflammatory pathways related to attenuation of nuclear factor–κB signaling, thereby alleviating cytokine-dependent resistance to the action of insulin [27]. We observed that telmisartan elicited a marked decrease in the serum soluble TNFα in all strains treated with telmisartan, especially in the CRDH group. Circulating TNFα in SHR animals was barely detectable in some studies [28], whereas initial levels of TNFα were prominently expressed in diabetic animal models, such as Zucker diabetic fatty rats [29,30] and Otsuka Long-Evans Tokushima fatty diabetic rats [31]. In our study, CRDH animals had initial levels of TNFα higher than both CDR and SHR animals, most probably because of the deleterious combination of hypertension and hyperglycemia. Treatment with rosiglitazone induced a similar reduction in TNFα in both CDR and CRDH animals. Notably, however, no decrease in TNFα was seen in the valsartan-treated group. This finding complements the report of Hong et al [32] who observed that TNFα decreased only by telmisartan and not by valsartan in hypertensive-diabetic patients.

The rise in adiponectin per se, seen in telmisartan- but not in valsartan-treated rats, which can lower inflammatory cytokines and TNFα signaling [33,34], could have further contributed to the lowering of TNFα and its downstream effects in our experiments. Two aspects of the rise in adiponectin in our study merit further examination. First, because adiponectin is expressed exclusively in adipocytes, the improved profile of adiponectin secretion with telmisartan in 3 different models concurs with the concept that telmisartan can favorably modulate adipocyte function and improve its secretory profile in vivo [18,35]. Second, this fat cell-dependent, metabolically beneficial effect of telmisartan is seen in 2 nonobese models of type 2 diabetes mellitus, without and with concomitant hypertension, and is apparently not attained through weight loss. A recent report indicated that telmisartan induced adipogenesis and activated PPAR-γ target genes, which could be prevented by the PPAR-γ antagonist GW9662 [36,37]. Furthermore, adiponectin secretion from adipocytes stimulated with pioglitazone was blocked by GW9662. Previous studies demonstrated that telmisartan up-regulated adiponectin

secretion in Zucker obese rats [38]; in overweight rats fed a high-fat, high-carbohydrate diet [39]; and in diet-induced obese mice [40]. Thus, these effects induced by telmisartan activation of PPAR-γ; lowering glucose levels in one side and averting the induced rise of adiponectin, indicate the involvement of PPAR-γ activation, most probably through adiponectin insulin sensitization and improved glucose uptake by tissue.

Telmisartan reduced the accumulation of visceral fat and decreased adipocyte size to a much greater extent than valsartan [8]. Adipocyte downsizing was significantly greater with telmisartan than valsartan [41], a finding consistent with our own observation in CRDH. Still, this finding could also be related, in part, to the fact that the final weight of the telmisartan-treated CRDH was somewhat less than that of the valsartan-treated group. Nevertheless, our study represents the only example to date that telmisartan can increase adiponectin in vivo in the absence of obesity.

Besides the increase in adiponectin and the fall in glucose, telmisartan treatment was also associated with a clear trend of leptin reduction in CRDH and CDR animals, whereas valsartan treatment increased leptin levels. Both circulating leptin and serum adiponectin apparently predict the development of metabolic syndrome independently of obesity [42,43]. In agreement with Matsubara et al [44], we observed a negative correlation between the leptin and adiponectin, although this was not consistently significant.

In conclusion, the increase in adiponectin in association with a decline in the proinflammatory and diabetogenic cytokine TNFα most likely contributed strongly to the improved glucose homeostasis attained with telmisartan, probably via enhancement of insulin sensitivity. This was seen in the absence of obesity, suggesting that fat tissue can be pharmacologically manipulated to generate therapeutic effects even in the nonobese state. Our finding that telmisartan induced permutation of adipocyte function, manifested by and operating through favorable changes in circulating adipokines, may constitute an important addition to its direct antihypertensive, vascular, and cardiac protective effects.

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