jraas

Journal of the Renin-Angiotensin-Aldosterone System
April-June 2016: 1–8

DOI: 10.1177/1470320316655005
jra.sagepub.com
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The effects of telmisartan on the nuclear factor of activated T lymphocytes signalling pathway in hypertensive patients

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Abstract

Hypothesis: Previous studies provide links between the nuclear factor of activated T lymphocytes (NFAT) signalling pathway and the development of hypertension. Our preliminary studies indicate that telmisartan can block Kv1.3 potassium channels and effectively inhibit potassium current densities, along with Kv1.3 mRNA and protein expression levels. This paper aims to investigate whether telmisartan has an inhibitory effect on the NFAT signalling pathway after activation and proliferation of peripheral blood T lymphocytes in Kazakh patients with essential hypertension (EH) from Xinjiang, China.

Materials and methods: T lymphocytes were isolated using the immunomagnetic cell sorting method (MACS). The mRNA expression of NFATc1, IL-6 and TNF-α was measured by quantitative polymerase chain reaction (qRT-PCR) and relative protein levels were evaluated by Western blot. T cell samples from 50 hypertensive Kazakh patients from Xinjiang were randomly divided into control, telmisartan, cyclosporin A (CsA), VIVIT, and 4-aminopytidine (4-AP) groups. Peripheral blood T lymphocytes were first activated and proliferated in vitro, then incubated for 48 h under different treatment conditions before determination of protein and mRNA expression of NFATc1, IL-6, and TNF-α by Western blot and qRT-PCR analyses, respectively.

Results: There were no significant differences in cardiovascular risk factors among the patients with samples assigned to the five groups ($p > 0.05$). Expression of NFATc1, IL-6, and TNF-α mRNA and protein was significantly reduced in T lymphocytes in all treatment groups (telmisartan, CsA, VIVIT, and 4-AP) compared with controls.

Conclusions: Antihypertensive function and inhibitory effects of telmisartan on the T lymphocyte NFAT signalling pathway are unlikely to affect the normal immune function of hypertensive patients. Telmisartan may exert anti-inflammatory effects by inhibition of the NFAT signalling pathway in the T lymphocytes of hypertensive patients.

Keywords

Xinjiang Kazakh, essential hypertension, T lymphocytes, nuclear factor of activated T lymphocytes signalling pathway, telmisartan

Date received: 28 January 2016; accepted: 13 May 2016

Introduction

Essential hypertension (EH) is one of the most common cardiovascular disorders and a major risk factor for cardiovascular disease. The pathogenesis of EH includes hereditary, dietary, and psychological factors, along with neuroendocrine mechanisms. However, increasing experimental and clinical evidence indicates that inflammation also plays a critical role in the pathogenesis and development of EH.[1–3] Indeed, it has been proposed that EH represents a form of chronic low-grade inflammatory disease.[4] Recent studies have shown that lymphocytes, especially T lymphocytes, are involved in the occurrence and development of hypertension and vascular remodelling.[5] De Miguel and co-workers[6] demonstrated that inhibition of T lymphocytes lowered the

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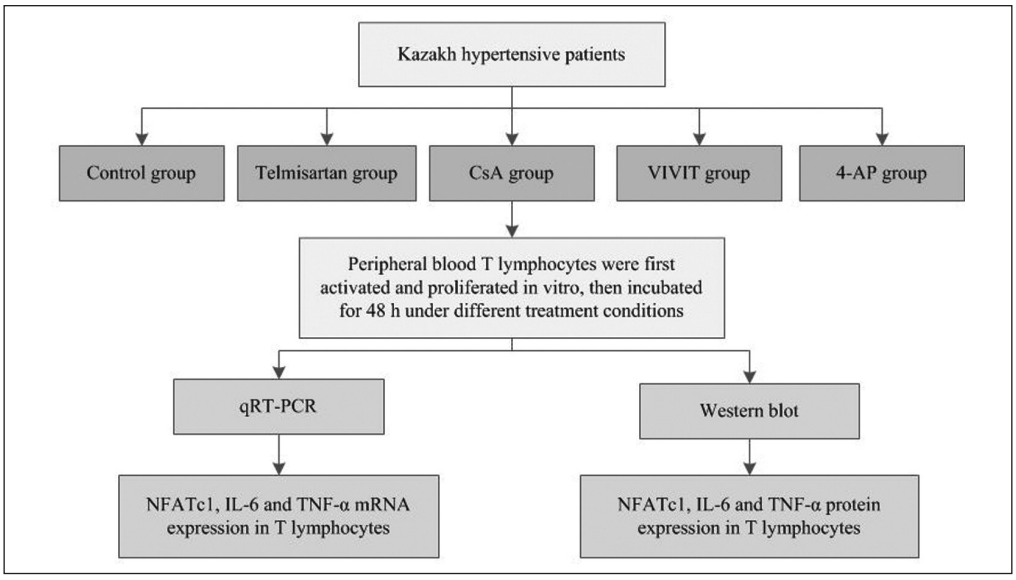

