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Tranilast treats cold-related hypertension by reducing the expression of NLRP3 inflammasome

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Abstract

Objective: Cold exposure is associated with increased prevalence of hypertension and the related severe cardiovascular events. Aberrant activation of the nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome plays an important role in the development of hypertension. Tranilast (TR), an inhibitor of NLRP3, provides a useful pharmacological probe for exploring the role of NLRP3 in pathogenesis associated with inflammation and its potential application as a therapeutic agent. This study was designed to examine the effects of TR on NLRP3 and hypertension in rats exposed to cold environment to simulate the frigid-zone conditions. Methods and results: Sprague Dawley (SD) rats were exposed to moderate cold temperature (4±1°C), and then were randomized to receive TR or vehicle for 3 weeks, while the control group was raised under rat room temperature (RT, 23±1°C). We found that cold exposure substantially increased blood pressure, NLRP3 inflammasome level, and fibrosis in aorta, which were reversed by TR. Conclusion: TR has an anti-hypertensive property in cold environment, and this beneficial action is likely conferred by its inhibitory effects on inflammation and fibrosis. These findings suggest TR as a potential drug for the treatment of cold-induced hypertension.

Keywords

cold exposure; blood pressure; NLRP3; tranilast

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1 Introduction

Mounting evidence has confirmed that cold exposure can cause various cardiovascular diseases, including hypertension[1]. Clinical data showed that systolic blood pressure in wintertime increases by around 10 mmHg relative to the levels in the other seasons. However, the mechanism of cold-induced hypertension is not fully understood.

Vascular inflammation is considered a major initiating factor for vascular remodeling in several vascular diseases including hypertension. Nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome is a protein complex formed by a member of the NOD-like receptor (NLR) family NLRP3, the adaptor protein apoptosis-associated speck-like protein containing CARD (ASC) and caspase-1, which is critical for provoking early inflammatory responses[2-3]. NLRP3 inflammasome has been implicated in the development of various cardiovascular diseases including atherosclerosis, myocardial fibrosis, vascular endothelial inflammation, hypertension and other pathological changes[4]. Of note, a latest research revealed that NLRP3 inflammasome was involved in the pathogenesis of hypertension, including inflammasome activation, vascular remodeling and phenotype switching in spontaneously hypertensive rats, and inhibition of NLRP3 effectively reversed the these detrimental alterations[5]. However, drugs targeting NLRP3 inflammasome for the treatment of hypertension are not yet available in clinic. Thus, there is an urgent need for developing and validating the potential applications of NLRP3 inflammasome inhibitors with a high safety profile in the preclinical setting.

Tranilast (N-[3',4'-dimethoxycinnamoyl]-anthranilic acid, TR), a small-molecule compound initially developed as an antiallergic drug, has been frequently employed for the clinical treatment of a variety of inflammatory diseases with an appreciable safety profile and tolerance[6]. TR was recently reported to be a specific inhibitor of NLRP3 inflammasome and is believed to elicit beneficial effects on cardiovascular diseases, especially on those associated with abnormal cell proliferation, fibrosis and inflammation[7-8]. The activation of NLRP3 inflammasome induces the release of downstream IL-1β and IL-18

inflammatory factors, eventually leading to vascular endothelial dysfunction[9-10]. Vascular endothelial dysfunction is considered an initiating step towards cardiovascular events, such as hypertension and atherosclerosis. In vivo studies showed that TR has significant preventive and therapeutic effects on NLRP3 inflammation-related diseases in mouse models[2, 11]. Whilst TR is known to possess anti-inflammatory property and the associated pathological process, whether it has a therapeutic effect on cold weather-related hypertension remains unknown. These findings suggest that the activation of NLRP3 inflammasome plays an important role in the development of hypertension, and NLRP3 inflammasome inhibitors might be new anti-hypertensive drugs. Here we sought to test this hypothesis in a rat model of cold exposure-induced hypertension using TR as a test agent.

2 Methods

2.1 Experimental design and animals

Thirty adult Sprague Dawley (SD) rats (weighing 180-200 g and aged 6-8 weeks) were purchased from Vital River (Beijing, China). The rats were individually housed under 12:12hour light-dark cycles with food and water available ad libitum. After one week of adaptive feeding, they were randomly divided into three experimental groups: normal control (n = 10), TR + cold (n = 10), and vehicle + cold (n = 10) groups. Rats in the normal group were maintained at room temperature (RT, 25±1°C) for 3 weeks. The two cold exposure groups were exposed to moderate cold environment (4±1°C) to induce hypertension in a temperature controlled and ventilated chamber. They were subsequently randomized to receive either TR (orally administered at 50 mg/kg/d; purchased from CPU-Pharma, Nanjing, China) or vehicle right at the beginning of cold exposure and maintained in the cold condition for three weeks.

