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ARTYKUŁ ORYGINALNY / *ORIGINAL ARTICLE*

Potassium channel changes of peripheral blood T-lymphocytes from Kazakh hypertensive patients in Northwest China and the inhibition effect towards potassium channels by telmisartan

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Abstract

Background and aim: Increasing evidence indicates that chronic inflammation is a direct or indirect manifestation of hypertension. Potassium channels are thought to be critical for lymphocyte activation, which suggests that hypertension may be an inflammatory disease initiated at the ion channel level.

Methods: This study investigated changes in interleukin (IL)-6, IL-17, and transforming *growth* factor beta (TGF-β1) expression in the blood of Kazakh hypertensive patients in Northwest China using ELISA technology. Whole-cell patch clamp technology was used to evaluate current changes associated with Kv1.3 and KCa3.1 in peripheral blood T lymphocytes of hypertensive patients, and to investigate current changes induced by telmisartan. We also investigated the effects of telmisartan on expression of Kv1.3 and KCa3.1 at mRNA and protein levels in peripheral blood T lymphocytes using real-time polymerase chain reaction and Western blot analysis.

Results: Expression of IL-6, IL-17 and TGF-β1 in the blood of Kazakh hypertensive patients in Northwest China was significantly higher than in healthy controls ($p < 0.05$). The current mediated by Kv1.3 and KCa3.1 and the corresponding expression at mRNA and protein levels in T lymphocytes were also higher in these hypertensive patients than in controls ($p < 0.05$). Telmisartan intervention for 24 h and 48 h inhibited the current and expression of Kv1.3 and KCa3.1 at mRNA and protein levels ($p < 0.05$).

Conclusions: These results indicated that the increase in functional Kv1.3 and KCa3.1 channels expressed in T lymphocytes of Kazakh patients with hypertension was blocked by telmisartan, resulting in a reduced inflammatory response. These results provide theoretical support for the treatment of hypertension at the cellular ion channel level.

Key words: Kazakh, hypertension, T lymphocyte, Kv1.3, KCa3.1, telmisartan

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INTRODUCTION

Essential hypertension is one of the most common cardiovascular diseases worldwide, but the pathogenic mechanisms involved in the condition remain largely unknown despite ongoing research. There is increasing evidence to suggest that inflammation may play a critical role in the initiation and development of hypertension [1, 2]. Therefore, some researchers have proposed that hypertension is a form of chronic inflammatory disease [3, 4].

Studies have shown that activation of inflammatory cells is involved in the progression of hypertension. In animal studies, marked activation of inflammatory cells was dem-

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onstrated in spontaneously hypertensive rats, and was more marked in adult rats than that in young rats [5]. Other studies have shown that upregulation of inflammatory mediators in hypertensive patients can be used to predict the development of hypertension. The inflammatory process in hypertensive patients is characterised by expression of cytokines (interleukin [IL]-6, IL-1, and tumour necrosis factor alpha [TNF-α]), monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and the corresponding activation of nuclear factor-κB (NF-κB) [6–8]. T lymphocytes are also thought to play a critical role in the pathogenesis of hypertension and vascular remodelling [9]. Recent data from experimental animal models found that T cells are associated with hypertension and inflammation [10]. It has been shown that potassium channels are essential for the activation of T lymphocytes, and that they are involved in multiple physiological processes, such as T lymphocyte differentiation, proliferation, and activation [11, 12]. The major potassium channels in T lymphocytes are the voltage-gated potassium channels Kv1.3 and the calcium-activated potassium channel KCa3.1 (also called IKCa1, KCNN4, hKCa4). The opening of Kv1.3 is essential for membrane depolarisation and the maintenance of resting potential. The opening of KCa3.1 channels increases cytoplasmic calcium levels and results in membrane hyperpolarisation. Potassium currents mediated by Kv1.3 and KCa3.1 provide sufficient cations to balance the calcium influx process. T lymphocyte potassium channels have been implicated in various human disorders. These two ion channels may therefore be potential therapeutic targets. However, little is known about the changes in T lymphocyte Kv1.3 and KCa3.1 channels in hypertension. Transcriptional factor Foxp3, which is expressed in T lymphocytes (including T helper cells [Th1, Th2, Th17] and suppressor T cells [including regulatory T cells]), has been reported to be critical for the production of angiotensin II, which plays a key role in the progression of essential hypertension [9]. Other studies have shown that the anti-inflammatory effects of angiotensin II receptor blocker (ARB) may be involved in the ability of these agents to lower blood pressure (BP) [6, 13, 14].

Northwest China is populated primarily by ethnic minorities, and the incidence of hypertension in Kazakh people (48.69%) is higher than that in Han people (33.42%) or other ethnic groups in the region. Furthermore, hypertension awareness, treatment, and control are significantly lower in Kazakh people than in Han or other populations in the same region.

In this study we explored the changes in expression of potassium channels and blood inflammatory factors in T lymphocytes of hypertensive Kazakh patients. Our results provide evidence suggesting that hypertension is an inflammatory disease at the level of cell ion channels and inflammatory factors. This forms a novel basis to research the pathogenesis of hypertension. In addition, we investigated the impact of telmisartan intervention on T lymphocyte potassium channels, highlighted theoretical mechanisms, and experimental evidence for the treatment of hypertension at the level of cellular ion channels.

METHODS

Research objective

The study included 30 hypertensive patients (15 male and 15 female) of Kazakh ethnicity, who were not receiving any form of drug treatment, were selected randomly from the Department of Hypertension, the First Affiliated Hospital, Xin Jiang Medical University and sought treatment between April 2012 and January 2013. Thirty healthy subjects of Kazakh ethnicity (15 males and 15 females) were selected over the same time period as a control group.

Diagnostic criteria were based on the World Health Organisation/International Society of Hypertension (2005) hypertension standard: all patients had a systolic BP ≥ 140 mm Hg (1 mm Hg = 0.133 kPa) and a diastolic BP ≥ 90 mm Hg. Exclusion criteria included the presence of secondary hypertension, coronary artery atherosclerosis, acute cerebrovascular disease, rheumatic heart disease, congenital heart disease, acute or chronic infection, systemic immune disease, diabetes, or organ failure as indicated by laboratory or clinical examination.

The study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all subjects prior to participation in the study. The protocol was approved by the Ethics Committee of the First Affiliated Hospital, XinJiang Medical University.

Sample collection

Peripheral venous blood samples (25 mL) were collected from each patient. The blood was divided into 2 × 10 mL and 1 × 5 mL portions. The samples were treated with heparin for anticoagulation.

Cell isolation and culture

Single cells isolated using lymphocyte isolation buffer (Sigma, USA) were cultured at 37°C in a 5% CO_2 incubator. After adherence incubation overnight, the mononuclear cells were removed. Then, T lymphocytes from hypertensive patients were isolated with immunomagnetic beads (Miltenyi Biotec, Germany) using a negative selection procedure. T lymphocytes isolated from one of the two 10-mL peripheral blood samples from hypertensive patients and healthy controls were used for subsequent experiments.