Figure 1. Experimental design and procedure.

level of renal angiotensin II (Ang II) and prevented occurrence of hypertension and renal damage in Dahl salt-sensitive rats. In addition, deficiency of T lymphocyte activity in mice can prevent the occurrence of hypertension,[7] indicating that T lymphocytes are involved in the pathogenesis of hypertension in this animal model. Moreover, Harrison et al.[8] demonstrated that T lymphocyte activation and accumulation in the adventitia and perivascular adipose tissue of both large and smaller resistance blood vessels is important in the pathogenesis and development of hypertension.

Nuclear factor of activated T lymphocytes (NFAT) is expressed in many immunocytes, including T and B lymphocytes, natural killer cells, mast cells, eosinophils, and monocytes. NFAT can selectively induce the transcription of cytokines and other immune-regulated genes, and has an essential role in the immune response process.[9] The NFAT signalling pathway is an important regulator of the inflammatory response, playing a pivotal role in T lymphocyte activation and stimulating the production of a large number of inflammatory cytokines, including interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α).[10] It is almost impossible to produce the cytokines required to stimulate T lymphocyte activation in the absence of sufficient NFAT.[11,12] In the current study, we investigated mRNA and protein expression levels of NFATc1, IL-6, and TNF-α in hypertensive Kazakh patients in Xinjiang, China.

Kazakh people comprise the second largest ethnic minority in the Xinjiang Uyghur Autonomous Region of China. Epidemiological studies indicate that the prevalence of EH is much higher among Xinjiang Kazakhs than other ethnic groups in Xinjiang, whereas the rates of disease awareness, cure, and control appear to be lower among Kazakhs compared with the other ethnic groups.[13]

Previous studies provide links between the NFAT signalling pathway and the development of hypertension. As one of the most frequently used antihypertensive drugs, the role of telmisartan in antihypertensive therapy has been intensively studied, although little is known about its effects on the NFAT signalling pathway. Our preliminary studies indicate that telmisartan can block Kv1.3 potassium channels and effectively inhibit potassium current densities, along with Kv1.3 mRNA and protein expression levels. Here, we investigated the effects of telmisartan on the NFAT signalling pathway and related molecular mechanisms after T lymphocyte activation and proliferation in the peripheral blood of Kazakh patients with EH. We used immunomagnetic cell sorting (MACS) to isolate T lymphocytes and quantitative reverse-transcribed polymerase chain reaction (qRT-PCR) and Western blot analysis to evaluate the mRNA and relative protein expression levels of NFATc1, IL-6, and TNF-α in hypertensive Kazakh patients. Our results suggest a new therapeutic target for the prevention and treatment of hypertension.

Materials and methods

We chose Kazakh hypertensive patients as the main subjects of our experiments. All patients were assigned to one of five groups: (1) Controls, (2) Telmisartan, (3) CsA, (4) VIVIT, and (5) 4-aminopytidine (4-AP). There were 10 individuals in each group (five males and five females). T lymphocytes in patient peripheral blood samples were first activated and proliferated in vitro, then incubated for 48 h under different treatment conditions. Next, we used MACS to isolate T lymphocytes and qRT-PCR and Western blot analyses to measure the relative mRNA and protein expression levels of NFATc1, IL-6 and TNF-α in the isolated T cells. The experimental design and procedure are illustrated in Figure 1.

