Telmisartan inhibits vasoconstriction via PPARγ-dependent expression and activation of endothelial nitric oxide synthase

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Aims
Telmisartan activates peroxisome proliferator-activated receptor-γ (PPARγ) in addition to serving as an angiotensin II type 1 receptor (AT1R) blocker. The PPARγ activity of telmisartan on resistance arteries has remained largely unknown. The present study investigated the hypothesis that telmisartan inhibited vascular tension in mouse mesenteric resistance arteries, which was attributed to an increased nitric oxide (NO) production through the PPARγ-dependent augmentation of expression and activity of endothelial nitric oxide synthase (eNOS).

Methods and results
Second-order mesenteric arteries were isolated from male C57BL/6J, eNOS knockout and PPARγ knockout mice and changes in vascular tension were determined by isometric force measurement with a myograph. Expression and activation of relevant proteins were analysed by Western blotting. Real-time NO production was measured by confocal microscopy using the dye DAF. Telmisartan inhibited 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin F2α (U46619)- or endothelin-1-induced contractions. An NOS inhibitor, Nα-nitro-L-arginine methyl ester (L-NAME), or an inhibitor of soluble guanylate cyclase, 1H-[1,2,4]-oxadizolo[4,3-a]quinoxalin-1-one (ODQ), prevented telmisartan-induced inhibition of U46619 contractions. A PPARγ antagonist, GW9662, abolished telmisartan-induced inhibition. Likewise, the PPARγ antagonist rosiglitazone attenuated U46619-induced contractions. The effects of telmisartan and rosiglitazone were prevented by actinomycin-D, a transcription inhibitor. In contrast, losartan, olmesartan, and irbesartan did not inhibit contractions. The inhibition was absent in mesenteric arteries from eNOS knockout or PPARγ knockout mice. Telmisartan augmented eNOS expression, phosphorylation, and NO production, which were reversed by the co-treatment with GW9662.

Conclusions
The present results suggest that telmisartan-induced inhibition of vasoconstriction in resistance arteries is mediated through a PPARγ-dependent increase in eNOS expression and activity that is unrelated to AT1R blockade.

Keywords
Telmisartan • Endothelial nitric oxide synthase • PPARγ • Mesenteric arteries

1. Introduction
The renin–angiotensin–aldosterone system plays a central role in the development of hypertension and diabetic vascular disease. Angiotensin II (Ang II) induces cellular oxidative stress by activating the Ang II type 1 receptor (AT1R) and NAD(P)H oxidase and subsequent production of superoxide anions in both endothelial and vascular smooth muscle cells. Elevated levels of reactive oxygen species (ROS) lower the bioavailability of nitric oxide (NO), thus contributing to endothelial dysfunction.1–3 Consequently, AT1R blockers (ARBs) such as losartan represent a major class of anti-hypertensive drugs which reduce ROS overproduction in the vascular wall through antagonizing the AT1R.

Peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand-activated nuclear transcription factor,4,5 which regulates adipogenesis and glucose metabolism.6 PPARγ is expressed in endothelial and vascular smooth muscle cells,7 and has been shown to enhance insulin sensitivity and mediate anti-inflammatory responses.8
Telmisartan induces and activates eNOS via PPARγ

Mutations of PPARγ in humans contribute to the pathogenesis of insulin resistance, diabetes, and hypertension.7 PPARγ agonists thiazolidinediones, such as rosiglitazone, are therapeutic choices for type 2 diabetes acting by enhancing the insulin sensitivity in liver, muscle, and adipose cells and by modulating lipid metabolism.10 Activation of PPARγ in endothelial and smooth muscle cells can produce cardio-protective benefit that is independent of insulin sensitivity.7 Rosiglitazone and pioglitazone have been reported to ameliorate endothelial dysfunction in diabetic mouse by reducing the NAD(P)H oxidase expression and ROS production,11,12 while accumulating clinical reports show that full PPARγ agonists are associated with deleterious side effects, such as congestive heart failure,13,14 exacerbation of fluid retention, causing oedema and weight gain15,16 as well as bone fractures in women with type 2 diabetes.14

