Angiotensin II receptor blocker telmisartan enhances running endurance of skeletal muscle through activation of the PPAR-δ/AMPK pathway

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Abstract

Clinical trials have shown that angiotensin II receptor blockers reduce the new onset of diabetes in hypertensives; however, the underlying mechanisms remain unknown. We investigated the effects of telmisartan on peroxisome proliferator activated receptor δ (PPAR-δ) and the adenosine monophosphate (AMP)-activated protein kinase (AMPK) pathway in cultured myotubes, as well as on the running endurance of wild-type and PPAR-δ-deficient mice. Administration of telmisartan up-regulated levels of PPAR-δ and phospho-AMPKα in cultured myotubes. However, PPAR-δ gene deficiency completely abolished the telmisartan effect on phospho-AMPKα in vitro. Chronic administration of telmisartan remarkably prevented weight gain, enhanced running endurance and post-exercise oxygen consumption, and increased slow-twitch skeletal muscle fibres in wild-type mice, but these effects were absent in PPAR-δ-deficient mice. The mechanism is involved in PPAR-δ-mediated stimulation of the AMPK pathway. Compared to the control mice, phospho-AMPKα level in skeletal muscle was up-regulated in mice treated with telmisartan. In contrast, phospho-AMPKα expression in skeletal muscle was unchanged in PPAR-δ-deficient mice treated with telmisartan. These findings highlight the ability of telmisartan to improve skeletal muscle function, and they implicate PPAR-δ as a potential therapeutic target for the prevention of type 2 diabetes.

Keywords: peroxisome proliferator activated receptor δ • adenosine monophosphate-activated protein kinase • telmisartan • skeletal muscle • running endurance

Introduction

The rising prevalence of diabetes mellitus requires new approaches to prevent the onset of diabetes among high-risk populations. Hypertension is commonly complicated by insulin resistance and type 2 diabetes. Several antihypertensive agents such as diuretics and β-adrenergic blockers may worsen insulin sensitivity and impair glucose tolerance; however, angiotensin II receptor blockers (ARBs) exert beneficial effects in hypertensives such as improving insulin sensitivity and preventing the onset of diabetes [1]. Some ARBs, such as telmisartan, can effectively activate the peroxisome proliferator activated receptor γ (PPAR-γ), a key regulator of adipocyte differentiation and adipose insulin sensitivity [2]. However, PPAR-γ is rarely expressed in skeletal muscle. In contrast, PPAR-δ (also referred to as PPAR-β) is expressed in a wide variety of tissues, with high levels in skeletal muscle [3]. In addition to pharmacological treatments, exercise plays an important role in the prevention of hypertension and diabetes. Skeletal muscle is the main tissue that is critical not only in endurance exercise but also in controlling glucose homeostasis. Recent studies have shown that PPAR-δ and AMPK regulate the metabolic and contractile characteristics of myofibres [4]. PPAR-δ overexpressing mice have a predominance of oxidative type I muscle fibres, enhanced whole-body insulin sensitivity, and greater endurance [5]. Activation of AMPK improved mouse exercise performance by 44% over that in controls [4]. Thus, activation of the PPAR-δ/AMPK pathway...
pathway may offer a promising strategy for enhancing running endurance and improving glucose homeostasis. Currently, specific PPAR-δ and AMPK agonists are not available for clinical use. Thus, it is important to determine whether an ARB (such as telmisartan) can affect PPAR-δ/AMPK and their related functions. Given the importance of skeletal muscle function in the development of type 2 diabetes, we suggested that telmisartan has direct effects on skeletal muscle function and remodelling via the PPAR-δ/AMPK pathway. Here, we present evidence that activation of the PPAR-δ/AMPK by telmisartan is a key target for enhancing the running endurance of skeletal muscle in mice.

Materials and methods

Animals and experimental procedures

Male C57BL/6J wild-type and PPAR-δ-deficient mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). The animals were housed in cages at a controlled temperature (22°C ± 1°C) and relative humidity (55% ± 15%) and kept in a 12 hrs light/12 hrs dark cycle. They were supplied with standard laboratory chow and tap water ad libitum until 8 weeks of age. Then the animals were randomly divided into telmisartan treated group (n = 10) and normal diet control group (n = 10). The control group was fed standard laboratory chow, which consists of 10% calories as fat and 68% as carbohydrate. The treated group was given telmisartan (5 mg/kg body wt) mixed in the chow. Daily food intake per mouse was recorded during the first 10 days after the start of telmisartan administration. Body masses were measured every 2 weeks throughout the experimental period. Other functional experiments were performed. Then during the 24th week, they were killed after having been made to fast for 16 hrs. Blood was obtained from the carotid arteries of mice. Serum glucose, triglyceride and insulin were measured using routine techniques. The gastrocnemius muscle was quickly removed, frozen and stored at –70°C for later analysis.