Subjects

Fifty Kazakh hypertensive patients (mean age 51.9 ± 3.0 years) attending the hypertension clinic of the First Affiliated Hospital of Xinjiang Medical University were enrolled into the study between January and December 2014. All patients were randomised into five groups according to a computer-generated randomisation list kept by a third party. There are exactly five males and five females in each group. Their mean blood pressure was (167.2 ± 7.0)/(102.6 ± 5.1) mm Hg (1 mm Hg = 0.133 Kpa), without antihypertensive therapy.

Inclusion criteria. The diagnostic criteria of hypertension used were those stated in the 2010 Chinese guidelines for the management of hypertension.[14] Briefly, systolic blood pressure (SBP) ⩾ 140 mm Hg and/or diastolic blood pressure (DBP) ⩾ 90 mm Hg, without treatment with antihypertensive medication, were considered diagnostic of hypertension.

Exclusion criteria. Patients with 10 types of disease were excluded from our study: secondary hypertension; cerebrovascular disease; coronary heart disease, rheumatic heart disease; congenital heart disease; acute or chronic infection; autoimmune disease; important organ failure; diabetes; carotid atherosclerosis. Patients included in the study were required both to satisfy the inclusion criteria and not exhibit any of the exclusion criteria. For example, a patient with both hypertension and atherosclerosis would be excluded from our experiments.

The study was conducted according to the World Medical Association Declaration of Helsinki. All procedures were approved by the Ethics Committee of First Affiliated Hospital of Xinjiang Medical University (approval number 20131011-2) and written informed consent was provided by every subject before inclusion in the study.

Cardiovascular risk factors data of subjects

Data for cardiovascular risk factors including age, SBP, DBP, smoking history, drinking history, body mass index (BMI), fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were collected, and compared regarding whether there were significant differences among the control, telmisartan, CsA, VIVIT and 4-AP groups. Smoking was defined as currently or previously smoking. Alcohol consumption was defined as drinking at least once per week for more than half a year.[15]

Reagents

Reagent used were: human lymphocyte isolation medium (Sigma, USA); Pan T Cell Isolation Kit (Milteni, Germany); 10% foetal bovine serum (FBS) (Hyclone, New Zealand); RPMI 1640 medium (Hyclone, New Zealand); rIL-2 (Sigma, US); FITC-CD3 (Becton, Dickinson and Company, USA); Trizol (Life Technologies, USA); Reverse transcription kit (Thermo Fisher, USA); RT-PCR kit (Qiagen, Germany); RIPA Lysis Buffer (Thermo Fisher, USA); Protease inhibitor (Thermo, USA); BCA Protein Assay Kit (Thermo, USA); SDS-PAGE (BIO-RAD, USA); anti-human monoclonal antibodies against NFATc1, IL-6, TNF-α, and β-actin, and goat anti-rabbit secondary antibodies (Abcam, UK); BCIP/NBT chromogenic reagent (Invitrogen, USA); telmisartan (Boehringer-Ingelheim, Germany); CsA (Novartis, Switzerland); VIVIT (Sigma, USA); 4-AP (Sigma, USA).

Instruments

Instruments used were: MACS separation column, MACS separators (Milteni, Germany); flow cytometer (Beckman Coulter, USA); centrifuges (Eppendorf, Germany); 5% CO_2 incubator (Thermo, USA); inverted fluorescent microscope (Leica, Germany); horizontal electrophoresis apparatus (LiuYi, China); real-time fluorescence quantification PCR amplification machine (BIO-RAD, US); vertical electrophoresis apparatus (BIO-RAD, USA); Quality image analysis system (BIO-RAD, USA).

