ORIGINAL RESEARCH

Telmisartan is effective to ameliorate metabolic syndrome in rat model – a preclinical report

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Background: Metabolic syndrome (MS) is known to be associated with hypertension, insulin resistance, and dyslipidemia, and it raises the risk for cardiovascular diseases and diabetes mellitus. Telmisartan is used in clinic as an angiotensin II receptor blocker and it is also identified as activating peroxisome proliferator-activated receptors δ (PPARδ). Activation of PPARδ produced beneficial effects on fatty acid metabolism and glucose metabolism. This study aims to investigate the effects of telmisartan on the modulation of MS in rats fed a high-fat/high-sodium diet.

Methods: Rats were fed with a high-fat/high-sodium diet and received injections of streptozotocin at low dose to induce MS. Then, rats with MS were treated with telmisartan. The weight, glucose tolerance, and insulin sensitivity were measured. The lipid profiles were also obtained. The weights of retroperitoneal and epididymal fat pads were determined. The role of PPARδ in telmisartan treatment was identified in rats pretreated with the specific antagonist GSK0660.

Results: The results showed that telmisartan, but not losartan, significantly reduced plasma glucose and plasma insulin, and improved insulin resistance in rats with MS. Telmisartan also decreased blood pressure and lipids more significantly than losartan. Moreover, GSK0660 effectively reversed the effects of telmisartan in the MS rats. In the MS group, telmisartan activated PPARδ to enhance the levels of phosphorylated GLUT4 in muscle or the expression of phosphoenolpyruvate carboxykinase (PEPCK) in the liver, which was also abolished by GSK0660. Telmisartan is useful to ameliorate hypertension and insulin resistance in rats with MS. Telmisartan improves the insulin resistance through increased expression of GLUT4 and down-regulation of PEPCK via PPARδ-dependent mechanisms.

Conclusion: Telmisartan has been proven to ameliorate MS, particularly in the prediabetes state. Therefore, telmisartan is suitable to develop for the management of MS in clinics.

Keywords: metabolic syndrome, telmisartan, PPARδ, GSK0660, diet

Introduction

Metabolic syndrome (MS) is a cluster of risk factors for metabolic abnormalities and cardiovascular disease. It includes abdominal obesity, dyslipidemia, hypertension, and hyperglycemia.[1] Prevalence of MS is rapidly increasing worldwide.[2] Approximately 31 % of the world's adult population is estimated to have MS.[3] Moreover, MS is associated with a 2.5-fold increase in cardiovascular- and diabetes-related mortalities.[4]

Diet is a potential factor that could be responsible for the rise in MS and the associated cardiovascular pathologies.[5] The diet pattern in Western countries is generally characterized by high intake of carbohydrates and saturated fat. The increase in calorific intake has been associated with many diet-induced complications, including MS,

cardiovascular diseases, and nonalcoholic fatty liver disease. High dietary fat intake is associated with oxidative stress and an activation of the proinflammatory transcription factors.[6]

High salt intake is also a significant environmental factor and is strongly associated with high blood pressure (BP). It has been previously indicated that essential hypertension is frequently related to insulin resistance and compensatory hyperinsulinemia.[7] MS patients also exhibit enhanced BP in response to sodium intake.[8] Insulin resistance could activate the renin-angiotensin system (RAS) by increasing the expression of angiotensinogen, angiotensin II (AT2), and angiotensin receptor (AR), which may contribute to the development of hypertension.[9] It has been recently discovered that adipocytes also produce aldosterone in response to AT2.[10]

The peroxisome proliferator-activated receptor δ (PPARδ) is a transcription factor that belongs to the superfamily of nuclear receptors.[11] Activation of PPARδ has beneficial effects on fatty acid and glucose metabolism.[12] Moreover, PPARδ could enhance fatty acid β-oxidation and attenuate MS.[13] PPARδ activation could prevent obesity and exert protective effects on hypertension and on the early manifestations of atherosclerosis in high-fat (HF) diet-fed mice.[14]

Telmisartan, an AR blocker (ARB), has the highest affinity for AT2 receptors among the available ARBs.[15] Telmisartan has a profound role in the improvement of glucose homeostasis in skeletal muscle, which is associated with activation of PPARδ.[16] Several studies have revealed that telmisartan improves insulin sensitivity in patients with hypertension or the early stages of diabetes mellitus.[17,18]

Many animal models were used to study disorders of MS[19] in a manner to mimic the major signs of MS. In the induction of animal models, various approaches were applied in rodents including dietary manipulation, genetic modification, and drugs.[19] However, limitations of each model were observed. Dietary approaches included the use of a single type of diet or a combination of diets, such as high-fructose,[20] HF,[21] high-fructose/HF,[22] which usually affects the whole-body metabolism, but the effects is limited[23] and the symptom did not include hypertension. The genetic models of MS included leptin-deficient (ob/ob) or leptin receptor-deficient (db/db) mice. Unlike humans with MS, ob/ob mice did not develop dyslipidaemia,[24] and both ob/ob and db/db mice did not show hypertension.[25,26] Although HF fed, spontaneously hypertensive rats show some symptoms of MS, they have genetically induced, rather than diet-induced, hypertension.[27] Models of drug-induced MS include glucocorticoid-

induced[28] and antipsychotic-induced[29] models, which seem more suitable for the research of specific diseases. In this study, we established a MS model based on environmental effects, which promoted blood glucose, blood pressure, and blood fat using the HF, high-sodium (HS) diet intake and a low-dose of streptozotocin (STZ) injection. The main aim of this study was to investigate the effects of telmisartan on insulin resistance, hyperlipidemia, and hypertension in rats with MS.