T lymphocytes isolated from the other 10-mL peripheral blood sample of hypertensive patients were cultured in 24-well plates using RPMI 1640 medium containing 10% FBS (Hyclone, New Zealand) and 50 U/mL rIL-2 (BD, USA). These cells were cultured at 37°C in a 5% CO_2 incubator for 48 h to promote the proliferation of T lymphocytes. The cultured lymphocytes were divided randomly into three groups: a blank control group,

a telmisartan-treated group, and a potassium channel blocker group (positive control group). Cells from 10 patients (5 male and 5 female) were included in each treatment group. No treatment was performed on the blank control group.

In the active treatment group, telmisartan (Boehringer Ingelheim Pharma GmbH, China) was dissolved and added to the medium at a final concentration of 100 μmol/L. In the positive control group cells were exposed to the Kv1.3 potassium channel blocker, 4-aminopyridine (Sigma, USA), and to the KCa1.3 potassium channel blocker, TRAM-34 (Sigma, USA). The blocking agent was added into culture medium at a final concentration of 3 mmol/L and 5 μmol/L, respectively. The T lymphocytes in each plate were cultured for another 0 h, 24 h, or 48 h and the cells were used for subsequent experiments at related time points.

ELISA Assay

Peripheral blood samples (5 mL) from hypertensive patients (n = 30) and healthy subjects (n = 30) were incubated at room temperature for 30 min and centrifuged at 3000 rpm for 10 min at 4°C. The supernatant serum was collected and stored at –80°C.

Levels of IL-6 (BOSTER, China), IL-17 (eBioscience, USA), and transforming *growth* factor beta (TGF-β1) (eBioscience, USA) were analysed using ELISA kits according to the manufacturer's instructions.

Electrophysiological analysis (patch clamp technology)

An EPC-10 amplifier (HEAK, Germany) was used to record Kv1.3 and KCa3.1 potassium currents in untreated T lymphocytes and in cells cultured with specific drug interventions. The current in control T lymphocytes was recorded with patch clamp technology within 4 h of isolation. The current in the cultured proliferating cells was recorded 2 h before and after drug intervention. The current was recorded in one or two cells from within each sample. The recording protocol for Kv1.3 currents was as follows:

— the membrane potential was maintained at –80 mV, and a > 1 GΩ seal was formed by approaching the cell using the glass pipette;
— a negative potential was applied to break through the cell membrane and achieve whole-cell recording;
— the cells were stimulated with ramp stimulation from –80 mV to +80 mV for 400 ms.

The recording protocol for KCa3.1 current was as follows:

— the membrane potential was maintained at –80 mV, and a > 1 GΩ seal was formed by approaching the cell using the glass pipette;
— a negative potential was applied to break through the cell membrane to achieve whole-cell recording;
— cells were stimulated with ramp stimulation from –120 mV to +50 mV for 200 ms.

The intracellular solution for Kv1.3 was: 150 mmol/L KCl, 1.0 mmol/L $CaCl_2$, 1.0 mmol/L $MgCl_2$,10 mmol/L HEPES, and 10 mmol/L EGTA (pH 7.2).

The intracellular solution for KCa3.1 was: 145 mmol/L K^+ asparate, 2.0 mmol/L $MgCl_2$, 8.5 mmol/L $CaCl_2$, 10 mmol/L HEPES, and 10 mmol/L EGTA (pH 7.2). The water microelectrode resistance was 3–6 MΩ.

The extracellular solution for Kv1.3 and KCa3.1 was: 150 mmol/L NaCl, 4.5 mmol/L KCl, 1.0 mmol/L $CaCl_2$, 1.0 mmol/L $MgCl_2$, and 10 mmol/L HEPES (pH 7.35).

All reagents were purchased from AMRESCO, USA except HEPES and K^+ aspirate, which were purchased from Sigma, United States.

Dissolved telmisartan, 4-aminopyridine, or TRAM-34 was added into extracellular solutions to achieve a final concentration identical to the intervention concentrations used for T lymphocyte culture and proliferation experiments.

The recording frequency was 10.0 kHz, and data were stored on hard disk prior to being transformed using Minianalysis software and prior to analysis using Clampfit 10.2 software.

RNA extraction and RT-PCR analysis

Total T lymphocyte RNA was extracted using Trizol (Invitrogen, USA). The A_{260}/A_{280} value for isolated RNA was in the range 1.8 to 2.0. A cDNA template was prepared using RNA isolated from the T lymphocytes using a reverse transcription kit (Thermo Scientific, USA). The following program was used: 42°C, for 60 min; 70°C for 5 min. The cDNA template was stored at –20°C for further study.

Polymerase chain reaction (PCR) and agarose gel electrophoresis were performed using the cDNA template, and a standard curve was established using PCR products. Real-time PCR was performed using 2 × Maxima SYBR Green mix (Thermo Scientific, USA) on a real-time fluorescence quantification PCR machine (BIO-RAD, USA). Target gene sequences in GenBank, including Kv1.3 (NM-002232.3), KCa3.1 (NM-002250.2), and β-actin (NM-001101.3), were used.

Premier 5.0 software was selected for primer design, and the primers were synthesised by Takara Biotechnology (Dalian) as follows:

— Kv1.3:
  - Kv1.3 forward: 5'- CCA GCA CCT CTC TTC AG-3'
  - Kv1.3 reverse: 5'-TCA CCA TAT ACT CCG ACT TAC TCA-3'
  - The length of the final PCR product was 80 bp;
— KCa3.1:
  - KCa3.1 forward: 5'-TGC ATG CAG AGA TGC TGT GG-3'
  - KCa3.1 reverse: 5'-GAA GGT GGA AAT GCT GAT CGT G-3'
  - The length of final PCR product was 86 bp;
— β-actin was used as internal reference:
  - β-actin forward: 5'-TGG CAC CCA GCA CAA TGA A-3'

- β-actin reverse: 5′-CTA AGT CAT AGT CCG CCT AGA AGC A-3′
- The length of final PCR product was 186 bp.

The amplification efficiency of the primers was between 90% and 100%. The real-time PCR data were analysed using the $2^{-\Delta Ct}$ method [15].

Western blot

T lymphocytes were washed three times with PBS at 4°C and centrifuged, and the supernatants were discarded. RIPA lysis buffer (Thermo Scientific, USA) pre-mixed with proteomic inhibitors (Thermo Scientific, USA) was added to re-suspend the cells. The cell suspension was placed on ice for 30 min with continuous shaking. The mixture was centrifuged with 12,000 rpm at 4°C for 15 min. The supernatants were collected and stored at –80°C. Protein concentration was measured using the BCA method (Thermo Scientific, USA). The SDS-PAGE gel electrophoresis system (BIO-RAD, USA) was used for protein analysis. Protein samples (60 μg) were added to each well, and after gel electrophoresis the proteins were transferred onto a PVDF membrane and blocked with 5% milk. The primary antibodies used were as follows:

— Kv1.3 (Abcam, USA) diluted at 1:200 with secondary antibody used at 1:4000;
— KCa3.1 (Abcam, USA) diluted at 1:500 with secondary antibody used at 1:3000.

β-actin was used as an internal reference. The primary antibody against β-actin (Abcam, USA) was diluted at 1:2000 with secondary antibody used at 1:4000.

The signals were detected using chemiluminescent method and detected using gel-imaging system (BIO-RAD, USA) to measure the absorbance value.