Experimental methods

T lymphocyte isolation. Using a heparinised vacutainer, 10 ml of peripheral venous blood was collected from each subject. Peripheral blood mononuclear cells were isolated using human lymphocyte isolation medium and density gradient centrifugation. Using the Pan T Cell Isolation Kit, human T lymphocytes were isolated by depletion of non-target T lymphocytes (negative selection). Non-target T lymphocytes were labelled with a cocktail of biotin-conjugated monoclonal antibodies and the Pan T Cell MicroBead Cocktail. The magnetically labelled non-target T lymphocytes were depleted by retaining them on a MACS separation column in the magnetic field of a MACS Separator, while the unlabelled T lymphocytes passed through the column. The purity of the enriched T lymphocytes was evaluated by flow cytometry; the cells were fluorescently stained with FITC-CD3 and analysed using the MACS Quant Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. The results showed that >95% of cells were T lymphocytes.

Cell culture. T lymphocytes were seeded in 24-well plates containing RPMI 1640 medium, 10% FBS and recombinant interleukin-2 (rIL-2), and cultured in an incubator at 37°C with 5% CO_2 for 48 h to promote T lymphocyte activation. The cultured T lymphocytes were treated as follows: telmisartan (final concentration, 100 μmol/l), CsA (final

Table 1. Primer sequences and amplicon size.

Gene	Sequences (5′–3′)	Annealing temperature (°C)	Product length (bp)
NFATC1	F: CAGACCATCAGGGCATCATA R: TCCAGCGTTCTCTTCATTCA	57	127
IL6	F: GGTACATCCTCGACGGCATCT R: GTGCCTCTTTGCTGCTTTCAG	59	202
TNF	F: GTGCTCCTCACCCACACCAT R: AAGACCCCTCCCAGATAGAT	58	152
ACTB	F: TGGCACCCAGCACAATGAA R: CTAAGTCATAGTCCGCCTAGAAGCA	55	186

concentration, 10 μmol/l), VIVIT (final concentration, 2 μmol/l), 4-AP (final concentration, 3 mmol/l) and an equal amount of dimethyl sulfoxide (DMSO) solution were added to the culture media of cells from the telmisartan, CsA, VIVIT, 4-AP, and control groups, respectively. The T lymphocytes were then cultured for a further 48 h, followed by collection of T lymphocyte suspensions and division of samples into two equal parts for extraction of RNA and protein.

RNA extraction and qRT-PCR analysis. Total RNA was extracted from T lymphocytes using Trizol and the A260/A280 values of the resulting RNA samples were approximately 1.8–2.0. Reverse transcription reactions were performed using 1 μg RNA at 42°C for 60 min, followed by 70°C for 5 min. The total volume of qRT-PCR reactions was 20 μl, including 10 μl SYBR Green PCR Master Mix (2×), 2 μl cDNA template, 0.5 μl each forward and reverse primers and 7 μl ddH2O. *NFATC1* (encoding NFATc1), *IL6* (encoding IL-6), and *TNF* (encoding TNF-α) gene sequences were obtained from GenBank. Primers were synthesised by Sangon Biotech (Shanghai). *ACTB* (encoding β-actin) was chosen as an internal reference gene (Table 1). PCR and agarose gel electrophoresis were performed with cDNA templates, and standard curves were established using PCR products. The qRT-PCR reaction program was 95°C for 5 min, followed by 39 cycles of 95°C for 10s and 60°C for 30s. Amplification efficiencies were approximately 90–100%. After qRT-PCR, amplicons were separated by 2% agarose gel electrophoresis. The data were analysed using the $2^{-\Delta\Delta Ct}$ method as follows: ΔCt = Ct target gene – Ct reference gene, ΔΔCt = ΔCt experimental group – ΔCt control group. The rate of inhibition by telmisartan was calculated as follows: Inhibitory rate % = (mRNA expression of control group – mRNA expression of intervention group) / mRNA expression of control group × 100%.