Methods
Animals
Male Sprague Dawley rats weighing 180–220 g were obtained from the National Animal Center (Taipei, Taiwan) and maintained in the animal center of Chi Mei Medical Center (Tainan, Taiwan). The animals were housed two rats per cage on a 12/12-hour light/dark cycle at a constant temperature (24 °C±1 °C) and humidity (60%±10%). This project was approved by the Institutional Animal Care and Use Committee of Chi Mei Medical Center (No. 105110330). The *Guide for the Care* was referred to during this study.

Rat model with MS
The rats were randomly fed either standard rat chow (13.43% kcal as fat; TestDiet®; Richmond, IN, USA) or HF/HS diet for 8 weeks. Custom HF diets (60% kcal as fat; LabDiet®; St Louis, MO, USA) were applied to prepare a HF/ HS (4% NaCl) diet.[30] All rats freely received normal tap water.

After an 8-week feeding of a HF/HS diet, rats were starved for 12 hours then injected intraperitoneally with STZ at low dose (30 mg/kg)[31] and were continued to be fed the same diet during the experiments. After 7 days of STZ injections, the rats had hyperglycemia (>200 mg/dL), hyperlipidemia (total cholesterol [TC] >110 mg/dL and triglyceride [TG] >150 mg/dL), an increase in body weight (8% of initial weight) or mean arterial BP >130 mmHg, and a marked decrease in high-density lipoprotein (HDL) cholesterol (<35 mg/dL) that were used to confirm the development of MS.[32] Rats with MS were allowed the HF/HS diet until the end of the study.

Treatment protocols
Once MS occurred, models and controls were treated by oral gavage with telmisartan (8 mg/kg/day; Boehringer Ingelheim, Ingelheim am Rhein, Germany)[10] or losartan (8 mg/kg/day; Zydus Pharmaceuticals, Pennington, NJ, USA) for 4 weeks. Moreover, PPARδ antagonist GSK0660 (10 mg/kg; Sigma-

Aldrich Co., St Louis, MO, USA) was intraperitoneally injected 30 minutes before telmisartan administration.

Food and water intake were measured daily. Body weight was monitored weekly. BP was determined at week 9 (before drug treatment) and week 13 (the end of 4-week periods of the drug treatment) using the tail-cuff method with a sphygmomanometer (Muromachi Kikai Co., Ltd., Tokyo, Japan).[33]

At week 14, insulin tolerance tests (ITTs) were performed in the rats fasting overnight. According to a previous report,[34] rats were intraperitoneally injected with 0.75 IU/kg of regular insulin. Blood was collected from the tail vein of rats under anesthesia before injection and after 15, 30, 60, 90, and 120 minutes.

At the end of the study, livers and soleus muscles were collected from the sacrificed rats. The weight of retroperitoneal and epididymal fat pads were also measured. All samples were immediately frozen in liquid nitrogen and kept at –80°C for further assays.

Biochemical measurements

Blood samples were collected from the tail vein of rats that were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and all efforts were made to minimize the animals' suffering. Blood glucose concentration was measured[35] using commercial kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum insulin concentrations were also measured using commercialized enzyme-linked immunosorbent assay kits (Mercodia AB, Uppsala, Sweden). The following formula was used to calculate the homeostasis model assessment for insulin resistance (HOMA-IR): (fasting insulin [μU/mL] × fasting glucose [mg/dL])/405. Additionally, the area under the curve was evaluated for the glucose concentrations determined at 0, 30, 60, 90, and 120 minutes. The lipid profile, including concentrations of TC, TG, and HDL, was estimated using laboratory kit reagents (Randox Laboratories, Crumlin, UK). The low-density lipoprotein (LDL) levels were then calculated using Frieldwann's equation.

Western blotting analysis

The Western blotting analysis was was performed according to the previous method.[52] Total protein lysates were extracted in lysis buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris [pH 7.5], and 5 mM ethylenediaminetetraacetic acid), containing a protease and phosphatase inhibitor cocktail (Sigma-Aldrich Co.). The protein concentration was determined with the BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The following primary antibodies were used at 4°C overnight: anti-PPARδ (1:1000) and anti-GLUT4 (1:1000) (Abcam, Cambridge, UK); and anti-phosphoenolpyruvate carboxykinase (anti-PEPCK) (1:1000) (EMD Millipore, Billerica, MA, USA) was used as an internal control. The next day, the blots were incubated in the secondary antibodies at room temperature for 1 hour. Protein bands were visualized using the enhanced chemiluminescence kit (PerkinElmer Inc., Boston, MA, USA). The optical densities of the bands were determined using software (Gel-Pro® Analyzer Version 4.0 software; Media Cybernetics Inc., Rockville, MD, USA).

Statistical analyses

All results are provided as mean ± SEM of each group. All statistical analyses were carried out by SPSS, Version 21 (IBM Corporation, Armonk, NY, USA). Differences between the two groups were determined by two-way repeated measures ANOVA. For comparisons between two independent groups, a Student's t-test was used. Significance was accepted at $p<0.05$.

Results

Telmisartan ameliorated hyperglycemia and insulin resistance in MS rats

Rats fed the HF/HS diet for 8 weeks followed by an injection of STZ (30 mg/kg) developed MS because they became hyperphagic, obese, hyperlipidemia, insulin resistant, and hypertensive. The MS group displayed moderate glucose intolerance characterized by a 2.0-fold increase in fasting serum glucose and 2.2-fold increase in HOMA-IR index. In ITT experiments, the MS group showed higher glucose levels in serum that were improved by telmisartan treatment compared with the normal control group.

Blood glucose was significantly reduced in MS rats treated with telmisartan compared with the vehicle-treated MS group (–28%, $P<0.05$) (Figure 1A). Similarly, the HOMA-IR was significantly attenuated in the telmisartan-treated group (–29%, $P<0.05$). However, losartan did not affect the glucose levels in MS rats (Figure 1C−E).

