Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

IJBMS

# The vasodilatory action of telmisartan on isolated mesenteric artery rings from rats

Xiao-ping Chen [1]*, Li-ren Qian [1]

[1] Department of Hematology, Navy General Hospital of PLA, Beijing, China

ARTICLE INFO

***Article type:***
Original article

***Article history:***
Received: Oct 19, 2014
Accepted: Sep 8, 2015

***Keywords:***
ARB
Potassium channel
Mesenteric arteries
Rat
Telmisartan
Vasodilation

ABSTRACT

***Objective(s):*** Angiotensin Ⅱ type 1 receptor blockers (ARBs) represent one of the widely used antihypertensive agents. In addition to anti-hypertension effect, some ARBs also show other molecular effects such as activating peroxisome proliferator-activated receptor-γ and so on. Here we studied the effects of telmisartan on the rat isolated mesenteric artery rings pre-contracted by phenylephrine (PE).

***Materials and Methods:*** Rat mesenteric artery rings were pre-contracted with 10 μM PE, and cumulative concentration-response curves to telmisartan were obtained. The endothelium-dependent mechanisms were investigated by mechanical removal of the endothelium. $K^+$ channels were investigated by pretreatment of the artery rings with various $K^+$ channel blockers.

***Results:*** Telmisartan produced concentration-dependent relaxation of the artery rings pre-contracted by 10 μM PE. Denudation of the endothelium did not affect the relaxant effect of telmisartan. Pretreatment with $BaCl_2$ nearly inhibited the relaxation induced by the 0.5, 1, 5 and 10 μM telmisartan, but did not affect the relaxation induced by the 50 and 100 μM telmisartan. While the relaxation induced by telmisartan was not affected by pretreatment with TEA, 4-AP and glibenclamide.

***Conclusion:*** These findings demonstrated that telmisartan produces concentration dependent vasodilation in isolated rat mesenteric artery rings with or without endothelium pre-contracted by PE. $K_{IR}$ channel may be involved in such a relaxant effect of telmisartan.

► *Please cite this article as:*
Chen XP, Qian LR. The vasodilatory action of telmisartan on isolated mesenteric artery rings from rats. Iran J Basic Med Sci 2015; 18:974-978.

## Introduction

Angiotensin Ⅱ is the major effector of the renin-angiotensin-aldosterone system, which takes effect through angiotensin Ⅱ type 1 and type 2 receptors (AT1 and AT2). AT1 mediates most of the well-known pathophysiological effects, which lead to hypertension, insulin resistance and so on (1, 2). The angiotensin Ⅱ type 1 receptor blockers (ARBs), which competitively bind AT1 antagonist with angiotensin Ⅱ have been recommended to lower blood pressure and prevent cardiovascular and kidney diseases by many guidelines. The blood pressure reducing effect of ARBs is obtained from the systemic vasodilation which is the result of antagonist with angiotensin Ⅱ. In addition, several basic experimental studies showed that some ARBs have specific molecular effects such as decreasing basal MCP-1 levels in human monocytes by irbesartan and losartan (3) and inducing adiponectin in adipocytes by irbesartan (4). Telmisartan is the long-acting ARB (5) and has been used clinically worldwide. Other than the classical anti-hypertensive effect through binding AT1 receptor, telmisartan also shows molecular effects such as activating peroxisome proliferator-activated receptor-γ (6), stimulating CYP11B2 expression in human adrenal H295R cells (7), blocking hKv1.5 potassium channels, which were expressed on Xenopus laevis oocytes (8), and stimulating adiponectin protein expression (4, 9). The above effects were obtained in the absence of angiotensin Ⅱ, which suggested that an AT1 receptor independent mechanism of action may be involved.

It is a common knowledge that telmisartan can reduce blood pressure through its vascular vasodilatory effect *in vivo*. However, it remains unclear whether telmisartan has dilatory action on isolated small artery rings *in vitro* in the absence of angiotensin Ⅱ. In this study, we tested the hypothesis that telmisartan can dilate the rat isolated mesenteric artery rings.

*Corresponding author: Xiao-ping CHEN. Department of Hematology, Navy General Hospital of PLA, Beijing, China. email: chenxpfj@126.com

## Materials and Methods

This study was performed with the permission of the Ethics Committee of the Navy General Hospital of PLA, three month old WKY rats weighing 250–260 g were used. The rats were housed under a 12-hr/12-hr day/night cycle and given tap water and standard chow *ad libitum*.