Western blots. Total proteins were extracted from T lymphocytes using RIPA lysis buffer supplemented with protease inhibitors. Protein concentration was measured by BCA protein assay and 5 μl loading buffer added to each sample before denaturation of proteins in a water bath at 95°C for 5 min and renaturation on ice for 5 min. Protein samples (20 μl = 30 μg) were added into each well of gels for SDS-PAGE. After electrophoresis, proteins were transferred to PVDF membranes and blocked with 5% skimmed milk powder for 1 h. Primary antibodies were diluted as follows: NFATc1, 1:2000; IL-6, 1:2000; TNF-α, 1:1000; β-actin, 1:5000. Blots were incubated with primary antibodies overnight at 4°C, with shaking. Blots were then washed with TBST, followed by incubation with alkaline phosphatase-conjugated Goat Anti-Rabbit secondary antibodies (1:2000) at room temperature for 1 h and washing with TBST. Visualisation was achieved by addition of 5 ml BCIP/NBT chromogenic reagent followed by analysis using a Quality image analysis system. The telmisartan inhibitory rate was calculated as follows: Inhibitory rate (%) = (protein expression of control group – protein expression of interventional group) / protein expression of control group × 100%.

Statistical methods

Data were statistically analysed using SPSS17.0 software and are expressed as mean ± standard deviation (SD) or percentage (%), as appropriate. Multiple data sets were compared by one-way analysis of variance or Chi-square tests. An LSD-t test was used for comparisons between two groups. $P < 0.05$ was considered to be statistically significant.

Results

Comparison of cardiovascular risk factors of individuals assigned to each of the five groups

There were no significant differences among the control, telmisartan, CsA, VIVIT, and 4-AP groups in age, SBP, DBP, smoking history, alcohol consumption history, BMI, FBG, TC, TG, HDL, or LDL ($p > 0.05$; Table 2).

Effect of telmisartan, CsA, VIVIT, and 4-AP on NFATC1, IL6, and TNF-α mRNA expression in T lymphocytes

The mRNA expression of *NFATC1, IL6*, and *TNF* in peripheral blood T lymphocytes was examined in the five experimental groups. Amplicons of the correct size were

Table 2. Comparison of cardiovascular risk factors among the control, telmisartan, CsA, VIVIT, and 4-AP groups (n = 50).

Group	Control	ARB	CsA	VIVIT	4-AP	p-value
Number	10	10	10	10	10	> 0.05
(male/female)	(5/5)	(5/5)	(5/5)	(5/5)	(5/5)	> 0.05
Age (years)	52.1 ± 3.3	52.5 ± 2.8	50.7 ± 3.2	51.3 ± 2.1	53.2 ± 2.2	> 0.05
SBP (mmHg)	168.3 ± 7.1	166.4 ± 5.5	167.2 ± 7.3	169.2 ± 6.7	165.2 ± 5.8	> 0.05
DBP (mmHg)	101.2 ± 3.8	104.1 ± 6.3	101.5 ± 5.1	103.4 ± 3.2	102.8 ± 4.5	> 0.05
Smoking (%)	48	50	50	49	48	> 0.05
Drinking (%)	55	53	50	52	50	> 0.05
BMI (kg/m^2)	26.6 ± 2.4	26.1 ± 3.3	25.9 ± 1.7	26.5 ± 2.3	26.4 ± 1.9	> 0.05
FBG (mmol/l)	4.6 ± 0.7	4.8 ± 0.5	4.2 ± 0.1	4.3 ± 0.2	4.4 ± 0.3	> 0.05
TC (mmol/l)	4.5 ± 0.2	4.6 ± 0.3	4.3 ± 0.5	4.7 ± 0.1	4.5 ± 0.8	> 0.05
TG (mmol/l)	1.8 ± 0.1	1.9 ± 0.2	1.8 ± 0.2	1.9 ± 0.3	1.8 ± 0.3	> 0.05
HDL (mmol/l)	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	1.3 ± 0.3	> 0.05
LDL (mmol/l)	3.3 ± 0.2	3.2 ± 0.4	3.4 ± 0.5	3.3 ± 0.7	3.2 ± 0.3	> 0.05

SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; FBG: fasting blood glucose; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol.

observed after agarose gel electrophoresis. The results demonstrated significantly decreased mRNA expression levels of all three genes in T lymphocytes from the telmisartan, CsA, VIVIT, and 4-AP groups compared with controls ($p < 0.05$). In addition, levels of all three genes were lower in the CsA, VIVIT, and 4-AP groups compared with those in the telmisartan group ($p < 0.05$). There was no statistically significant difference in expression of the three genes among the CsA, VIVIT, and 4-AP groups ($p > 0.05$) (Figure 2). Telmisartan inhibited mRNA expression of *NFATC1, IL6*, and *TNF* in T lymphocytes by 50.0%, 73.5%, and 50.0%, respectively.