Effects of telmisartan on body weight, food intake and white adipose mass in MS rats

The MS group showed a 14% increase in body weight compared with the control group. Also, the MS group significantly increased retroperitoneal fat mass and epididymal fat mass in a different way to the control group (Table 1). In addition, the
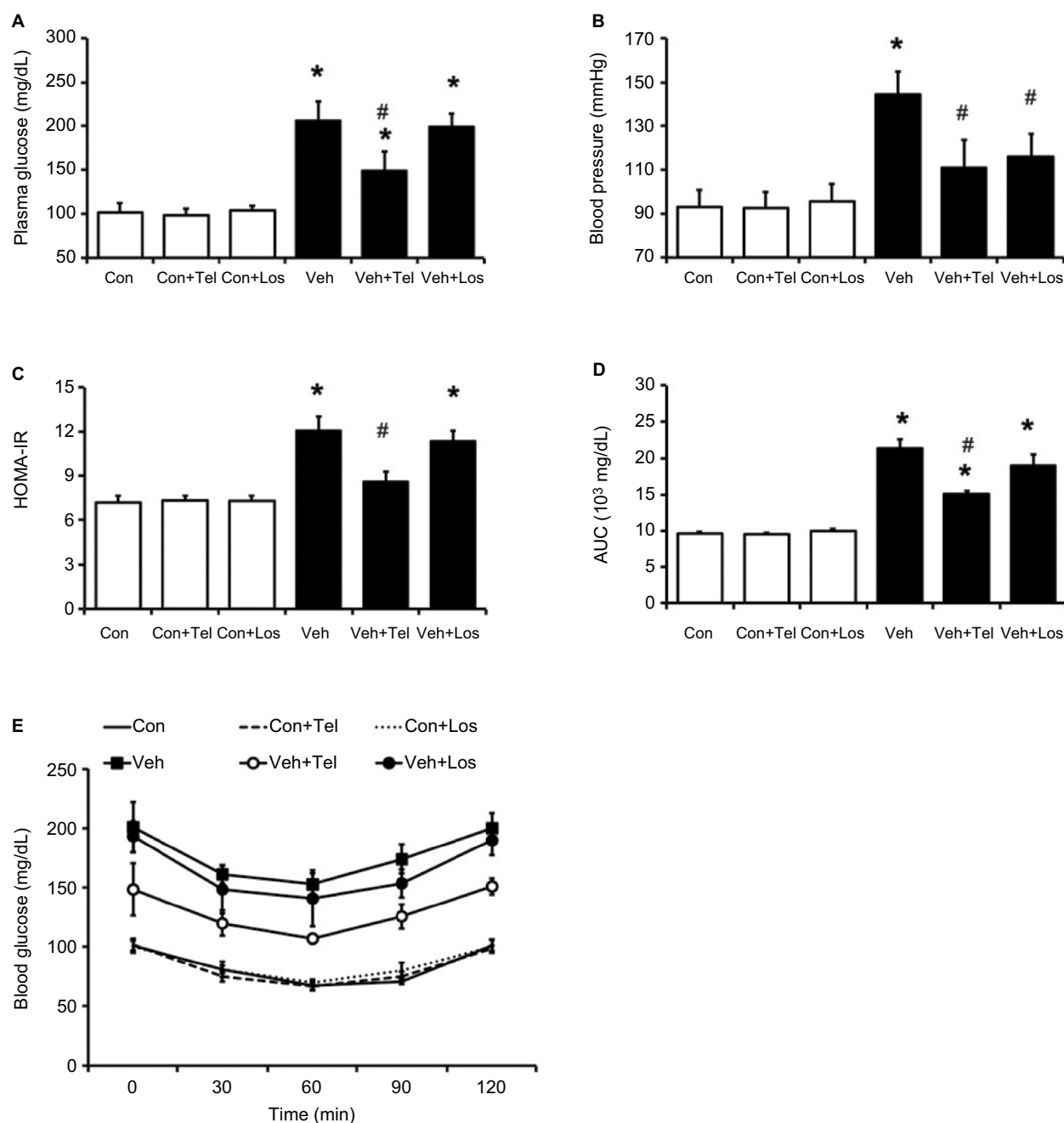
Figure 1 Effects of telmisartan or losartan on MS rats.
Notes: The change in (**A**) blood glucose; (**B**) mean arterial blood pressure; (**C**) HOMA-IR; (**D**) AUC of HOMA-IR; and (**E**) ITT in normal rats and MS rats after 4 weeks of experimental period. Con (white column), SD rats fed normal chow diet; Con+Tel (white column), SD rats fed normal chow diet and administered with telmisartan (8mg/kg); Con+Los (white column), SD rats fed normal chow diet and administered with losartan (8mg/kg); Veh (black column), MS rats received with vehicle; Veh+Tel (black column), MS rats received administered with telmisartan (8mg/kg) for 4 weeks; Veh+Los (black column), MS rats received administered with losartan (8mg/kg) for 4 weeks. Data are expressed as mean ± SEM (n = 6). *$P<0.05$ vs Con; #$P<0.05$ vs Veh.
Abbreviations: AUC, area under the curve; Con, control; HOMA-IR, homeostatic model assessment for insulin resistance; ITT, insulin tolerance test; Los, losartan; MS, metabolic syndrome; SD, Sprague Dawley; Veh, vehicle; Tel, telmisartan.

average daily food intake and water intake were elevated in MS groups more markedly than in the control group (Table 2).