Oxygen consumption analysis

Oxygen consumption was measured in PPAR-δ-deficient mice and wild-type C57BL/6J mice using the OUBIT systems respiration package (Oubit Systems, Inc., Ontario, Canada) in basal and post-exercise conditions. In the post-exercise condition the animals ran on the treadmill at 10 m/min. for 10 min. before being measured. Animals were placed individually in chambers. Results are expressed as millilitre per kilogram per minute.

Metachromatic fibre typing of skeletal muscle

Fibre typing was performed with cryostat sections of the gastrocnemius muscle using the metachromatic dye-ATPase method as described previously [7]. Muscle sections were treated in pre-incubation buffer (50 mM potassium acetate, pH 4.4, 17 mM CaCl2) for 8 min., followed with three washes in Tris rinse buffer (300 mM Tris-HCl, pH 7.8, 53 mM CaCl2). Muscle slides were then put into incubation buffer (53 mM glycine, pH 9.4, 28 mM CaCl2, 65 mM NaCl, 47.5 mM NaOH, 4 mM ATP) for 25 min. and rinsed three times in 1% (w/v) CaCl2 solution. Then, 0.1% toluidine blue was used to stain muscle fibres for 1 min. The sections were rinsed in H2O and dehydrated with ethanol. All steps were carried out at room temperature. The proportion of type I slow fibres were analysed with NIS-Elements imaging software (Nikon Corporation, Tokyo, Japan).

Semi-quantitative conventional polymerase chain reaction

mRNA levels of PPAR-δ, PPAR-γ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control were measured in cultured primary skeletal muscle cells or skeletal muscle tissue using standard techniques as we have reported previously [8]. Total RNA was isolated with Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). RNA was used to synthesize first-strand cDNA using the Reverse Transcription System (Promega, Madison, WI, USA); 3 μg of purified mRNA was reverse-transcribed with an RT mixture consisting of oligo dT (12 to 18) and 5 U avian myeloblastosis virus (AMV) reverse transcriptase at 42°C for every 48 hrs. After confluence, cells were given DMEM without FBS. Telmisartan was dissolved in dimethyl sulfoxide (DMSO) and added to the media within 0.1% of volume.

Assessment of running endurance

PPAR-δ-deficient mice and wild-type mice were subjected to a treadmill running test, conducted following a protocol modified from that of Wang [5]. Briefly, prior to the exercise performance test, the mice were acclimated to the treadmill with a 5-min. run at 7 m/min. once per day for 2 days. For the test, the treadmill was started at 10 m/min. for the first 60 min., and then the speed was incrementally increased 1 m/min. every 15 min. until the mouse reached exhaustion. Exhaustion was defined as when a mouse was unable to avoid repetitive electrical shocks. Running endurance was estimated by the following two parameters: the duration of the run (in minutes) and the distance run (in metres).
60 min., followed by heating to 95°C for 5 min. The single-stranded cDNA was amplified by PCR using Taq DNA polymerase (Sangon Biological Engineering, Shanghai, China). The sense and antisense primers for the coding regions of PPAR-δ, PPAR-γ or GAPDH genes were the following: PPAR-δ (Reference Sequence [Ref Seq] database accession number: NM_001473623), forward, 5'-CGACGCTTTGCTACCCAC-3'; reverse, 5'-CGACCTTGGCCATCT-3'; expected size, 597 bp; PPAR-γ (Ref Seq NM_011146), forward, 5'-CTGACCAAGCCAGAGGAGG-3'; reverse, 5'-TTCAGCAGGCGGGATCCCT-3'; expected size, 523 bp; GAPDH (Ref Seq BC095932), forward, 5'-ACCTCAACTACATGGTCTAC-3'; reverse, 5'-TTGTCAATTGAGAGCAATGCC-3'; expected size, 802 bp. The reaction was initiated at 94°C for 4 min. and followed by 32 cycles of denaturation at 94°C for 30 sec., annealing at 60°C for 30 sec. and extension at 72°C for 40 sec. The reaction was achieved with a final extension at 72°C for 5 min. Each sample was run in parallel with the housekeeping gene. Control experiments in which reverse transcriptase was omitted were performed in order to exclude the possibility that the signal detected could come from the genomic DNA. A total of 14 μl of the PCR products were size fractionated on an agarose gel (1.5%) and quantified using a Gel Doc 2000 Imager (Bio-Rad, CA, USA).