2.2 Blood pressure measurements

Blood pressure (BP) was measured twice at 0 and 3 weeks, respectively, using tail-cuff plethysmography (IITC Life Science MRBP Blood Pressure System IITC Life Science Inc., CA). Parameters were set according to the instructions; after the animals had been adapted to the animal sphygmomanometer for 1 hour, BP was measured rapidly for consecutive 10 times, and the average value from the 10 measurements was used for data analysis. After 3 weeks, rats were killed by decapitation under deep anesthesia. Blood samples were collected from the abdominal aorta, and the thoracic aorta was sampled for subsequent analysis. The experimental procedure was performed in accordance with the Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee at the Harbin Medical University.

2.3 Assessments of NLRP3, ASC, and Casp1-p20 by western analysis

Western blot procedure was performed as described previously[12]. Briefly, thoracic aorta tissues were homogenized in the Radio Immunoprecipitation Assay (RIPA) lysis buffer. Protein samples were size separated on 10-12% sodium dodecyl sulfate polyacrylamide and then transferred to polyvinylidene difluoride membranes. The membranes were blocked with 10% nonfat milk for 1 hour and incubated at 4°C overnight with primary antibodies against NLRP3 (NLRP3, 1:500; Abcam, Cambridge, United Kingdom, ab167161), ASC (Tjp1, 1:500; Proteintech Group, Inc, Chicago, IL, 21773-1-AP), Casp1-p20 (1:500; Cell signaling technology, #9570), or GAPDH (1:1000; Kangcheng, Shanghai, China). After washing, the membranes were incubated with a secondary antibody for 1 hour. The protein bands were processed by enhanced chemiluminescence reagents and analyzed using Quantity One software (Bio-Rad, Hercules, CA).

2.4 Hematoxylin and eosin staining

The aortas were imbedded in paraffin and cut into 5-mm sections. Then, the paraffin embedded tissues were stained with hematoxylin and eosin (HE) to observe the transverse section of thoracic aorta. Light microscopy was used to measure lumen radius and lumen thickness at a 20× magnification.

2.5 Masson trichrome staining

The relative collagen content and interstitial fibrotic areas in aorta were evaluated by Masson trichrome staining and calculated with an image analysis software (Image-pro plus 6.0; Meida Cybernetics LP).

2.6 Statistical analysis

Unpaired Student t-test was applied for two group comparisons. The statistical analysis was conducted with the GraphPad Prism 8.0 Software, and data are expressed as mean±SEM with $P < 0.05$ being considered statistically significant.

3 Results

3.1 Cold exposure increases blood pressure in rats

Accumulating evidence indicates that lower ambient temperature promotes cardiovascular diseases[13-14]. There was no significant difference in basal BP between the RT group and

the cold group (P > 0.05). The systolic blood pressure (SBP) of rats in the RT group and cold group were 112±8 mmHg and 113±9 mmHg, respectively, and diastolic BP (DBP) were 88±6 mmHg and 84±8mmHg, respectively. However, after three-weeks exposure to low temperature, BP was significantly elevated compared with RT rats. The SBP and DBP of the rats in the RT group were 115±10 mmHg and 83±9 mmHg, respectively, and those in the cold group were 151±9 mmHg and 94±10 mmHg, respectively. These differences were all statistically significant (P < 0.05) (Fig. 1A and 1B).

3.2 Cold exposure increases blood pressure in rats by activating the NLRP3 inflammasome

Previous studies have documented that cold exposure can increase the levels of a variety of inflammatory factors[15-16], and hypertension is a disease characterized by low-grade chronic inflammation[17]. To verify whether cold exposure can cause inflammation of the aortic endothelium and increase BP in cold-exposed rats, we first tested the expression levels of NLRP3 inflammasome in aorta. NLRP3 is an important part of innate immunity[18], it has been confirmed that NLRP3 inflammasome is closely related to hypertension. Compared with the RT group, NLRP3 and Casp1-p20 protein levels in the aorta were significantly increased in the cold exposure group (P = 0.031 3 and P = 0.012 6, respectively). However, no significant difference was observed in the expression of ASC protein between the two groups (Fig. 2A and .2B).