Telmisartan significantly reduced the rise in body weight of MS rats compared with the vehicle-treated MS rats. Retroperitoneal fat mass and epididymal fat mass were also noted

to be reduced by telmisartan in the MS group at the end of experiments. However, a similar change was not observed in MS rats administered with losartan. Additionally, telmisartan or losartan did not influence body weight or fat mass in rats that received normal chow diet (Table 1).

Table 1 Effects of telmisartan (Tel) or losartan (Los) on the metabolic parameters in normal rat and MS rats

	Con	Con+Tel	Con+Los	MS +Veh	MS +Tel	MS +Los
TG (mg/dL)	70.5±7.21	72.88±8.13	73.75±7.05	131.00±9.74*	105.63±8.43*#	122.25±8.31*
TC (mg/dL)	57.75±4.53	57.63±5.45	58.63±4.66	74.13±5.59*	67.13±5.17#	70.63±6.55*
HDL(mg/dL)	25.00±2.39	25.75±2.19	25.88±1.73	20.50±1.31*	24.25±2.66#	21.25±1.75*
LDL(mg/dL)	17.90±4.82	17.30±6.07	18.00±4.77	27.43±6.28*	18.55±7.94#	24.93±5.96*
Retroperitoneal fat mass (g)	4.54±0.75	4.49±0.66	4.71±0.45	6.78±0.75*	5.66±0.78#	6.71±0.55*
Epididymal fat mass (g)	4.23±0.53	4.33±0.69	4.58±0.57	7.21±0.95*	5.98±0.96*#	6.44±0.56*
Body weight (g)	272.63±10.01	276.5±7.83	279.75±8.71	311.38±13.70*	289.25±6.84#	314.13±16.25*
The increase of body weight (Compared with Con,%)	≈0	≈0	≈0	14.36±1.72*	4.43±1.55#	15.14±1.20*

Notes: Control (Con), SD rats fed normal chow diet; Metabolic Syndrome (MS), SD rats were induced MS model as described in methods; Vehicle (Veh), MS rats received vehicle. Rats were daily administered with telmisartan (8 mg/kg) or losartan (8 mg/kg) for 4 weeks. Data shown mean ± SEM (n = 6). *$P<0.05$ vs. Con; #$P<0.05$ vs. Veh.
Abbreviation: SD, Sprague Dawley; Con, control; Los, losartan; MS, metabolic syndrome; Veh, vehicle; Tel, telmisartan.

Table 2 Effects of telmisartan or losartan on food intake and water intake in normal rats and MS rats

	Con	Con+Tel	Con+Los	MS+Veh	MS+Tel	Con+Los
Food intake (g/day)	36.88±2.47	39.13±3.76	38.38±1.89	47.88±1.35*	49.50±1.77*	48.75±4.20
Water intake (g/day)	30.88±2.47	30.75±2.25	29.25±1.84	41.88±3.489	43.50±5.13*	45.75±4.49

Notes: Average daily food and water intakes were calculated. Con, SD rats fed normal chow diet; MS, MS rat model using SD rats as described in Methods; Veh, MS rats received vehicle. Rats were administered daily with telmisartan (8 mg/kg) or losartan (8mg/kg) for 4 weeks. Data are expressed as mean ± SEM (n = 6). *$P<0.05$ vs Con; #$P<0.05$ vs Veh.
Abbreviations: Con, control; Los, losartan; MS, metabolic syndrome; SD, Sprague Dawley; Tel, telmisartan; Veh, vehicle.

Telmisartan improved lipid metabolism in MS rats

Lipids in normal control or MS rats treated with telmisartan or losartan shown in Table 1. Significant increases in TC, TG, HDL, and LDL cholesterol concentrations were observed in MS rats.

Telmisartan attenuated the plasma TC, TG and LDL levels significantly and increased the HDL levels. However, losartan produced a little improvement in the lipids in MS rats compared with telmisartan-treated MS rats.