Substantial evidence suggests that telmisartan, an ARB approved for the treatment of hypertension, is a partial PPARγ agonist.17,18 Clinical studies show that telmisartan is well tolerated and does not cause oedema and weight gain.19,20 In addition, telmisartan may be clinically more effective than losartan in lowering blood pressure in hypertensive patients.21 On the other hand, telmisartan can activate PPARγ partly due to its highly lipophilic nature22 and its ability to bind to the PPARγ ligand-binding domain.17 This unique nature of being a dual ARB/PPARγ agonist makes telmisartan a more promising drug in treating cardiovascular diseases in a safer manner. However, it remains unknown how the PPARγ property of telmisartan alters vascular reactivity. The present study examined the hypothesis that telmisartan could enhance the expression of endothelial nitric oxide synthase (eNOS) via a PPARγ-dependent mechanism to reduce ligand-induced contractions in mouse mesenteric resistance arteries.

2. Methods

2.1 Animals

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). This study was approved by the Experimental Animal Ethics Committee, the Chinese University of Hong Kong. Male C57BL/6j mice aged between 12 and 13 weeks and age-matched eNOS knockout (eNOS KO) mice were supplied by the Laboratory Animal Services Center, the Chinese University of Hong Kong. PPARγ KO mice were provided by Dr Jun Yu, the Chinese University of Hong Kong. The animals were housed in a temperature-controlled room (22 ± 1°C) under a 12 h light/dark cycle and had free access to water and the standard chow diet.

2.2 Artery preparation

Mice were sacrificed by CO2 inhalation. Second-order mesenteric arteries and aortae were dissected out and placed in a dissecting dish containing the ice-cold phosphate buffered saline (PBS) in which the adhering connective tissues were carefully removed. The arterial tissues were cut into 1.0–1.3 mm rings for tissue culture.

2.3 Tissue culture of arteries

Pre-warmed Dulbecco’s modified Eagle medium supplemented with 10% FBS and 100 U mL−1 penicillin plus 100 mg mL−1 streptomycin was added into the wells of the 24-well plate. Different concentrations of telmisartan (0.1–10 μmol L−1) or other ARBs (losartan, olmesartan, and irbesartan; all used at 10 μmol L−1) were added into the wells. GW9662 (PPARγ antagonist, 300 nmol L−1) was added in addition to 10 μmol L−1 telmisartan in separate experiments. The plate was gently shaken to allow thorough mixing of drugs before arteries were transferred to the wells for a 24 h incubation at 37°C.

2.4 Isometric force measurement

Tension changes of the cultured mesenteric arteries and aortae were recorded as previously described.23 Briefly, the arteries were suspended by two stainless steel wires in the 5 mL chamber of a Multi Myograph (Danish, Myo Technology A/S, Denmark) with Krebs solution constantly bubbled with 95% O2–5% CO2 and maintained at 37°C. After 30 min equilibration, rings were first contracted by 30 nmol L−1 U46619, and then relaxed by 3 μmol L−1 acetylcholine (ACh). Arteries with relaxations over 80% were regarded as those with intact endothelium. After several washes, the telmisartan-treated arteries were cumulatively contracted by U46619 (1–300 μmol L−1) or endothelin-1 (ET-1, 0.3–30 nmol L−1). Results were compared between control and 30 min incubation of L-NAME (100 μmol L−1) or 1H-[1,2,4]oxadiazolo [4,3-a]quinoxalin-1-one (ODQ) (5 μmol L−1). To examine the changes in NO-mediated endothelium-dependent relaxations, the arteries were pre-contracted with 3 μmol L−1 phenylephrine and relaxed by cumulative additions of ACh (3 nmol L−1–1 μmol L−1) in the presence of 20 mmol L−1 KCl which eliminated the effect from endothelium-derived hyperpolarizing factors.