### *Dissociation of rat mesenteric artery ring*

At the beginning of the study, rats were decapitated and the mesenteric vessels of the small intestine were removed and placed in cold (4 ˚C) physiological salt solution (PSS) which was oxygenated (95% $O_2$, 5% $CO_2$). The composition of PSS was as follows (in mmol/L): KCl 4.7, NaCl 119.0, $NaHCO_3$ 25.0, $KH_2PO_4$ 0.4, $MgSO_4$ 1.17, $CaCl_2$ 2.5, and glucose 5.5. The fat and connective tissues surrounding the second-order mesenteric arterioles were removed; each artery was cut into cylindrical segments, which was 2.5 mm in length. The inner diameter of the arterial rings ranged from 100 to 150 μm.

### *Record of vascular tone*

According to the method described by Huang *et al* (10), arterial rings isolated from rats were placed in a multi-myograph system (Danish Myo Technology A/S), and changes of the tension of the vessel were recorded by Power lab data recording system (AD Instrument). The arterial rings were bathed in PSS solution, which was changed every 20 min, with 95% $O_2$ plus 5% $CO_2$ at 37 ˚C (pH 7.40). The arterial rings were mounted under an optimal resting tension. This optimal resting tension was the minimum level of stretch that gave the largest force after administration of 60 mM KCl. The rings were allowed to stabilize at optimal resting tension for 90 min before the start of the experiments.

### *Effect of telmisartan on contractions induced by phenylephrine (PE)*

After 90 min of stabilization, intact endothelium mesenteric artery ring was pre-contracted with PE (10 μM). Cumulative concentration-response curves to 0.5, 1, 5, 50, and 100 μM telmisartan (TM) were recorded. Relaxing responses were measured as percentages of the contraction induced by PE. The curves of concentration response to DMSO, which is the solvent of telmisartan, were also obtained in endothelium-intact mesenteric arterial rings.

### *Role of endothelium in telmisartan-induced relaxation*

In order to examine the role of endothelium involvement in telmisartan-mediated relaxation, response to telmisartan was studied in endothelium-intact and endothelium-denuded rings pre-contracted by PE (10 μM). At the end of relaxation, 60 mM KCl was added into the slot to test the activity of arterial rings and to study the role of depolarization in such relaxation. The presence of functional endothelium was assessed by the ability of acetylcholine (ACh, 10 μM) to induce more than 90% relaxation of pre-contracted rings with PE (10 μM) and the absence, less than 10% of relaxation induced by ACh.

### *Role of $K^+$ channels in telmisartan vasodilation*

To examine the role of $K^+$ channels in vasodilation, the endothelium-intact ring was used for this determination by preincubation with one of the following $K^+$ channel blockers: 10 mM tetraethyl-ammonium (TEA), 1 mM 4-amino-pyridine (4-AP), 10 μM glibenclamide (Gli), and 30 μM $BaCl_2$ for 30 min before PE (10 μM) pre-contracted. Then, the cumulative concentration response of telmisartan at the concentrations of 0.5, 1, 5, 50, and 100 μM was directly added.

### *Statistical analysis*

Data are presented as the mean ± SEM, and n stands for the number of rings prepared from different rats. The curves of concentration response to telmisartan were based on the percent relaxation of the PE-induced contraction. The results were analyzed by Student's t-test. Two-sided $P<0.05$ was considered statistically significant.

## Results

### *Effect of telmisartan on PE-induced contractions*

The contraction induced by 10 μM PE in rat mesenteric artery rings was 20.41±3.36 mN. Telmisartan 0.5, 1, 5, 50 and, 100 μM concentration dependently relaxed the pre-contractions with the maximum relaxation being 100% for 10 μM PE-induced contractions. Endothelium-denudation did not affect the relaxant effect of telmisartan. In contrast, DMSO did not affect the contractions induced by PE. 100 μM telmisartan relaxed the contractions induced by 10 μM PE completely. At the end of relaxations, 60 mM KCl was added into the slot, which induced 16.25±2.73 mN contractions of the mesenteric artery rings again (Figure 1).

### *Role of $K^+$ channels on telmisartan induced relaxation*

After pre-incubation with TEA (10 mM), 4-AP (1 mM), Gli (10 μM), and $BaCl_2$ (30 μM) for 30 min, the tensions induced by 10 μM PE were 19.43±2.76 mN, 22.53±3.12 mN, 21.72±4.26 mN, and 18.54±3.79 mN in isolated arterial rings, respectively. Pretreatment with $BaCl_2$ nearly inhibited the relaxation induced by the 0.5, 1, 5, and 10 μM concentration of telmisartan, but $BaCl_2$ did not affect the relaxation induced by the 50 and 100 μM telmisartan. While the relaxation induced by telmisartan was not affected by pretreatment with TEA, 4-AP and Gli (Figure 2).