3.3 TR decreases blood pressure in cold exposed rats

We then continued to investigate the effect of TR on BP in cold exposed rats. There was no significant difference in the basal BP between the cold exposure group and cold+TR group (P > 0.05) right before TR administration (at zero time point). After three weeks of cold exposure (4±1°C), however, both SBP and DBP were remarkably dropped by TR (P > 0.05) (Fig. 3A and 3B).

3.4 TR reduces cold exposure-induced formation of NLRP3 inflammasome

Having confirmed the anti-hypertensive property of TR in cold-exposed rats, we turned to detect the inflammations in aortic using Western blot analysis. We found considerable downregulation of NLRP3 and Casp1-p20 protein levels in the cold+TR rats relative to non-TR-treated counterparts exposed to cold environment (P = 0.002 2 and P = 0.011 9, respectively). TR also diminished the protein level of ASC, but the effect did not reach statistical significance (Fig. 4A and 4B). These results suggest that TR abrogated the cold exposure-stimulated increases in BP likely by suppressing aortic inflammation.

3.5 TR could improve aortic fibrosis in cold exposed rats

The results of HE staining and Masson staining in the aorta from cold-exposed rats showed apparent uneven distribution of collagen fibers, partial proliferation of vascular smooth muscles, disarrangement of endothelial cells, and distortion and disorderliness of the elastic layer of tunica media. Compared with the cold exposure group without TR treatment, the above-mentioned pathological phenotypes were markedly improved by TR with reduced collagen fibers in aortic wall (P = 0.016 6) and proliferation and uneven arrangement of vascular smooth muscle cells (Fig. 5A and 5B).

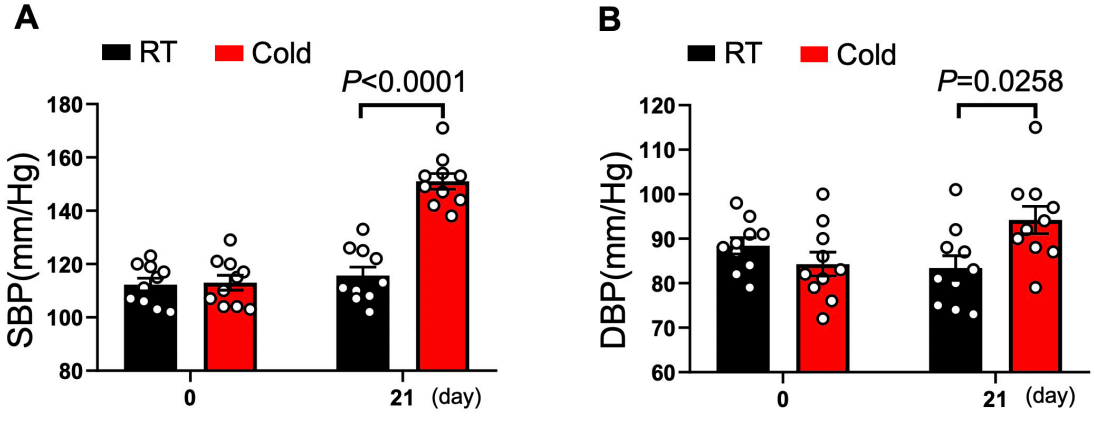

Figure 1. Cold exposure increases blood pressure in rats. (A and B) SDP and DBP change among the groups. P < 0.0001, P = 0.0258 vs. RT group, n = 10 per group. RT, room temperature.