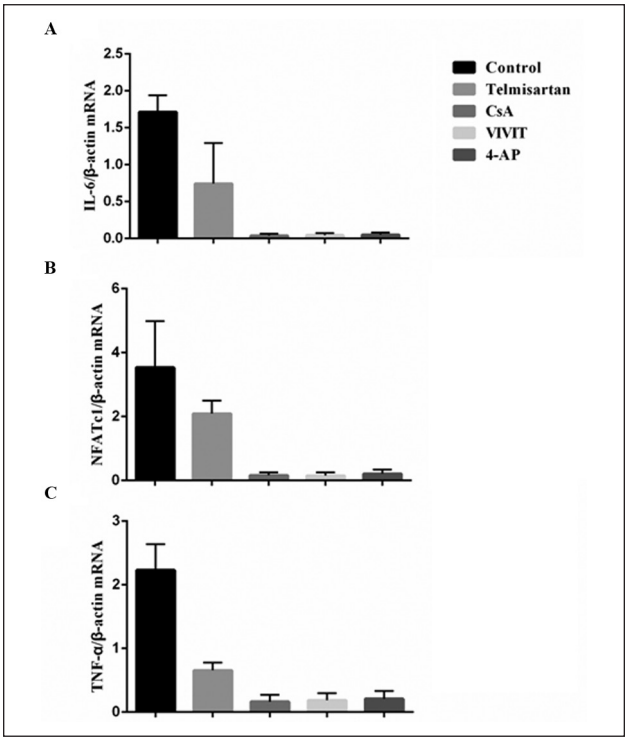

Figure 2. mRNA expression of (A) *NFATC1*, (B) *IL6*, and (C) *TNF*, relative to *ACTB* in samples extracted from activated peripheral blood T lymphocytes from Xinjiang Kazakh patients with EH, determined by qRT-PCR. Cells were treated with DMSO (control); telmisartan (100 μmol/l); cyclosporin A (10 μmol/l); VIVIT (2 μmol/l); and 4-AP (3 mmol/l). N = 10 in each treatment group.

Effect of telmisartan, CsA, VIVIT, and 4-AP on NFATc1, IL-6, and TNF-α protein expression in T lymphocytes

Western blot analysis revealed that NFATc1, IL-6, and TNF-α proteins were expressed in peripheral blood T lymphocytes of all five groups. Levels of NFATc1, IL-6, and TNF-α were significantly lower in T lymphocytes from the telmisartan, CsA, VIVIT, and 4-AP groups compared with those in the control group ($p < 0.05$). In addition, expression levels of all three proteins were significantly decreased in the CsA, VIVIT, and 4-AP groups compared with that in the telmisartan group ($p < 0.05$). There were no statistically significant differences among the CsA, VIVIT, and 4-AP groups ($p > 0.05$) (Figures 3 and 4). Telmisartan inhibited protein expression of NFATc1, IL-6, and TNF-α in T lymphocytes by 47.5%, 47.9%, and 21.1%, respectively.

Discussion

Based on the above results, we conclude that the inhibitory effect of telmisartan on the NFAT signalling pathway and related molecules, including IL-6 and TNF-α, in T lymphocytes from hypertensive patients was less than that of CsA, VIVIT, and 4-AP, indicating that the antihypertensive function and inhibitory effects of telmisartan on the T lymphocyte NFAT signalling pathway are unlikely to affect the normal immune function of hypertensive patients.