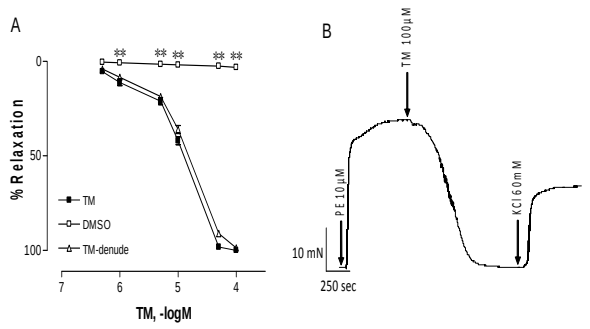


**Figure 1**. Relaxation effect of telmisartan (TM, 0.5, 1, 5, 50, and 100 μM) on pre-contractions induced by 10 μM phenylephrine (PE) in rat isolated mesenteric artery rings with an intact or denuded endothelium. Telmisartan 0.5, 1, 5, 10, 50, and 100 μM concentration dependently relaxed the pre-contractions with the maximum relaxation being 100% for 10 μM PE-induced contractions. Denudation of the endothelium did not affect the relaxant effect of telmisartan. In contrast, the vehicle of DMSO did not affect the contractions induced by PE (A). 100 μM of telmisartan relaxed the contractions induced by 10 μM PE completely. Until a stable tone plateau was reached, 60 mM of KCl was added, which induced 16.25±2.73 mN contractions of the artery rings again (B). Each point represents the mean±SEM for 6 artery rings obtained from separate rats. ***P*<0.01 vs TM and TM-denude

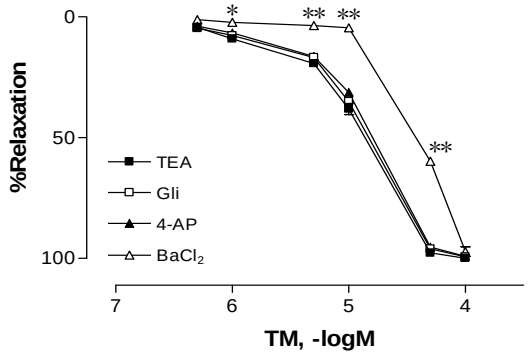


**Figure 2.** Role of $K^+$ channels on the relaxation induced by telmisartan (TM, 0.5, 1, 5, 10, 50, and 100 μM) in rat mesenteric artery rings pre-contracted with 10 μM phenylephrine (PE). After pre-incubation with tetraethylammonium (TEA, 10 mM), 1 mM 4-aminopyridine (4-AP), 10 μM glibenclamide (Gli) and 30 μM $BaCl_2$ for 30 min, the tension induced by 10 μM PE were 19.43±2.76 mN, 22.53±3.12 mN, 21.72±4.26 mN and 18.54±3.79 mN in rat mesenteric artery rings, respectively. Pretreatment with $BaCl_2$ nearly inhibited the relaxation induced by the 0.5, 1, 5 and 10 μM concentration of telmisartan, but did not affect the relaxation induced by the 50 and 100 μM telmisartan. While the relaxation induced by telmisartan were not affected by pretreatment with TEA, 4-AP and glibenclamide. Each point represents the mean± SEM for 6 mesenteric artery rings obtained from separate rats. * *P*<0.05, ***P*<0.01 vs TEA, Gli and 4-AP groups

## Discussion

Since 1970s, the deleterious effects of angiotensin Ⅱ on the cardiovascular and renal systems have been recognized (11, 12), making the blockade of the renin-angiotensin system an effective therapeutic approach in the treatment of hypertension, chronic renal dysfunction, cardiac insufficiency, and so on. ARBs, which exhibit highly selective binding and antagonistic activity for the AT1 receptor, have become a major drug class in the treatment of cardiovascular and renal disease (13). The effects of ARBs are due to the antagonistic effect, which diminishes the harmful role of angiotensin Ⅱ *in vivo*, however, more and more studies have shown that in addition to their AT1 receptor blocking activity, some ARBs may also activate peroxisome proliferator-activated receptor γ and affect currents through various ion channels expressed in cells (14) *in vitro*. Telmisartan exhibits inhibition effect on several ion channels, such as cloned Kv 1.3 and Kv 1.5 voltage-gated potassium channels expressed in X. Laevis oocytes (8, 15), Kv 1.3 expressed in rat lymphocytes (16), and HERG channels expressed in X. Laevis oocytes (8). However, such effects were typically observed at high concentrations in micromolar range. Our present study found that telmisartan can relax the rat isolated mesenteric artery rings which were precontracted with PE. Such a phenomenon may not be associated with angiotensin Ⅱ, for it was observed *in vitro*. Next, we further investigated the probable mechanisms involved in the telmisartan induced rat mesenteric artery ring relaxation.