Effects of telmisartan and losartan on BP

Mean arterial BP was significantly increased in MS rats and mild hypertension was observed compared with the control group (144.3±10.7 vs 92.9±7.9 mmHg, $P<0.05$). A significant reduction in BP was observed in MS rats treated with telmisartan or losartan (telmisartan: 110±12.7 mmHg and losartan: 115.8±10.6 mmHg) compared with those not treated (144.3±10.7 mmHg, $P<0.05$) (Figure 1B).
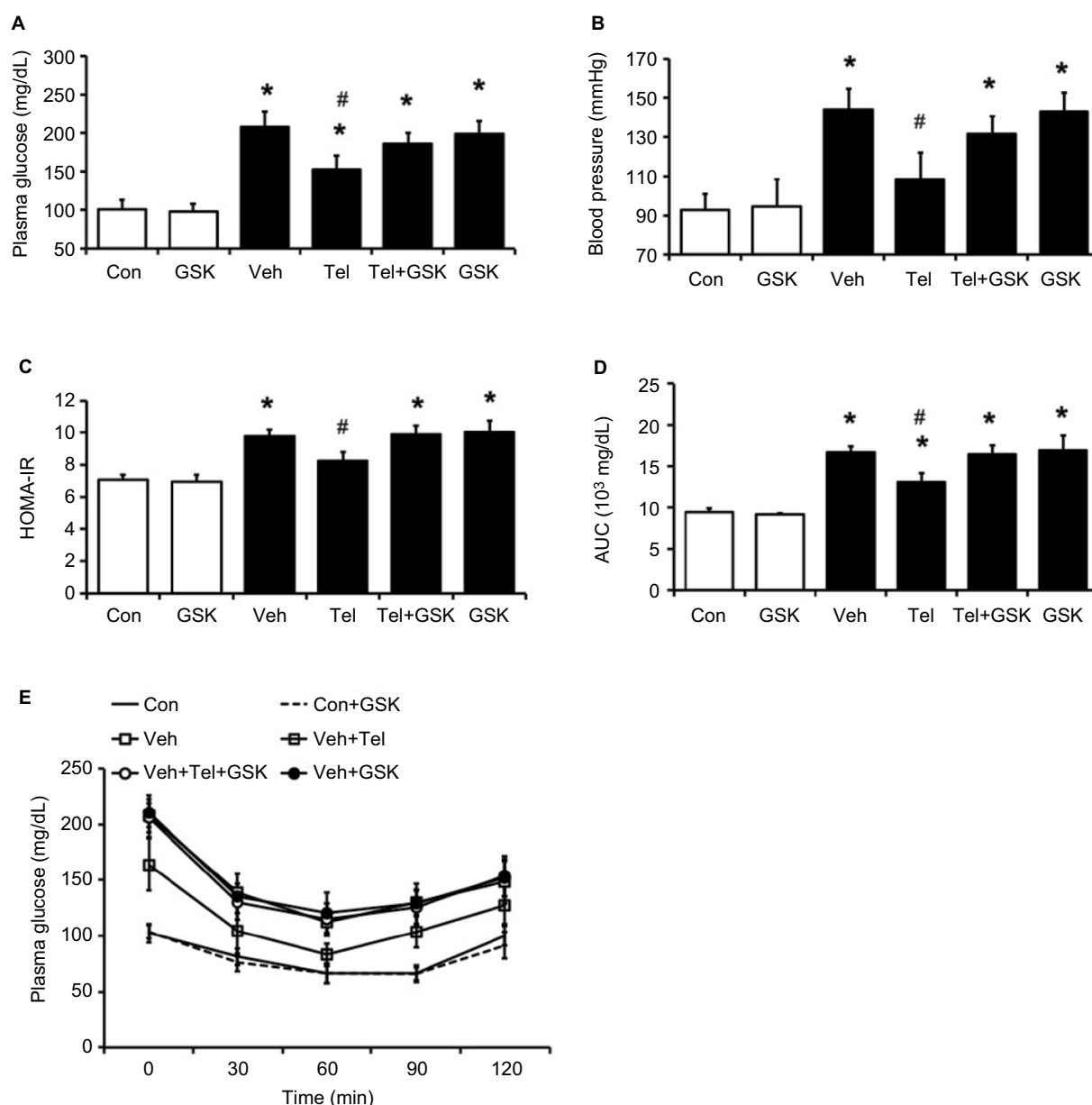
PPARδ antagonist inhibited the effects of telmisartan in MS rats

To investigate the role of PPARδ in the effects of telmisartan in MS rats, the selective PPARδ antagonist GSK0660 was pretreated with telmisartan.

GSK0660 significantly reduced the beneficial effects of telmisartan in blood glucose (from 153.0±17.1 mg/dL to 185.6±14.2 mg/dL, $P<0.05$) (Figure 2A). Additionally, TG and TC levels in the telmisartan-treated MS group were reversed markedly (Table 3). Moreover, pretreatment with GSK0660 reversed the reduction of HOMA-IR index and the improvement of ITT, which were induced by telmisartan in MS rats ($P<0.05$) (Figure 2C−E). In addition, the BP-lowering effect of telmisartan was also inhibited by GSK0660; GSK0660 reversed BP from 137±1 mmHg to 152±1 mmHg ($P<0.05$) showing ~30% recovery in the telmisartan-treated MS group (Figure 2B). Administration of GSK0660 also reversed the glucose and lipid profiles modified by telmisartan in MS rats but not in the control group. In addition, GSK0660 also reversed the body weight and adipose mass in MS rats attenuated by telmisartan (Table 3).

Telmisartan ameliorates MS through PPARδ-dependent mechanisms in liver and skeletal muscle

The protein levels of GLUT4 in skeletal muscle and PEPCK in the liver of MS rats were determined. As shown in Figure 3A, the decreased GLUT4 and PPARδ expression in the soleus muscle of MS rats were reversed by telmisartan. Additionally, the increased hepatic PEPCK level in MS rats was also markedly reduced by telmisartan (Figure 3B). But similar changes were not observed in losartan-treated MS

Figure 2 PPARδ antagonist GSK0660 inhibited the effects induced by chronic telmisartan treatment in MS rats.
Notes: The changes in (**A**) blood glucose; (**B**) mean arterial blood pressure; (**C**) HOMA-IR; (**D**) AUC of HOMA-IR; and (**E**) ITT. Con (white column), SD rats fed normal diet; Con+Tel (white column), SD rats fed normal diet and administered with telmisartan (8 mg/kg); Veh (black column), MS rats received telmisartan (8 mg/kg) for 4 weeks and GSK0660 were pretreated 30 minutes before telmisartan treatment; Veh+GSK (black column) MS rats received administered with vehicle and GSK0660 for 4 weeks. Data are expressed as mean ± SEM (n = 6). *$P<0.05$ vs Con; #$P<0.05$ vs Veh.
Abbreviations: AUC, area under the curve; Con, control; HOMA-IR, homeostatic model assessment; ITT, insulin tolerance test; MS, metabolic syndrome; PPARδ, peroxisome proliferator-activated receptor delta; SD, Sprague Dawley; Tel, telmisartan; Veh, vehicle.

rats. Moreover, the effects of telmisartan were attenuated by GSK0660 in MS rats (Figure 4). Therefore, the results suggested that the effects of telmisartan in liver and skeletal muscle were PPARδ dependent.

Discussion

A rat model was successfully developed for MS using the modified diet and pancreatic toxin described in this study.

Male SD rats received a HF/HS diet for 8 weeks, followed by a low-dose STZ (30 mg/kg) injection. The MS rats showed insulin resistance, impaired glucose tolerance, obesity, dyslipidemia, and hypertension. Moreover, this MS model mimics the main changes that occur in humans.