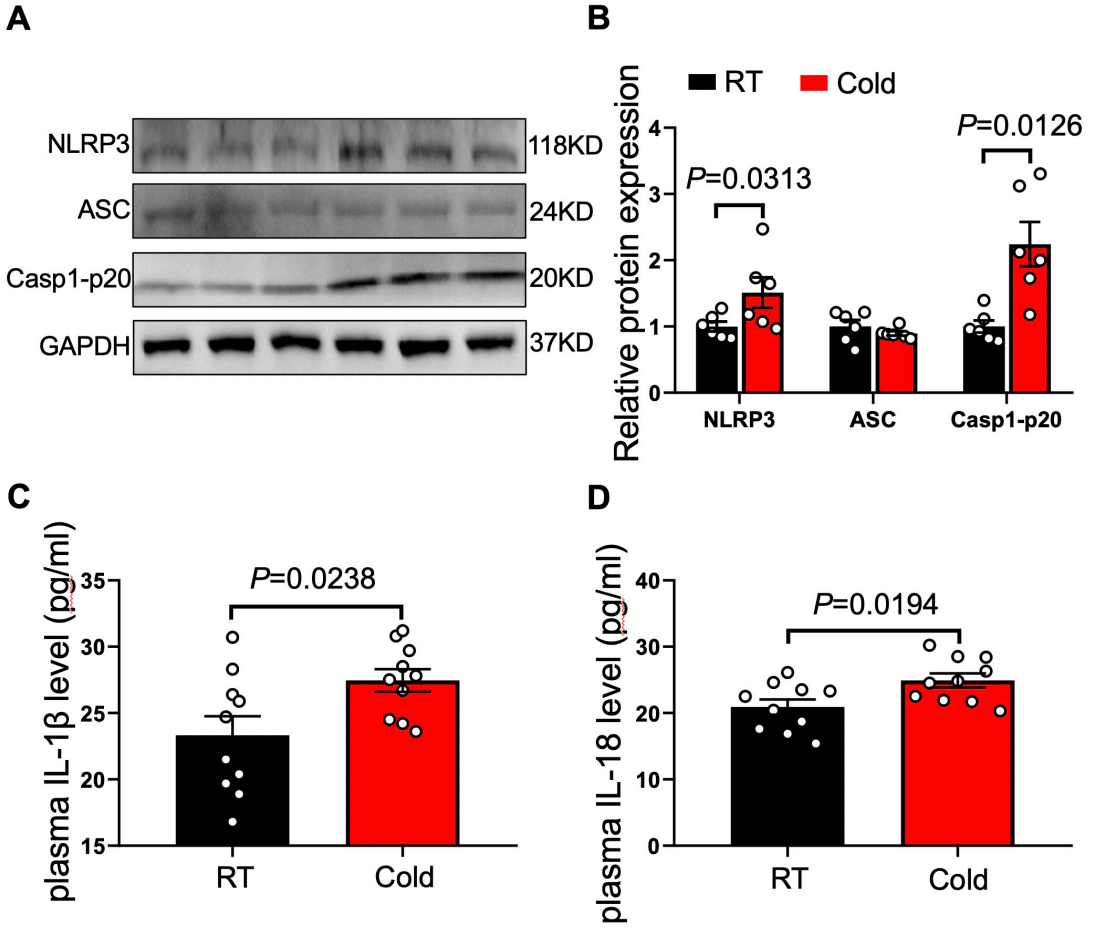

Figure 2. Cold exposure increases blood pressure in rats by activating the NLRP3 inflammasome. (A and B) Western blotting for NLRP3, ASC and Casp1-p20 proteins in aorta isolated from rats in the RT and cold groups. *P* = 0.031 3, *P* = 0.012 6 vs. RT group, *n* = 10 per group. RT, room temperature. (C and D) ELISA for the plasma levels of IL-1β and IL-18 in the RT and cold groups. *P* = 0.023 8, *P* = 0.019 4 vs. RT group, *n* = 10 per group.

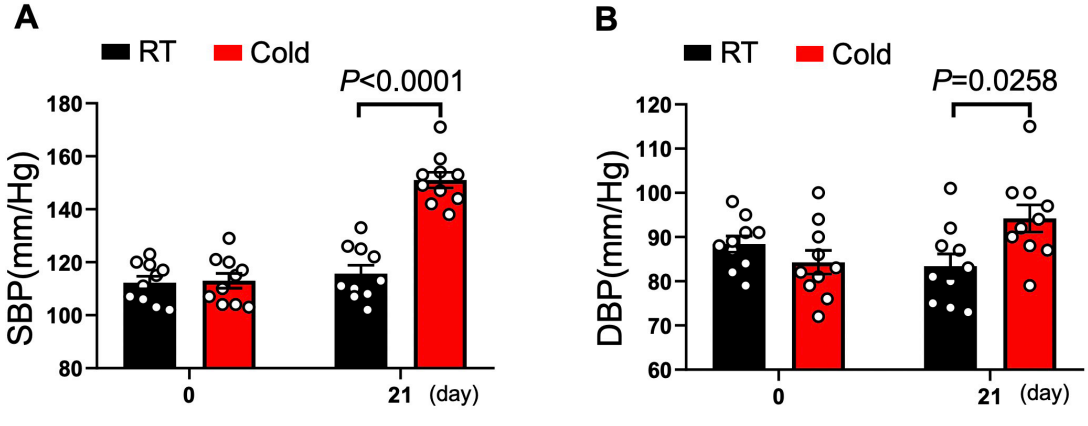

Figure 3. Tranilast decreases blood pressure in cold exposed rats. (A and B) Comparisons of SDP and DBP values between cold exposed rats with and without tranilast treatment. *P* < 0.000 1 vs. cold group, *n* = 10 per group. TR, tranilast.