To our knowledge, endothelium dependent or independent mechanism was involved in vascular relaxation. In the endothelium dependent mechanism, the intact endothelium cells secrete vascular dilators such as nitric oxide, prostacyclin and hyperpolarizing factor (17, 18). The present study showed that telmisartan induced vasodilation in the isolated endothelium-denuded rat mesenteric artery rings was not significantly different as compared with that in the endothelium intact artery rings. Such results suggest that telmisartan has an endothelium independent vasodilatory effect on the isolated rat mesenteric artery.

The increasing potassium efflux through membrane $K^+$ channels, blocking of extracellular $Ca^{2+}$ influx through membrane $Ca^{2+}$ channels, as well as other mechanisms participate in the process of endothelium independent vasodilation (19). Up to now, four $K^+$ channels have been found in vascular smooth muscle cells, which include ATP-sensitive ($K_{ATP}$), calcium-activated ($BK_{Ca}$), delayed rectifier ($K_V$), and inward rectifier ($K_{IR}$) potassium channels (20). Potassium ion efflux induced by activating the above $K^+$ channels leads to hyperpolarization of the vascular muscle cell membrane, inhibits calcium ion influx through voltage-operative $Ca^{2+}$ channel and causes vascular dilation. In order to investigate the role of $K^+$ channels in the telmisartan induced vasodilation in rat mesenteric artery rings, we used selective blocker of $K^+$ channels to pretreat rat mesenteric artery rings. We observed that the relaxant effect of telmisartan in the pre-contracted mesenteric artery rings was not affected by pretreatment with 10 mM TEA, 10 μM Gli and 1 mM 4-AP, which are the selective blockers of $BK_{Ca}$, $K_{ATP}$ and $K_V$ channels, respectively. But 30 μM $BaCl_2$, a

selective blocker of inward rectifier potassium channels ($K_{IR}$), significantly inhibited the vasodilation induced by 0.5, 1, 5 and, 10 μM telmisartan, which indicated that $K_{IR}$ channels may play a role in the vasodilation of telmisartan. With the increase of telmisartan concentration, a counter result has been observed: 50 μM and 100 μM telmisartan can reverse the inhibited effect of $BaCl_2$. This result may be explained by the possibilities that telmisartan competes with $BaCl_2$ to act on $K_{IR}$ channels or telmisartan may act in other ion channels. After telmisartan completely dilated the PE induced pre-contraction of rat mesenteric artery rings, 60 mM KCl can significantly make the dilated artery rings re-contracted, considering the vascular contracting effect of 60 mM KCl is related with voltage-operative $Ca^{2+}$ channel (21), the result indicates that voltage-operative $Ca^{2+}$ channel is not involved in the vascular relaxant effect of telmisartan.

## Conclusion

The present study demonstrated for the first time that telmisartan produces concentration dependent vasodilation in isolated rat mesenteric artery rings with or without endothelium pre-contracted by PE. $K_{IR}$ channel may be involved in this relaxant effect of telmisartan. Our research only observed the relaxant effect of telmisartan in the isolated rat mesenteric artery rings *in vitro* and the maximum plasma concentration of telmisartan was 0.04 to 1.15 μM after daily oral 20 mg to 120 mg administration (5). Whether such concentrations of telmisartan in plasma also play a role in vasodilation in rat mesenteric artery *in vivo* need to be further investigated. However, the effects of ARBs on ion channels typically occur at the supratherapeutic concentrations in micromolar range; clinical symptoms associated with such effects of ARBs have not been reported.

## Acknowledgment

This study was financially supported by a grant from the Innovation Promoted Foundation of Navy General Hospital, Beijing, China (grant no. CXPY201309).