Statistical analysis

Statistical analysis was undertaken using SPSS version 17.0 software. Data are presented as means and standard deviation (or standard error of the mean [SEM]). Differences among groups were analysed using repeated measure analysis of variance. Paired tests were performed using the least square difference t test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Baseline characteristics

As shown in Tables 1 and 2 there were no significant differences in baseline characteristics between hypertensive patients and control subjects.

Table 1. Baseline characteristic of hypertensive and control subjects

Variable	Hypertensive	Healthy
Age [years]	49.1 ± 2.8	48.8 ± 4.5
Smokers	50%	50%
Alcohol	50%	50%
Body mass index [kg/m^2]	26.5 ± 1.8	26.0 ± 1.4
Fasting blood-glucose [mmol/L]	4.5 ± 0.4	4.7 ± 0.6
C-reactive protein [mg/L]	8.2 ± 0.9	7.8 ± 0.7
Triglyceride [mmol/L]	1.8 ± 0.1	1.7 ± 0.2
LDL-C [mmol/L]	3.6 ± 0.6	3.5 ± 0.9
HDL-C [mmol/L]	1.1 ± 0.1	1.3 ± 0.2

No significant differences were observed between hypertensive patients and healthy persons; $p > 0.05$; HDL-C — high density lipoprotein cholesterol; LDL-C — low-density lipoprotein cholesterol

Changes in expression of peripheral blood inflammatory factors (IL-6, IL-17, and TGF-β1)

Expression levels of inflammatory factors IL-6, IL-17, and TGF-β1 in hypertensive patients and healthy subjects were analysed using ELISA technology. Expression of inflammatory factors IL-6, IL-17, and TGF-β1 were significantly higher in the peripheral blood of hypertensive patients than in healthy controls (Fig. 1). The relative differences were 48.0%, 130.9%, and 99.3%, respectively.

Changes in T lymphocyte of potassium current mediated by Kv1.3 and KCa3.1

Whole-cell patch clamp technology was used to analyse the changes of current mediated by Kv1.3 and KCa3.1 in hypertensive patients, healthy subjects, and in vitro-activated T cells of hypertensive patients exposed to different drug interventions.

The peak current density mediated by Kv1.3 and KCa3.1 in T lymphocytes was significantly higher (56.3% and 58.9%, respectively) in hypertensive patients than in healthy subjects (Figs. 2, 3). These results suggest that more functional Kv1.3 and KCa3.1 potassium channels are present in T lymphocytes of hypertensive patients than in healthy controls.

The potassium blockers, 4-aminopyridine and TRAM-34, as well as telmisartan blocked the potassium current mediated by Kv1.3 and KCa3.1 in activated proliferating T lymphocytes. The inhibitory effect was enhanced with an extended intervention time (Figs. 4, 5). Telmisartan intervention for 24 h or 48 h reduced Kv1.3-mediated current in activated proliferating T lymphocytes by 46.7% and 61.4%, respectively. It inhibited the KCa3.1-mediated current by 45.1% and 60.1%, respectively, after 24 h and 48 h.

Kv1.3 and KCa3.1 expression at mRNA and protein levels in T lymphocytes

Real time-PCR was used to analyse the mRNA changes in potassium channels mediated by Kv1.3 and KCa3.1 in hypertensive patients, healthy subjects, and in vitro-activated T cells from hypertensive patients exposed to different drug interventions. The results indicate that that T lymphocyte

Table 2. Baseline characteristics of different drug intervention groups of hypertensive patients

Variable	Controls	Telmisartan	4-AP
Age [years]	49.3 ± 2.3	49.2 ± 2.7	49.7 ± 3.3
Systolic blood pressure [mm Hg]	167.4 ± 7.8	165.8 ± 7.7	169.2 ± 7.3
Diastolic blood pressure [mm Hg]	101.2 ± 5.9	102.8 ± 3.8	102.0 ± 3.9
Smokers	50%	50%	50%
Drinkers	50%	50%	50%
Body mass index [kg/m^2]	26.1 ± 1.5	26.9 ± 1.6	26.5 ± 2.3
Fasting blood-glucose [mmol/L]	4.6 ± 0.4	4.8 ± 0.3	4.1 ± 0.5
C-reactive protein [mg/L]	8.0 ± 0.8	7.7 ± 0.7	8.9 ± 1.2
Triglyceride [mmol/L]	1.7 ± 0.1	1.9 ± 0.1	1.8 ± 0.1
HDL-C [mmol/L]	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
LDL-C [mmol/L]	3.6 ± 0.6	3.6 ± 0.5	3.4 ± 0.7

No significant differences were observed between hypertensive patients and healthy persons; $p > 0.05$; HDL-C — high-density lipoprotein cholesterol; LDL-C — low-density lipoprotein cholesterol; 4-AP — 4-aminopyridine

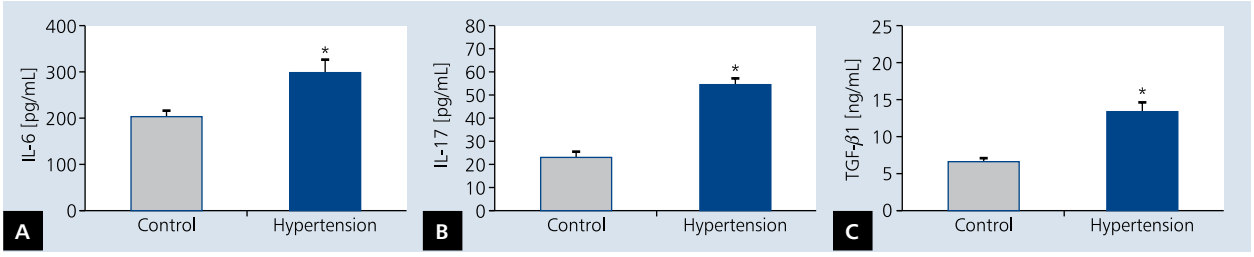

Figure 1. Levels of inflammatory factors interleukin (IL)-6, IL-17 and transforming *growth* factor beta (TGF-β1), determined using ELISA technology; **A**. Expression of IL-6 in hypertensive patients and healthy controls (*$p = 0.002$); **B**. Expression of IL-17 in hypertensive patients and healthy controls (*$p < 0.001$); **C**. Expression of TGF-β1 in hypertensive patients and healthy controls (*$p < 0.0001$). Data are shown as mean ± standard error of the mean [SEM], n = 30 per group