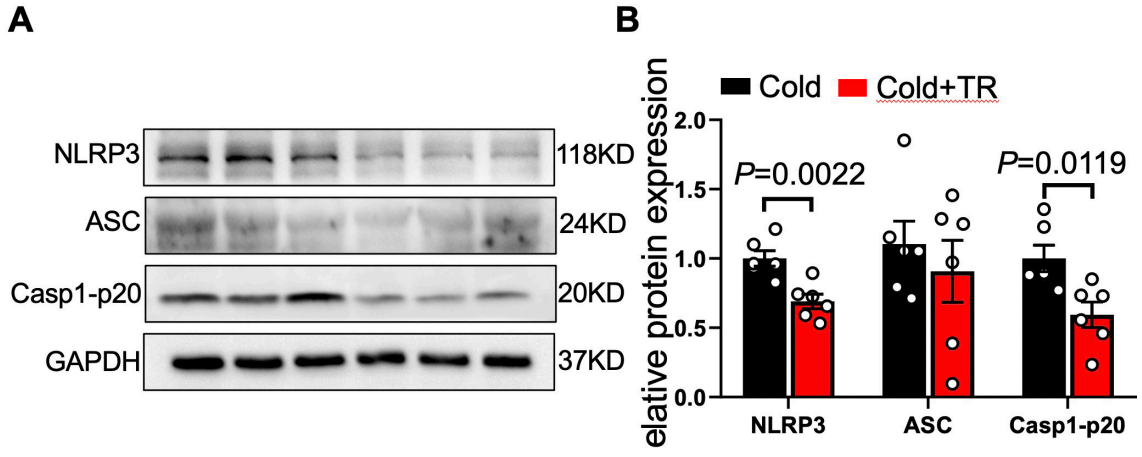

Figure 4. Tranilast reduces the expression of NLRP3 inflammasome induced by cold exposure. (A and B) Western blot results for NLRP3, ASC and Casp1-p20 proteins of aorta in the cold and cold + TR groups. *P* = 0.002 2, *P* = 0.002 2 vs. cold group, *n* = 10 per group. TR, tranilast.