## References

1. Ferrario CM, Strawn WB. Role of the renin-angiotensin-aldosterone system and proinflammatory mediators in cardiovascular disease. Am J Cardiol 2006;98:121-128.
2. Underwood PC, Adler GK. The renin angiotensin aldosterone system and insulin resistance in humans. Curr Hypertens Rep 2013; 15:59-70.
3. Proudfoot JM, Croft KD, Puddey IB, Beilin LJ. Angiotensin II type 1 receptor antagonists inhibit basal as well as low-density lipoprotein and platelet-activating factor-stimulated human monocyte chemoattractant protein-1. J Pharmacol Exp Ther 2003; 305:846-853.
4. Clasen R, Schupp M, Foryst-Ludwig A, Sprang C, Clemenz M, Krikov M, *et al.* PPARgamma-activating angiotensin type-1 receptor blockers induce adiponectin. Hypertension 2005; 46:137-143.
5. Stangier J, Su CA, Roth W. Pharmacokinetics of orally and intravenously administered telmisartan in healthy young and elderly volunteers and in hypertensive patients. J Int Med Res 2000; 28:149-167.
6. Benson SC, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, Pravenec M, *et al*. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. Hypertension 2004; 43:993-1002.
7. Matsuda K, Uruno A, Kogure N, Sugawara K, Shimada H, Nezu M, *et al.* Angiotensin II receptor blockers differentially affect CYP11B2 expression in human adrenal H295R cells. Mol Cell Endocrinol 2014; 383:60-68.
8. Tu DN, Liao YH, Zou AR, Du YM, Run Q, Wang XP, *et al.* Electropharmacological properties of telmisartan in blocking hKv1.5 and HERG potassium channels expressed on Xenopus laevis oocytes. Acta Pharmacol Sin 2008; 29:913-922.
9. Yamada S, Ano N, Toda K, Kitaoka A, Shiono K, Inoue G, *et al*. Telmisartan but not candesartan affects adiponectin expression *in vivo* and *in vitro*. Hypertens Res 2008; 31:601-606.
10. Huang Y, Chan FL, Lau CW, Tsang SY, Chen ZY, He GW, *et al*. Roles of cyclic AMP and $Ca^{2+}$-activated $K^+$ channels in endothelium-independent relaxation by urocortin in the rat coronary artery. Cardiovasc Res 2003; 57:824-833.
11. Gavras H, Lever AF, Brown JJ, Macadam RF, Robertson JI. Acute renal failure, tubular necrosis, and myocardial infarction induced in the rabbit by intravenous angiotensin II. Lancet 1971; 2:19-22.
12. Brunner HR, Laragh JH, Baer L, Newton MA, Goodwin FT, Krakoff LR, *et al.* Essential hypertension: renin and aldosterone, heart attack and stroke. N Engl J Med 1972; 286:441-449.
13. Mallat SG. Dual renin-angiotensin system inhibition for prevention of renal and cardiovascular events: do the latest trials challenge existing evidence? Cardiovasc Diabetol 2013; 12:108.
14. Michel MC, Foster C, Brunner HR, Liu L. A systematic comparison of the properties of clinically used angiotensin II type 1 receptor antagonists. Pharmacol Rev 2013; 65:809-848.
15. Li MW, Wang XP, Gao CY, Zou AR. Effects of telmisartan on voltage-gated Kv1.3 and Kv1.5 potassium channels expressed in Xenopus oocytes. Zhonghua Xin Xue Guan Bing Za Zhi 2009; 37:165-168.
16. Luo J, Ma KT, Zhang YM, Si JQ, Liang P, Li J. Effects of telmisartan on 4-Aminopyridine-sensitive voltage dependant potassium channel of lymphocyte derived from spontaneously hypertensive rat. Zhonghua Xin Xue Guan Bing Za Zhi 2010; 38:751-754.
17. Mitchell JA, Ali F, Bailey L, Moreno L, Harrington LS. Role of nitric oxide and prostacyclin as vasoactive hormones released by the endothelium. Exp Physiol 2008; 93:141-147.
18. Nagao T, Vanhoutte PM. Endothelium-derived hyperpolarizing factor and endothelium-dependent relaxations. Am J Respir Cell Mol Biol 1993; 8:1-6.
19. Chen GP, Ye Y, Li L, Yang Y, Qian AB, Hu SJ. Endothelium-independent vasorelaxant effect of

sodium ferulate on rat thoracic aorta. Life Sci 2009; 84:81-88.
20. Ko EA, Han J, Jung ID, Park WS. Physiological roles of $K^+$ channels in vascular smooth muscle cells. J Smooth Muscle Res 2008; 44:65-81.
21. Rembold CM. Regulation of contraction and relaxation in arterial smooth muscle. Hypertension 1992; 20:129-137.