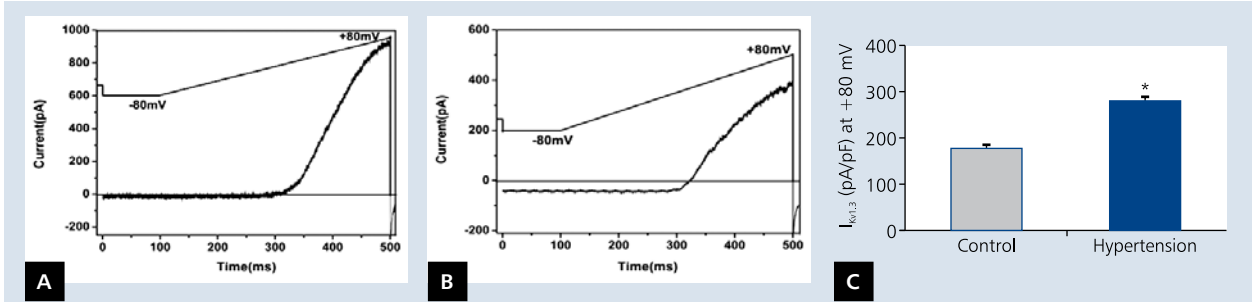

Figure 2. Kv1.3-mediated potassium current in T lymphocytes recorded with whole-cell patch clamp technology. Membrane potential was maintained at –80 mV and cells were stimulated with ramp stimulation from –80 mV to +80 mV for 400 ms (the ramp curve is shown above the recorded current trace). Recorded numbers of T lymphocytes were $N_{healthy}$:$N_{hypertension}$ = 38:40. **A**. The Kv1.3-mediated current curve in T lymphocytes of hypertensive patients; **B**. Corresponding current in healthy subjects; **C**. Comparison of peak current density at +80 mV in healthy subjects and hypertensive patients; *$p < 0.0001$. Data are shown as mean ± standard error of the mean [SEM], n = 30 per group

mRNA expression of Kv1.3 (Fig. 6A) and KCa3.1 channels (Fig. 6B) in hypertensive patients (n = 30) was higher than in control subjects (n = 30). The differences were 1.5- and 18.3-fold, respectively.

A significant inhibitory effect on Kv1.3 (Fig. 6C) and KCa3.1 (Fig. 6D) mRNA expression was observed in T lymphocytes exposed to both telmisartan and potassium channel blockers. Expression of Kv1.3 mRNA was downregulated by

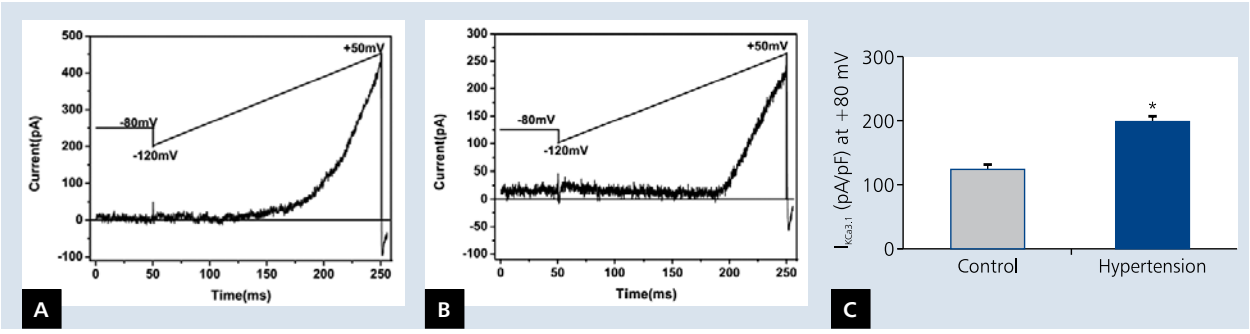

Figure 3. KCa3.1-mediated potassium current in T lymphocytes recorded with whole-cell patch clamp technology. Membrane potential was maintained at –80 mV and cells were stimulated with ramp stimulation from –120 mV to +50 mV for 200 ms (the ramp curve is shown above the current trace). Recorded numbers of T lymphocytes were $N_{healthy}$:$N_{hypertension}$ = 36:38. **A**. KCa3.1-mediated current curve in T lymphocytes of hypertension patients; **B**. Current in healthy subjects; **C**. Comparison of peak current density at +50 mV between healthy subjects and hypertensive patients, *p < 0.0001. Data are shown as mean ± standard error of the mean [SEM], n = 30 per group

50.0% and 73.5% after exposure to telmisartan for 24 h or 48 h. KCa3.1 mRNA was downregulated by 70.5% or 76.9% after 24 h or 48 h exposure to telmisartan.

We further analysed expression of protein levels using an Immunoblot assay. The protein expression of the KV1.3 and KCa3.1 channels in hypertensive patients (n = 30) was 1.0- and 0.8-fold that of control subjects (n = 30) (Fig. 7). Following exposure to telmisartan or potassium channel blockers for 24 h or 48 h, protein expression of Kv1.3 and KCa3.1 was reduced significantly compared to the blank control group (Fig. 8). Kv1.3 expression was downregulated by 49.3% and 67.9%, and KCa3.1 expression was downregulated by 32.1% and 56.8% after exposure for 24 h or 48 h, respectively.

DISCUSSION

Herein, we have shown that expression levels of inflammatory factors, including IL-6, IL-17, and TGF-β1 in peripheral blood are higher in hypertensive Kazakhstani patients than in non-hypertensive control Kazakhstani subjects from Northwest China. In addition, we have demonstrated the increased expression of functional Kv1.3 and KCa3.1 channels in peripheral blood T lymphocytes in hypertensive patients and have shown that the ARB type antihypertensive drug telmisartan inhibited T lymphocyte Kv1.3 and KCa3.1 expression at both mRNA and protein levels. The currents mediated by these two potassium channels were blocked by telmisartan.

Hypertension is characterised by the activation of lymphocytes and monocytes resulting in chronic inflammation. It is thought that T lymphocyte activation generates abundant pro-inflammatory factors in hypertensive patients. These activated T lymphocytes produce multiple cytokines, including IL-6, IL-17, and TGF-β1, depending on the underlying conditions. Furthermore, we found that the levels of IL-6, IL-17, and TGF-β1 in peripheral blood were higher in hypertensive patients than in control subjects.

Blood levels of IL-6 have been shown to be correlated with increased systolic and diastolic BP [16, 17]. In addition, IL-6 has been shown to be the key cytokine leading to the acute-phase inflammatory responses in hypertensive patients [18]. IL-6 enhances calcium-dependent potassium efflux and increases membrane permeability to calcium in red blood cells of hypertensive patients. These changes result in increased calcium influx, and finally in elevated BP [19]. However, it remains unclear as to whether IL-6 enhances potassium efflux and calcium influx in T lymphocytes of hypertensive patients.

IL-17 is predominantly secreted by Th17 cells and may act synergistically with variant cytokines to amplify the inflammatory responses. For example, it is possible that IL-17 works together with TNF-α to promote nitric oxide production, or with IL-1β to synergistically activate chemokine CXC. It has also been reported that IL-17 plays a critical role in the development of hypertension and blood vessel dysfunction mediated by angiotensin II [20].

Mutual regulation is thought to exist between TGF-β1 and the renin–angiotensin system (RAS). Research has shown that TGF-β1 stimulates renal juxtaglomerular cells to produce renin, finally resulting in upregulation of angiotensin II expression and elevated BP [21]. Another study reported significantly higher TGF-β1 levels in peripheral blood and mononuclear cells in African-American and Caucasian-American hypertensive patients than in non-hypertensive controls [22]. These findings, coupled with our own results, indicate that TGF-β1 plays and important part in the development and progression of hypertension. TGF-β1 and IL-6 may act synergistically to stimulate $CD4^+$ T cells to activate the STAT3. This would result in the induction of RORγt expression and differentiation of $CD4^+$ T cells to Th17 cells. It would also inhibit the differentiation of Treg cells, characterised by $Foxp3^+$ mRNA, and induces the transcription of IL-17, thereby promoting an inflammatory response.