Western blot analyses

Western blot analyses of PPAR-δ, PPAR-γ, AMPKα, phospho-AMPKα, angiotensin II type 1 (AT1) receptor, Troponin I-ss and fl-actin from cultured primary skeletal muscle cells or skeletal muscle tissue were performed with standard techniques as reported by our group previously [8]. Briefly, total protein of the skeletal muscle cells in primary culture or the gastrocnemius muscle was extracted and measured by the Bradford method. Equal amounts of protein samples (50 μg) were separated by SDS-PAGE, transferred to polyvinylidene difluoride (PVDF) membrane, blocked with 5% (w/v) dry milk in phosphate-buffered saline and incubated with primary antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) overnight.
at 4°C. After incubation with the secondary antibodies for 1 hr, the proteins were detected by enhanced chemiluminescence and quantified using a Gel Doc 2000 Imager (Bio-Rad).

**Statistical analysis**

Data were expressed as mean ± S.E.M. Statistical significance of differences between groups was evaluated by Student’s t-test or ANOVA with Bonferroni’s multiple comparison post hoc tests, as appropriate (SPSS Inc., Chicago, IL, USA). Two-sided P-values less than 0.05 were considered to be statistical significance.

**Results**

**Telmisartan enhances PPAR-δ expression in cultured myotubes**

First, we examined whether telmisartan affects PPAR-γ and PPAR-δ levels in cultured myotubes. After stimulation with telmisartan for 24 hrs, PPAR-δ mRNA and protein expression increased 105% and 102%, respectively, in cultured myotubes from wild-type mice. However, telmisartan did not affect the PPAR-γ mRNA or protein levels in cultured myotubes either from wild-type or PPAR-δ-deficient mice (Fig. 1A–D).

**Telmisartan up-regulates p-AMPK levels through PPAR-δ**

We found that telmisartan significantly increased phospho-AMPK α levels in cultured myotubes from wild-type mice but not in those from PPAR-δ-deficient mice (Fig. 2A). Importantly, telmisartan had no effect on AT1 receptor expression in cultured myotubes from either wild-type or PPAR-δ-deficient mice (Fig. 2B).

**Chronic treatment with telmisartan increases PPAR-δ expression in skeletal muscle**

We next asked whether the effect of telmisartan on PPAR-δ expression in vitro would extend to the in vivo setting. Wild-type and PPAR-δ-deficient mice were treated with telmisartan (5 mg/kg) for 6 months. Compared with control mice, telmisartan increased PPAR-δ mRNA and protein levels (P < 0.01 and P < 0.05, respectively), but did not significantly affect PPAR-γ levels (P > 0.05) in skeletal muscle of wild-type mice (Fig. 3A and C). PPAR-γ mRNA and protein levels in skeletal muscle were unaffected in PPAR-δ-deficient mice treated with or without telmisartan (Fig. 3B and C).

**Effect of telmisartan on metabolic profile in vivo**

To evaluate changes of phenotype after telmisartan treatment, body weight, food intake and blood metabolic parameters were examined. Our study revealed that long-term telmisartan reduced weight gain in wild-type mice (P < 0.05) but not in PPAR-δ-deficient mice (Fig. 4A and B). Furthermore, telmisartan significantly reduced the weight of visceral and subcutaneous fat in wild-type mice, but this effect was absent in PPAR-δ-deficient mice (Fig. 4G and H). Food intake was not affected in either group (Fig. 4C). Also serum insulin, triglyceride and glucose levels did not differ between mouse strains with or without telmisartan treatment (Fig. 4D–F).
Telmisartan enhances AMPK phosphorylation through PPAR-δ in skeletal muscle

Results in vitro showed that up-regulation of phospho-AMPK α levels by telmisartan occurred in a PPAR-δ-dependent manner. Long-term administration of telmisartan increased phospho-AMPK α protein levels in skeletal muscle of wild-type mice (P < 0.05), but not in that of PPAR-δ-deficient mice (Fig. 5A and B). Interestingly, telmisartan did not influence AT1 receptor expression in skeletal muscle from either wild-type or PPAR-δ-deficient mice (Fig. 5A and B).