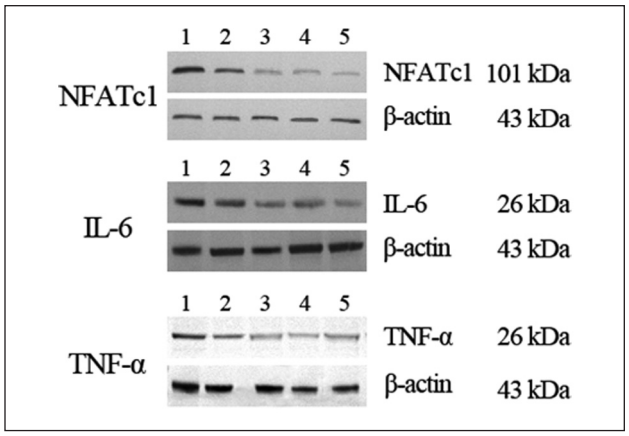

Figure 3. Representative Western blots of protein samples extracted from activated peripheral blood T lymphocytes from Xinjiang Kazakh patients with EH. Cells were treated with: 1, DMSO (Control); 2, telmisartan (100 μmol/l); 3, cyclosporin A (10 μmol/l); 4, VIVIT (2 μmol/l); and 5, 4-AP (3 mmol/l). Blots were probed with antibodies against NFATc1, IL-6, TNF-α, and β actin.

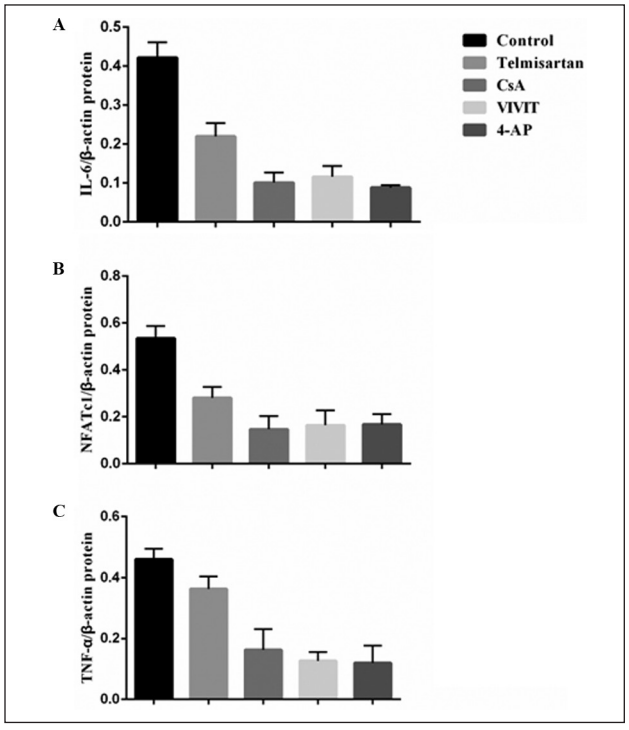

Figure 4. Protein expression of (A) NFATc1, (B) IL-6, and (C) TNF-α, relative to β actin in samples extracted from activated peripheral blood T lymphocytes from Xinjiang Kazakh patients with EH, determined from Western blots. Cells were treated with DMSO (control); telmisartan (100 μmol/l); cyclosporin A (10 μmol/l); VIVIT (2 μmol/l); and 4-AP (3 mmol/l). *N* = 10 in each treatment group.

Several studies have provided evidence that activation of the NFAT signalling pathway is dependent on intracellular Ca^{2+} concentration. NFAT, either alone or together with various other nuclear transcription factors (AP1, GATA, FOXP3), can induce transcription of target genes, including *IL6, IL2, IL4*, and *TNF*, leading to their specific expression.[16–24] Activation of the NFAT signalling pathway mediates T lymphocyte proliferation and release of inflammatory cytokines to promote the development of hypertension. A large amount of IL-6 is released into the extracellular fluid on T lymphocyte activation, influencing lymphocyte differentiation and migration, activating the NFAT signalling pathway, and leading to further lymphocyte activation. Activated lymphocytes generate additional inflammatory cytokines, such as IL-17, and promote Ang II release. This inflammatory environment, including IL-6, IL-17, and Ang II, along with other cytokines, promotes sodium retention in the renal system and blood vessels, leading to vasoconstriction and vascular remodelling, and ultimately hypertension.[25]