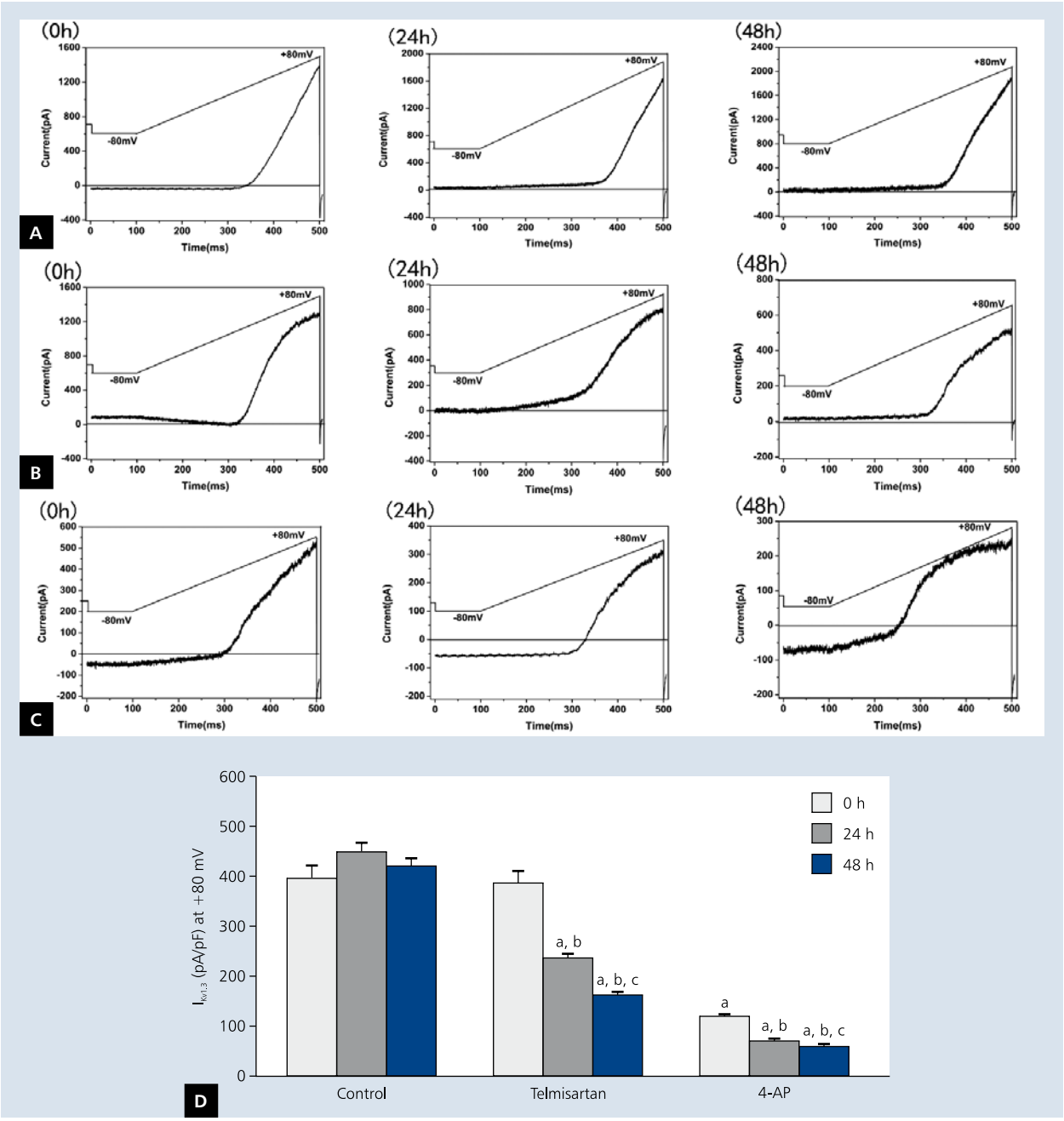

Figure 4. Kv1.3-mediated potassium current in proliferating T lymphocytes of hypertensive patients treated with specific drugs in vitro recorded with whole-cell patch clamp technology. Membrane potential was maintained at –80 mV and cells were stimulated with ramp stimulation from –80 mV to +80 mV for 400 ms (the ramp curve is shown above the recorded current trace). Recorded numbers of T lymphocytes were $N_{\text{blank control group}}$:$N_{\text{telmisartan group}}$:$N_{\text{4-AP group}}$ = 25:26:10. **A**. Kv1.3-mediated current curves in T lymphocytes of control hypertensive patients cultured for 0 h, 24 h, or 48 h; **B**. Corresponding results for the telmisartan-treated group (100 μmol/L); **C**. Results for the positive control 4-AP-treated group (3 mmol/L); **D**. Paired comparison of peak current density at +80 mV among three groups; statistically significant differences were observed between telmisartan- and 4-AP-treated group and the blank control group ($^{a}p < 0.0001$). Within each group, statistically significant differences were observed between results at 24 h and 48 h compared with results at 0 h ($^{b}p < 0.0001$). In the telmisartan-treated group, significant differences were detected between cells treated for 24 h and 48 h ($^{c}p < 0.0001$). Data are shown as mean ± standard error of the mean [SEM], n = 10 per group; 4-AP — 4-aminopyridine