2.5 Western blotting

Western blotting was performed as described elsewhere.24 Mouse aortae harvested upon a 24 h incubation protocol were snap-frozen in liquid nitrogen. The tissues were homogenized in ice-cold radio-immunoprecipitation assay lysis buffer containing a cocktail of protease inhibitors (leupeptin, 1 μg mL−1; aprotonin, 5 μg mL−1; PMSF, 100 μg mL−1; sodium orthovanadate, 1 mmol L−1; ethylene glycol tetraacetic acid, 1 mmol L−1; EDTA, 1 mmol L−1; NaF, 1 mmol L−1; and β-glycerophosphate, 2 mg mL−1). The lysates were incubated on ice for 30 min and then centrifuged at 20,000 g for 20 min. The supernatant was collected and analysed for protein concentration using the Lowry method (Bio-Rad, USA). For each sample, 25 μg of the total protein was electrophoresed on an immobilon-P polyvinylidene difluoride membrane (Millipore, USA) using wet transfer containing 10 V for 70 min at 4°C. The membranes were blocked with 1% bovine serum albumin in 0.5% Tween-20 phosphate buffered saline (PBS) for 60 min before an overnight incubation with either polyclonal rabbit anti-PPARγ antibody (1:1000, Cell Signaling), polyclonal rabbit anti-eNOS antibody (1:500, BD Transduction Laboratories), or polyclonal rabbit anti-phosphorylated eNOS antibody (1:1000, Upstate) at 4°C. The membranes were then probed with a horse-radish peroxidase-conjugated swine anti-rabbit secondary antibody at a dilution of 1:3000 for 1 h at room temperature. The membranes were developed with an enhanced chemiluminescence detection system (ECL reagents; Amersham PharmaciaBiotech, Bucks, UK) and then exposed to X-ray films. The signal intensities were quantified using a gel documentation system (GBOX-CHEM1-HR16, SynGene) and normalized with the housekeeping protein glyceraldehyde 3-phosphate dehydrogenase.

2.6 Real-time measurement on NO production in endothelial cells

Human aortic endothelial cells (HAEC) were purchased from Cascade Biologics (Oregon, USA) and cultured in Medium 200 supplemented with Low Serum Growth Supplement (Cascade Biologics) until 80% confluence upon which they were plated on coverslips. Cells were incubated with telmisartan (10 μmol L−1) for 24 h with or without 30 min pre-treatment of GW9662 (300 nmol L−1) and then imaged as described earlier.23 Briefly, cells after 24 h incubation were rinsed in NPSS (140 mmol L−1 NaCl, 5 mmol L−1 KCl, 1 mmol L−1 CaCl2, 1 mmol L−1 MgCl2, 10 mmol L−1 glucose, and 5 mmol L−1 HEPES, pH 7.4) and
incubated with 1 μmol L\(^{-1}\) DAF-FM diacetate (4-amino-5-methylamino-2',7'-difluorescein, Invitrogen) for 10 min at room temperature. The cells were then excited at 495 nm with emission wavelength of 515 nm using the Olympus Fluoview FV1000 laser scanning confocal system (Olympus America, Inc., Melville, NY, USA) mounted on an inverted IX81 Olympus microscope. Basal NO levels were determined from the initial reading and real-time changes in ACh (10 μmol L\(^{-1}\))-stimulated NO production were recorded and presented as a ratio of fluorescence relative to the initial intensity (time = 0 min, before ACh addition).

2.7 Chronic treatment with telmisartan

C57BL/6j mice were orally treated with either telmisartan (1 mg kg\(^{-1}\) day\(^{-1}\)) or water for 14 days, after which mesenteric arteries were dissected for isometric force measurement.