Data from our preliminary studies[26,27] indicate that the expression and activation of Kv1.3 potassium channels were increased in peripheral blood T lymphocytes from Xinjiang Kazakh hypertensive patients compared with that in healthy subjects. We also found that the angiotensin receptor blocker, telmisartan, which is widely used to treat hypertension, can block Kv1.3 potassium channels in lymphocytes of spontaneously hypertensive rats in a concentration-dependent manner,[28] consistent with other studies.[29–32] In addition, telmisartan effectively inhibits potassium current densities, along with Kv1.3 mRNA and protein expression levels in peripheral blood T lymphocytes of Kazakh patients with EH in a time-dependent manner.[33] Hence, angiotensin receptor blockers, including telmisartan, have the potential to exert potent anti-inflammatory effects by blocking Kv1.3 potassium channels on T lymphocytes. Activated T lymphocyte potassium channels can enhance potassium ion efflux, which increases the electric potential gradient on both sides of the cytomembrane, generating hyperpolarisation to promote calcium ion influx, causing a transient increase in calcium concentration and subsequent Calcineurin (CaN)-mediated dephosphorylation of NFAT and activation of the NFAT signalling pathway. In this experiment, we found that telmisartan can effectively inhibit mRNA and protein expression of NFATc1, IL-6, and TNF-α in peripheral blood T lymphocytes of hypertensive Kazakh patients. These data provide an important link between the NFAT signalling pathway and the Kv1.3 potassium channel.

Nataraj et al.[34] demonstrated that the angiotensin type I receptor (AT1R) is strongly expressed on T lymphocytes. AT1R can increase intracellular Ca^{2+} concentration by activating T lymphocyte potassium channels and thus trigger Ca^{2+} mediated activation of the NFAT signalling pathway, leading to T lymphocyte activation and proliferation. Telmisartan can inhibit T lymphocyte potassium channels, resulting in decreased influx of extracellular Ca^{2+} into the cytoplasm and a consequent decrease in intracellular Ca^{2+}

concentration and inhibition of the NFAT signalling pathway. This leads to strong suppression of activation and proliferation of T lymphocytes, promoting an anti-inflammatory environment. Therefore, we used the angiotensin blocker, telmisartan, to inhibit the NFAT signalling pathway in T lymphocytes and obtained preliminarily data indicating that telmisartan can have an anti-inflammatory effect by inhibiting the NFAT signalling pathway through blocking T lymphocyte Kv1.3 potassium channels. We conclude that increased expression of potassium channels on T lymphocytes enhances potassium ion efflux, and that the increased electric potential gradient on both sides of the cytomembrane generates hyperpolarisation to promote calcium ion influx, cause a transient increase in calcium concentration, and subsequent CaN-mediated dephosphorylation of NFAT and activation of the NFAT signalling pathway.

Similarly, both cyclosporin A (CsA) and VIVIT are immunosuppressants; CaN and NFAT are their respective target enzymes. These two inhibitors can block the NFAT signalling pathway by suppressing the activities of CsA and VIVIT, and consequently nuclear translocation of transcription factors and activation of some inflammatory cytokines are also inhibited, preventing activation and proliferation of T lymphocytes.[12] 4-aminopytidine (4-AP) is a blocker of the Kv1.3 potassium channel, and its action leads to strong suppression of transmembrane potential formation and suppression of extracellular Ca^{2+} influx to the cytoplasm, with an ultimately anti-inflammatory effect.

We investigated the effects of telmisartan on the NFAT signalling pathway and related molecular expression in peripheral blood T lymphocytes of Kazakh patients with EH and found that telmisartan could effectively inhibit NFATc1, IL-6, and TNF-α mRNA and protein expression. However, the specific mechanism by which this occurs is not entirely clear and requires further study.

Taken together, our results indicate that telmisartan can not only lower blood pressure directly by blocking the renin–angiotensin–aldosterone system, but also regulate hypertensive inflammation indirectly by inhibiting the NFAT signalling pathway of T lymphocytes. It is conceivable that specific targeted inhibition of related signalling pathways in T lymphocytes could be used to treat hypertension in humans; however, additional studies are needed in this area.

Acknowledgements

Thanks are due to TX Ding, CL Lv and Z Han for assistance with the experiments and to JL Huang and CX Lv for valuable discussion.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Natural Science Foundation of China (grant number 81160039).

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