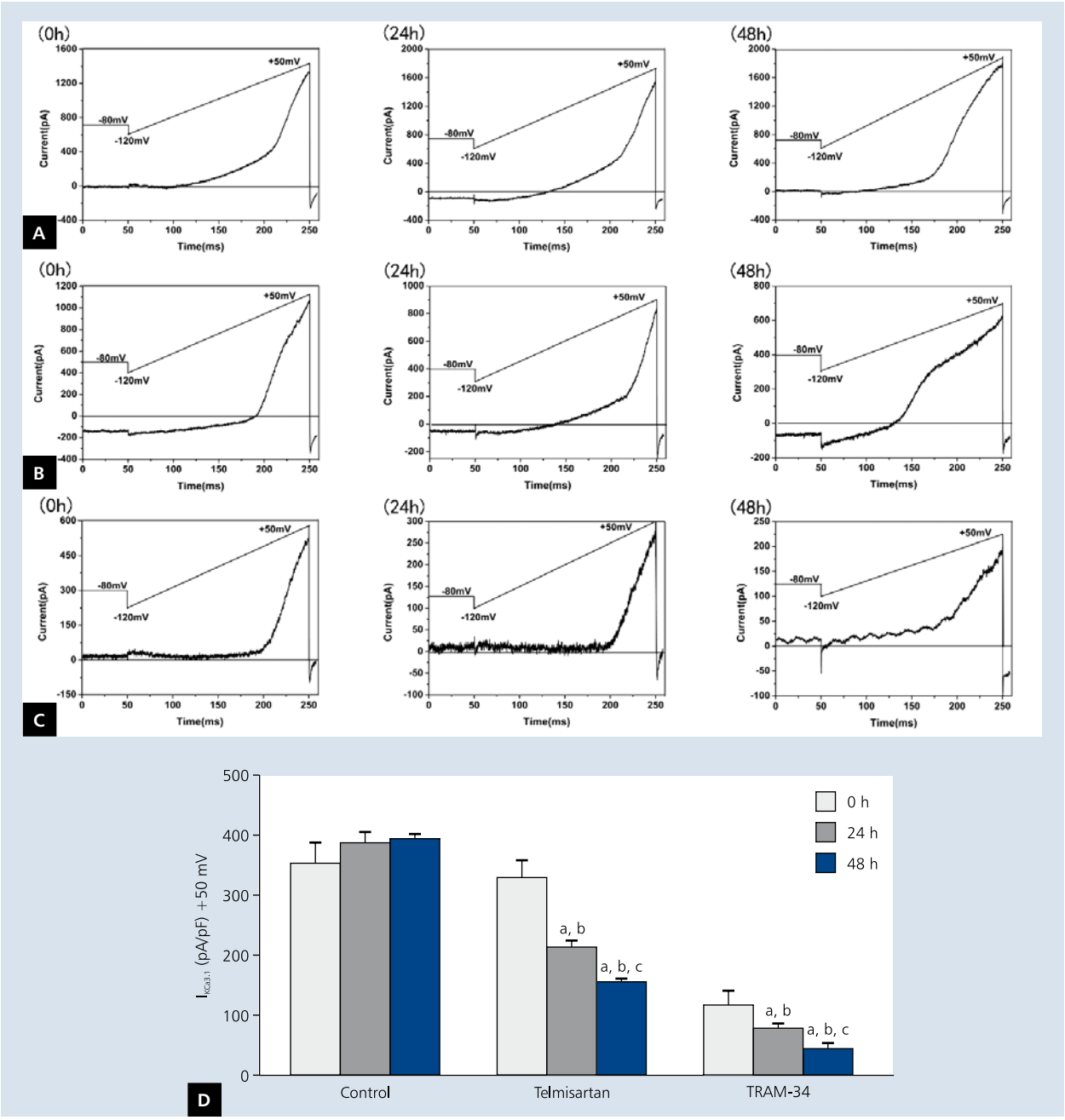

Figure 5. KCa3.1-mediated potassium current in proliferating T lymphocytes of hypertensive patients treated with specific drugs in vitro recorded with whole-cell patch clamp technology. Membrane potential was maintained at –80 mV and cells were stimulated with ramp stimulation from –120 mV to +50 mV for 200 ms (the ramp curve is shown above the recorded current trace). Recorded numbers of T lymphocytes were $N_{\text{blank control group}}$:$N_{\text{telmisartan group}}$:$N_{\text{TRAM-34 group}}$ = 25:35:10. **A**. KCa3.1-mediated current curves in T lymphocytes of blank control group cultured for 0 h, 24 h, or 48 h; **B**. Corresponding currents for the telmisartan-treated (100 μmol/L); **C**. Results for the positive control TRAM-34-treated group (5 μmol/L); **D**. Paired comparison of peak current density at +50 mV among three. Statistically significant differences were observed between telmisartan- and TRAM-34-treated groups and the blank control group ($^{a}p < 0.0001$). Within in each group, statistically significant differences were observed between 24-h- and 48-h-treated cells and the 0-h-treated cells ($^{b}p < 0.0001$); in the telmisartan- and TRAM-34-treated groups, significant differences were detected between cells treated for 24 h and those treated for 48 h ($^{c}p = 0.001$ and $^{c}p = 0.006$, respectively). Data are shown as mean ± standard error of the mean [SEM], n = 10 per group; TRAM-34 — triarylmethane-34

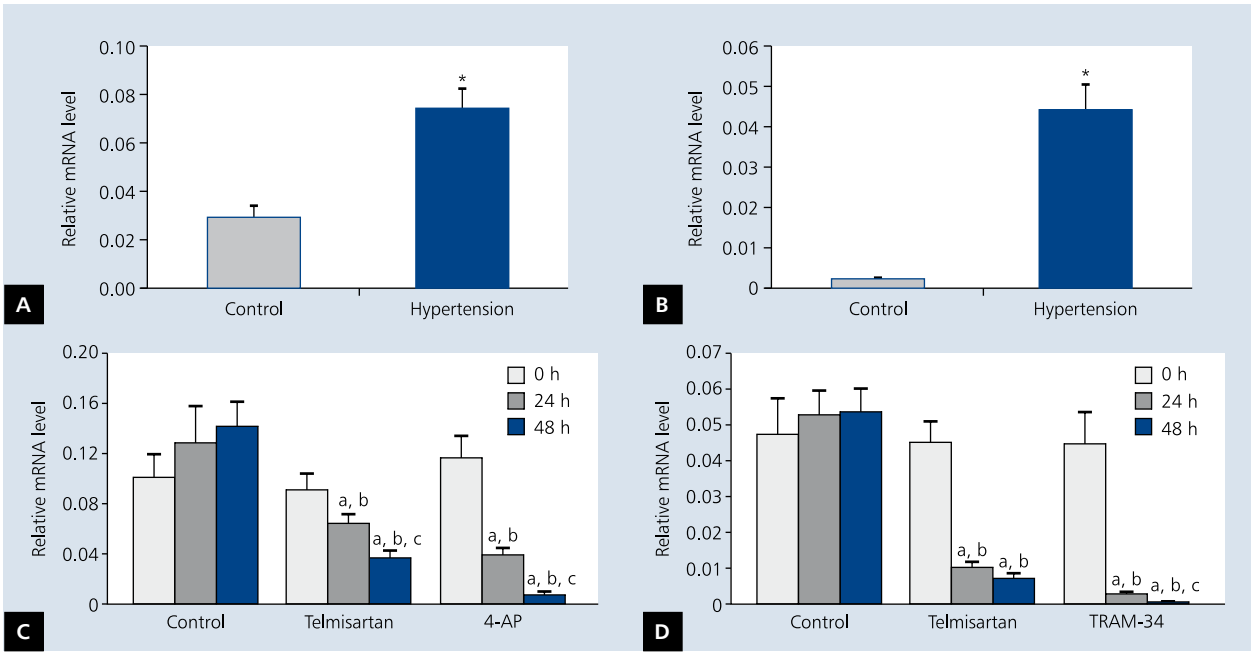

Figure 6. Expression changes of Kv1.3 (80 bp) and KCa3.1 (86 bp) at mRNA level analysed by real time-polymerase chain reaction. β-actin (186 bp) was used as an internal control. **A**, **B**. Expression of Kv1.3 and KCa3.1 compared in the hypertensive group (n = 30) and healthy control group (n = 30) (*p = 0.000); **C**, **D**. Expression of Kv1.3 and KCa3.1 at the mRNA level in activated proliferating T lymphocytes isolated from hypertension patients treated with different drugs. Significant differences were observed between drug-treated groups and the blank control group ($^a p < 0.0001$) (n = 10 per group). In each group, significant differences were detected between 24-h- or 48-h-treated cells and the 0-h-treated cells ($^b p < 0.05$). In the drug intervention group, differences were significant between 24-h-treated and 48-hour-treated cells ($^c p < 0.05$). Data are shown as mean ± standard error of the mean [SEM]; 4-AP — 4-aminopyridine; TRAM-34 — triarylmethane-34