2.8 Chemicals

Actinomycin-D, ODQ and N\(^{\circ}\)-nitro-L-arginine methyl ester (L-NAME) were purchased from Tocris (Avonmouth, UK). 9,11-Dideoxy-11α,9α-epoxymethanoprostaglandin \(F_{2\alpha}\) (U46619), Ach, and GW9662 were purchased from Sigma-Aldrich (St Louis, MO, USA). Rosiglitazone was purchased from GLAXOSMITHKLINE and olmesartan from Pfizer. Losartan was purchased from Cayman Chemical (Ann Arbor, MI, USA). Telmisartan was purchased from Changzhou Yabang Pharmaceutical Co., Ltd, Jiangsu Province, China and irbesartan from Zhejiang Apeloa Jiayuan Pharmaceutical Co, Ltd, Zhejiang Province, China. ACh and L-NAME were prepared in distilled water, while other chemicals were dissolved in dimethyl sulphoxide (DMSO, Sigma).

2.9 Statistical analysis

Data are means ± SEM of \(n\) experiments and analysed with Student’s \(t\)-test or two-way ANOVA followed by Bonferroni posthoc tests when more than two groups were compared. pD\(_2\) is the negative logarithm of the vasoconstrictor concentration needed to produce half of the maximal contraction as determined by non-linear regression curve fitting (Graphpad Prism Software, version 4.0). \(P < 0.05\) was considered statistically significant.

3. Results

3.1 Telmisartan inhibits U46619-induced contractions

Telmisartan concentration-dependently inhibited U46619-induced contractions in the mesenteric arteries from C57BL/6j mice. Telmisartan at 10 μmol L\(^{-1}\) attenuated the contractions (pD\(_2\): 7.89 ± 0.04 for control and 7.40 ± 0.07 for telmisartan 10 μmol L\(^{-1}\), \(P < 0.001\), Figure 1A, Supplementary material online, Figure 1A and Table 1). In contrast, losartan, olmesartan, and irbesartan (all used at 10 μmol L\(^{-1}\)) did not alter the U46619-induced contractions (Supplementary material online, Figure 2A). Telmisartan also enhanced ACh-induced endothelium-dependent relaxations (Supplementary material online, Figure 3A). Similar findings were also obtained in the mouse aortae, in which telmisartan, but not losartan, olmesartan, and irbesartan, inhibited contractions to U46619 (Supplementary material online, Figure 4A). Telmisartan also potentiated the ACh-induced relaxations in the mouse aortae (Supplementary material online, Figure 4D).

3.2 Inhibitory effect of telmisartan on contractions is mediated by NO

L-NAME (100 μmol L\(^{-1}\)) abolished the inhibitory effect of telmisartan (10 μmol L\(^{-1}\)) on U46619-induced contractions (Figure 1B, Supplementary material online, Figure 1B and Table 1). ODQ (5 μmol L\(^{-1}\)) also prevented telmisartan-induced effect (Supplementary material online, Figure 2B and Table 1). In addition to U46619, telmisartan also suppressed contractions induced by endothelin-1 and this effect was abrogated by L-NAME (Figure 1C, Table 1). In control rings without telmisartan treatment, L-NAME and ODQ enhanced the contractions to U46619 and endothelin-1 (Figure 1B and C). L-NAME abolished the enhanced ACh-induced relaxations in the mesenteric arteries (Supplementary material online, Figure 3A) and reversed the telmisartan-induced inhibition on U46619-induced contractions in the aortae (Supplementary material online, Figure 4B). Mesenteric arteries from C57BL/6j mice orally treated with telmisartan exhibited smaller U46619-induced contractions compared with those from vehicle-treated control mice. L-NAME restored and even potentiated U46619-induced contractions (Supplementary material online, Figure 5).