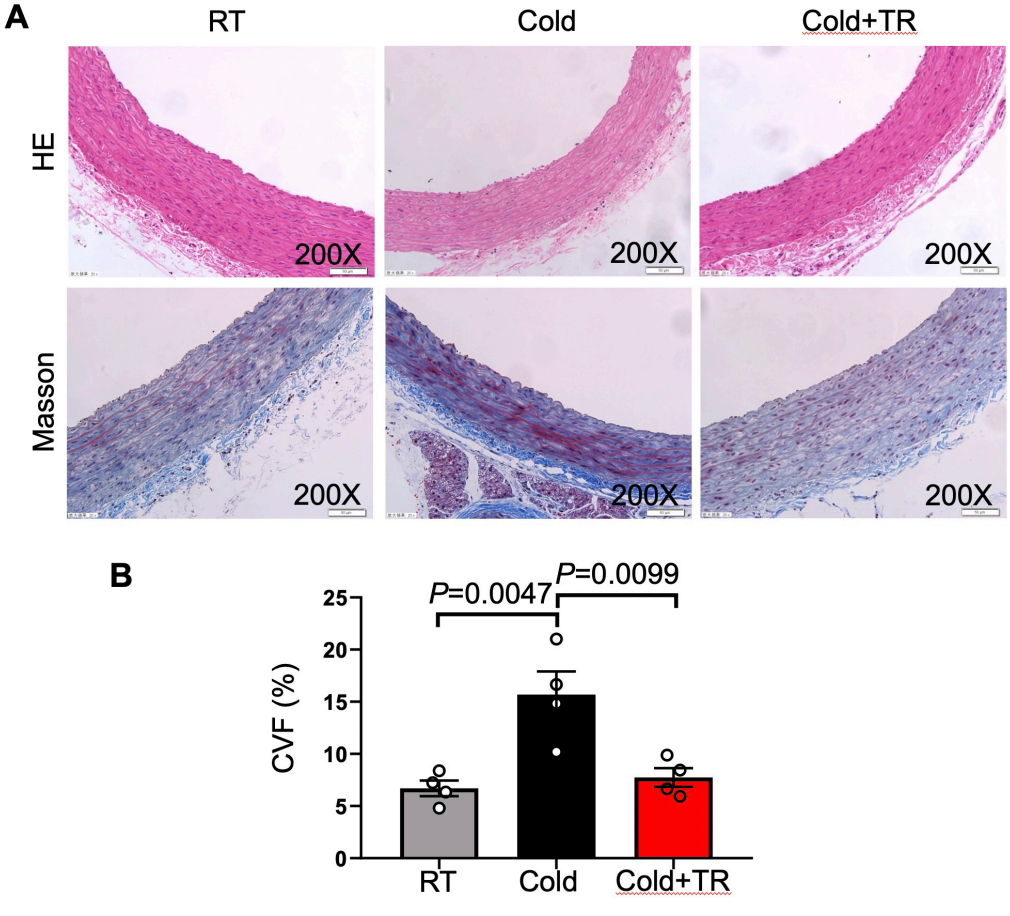

Figure 5. Tranilast alleviates aortic fibrosis in cold exposed rats. (A) Hematoxylin and eosin (H&E) staining (200× magnification; scale bar: 50 μm. (B) Masson's trichrome staining (200× magnification; scale bar: 50 μm). (C) Collagen volume fraction (CVF) per field in the atria of rats from each group. TR, tranilast.

4 Discussion

The results of this study revealed three important findings. First, we found that SBP and DBP were significantly elevated in rats subjected to 3-weeks cold exposure (4°C), relative to the levels recorded in the animals maintained at room temperature (25°C). The results are consistent with previous reports in the literature[19]. Second, we observed that the protein levels of NLRP3 and Casp1-p20 were substantially elevated in rats following cold exposure, suggesting a link between NLRP3 inflammasome level and hypertension in the setting of cold exposure. Finally and most importantly, Tranilast (TR) reversed the cold exposure-induced abnormal increases in SBP and DBP and in the levels of NLRP3 and Casp1-p20 proteins.

In the present study, we reproduced the abnormal elevations of both systolic and diastolic BP in rats maintained in cold environment as in humans exposed to cold ambient temperature which has been well documented in the literature[20-21]. Our data also demonstrated that cold exposure increased the formation of vascular NLRP3 inflammasome which is known to be a critical factor for the development of hypertension[22]. Moreover, the present study unraveled for the first time TR, a direct inhibitor of NLRP3 inflammasome and a clinically used antiallergic drug, mitigated these detrimental alterations. Our findings highlight the possibility of TR as a potential therapeutic drug for the treatment of hypertension and associated cardiovascular diseases caused by cold exposure.

Hypertension can be viewed as a chronic low-grade inflammatory disease in which a variety of pro-inflammatory factors and chemokines is secreted to cause infiltration of inflammatory cells such as macrophages, leading to vascular endothelial dysfunction[23-25]. NLRP3 inflammasome can also damage the vascular endothelium to induce and promote the development of hypertension[26]. On the other hand, hypertension increases the release of IL-1β by promoting the formation and activation of NLRP3 inflammasome in macrophages and promotes the infiltration of these macrophages into cardiac tissue, leading to myocardial damage and fibrosis[27]. Consistently, our pathological results showed that the aorta became fibrotic following cold exposure, which was mitigated by TR. Of note, the NLRP3 inflammasome-induced hypertension in the cold environment is reversible upon inhibition of this pro-inflammatory complex, in agreement with the published studies showing the effectiveness of NLRP3 inflammasome inhibition in alleviating hypertension[22,28]. Therefore, TR holds the potential to become a new drug for the treatment of hypertension after more rigorous research and development, which is worthy of further pre-clinical investigations and clinical trials.

TR was originally used as an anti-allergic drug with confirmed excellence of safety, tolerability and efficacy[29-30]. It has also been approved for the treatment of many other diseases.Experiments in animal models have confirmed the effectiveness of TR in the treatment of cardiovascular disease[31-32], but no clinical trials have been reported on this agent for its efficacy in cardiovascular diseases and safety profile in humans.

Clinical research on the correlation between NLRP3 inflammasome and hypertensive patients indicate that the expression level of NLRP3 inflammasome and its downstream inflammatory factors can be used to predict the severity of hypertension[33]. Several studies proved that the NLRP3 inflammasome could be a potential therapeutic target for early stage of hypertension[34].

Ethical approval

All procedures performed in the animal studies were in accordance with the institutional guidelines for the care and use of animals.

Conflicts of interest

Yue Li is an Editorial Board Member of the journal. The article was subject to the journal's standard procedures, with peer review handled independently of this editor and his research groups.

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