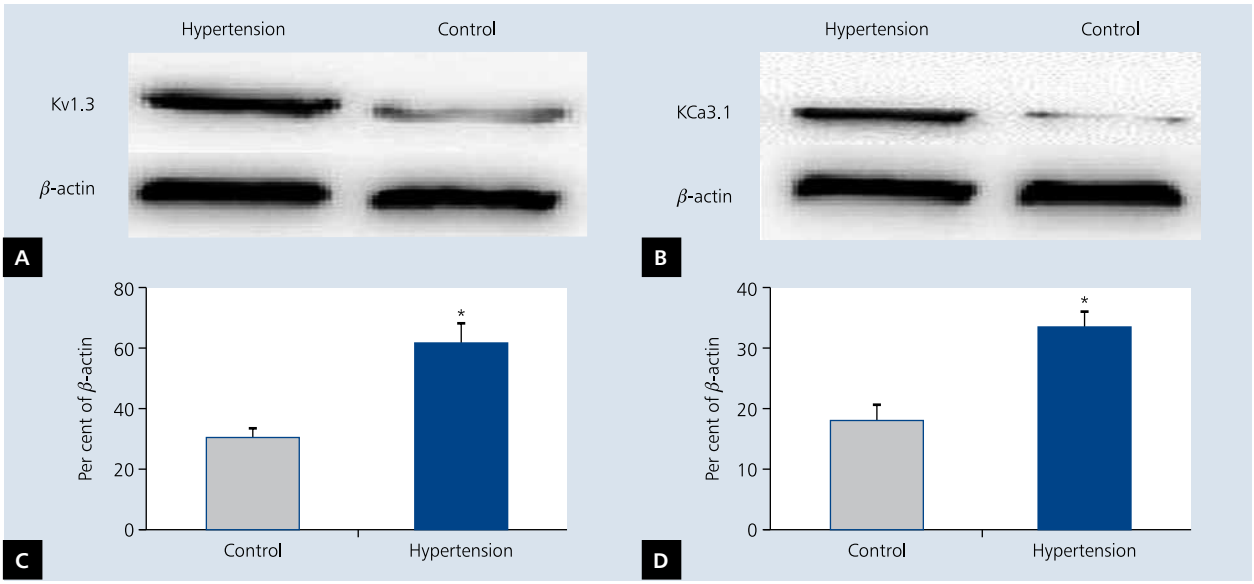

Figure 7. Western blot analysis of Kv1.3 (64 kDa) (**A**) and KCa3.1 (47 kDa) (**B**) expression in hypertensive patients and healthy control subjects. β-actin (42 kDa) was used as an internal control; **C**. Expression changes of Kv1.3 potassium protein between hypertension group and healthy group (*p < 0.0001); **D**. Expression changes in KCa3.1 (*p = 0.001). Data are shown as mean ± standard error of the mean [SEM], n = 30

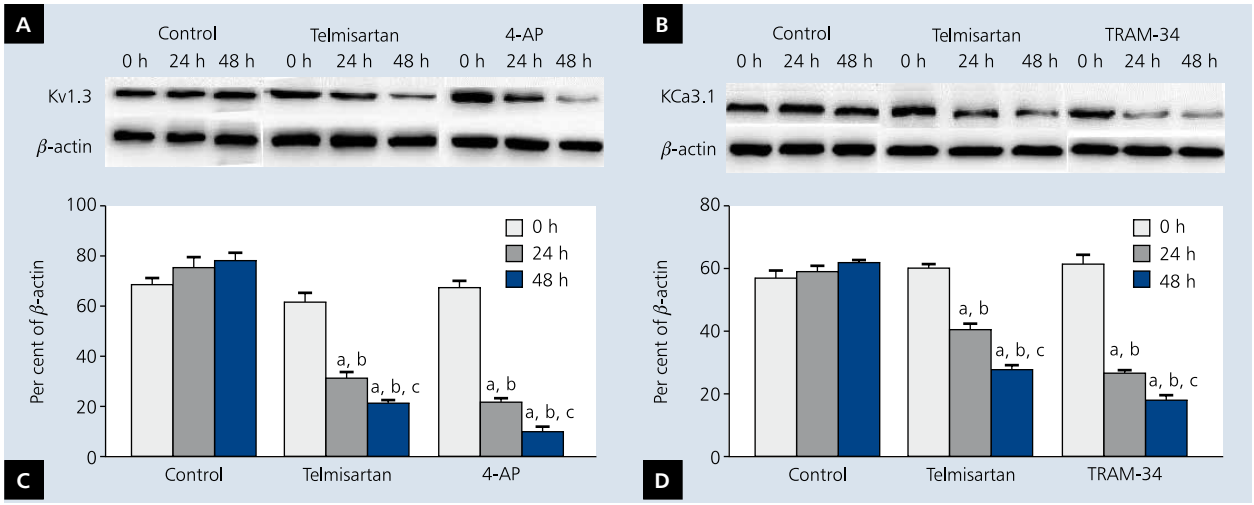

Figure 8. Western blot analysis of the Kv1.3 and KCa3.1 channels expression changes of different groups. T lymphocytes of hypertension patients were proliferated in vitro and divided into different groups. The expression changes of Kv1.3 and KCa3.1 channels at different time points were analysed using western blot. **A**. Kv1.3 (64 kDa); **B**. KCa3.1 (47 kDa). β-actin (42 kDa) was used as an internal control. Comparison with blank control group ($^{a}p < 0.0001$); paired comparison with subgroup of 0-h treatment in experimental group ($^{b}p < 0.05$); comparison between 48-h-intervention group and 24-h-intervention group ($^{c}p < 0.05$). Data are shown as mean ± standard error of the mean [SEM], n = 10 per group; 4-AP — 4-aminopyridine; TRAM-34 — triarylmethane-34

Potassium channels are important for the activation of T lymphocytes, and Kv1.3 and KCa3.1 channels provide necessary cations to balance the sustained calcium influx. The key phosphatase for T lymphocyte activation is calcium-dependent calcineurin. Potassium channels opening increases the negative transmembrane potential and promotes calcium influx, resulting in activation of calcineurin and T lymphocytes.

It has previously been reported that calcium signalling is involved in the activation of transcriptional factor NF-AT (nuclear factor for T lymphocyte activation), which is phosphorylated by calcium-calmodulin-dependent phosphatase. This results in nuclear accumulation and binding to promoter elements of the IL-2 gene with subsequent activation and expression of IL-2. IL-2 has been shown to stimulate the proliferation of T lymphocytes without any specific antigens [23]. These studies indicated that potassium channels may be essential for the activation of T lymphocytes.

In our study, we have demonstrated that the expression of Kv1.3 and KCa3.1 channels at both mRNA and protein levels were significantly higher in hypertensive patients than in non-hypertensive controls. In addition, we showed that augmented expression of Kv1.3 and KCa3.1 channels resulted in significant hyperpolarisation of activated T lymphocytes, and that accelerated calcium influx provided sufficient cytoplasmic calcium to modulate the calcium signal amplitude and guarantee efficient gene transcription and subsequent T lymphocyte proliferation. Further investigation shows that these currents increased significantly in hypertension patients, suggesting the presence of increased numbers of functional Kv1.3 and KCa3.1 potassium channels in T lymphocytes of hypertensive patients. The opening of these channels augmented extracellular calcium influx, resulting in activation and proliferation of T lymphocytes and exacerbation of anti-inflammation effects. Thus, our results provide further evidence to support hypertension as an inflammatory disease at cellular ion channel levels.