Chronic telmisartan treatment enhances running endurance, reduces fat mass and remodels myofibres

Skeletal muscle fibres are generally classified as type I (oxidative/slow) or type II (glycolytic/fast) fibres. Recent studies showed that PPAR-δ/AMPK activation induces a transformation from fast glycolytic to slow oxidative fibres [4, 5]. To determine whether telmisartan has a similar effect, we assessed running time, running distance, basal and post-exercise oxygen consumption. Compared with control mice, wild-type mice treated with telmisartan...
had greater running endurance and more post-exercise oxygen consumption (Fig. 6A–D). However, PPAR-δ-deficient mice treated with telmisartan displayed no phenotypic change relative to untreated PPAR-δ-deficient mice. The skeletal muscle mass was not different between mice with and without telmisartan treatment (Fig. 6E), but the ratio of skeletal muscle weight to whole body weight was significantly higher in mice treated with telmisartan than untreated mice (Fig 6F). We further analysed the myofibre composition of gastrocnemius muscle and found that the proportion of type I slow fibres was significantly higher in telmisartan-treated wild-type mice than in control wild-type mice, whereas there were no difference between two groups of PPAR-δ-deficient mice (Fig. 6G and H). In accordance with the fibre composition, the biomarker for slow skeletal muscle fibre (troposin I-ss) was significantly up-regulated in wild-type mice receiving telmisartan but not in telmisartan-treated PPAR-δ-deficient mice (Fig. 5A and B).

Discussion

Previous studies have highlighted a potential role for ARBs in activation of PPAR-γ in adipose tissue and the vasculature [9–11].
The present study provides novel evidence that the effect of telmisartan on skeletal muscle function is PPAR-\(\delta\) dependent. In vitro, telmisartan up-regulated levels of PPAR-\(\delta\) and phospho-AMPK\(\alpha\) in cultured myotubes, but this effect was absent in cultured myotubes from PPAR-\(\delta\)−/− deficient mice. In vivo, phospho-AMPK\(\alpha\) levels in skeletal muscle were up-regulated in wild-type mice treated with telmisartan. In contrast, AMPK\(\alpha\) expression in skeletal muscle was unchanged in PPAR-\(\delta\)−/− deficient mice treated with telmisartan. Chronic administration of telmisartan remarkably prevented weight gain, enhanced running endurance and post-exercise oxygen consumption and promoted the production of slow-twitch skeletal muscle fibres in wild-type mice but not in PPAR-\(\delta\)−/− deficient mice. The present findings suggest that activation of the PPAR-\(\delta\) / AMPK pathway could be considered as a novel therapeutic target for the prevention of diabetes.

Several large-scale clinical trials showed a 14–34% reduction in the incidence of type 2 diabetes in hypertensive patients treated with either ACE inhibitors or ARBs for 3–6 years compared with those treated with thiazide diuretic, \(\beta\)-adrenergic blocker and/or the calcium channel antagonist [12–14]. From a clinical perspective, these studies form the basis of a new strategy to reduce the ongoing epidemic and burden of type 2 diabetes. However, the mechanisms whereby inhibition of the rennin-angiotensin system improves glucose homeostasis are complex. They may include improvement of blood flow through the microcirculation of skeletal muscles, protection of the pancreatic islets from glucotoxicity and restoration of \(\beta\)-cell mass, and inhibition of oxidative stress [15–18]. In addition, ARBs are known to activate PPAR-\(\gamma\), which is responsible for their insulin-sensitizing effects [19]. PPAR-\(\gamma\) is abundant in adipose tissues, functioning as the key transcription factor for adipocyte differentiation and lipid storage [10]. However, PPAR-\(\delta\) is the predominant PPAR isoform in skeletal muscle [3]. Activation of PPAR-\(\delta\) causes several changes in metabolism, including enhanced fatty acid oxidation, improved lipid profiles and reduced adiposity [20, 21]. It also regulates CB1 expression in adipose tissue [22], increases glucose metabolism and promotes gene regulatory responses in cultured human skeletal muscle [6, 23]. Muscle-specific PPAR-\(\delta\) overexpression results in a profound change in fibre composition due to hyperplasia and/or a shift to more oxidative fibres, resulting in increased enzymatic activity and running endurance [5].

Although telmisartan is regarded as a partial PPAR-\(\gamma\) agonist [2], little is known about whether telmisartan has an effect on PPAR-\(\delta\). Our study shows for the first time that telmisartan can up-regulate PPAR-\(\delta\) expression in skeletal muscle. First, we demonstrated that telmisartan can up-regulate PPAR-\(\delta\) without affecting PPAR-\(\gamma\) in cultured myotubes. Second, long-term administration of telmisartan markedly increased PPAR-\(\delta\) levels but not PPAR-\(\gamma\) levels in skeletal muscle of wild-type mice. Third, no telmisartan stimulatory effect was observed in skeletal muscle from PPAR-\(\delta\)-deficient mice. This evidence strongly supports the notion that telmisartan directly acts on PPAR-\(\delta\) in skeletal muscle.