In this study, we found that telmisartan improved glucose and/or lipid profiles in MS rats through PPARδ activation. Telmisartan significantly decreased the plasma insulin
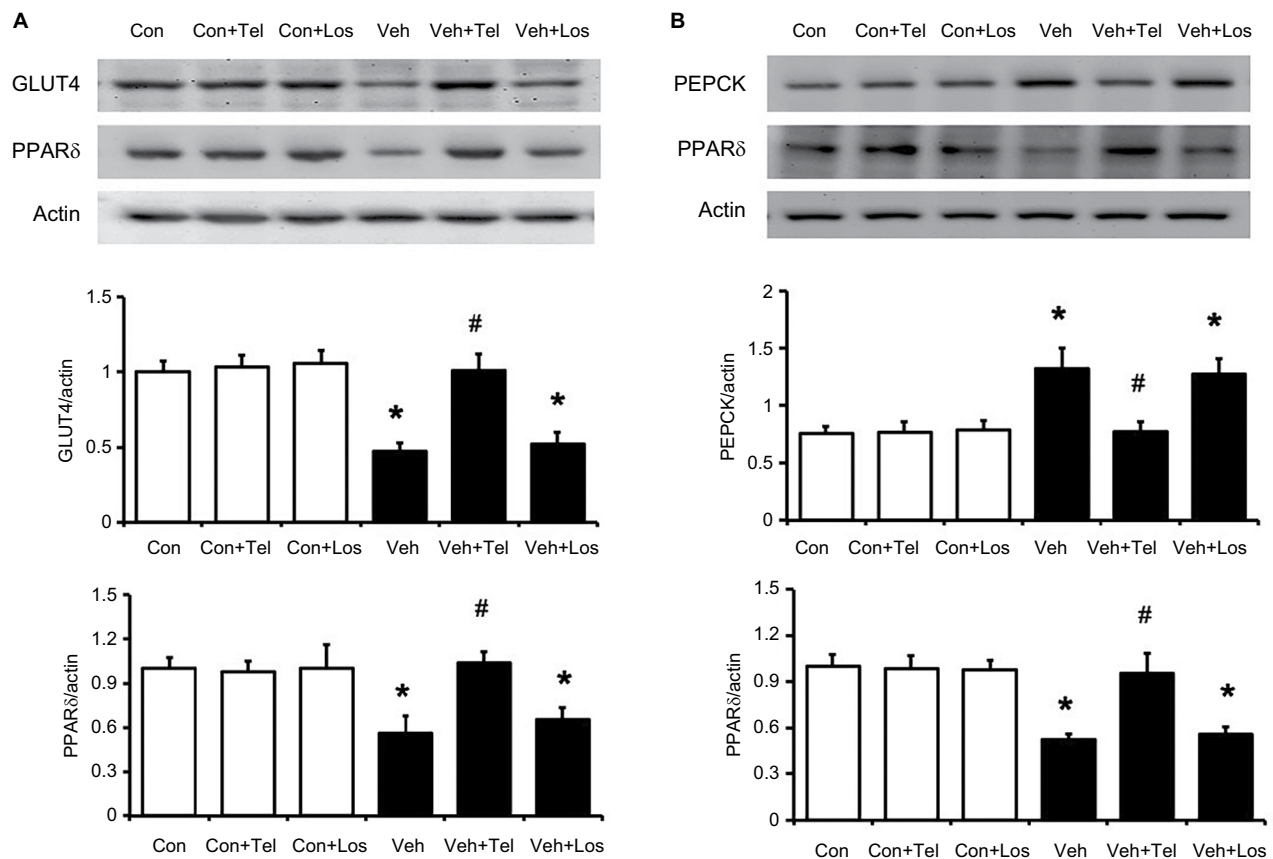
Figure 3 Effects of telmisartan or losartan on PPARδ and related signal expression in skeletal muscle and liver.
Notes: (**A**) Representative immunoblots are shown in the upper part of the figure, and the relative expression levels of GLUT4 and PPARδ expression in soleus muscle are indicated in the lower. (**B**) Representative immunoblots are shown in the upper part of the figure, and the relative expression levels of PEPCK and PPARδ in liver are indicated in the lower. Con (white column), SD rats fed normal diet; Con+Tel (white column), SD rats fed normal diet and administered with telmisartan (8mg/kg); Con+Los (white column), SD rats fed normal diet and administered with losartan (8mg/kg); Veh (black column), MS rats received with vehicle; Veh+Tel (black column), MS rats received administered with telmisartan (8mg/kg) for 4 weeks; Veh+Los (black column), MS rats received administered with losartan (8mg/kg) for 4 weeks. Data are expressed as mean ± SEM (n = 4). *$P<0.05$ vs Con; #$P<0.05$ vs Veh.
Abbreviations: Con, control; PEPCK, phosphoenolpyruvate carboxykinase; PPARδ, peroxisome proliferator-activated receptor delta; Los, losartan; MS, metabolic syndrome; SD, Sprague Dawley; Tel, telmisartan; Veh, vehicle.