Angiotensin II is the critical bioactive product of RAS and is essential for the regulation of BP and electrolyte balance, as well as being involved in cardiovascular physiology. An increasing body of data indicates that inflammation is closely related to the development of essential hypertension. RAS activation and angiotensin II elevation are both involved in the pathogenesis of hypertension-related inflammation. There is substantial evidence to show that multiple critical components of RAS are expressed in inflammatory cells and that angiotensin II receptors (AT1 receptor) are expressed in T lymphocytes. It has been reported that AT1 receptor-mediated T lymphocyte activation is achieved by the augmented cellular levels of calcium mediated by potassium channels. It has been proposed that calcium signals are triggered by AT1 receptors to stimulate calcineurin and NF-AT activation. Signal transduction results in proliferation of T lymphocytes and of AT1 receptor blockers, which in turn inhibit the AT1 receptor signals in T lymphocytes and macrophages. Thus, AT1 receptor antagonists are considered effective anti-inflammatory and immunosuppressive drugs [24].

Consequently, chronic inflammation may play an important role in the pathogenesis of hypertension, and

inflammation could serve as a potential target for hypertension treatment, Investigation on immunity, including the role of T-lymphocytes, supports the development of new therapeutic targets that may improve outcomes in hypertension and cardiovascular disease, leading to the discovery of novel approaches towards the treatment of hypertension and vascular disease [25]. Opening voltage-dependent Kv1.3 and calcium-dependent KCa3.1 channels in T lymphocytes results in potassium efflux and subsequent calcium influx, which sustain the calcium concentration required for T lymphocyte activation [17]. Numerous studies have focused on small molecules that block the opening of Kv1.3 and KCa3.1 channels, but the development of effective compounds that target these potassium channels is ongoing and clinical trials have not begun.

Numerous studies have shown that AT1 receptor antagonists not only reduce BP, but also display marked anti-inflammatory properties [6, 14]. Since hypertension-related chronic inflammation might be closely associated with cardiovascular risk, we explored the impact of the AT1 receptor antagonist telmisartan on Kv1.3 and KCa3.1 potassium channels in T lymphocytes. We demonstrated that exposure of activated proliferating T lymphocytes to telmisartan for 0 h, 24 h, or 48 h significantly inhibited the potassium current mediated by Kv1.3 and KCa3.1. The mRNA expression of Kv1.3 and KCa3.1 was also inhibited by telmisartan. A similar inhibitory effect of telmisartan on both channels was observed at the protein level. The magnitude of the inhibitory effect of telmisartan on cell current mediated by Kv1.3 and KCa3.1 and on mRNA and protein expression of Kv1.3 and KCa3.1 was similar. We showed that telmisartan inhibited the opening of potassium channels directly to block the calcium influx. We also showed that it interacted with AT1 receptor to inhibit the opening of potassium channels in T lymphocytes, reduce calcium influx, downregulate cytoplasmic calcium concentration, and inhibit calcium-dependent calcineurin and NF-AT activation. These changes are thought to reduce the expression of potassium channels at mRNA and protein levels, and result in its anti-hypertensive and anti-inflammatory effect.

In our study, the inhibitory effects of 4-AP and TRAM-34 against Kv1.3 and KCa3.1 were even more robust. 4-AP and TRAM-34 inhibit the opening of potassium channels directly and then block the formation of membrane potential between the inside and outside of a cell, thereby reducing calcium influx and suppressing potassium channels at the mRNA and protein levels.

Limitations of the study

Limitations exist in our study as the experiments were performed using peripheral blood in vitro. Since this was an exploratory clinical study, the primary aim was to prove the efficacy of telmisartan as an antihypertensive-and an anti-inflammation agent. Therefore, we performed an in vitro study first, since this type of study would not violate moral principles and would also be a cost saving. In addition, the relatively small sample size means that further studies in larger populations are required to confirm our findings. Such studies could use non hypertensive controls selected from healthy Han or Uyghur populations.

CONCLUSIONS

Our results show that increased numbers of functional Kv1.3 and KCa3.1 channels are present in T lymphocytes of hypertensive patients, suggesting that inflammation plays an important role in the pathogenesis of hypertension and providing direct evidence for T lymphocyte activation in hypertension. We demonstrated that telmisartan blocks the opening of potassium channels in T lymphocytes of hypertensive patients, resulting in anti-inflammatory activity. These findings provide experimental support for treating hypertension at the cellular ion channel level.

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Conflict of interest: *none declared*

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Zmiany kanałów potasowych w limfocytach T krwi obwodowej pacjentów pochodzenia kazachskiego mieszkających w północno-zachodnich Chinach i hamujący wpływ telmisartanu na kanały potasowe

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Streszczenie

Wstęp i cel: Coraz więcej danych naukowych wskazuje, że przewlekły stan zapalny jest zaburzeniem związanym bezpośrednio lub pośrednio z nadciśnieniem tętniczym. Uważa się, że kanały potasowe odgrywają decydującą rolę w aktywacji limfocytów, co sugeruje, że nadciśnienie tętnicze może być chorobą zapalną zapoczątkowaną na poziomie kanałów jonowych.

Metody: W badaniu oceniano zmiany ekspresji interleukiny (IL)-6 i IL-17 oraz transformującego czynnika wzrostu (TGF-β1) we krwi chorych z nadciśnieniem tętniczym pochodzenia kazachskiego mieszkających w północno-zachodniej części Chin, stosując testy ELISA. Do oceny zmian prądu jonowego związanego z kanałami Kv1.3 i KCa3.1 w limfocytach T krwi obwodowej chorych z nadciśnieniem tętniczym pod wpływem telmisartanu zastosowano technologię *„whole-cell patch clamp"*. Autorzy zbadali również wpływ telmisartanu na ekspresję kanałów Kv1.3 i KCa3.1, oceniając stężenia mRNA i białek w limfocytach T krwi obwodowej z zastosowaniem reakcji łańcuchowej polimerazy w czasie rzeczywistym oraz analizy *Western blot*.

Wyniki: Ekspresja IL-6, IL-17 i TGF-β1 we krwi pacjentów z nadciśnieniem tętniczym pochodzenia kazachskiego mieszkających w północno-zachodniej części Chin była istotnie wyższa niż u zdrowych osób z grupy kontrolnej ($p < 0,05$). Prąd przepływający przez kanały Kv1.3 i KCa3.1 oraz odpowiadająca mu ekspresja na poziome mRNA i białek w limfocytach T były również wyższe u osób z nadciśnieniem tętniczym niż w grupie kontrolnej ($p < 0,05$). Podawanie telmisartanu przez 24 h i 48 h spowodowało inhibicję prądu jonowego i ekspresji kanałów Kv1.3 i KCa3.1 ocenianej na podstawie analizy mRNA i białek ($p < 0,05$).

Wnioski: Wyniki badania wskazują, że telmisartan wpływał hamująco na zwiększenie aktywności czynnościowych kanałów Kv1.3 i KCa3.1 wykazujących ekspresję w limfocytach T osób z nadciśnieniem tętniczym pochodzenia kazachskiego, co prowadziło do zmniejszenia odpowiedzi zapalnej. Te obserwacje stanowią teoretyczne uzasadnienie terapii nadciśnienia tętniczego działających na poziomie komórkowych kanałów jonowych.

Słowa kluczowe: Kazach, nadciśnienie tętnicze, limfocyt T, Kv1.3, KCa3.1, telmisartan

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