3.3 Telmisartan up-regulates eNOS expression and phosphorylation

Twenty-four hour incubation of telmisartan concentration-dependently up-regulated the eNOS expression (Figure 2A) and its phosphorylation at Ser\(^{1177}\) (Figure 2B). The positive role of eNOS was further verified by eNOS KO mice, in which eNOS protein was not expressed in aortae (Figure 2C). L-NAME did not enhance the contractions to U46619 and telmisartan (10 μmol L\(^{-1}\)) failed to inhibit contractions in mesenteric arteries from the eNOS KO mice (Figure 2D). In addition, the inhibitory effect of telmisartan to

### Figure 1

(A) Telmisartan (0.1–10 μmol L\(^{-1}\)) concentration-dependently attenuated U46619-induced contractions. Inhibitory effect of telmisartan (Tel, 10 μmol L\(^{-1}\)) to (B) U46619- and (C) endothelin-1-induced contractions was abolished in the presence of L-NAME (100 μmol L\(^{-1}\)). Data are from 5 to 6 experiments. *\(P < 0.05\) vs. control; \#\(P < 0.05\) vs. Tel without L-NAME.
3.4 Telmisartan elevates PPARγ expression via PPARγ activity

Telmisartan concentration-dependently increased the PPARγ expression (Figure 3B). Telmisartan (10 μmol L⁻¹)-induced up-regulation of PPARγ expression was prevented by a PPARγ antagonist GW9662 (300 nmol L⁻¹). Likewise, rosiglitazone, a PPARγ agonist, also increased the GW9662-sensitive PPARγ expression (Figure 3C). In contrast, losartan (10 μmol L⁻¹) did not affect the expression of PPARγ (Figure 3C).

3.5 Telmisartan-induced eNOS expression and activation are PPARγ-dependent

GW9662 (300 nmol L⁻¹) antagonized telmisartan-induced inhibition on U46619-induced contractions and prevented the potentiation of ACh-induced relaxations in both mesenteric arteries and aortae, while GW9662 per se did not modify U46619-induced contractions and ACh-induced relaxations (Figure 4A and B; Supplementary material online, Figures 3B, 4C and D; Table 1). Likewise, rosiglitazone (1 μmol L⁻¹) attenuated U46619-induced contractions, and this attenuation was reversed by GW9662 (Supplementary material online, Figure 2C) and prevented by actinomycin-D (Supplementary material online, Figure 2D).

Telmisartan (10 μmol L⁻¹) and rosiglitazone (1 μmol L⁻¹)-induced increases in eNOS expression (Figure 4C) and phosphorylation (Figure 4D) were prevented by GW9662 (300 μmol L⁻¹). On the contrary, losartan did not increase eNOS expression and only caused a slight elevation in eNOS phosphorylation, which was insensitive to GW9662 (Figure 4C and D).

PPARγ was not expressed in the PPARγ KO mice (Figure 5A). In the mesenteric arteries of PPARγ KO mice, the inhibitory effects of telmisartan (10 μmol L⁻¹) on U46619-induced contractions were diminished compared with those in C57BL/6j control mice (Figure 5B). Telmisartan did not augment eNOS expression (Figure 5C) and phosphorylation (Figure 5D) in the aortae of PPARγ KO mice.

3.6 Telmisartan increases intracellular NO levels in cultured endothelial cells

HAEC treated with telmisartan for 24 h exhibited higher un-stimulated (i.e. basal) NO level, which was prevented by co-treatment of GW9662 or exposure to l-NAME (Figure 6A, before ACh addition at 0 min, Figure 6B). Addition of ACh to HAEC stimulated NO production. The increase in intracellular NO level was remarkably higher in the telmisartan-treated cells compared with that of the untreated control. Cells co-treated with GW9662 exhibited a rise of NO level similar to that of the control and acute l-NAME treatment inhibited NO production (Figure 6A and C).