Table 3 GSK0660 inhibited the effects of telmisartan on various metabolic parameters in MS rats

Parameters	Con	Con+GSK	MS+Veh	MS+Tel	MS+Tel+GSK	MS+GSK
TG (mg/dL)	70.50±7.21	72.38±9.68	131.00±9.07*	105.63±8.43*#	135.88±10.80*	138.00±12.33*
TC (mg/dL)	57.75±4.53	59.63±4.21	74.13±5.59*	67.13±5.17#	77.13±8.48*	77.25±8.60*
HDL (mg/dL)	25.00±2.39	24.25±2.38	20.50±1.31*	24.25±2.66#	21.38±1.85*	20.63±2.83*
LDL (mg/dL)	17.90±4.82	19.90±6.15	27.43±6.28*	18.55±7.94#	28.58±10.53*	29.03±9.54*
Retroperitoneal fat mass (g)	4.54±0.75	4.62±0.59	6.78±0.75*	5.66±0.78#	7.01±0.65*	6.85±0.42*
Epididymal fat mass (g)	4.23±0.53	4.31±0.73	7.21±0.95*	5.98±0.96*#	6.92 ±0.58*	6.44±0.56*
Body weight (g)	272.63±10.01	268.4±8.83	311.38±13.70*	289.25±6.84#	320.51±13.31*	318.87±14.58*
The increase of body weight (compared with Con, %)	≈0	≈0	14.36±1.72*	4.43±1.55#	16.19±2.51*	15.74±2.32*

Notes: Con, SD rats fed normal chow diet; MS, MS rat model using SD rats as described in Methods; Veh, MS rats received the vehicle. Rats were administered with telmisartan (8 mg/kg) or losartan (8mg/kg) for 4 weeks. GSK0660 were pretreated 30min before telmisartan administration. Data are expressed as mean ± SEM (n = 6). *$P<0.05$ vs Con; #$P<0.05$ vs Veh.
Abbreviations: Con, control; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MS, metabolic syndrome; SD, Sprague Dawley; TC, total cholesterol; Tel, telmisartan; TG, triglyceride; Veh, vehicle.

and markedly reduced plasma glucose. Telmisartan also alleviated the impaired insulin resistance and ameliorated the responses of ITT and HOMA-IR in MS rats. Addition-

ally, telmisartan significantly attenuated the increased adipose mass and reduced increased plasma TC and TG concentrations that may be due to modification of adipocyte
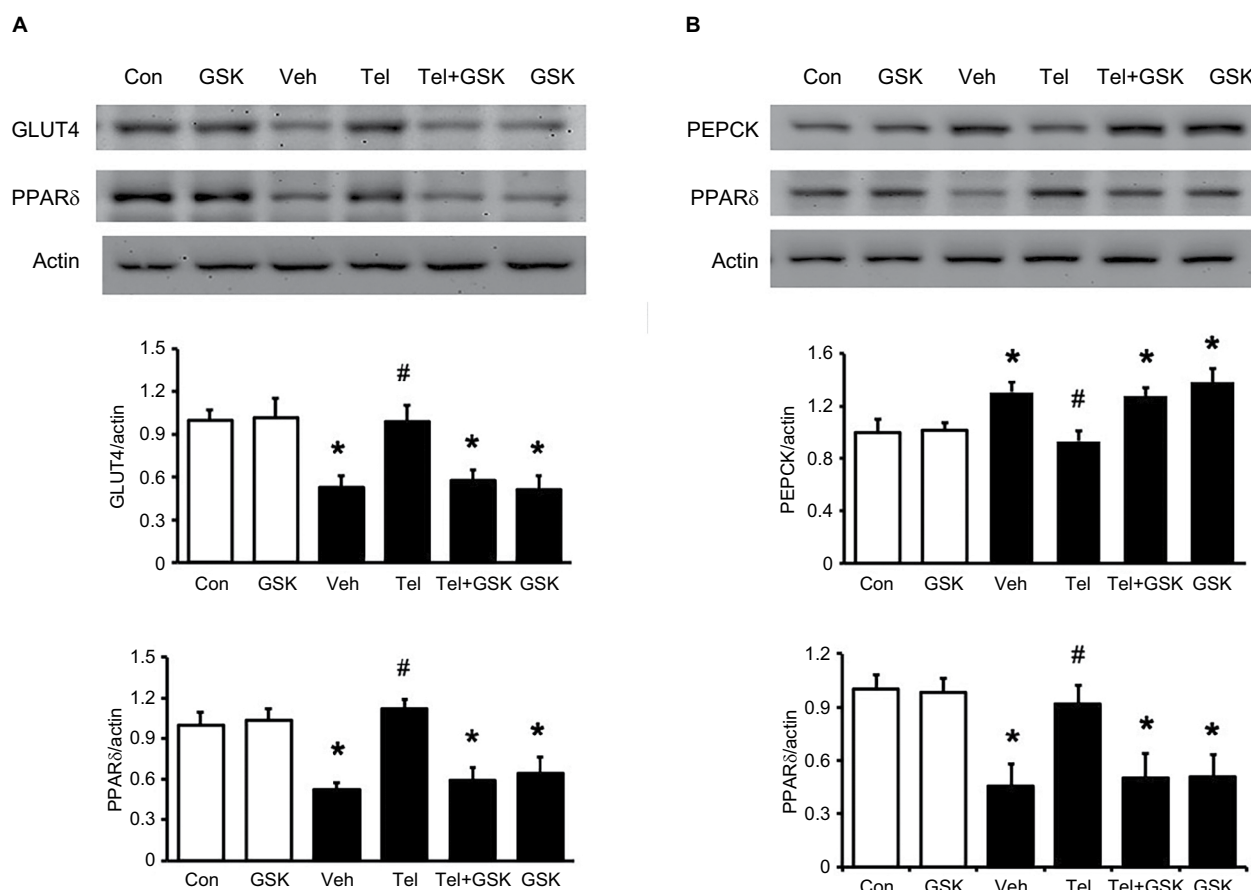
Figure 4 Effects of the PPARδ antagonist GSK0660 on expressions of PPARδ and related signals in skeletal muscle and liver isolated from the telmisartan-treated MS rats.
Notes: (**A**) Representative immunoblots are shown in the upper part of the figure, and the relative expression levels of GLUT4 and PPARδ in soleus muscle are indicated in the lower. (**B**) Representative immunoblots are shown in the upper part of the figure and the relative expression levels of PEPCK and PPARδ in the liver are indicated in the lower. Con (white column), SD rats fed normal diet; Con+Tel (white column), SD rats fed normal diet and administered with telmisartan (8mg/kg); Veh (black column), MS rats received with vehicle; Veh+Tel (black column), MS rats received administered with telmisartan (8mg/kg) for 4 weeks; Veh+Tel+GSK (black column), MS rats received administered with telmisartan (8mg/kg) for 4 weeks and GSK0660 were pretreated 30 minutes before telmisartan treatment; Veh+GSK (black column) MS rats received administered with GSK0660 and vehicle for 4 weeks. Data are expressed as mean ± SEM (n = 4). *$P<0.05$ vs Con; #$P<0.05$ vs Veh.
Abbreviations: Con, control; GSK, GSK0660; PEPCK, phosphoenolpyruvate carboxykinase; PPARδ, peroxisome proliferator-activated receptor delta; MS, metabolic syndrome; SD, Sprague Dawley; Tel, telmisartan; Veh, vehicle.