In mammals, AMPK is known to contribute to glucose homeostasis, appetite and exercise physiology [4, 24, 25]. Activation of AMPK in skeletal muscle increases glucose uptake, fatty acid oxidation and mitochondrial biogenesis by increasing gene expression in these pathways [26]. Administration of PPAR-\(\delta\) agonist or AMPK agonist increased oxidative fibre content and running endurance [4, 5]. In this study, we showed that telmisartan promoted AMPK phosphorylation through PPAR-\(\delta\) activation. A
recent study showed that PPAR-δ agonist AICAR can increase AMPK phosphorylation and expression [27]. Our results in vitro indicated that telmisartan increases phospho-AMPK expression in cultured myotubes from wild-type mice but not in myotubes from PPAR-δ-deficient mice. Similar findings were demonstrated in vivo. Chronic telmisartan treatment increased phospho-AMPK in the skeletal muscle of wild-type mice but not in PPAR-δ-deficient mice. The lack of an effect of telmisartan on the AT1 receptor in cultured myotubes and skeletal muscle is noteworthy. These results indicate that telmisartan stimulates the PPAR-δ/AMPK pathway in skeletal muscle by a mechanism independent of AT1 receptor blockade.

An important aspect of the present study was the finding that telmisartan’s effect on the PPAR-δ/AMPK pathway is linked with the skeletal muscle composition and function in vivo. In wild-type mice, chronic administration of telmisartan enhanced running endurance, increased post-exercise oxygen consumption, reduced body fat and increased the proportion of type I fibres in skeletal muscle. In contrast, telmisartan had no effect on body fat accumulation and skeletal muscle function or remodelling in PPAR-δ-deficient mice.
Post-exercise oxygen consumption is accompanied by an elevated consumption of fuel [28]. In response to exercise, fat stores are broken down and free fatty acids are released into the blood. In recovery, the direct oxidation of free fatty acids as fuel and the energy consuming reversion of free fatty acids back into fat stores both take place [29].

Although telmisartan does not influence serum glucose directly, it affects several pathways related to glucose haemostasis. Besides improvement of skeletal muscle function, telmisartan also improves blood flow through the microcirculation of skeletal muscles, protects the pancreatic islets from glucoxicity and restores β-cell mass, inhibits oxidative stress, prevents weight gain and activates PPAR-γ. These factors are critical in the development of insulin resistance and type 2 diabetes [15–19, 30].

Skeletal muscle is the largest organ in the human body. The mass and composition of skeletal muscle are critical for its functions and the whole body energy metabolism, such as exercise, energy expenditure and glucose metabolism [31–33]. In human beings, three major muscle fibre types are distinguished, including type I, type IIA and type IIB. However, human type IIB gene is very homologous to rat type IIX gene [34].

Type I fibres are mitochondria-rich and mainly use oxidative metabolism for energy production, which provides a stable supply of ATP, and thus are fatigue resistant. In mice, type II fibres comprise three subtypes, IIA, IIX and IIB. Type IIB fibres have the lowest levels of mitochondrial and oxidative enzymes, rely on glycolytic metabolism and are susceptible to fatigue, whereas the oxidative and contraction functions of type IIA and IIX lie between type I and IIB [5]. However, we did not successfully verify IIA, IIX, IIB fibre subtypes of skeletal muscle in mice because of the methodological problem. This weakness limits us to further evaluate the skeletal muscle function. Mass, fibre size and fibre composition are regulated in adult skeletal muscle in response to changes in physical activity, environment or pathological conditions [35]. Skeletal muscle in obese and diabetic patients had a lower oxidative capacity, increased glycolytic capacity and a decreased percentage of type I fibres [36, 37]. Therefore, PPAR-δ activation induced by telmisartan not only increases oxidative fibres and running endurance, but also substantially contributes to enhanced mitochondria function and lipid oxidation.

In summary, these findings support the notion of a previously unrecognized role of telmisartan in skeletal muscle metabolism. We provide evidence for the first time that telmisartan increases running endurance through activation of the PPAR-δ/AMPK pathway, and this may have the additional benefit of preventing diabetes.

From a clinical point of view, it is tempting to speculate that our data provide the molecular basis for the prevention of diabetes in hypertensives using ARBs. A randomized clinical trial is needed to verify whether ARBs show additional benefits in improving skeletal muscle function in hypertensive patients.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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