Table 1 pD₂ and E_max (%) of telmisartan-induced inhibition on contractions

<table>
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<th>Treatment</th>
<th>pD₂</th>
<th>E_max (%)</th>
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<td>C57BL/6j arteries</td>
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<tr>
<td>U46619 (Control)</td>
<td>7.89</td>
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<td>Tel 0.1 μmol L⁻¹</td>
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<td>7.40</td>
<td>118.38 ± 5.17</td>
<td>6</td>
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<tr>
<td>Control</td>
<td>7.87</td>
<td>138.03 ± 4.67</td>
<td>6</td>
</tr>
<tr>
<td>Tel 10 μmol L⁻¹</td>
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<td>113.09 ± 4.62</td>
<td>6</td>
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<tr>
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<tr>
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<td>6</td>
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<tr>
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<td>6</td>
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<td>139.76 ± 5.06</td>
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Significant difference between control and treatment groups is indicated by *p < 0.05; **p < 0.01; ***p < 0.001 vs. control; *p < 0.05; **p < 0.01; ***p < 0.001 vs. tel. Data are means ± SEM of n experiments.
4. Discussion
The present study demonstrated for the first time that telmisartan attenuates receptor-dependent contractions in the mesenteric resistance arteries and aortae from C57BL/6J mice via the activation of PPARγ and a subsequent increase in eNOS expression and activity. The concentration-dependent inhibition of telmisartan on U46619-induced contractions implies its therapeutic potential in reducing over-constrictions caused by TP receptor activation in vascular complications of hypertension and diabetes.26,27

Thiazolidiones, such as rosiglitazone, have been reported to ameliorate endothelial dysfunction by inhibiting ROS production and stimulating NO generation via a PPARγ-mediated pathway.11,12 Full PPARγ agonists, pioglitazone, and troglitazone, increase NO production by up-regulating eNOS expression and eNOS-Ser1177 phosphorylation in a rabbit model of myocardial infarction,28 bovine endothelial cells,29 and human umbilical vein endothelial cells (HUVEC).30 We therefore hypothesized that telmisartan, acting as a partial agonist of PPARγ, could also augment eNOS expression and activity in a PPARγ-dependent manner, thereby inhibiting...
vasoconstriction. The present study shows that telmisartan-induced inhibition on U46619-induced contractions was attributed to the increase in NO production via an augmentation of eNOS expression and activity, evidenced by the abolishment of such inhibition by l-NAME and ODQ, as well as by the absence of the inhibition in arteries from eNOS KO mouse. Indeed, the potentiation of U46619- or ET-1-induced contractions by acute treatment of L-NAME or ODQ already suggests that basal NO can suppress vasoconstriction, in which the role of NO is further exaggerated by telmisartan treatment. The in vitro studies were supported by chronic oral administration of telmisartan to C57BL/6J mice, from which mesenteric arteries exhibited less U46619-induced contractions, and were markedly potentiated by L-NAME, indicating a significant increase of basal NO after telmisartan treatment. Telmisartan also enhanced the NO-mediated endothelium-dependent relaxations in both mesenteric arteries and aortae resulting from the increase in ACh-stimulated NO production.

Telmisartan-induced inhibition of contractions was significantly attenuated by actinomycin-D, confirming that transcriptional event was involved. In the present study, telmisartan increased eNOS phosphorylation at Ser^{1177} as revealed by Western blot analysis on the mouse aortae, which exhibited similar telmisartan-induced effects and thus acted as the surrogate for molecular studies in regards to the very limited protein amount from the second-order mouse mesenteric arteries. In fact, eNOS is not only regulated at its expression level, but also its activity modified by phosphorylation and post-translational mechanisms including the interaction of eNOS with other regulatory proteins. Increased eNOS phosphorylation may result from an increased eNOS expression by telmisartan and the elevated expression of other eNOS-interacting proteins. Telmisartan was reported to improve endothelial function by augmenting the vascular level of tetrahydrobiopterin (BH₄, an eNOS cofactor) in aortae of Dahl salt-sensitive rats. In addition, telmisartan up-regulates a BH₄-synthesizing enzyme GTP cyclohydrolase I, which reduces eNOS uncoupling in diabetic rats. Polikandriotis et al. showed that rosiglitazone elevates endothelial NO production by increasing heat shock protein 90 (hsp90) in HUVEC, while hsp90 was identified to strengthen eNOS activities by promoting eNOS-Ser^{1177} phosphorylation. These observations may explain part of mechanisms by which telmisartan increases the eNOS activity in vasculatures.