biology and metabolism.[36] This resulted in an increase of energy expenditure and decrease of dietary-induced obesity and/or accumulation of visceral fat.[37,38] It is consistent with a previous report that telmisartan activates PPARδ expression and reduces weight gain and HF-induced obesity.[39] In this study, telmisartan produced a beneficial effect on hyperglycemia, insulin resistance, and lipid metabolism more effectively than losartan in the MS model, which is consistent with a previous report.[40] In clinics, telmisartan showed effects on metabolic parameters to a greater degree than losartan in hypertensive patients with MS.[41] A large-scale clinical study reported that hypertensive patients with type 2 diabetes had reduced plasma glucose and serum TG concentrations after 6 months' treatment with telmisartan compared with baseline values.[42] Telmisartan may induce

beneficial effects on MS by direct blockade of the AT1 receptor. Some studies have established that AT1 receptor stimulation by AT2 contributes to insulin resistance and its associated deleterious metabolic profile.[43,44] Therefore, the AT1 receptor blockade ameliorates the disorders and partially explains the beneficial effects of telmisartan on insulin resistance. A recent study indicated that the prevention of weight gain by telmisartan is partly attributed to an Ang-(1-7)-dependent mechanism.[45] However, it also indicated that lowering of BP in fructose-fed rats by the use of other antihypertensive drugs, such as calcium channel blockers,[46,47] failed to show a metabolic impact, which suggests that telmisartan improved insulin resistance via BP-independent mechanisms. Moreover, our study showed that the beneficial effects of telmisartan on MS were markedly

reversed by GSK0660 at the dose sufficient to block PPARδ. Therefore, telmisartan may activate PPARδ to improve glucose and lipid metabolism and prevent the increase of insulin resistance induced by diet.[48]

Increase in insulin sensitivity is mainly induced by the enhancement of insulin signals. PPARδ is the most abundant isoform among the three PPARs in skeletal muscle.[49] Alternatively, Ang II (via AT receptor) is the predominant component of the RAS, which appears to be antagonistic to insulin action and contributes to insulin resistance.[50] Ang II impairs the insulin-induced activation of IRS1 and Akt in addition to GLUT4 membrane translocation in skeletal muscle cells.[51] Although it is an ARB, telmisartan could activate PPARδ to increase the oxidative capacity and result in the usage of glucose or breakdown of fat.[52] Insulin induces GLUT4 translocation to the cellular membrane to facilitate glucose uptake in skeletal muscle. Insulin resistance leads to defective PI3K/Akt signaling, reduced GLUT4 expression, and impaired insulin-stimulated glucose uptake.[49] The present study demonstrated that telmisartan activates PPARδ in the skeletal muscle of MS rats, which is consistent previous research.[16] Moreover; telmisartan attenuated the increased expression of hepatic PEPCK in a dose-related manner. It has been documented that PPARδ functions as a nuclear sensor of dietary fats, capable of modulating immune response through regulation of metabolic programs in the liver.[53] Therefore, telmisartan could activate PPARδ to alter peripheral insulin sensitivity and improve pancreatic β-cell function.

Elevated BP is associated with metabolic disorders.[54] In this study, HS intake was an important factor associated with the exacerbation of hypertension.[55] Excessive salt intake may stimulate ROS production to increase the oxidative stress in various organs including muscle, liver and fat tissues in rats.[56,57] HS diet also causes a decrease in the activity of circulating RAS to lower Ang II levels, which may induce the compensatory upregulation of AT receptors.[58] Telmisartan, as a long-acting ARB, showed the antihypertensive effect more effectively than losartan, which is consistent with a previous report.[59] A 3-year study confirmed the advantage of telmisartan in controlling BP and reducing the risk of MS.[60] Telmisartan may cause an AT receptor blockade to result in a fall of peripheral resistance[59] or a PPAR-dependent increase in eNOS expression and activity.[61] PPARδ has been suggested as a potential therapeutic target in the treatment of hypertensive subjects with insulin resistance. We also confirmed that systemic blockade of PPARδ seems to be associated with the elevation of BP in MS rats. Chronic PPARδ agonist administration in the hypertensive rats induced a marked decrease in BP.[58] In addition, the PPARδ agonist also induced the upregulation of hepatic lipid oxidation processes to suppress Ang II-induced dysfunctional adipogenesis and lipid accumulation.[61]

Conclusion

In summary, we have provided experimental evidence that telmisartan is effective in ameliorating hypertension, hyperinsulinemia, and hypertriglyceridemia through activation of PPARδ in rats with MS. Therefore, the preclinical data support that treatment with telmisartan is suitable for managing patients with MS after clinical trials in the future.

Author contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflict of interests in this work.

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