The critical role of PPARγ in the telmisartan-induced effects in the arteries is pinpointed by the following observations. First, the attenuated U46619-induced contractions and the enhanced ACh-induced relaxations in the telmisartan-treated arteries, augmentation of aortic eNOS expression and phosphorylation, and elevated basal and ACh-stimulated NO levels in HAEC were all reversed by GW9662 (300 nmol L⁻¹), a PPARγ antagonist applied at a much higher concentration in the acute treatment experiments.
lower concentration in the present study to avoid its non-specific antagonism on PPARα or PPARβ when used in high concentrations (10 μmol L\(^{-1}\)) as shown in previous reports.\(^{37,38}\) Second, the inhibition of U46619-induced contractions by telmisartan was markedly reduced in the mesenteric arteries from PPARγ KO mice. It is reported that interference with PPARγ signalling decreases endothelium-dependent but not endothelium-independent vasodilatation in small cerebral arteries,\(^{39}\) suggesting that PPARγ is likely to act on the endothelium. We confirmed that the lack of inhibitory effect of telmisartan in the arteries of PPARγ KO mice is due to the absence of PPARγ and therefore the PPARγ-dependent eNOS expression and phosphorylation were not up-regulated. Third, losartan and olmesartan, two classical ARBs, did not exhibit the telmisartan-like effects. Even irbesartan, an ARB that has been reported to slightly activate PPARγ, failed to modify the U46619-induced contractions. Benson et al.\(^{17}\) compared the PPARγ activation capability of a variety of ARBs and found that telmisartan exhibits the strongest PPARγ agonistic action (>20-fold activation), second by irbesartan which has only about two to three-fold slight activation, while none of the others (candesartan, valsartan, olmesartan, eprosartan, and EXP 3174, the active metabolite of losartan) can activate PPARγ. Collectively, the unique PPARγ-stimulating activity of telmisartan is essential in mediating the anti-contraction effects in the arteries, possibly independent of AT\(_1\)R antagonism. The exceptional lipophilic nature of telmisartan allows it to behave as a PPARγ agonist beyond its ARB activity.\(^{22}\) In fact, telmisartan has the highest lipophilicity index (+3.20) whereas EXP 3174, an active metabolite of losartan, has the lowest (−2.45) of the ARBs.\(^{40}\) Telmisartan has a high volume of distribution compared with other ARBs as revealed by a test determining the ability of telmisartan to penetrate into tissues.\(^{41}\) It is therefore thought that telmisartan possesses a greater capacity to penetrate cell membranes and thereby interacts with the nuclear PPARγ. More importantly, molecular modelling reveals that telmisartan binds to helices H3, H6, and H7 of the key PPARγ ligand-binding domain but other ARBs can only bind to helix H3.\(^{17}\) Such difference may account for the substantially different potentials for activation of PPARγ between telmisartan and other ARBs. Telmisartan increased PPARγ expression as its full agonist rosiglitazone did, suggesting a novel positive feedback mechanism.

In conclusion, besides acting as an ARB, telmisartan uniquely and robustly activates PPARγ. The present study demonstrates that telmisartan inhibits vasoconstriction through increased NO production arising from the elevated eNOS expression and phosphorylation at Ser\(^{1177}\) via a PPARγ-dependent mechanism. Compared with other ARBs, telmisartan, with the dual ARB/PPARγ-agonistic properties, shall be a more promising drug with higher therapeutic value against vascular disorders associated with hypertension and type 2 diabetes.

Supplementary material
Supplementary material is available at Cardiovascular Research online.

Conflict of interest: none declared.

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