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The involvement of *AQP1* in myocardial edema induced by pressure overload in mice

D. SONG[1], Y. YANG[1], N. HE[2], X. TIAN[3], D.-S. SANG[3], Y.-J. LI[4]

[1]Department of Cardiology, Hebei Medical University, Shijiazhuang, China
[2]Department of Cardiology, Central Hospital, Bureau of Geophysical Prospecting INC, China National Petroleum Corporation, Zhuozhou, China
[3]Department of Cardiology, Baoding First Central Hospital, Baoding, China
[4]Department of Cardiology, The Second Hospital of Hebei Medical University, Shijiazhuang, China

Abstract. – OBJECTIVE: To investigate the effect of aquaporin-1 (*AQP1*) on heart edema induced by transverse aortic constriction (TAC) in mice, and to explore whether inhibiting the expression of *AQP1* could attenuate myocardial edema and improve cardiac function.

MATERIALS AND METHODS: The C57BL/6 mice were divided into four groups: (1) the sham group; 2) the sham + acetazolamide group: mice were orally gavaged with acetazolamide (20 mg/kg/day) after sham operation; (3) the TAC group: a mouse model of pressure overload induced by TAC for two weeks; (4) the TAC + acetazolamide group: mice were orally gavaged with acetazolamide (20 mg/kg/day) after TAC. Cardiac function was detected by echocardiography after 2 weeks' TAC. The ratio of heart weight to body weight (HW/BW) and myocardial water content were calculated. The mRNA and protein expressions of *AQP1* were measured by reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blot, respectively.

RESULTS: Significant myocardial hypertrophy and dysfunction were found in TAC mice. The ratio of HW/BW, myocardial water content, and the mRNA and protein expression of *AQP1* of the TAC group were markedly higher than those of the sham group. By contrast, acetazolamide administration reduced the ratio of HW/BW and myocardial water content, whereas improved cardiac dysfunction induced by TAC. Moreover, acetazolamide reduced the mRNA and protein expression of *AQP1* in TAC mice.

CONCLUSIONS: The expression of *AQP1* was closely related to myocardial edema induced by TAC. The inhibition of *AQP1* could reduce myocardial edema and improve cardiac dysfunction.

Key Words:
Myocardial hypertrophy, Myocardial edema, Aquaporin protein 1, Acetazolamide.

Introduction

Pathological cardiac hypertrophy is usually developed as a response to pathological stimuli, including pressure stress, inflammatory cytokines, and oxidative stress. It is commonly performed as the increase of heart weight and volume. In the early stage, compensatory cardiac hypertrophy is the mainly manifestation of maintaining cardiac function. However, persistent cardiac hypertrophy can lead to cardiac dysfunction and energy metabolism disorder, which may eventually result in a significant increase in the occurrence of cardiovascular accidents such as sudden death, heart failure, and arrhythmia. Therefore, pathological myocardial hypertrophy is recognized as an independent risk factor for cardiovascular diseases[1]. Cardiomyocyte edema is the most common pathophysiological performance during the development of cardiac hypertrophy. Moreover, chronic and long-term edema will further aggravate cardiac dysfunction[2]. Therefore, the inhibition of myocardial edema may have an important role in attenuating cardiac hypertrophy and improving cardiac function.

Aquaporin (*AQPs*) is a family of transmembrane channel proteins that possess high permeability to water molecules. *AQPs* are reported to be closely associated with cellular edema. Under the pressure gradient of osmotic pressure, *AQPs* can promote the transmembrane transport of water molecules rapidly. It is known to all that AQP1 is widely expressed in multiple cell types, including endothelial cells and cardiomyocytes[3,4]. In myocardium, *AQP1* is mainly expressed in capillary endothelial cells and the cardiomyocyte membrane[4,5]. Ding et al[6] have reported that the expression of *AQP1* is closely related to cardiac edema after

Corresponding Author: Yongjun Li, Ph.D; e-mail: paish428826@163.com

cardiopulmonary bypass. However, the association between the expression of *AQP1* and myocardial edema induced by aortic constriction has not yet been confirmed. In this study, we constructed a mouse model of pressure overload induced by TAC to investigate the effect of *AQP1* in myocardial edema and to explore whether inhibiting the expression of *AQP1* could attenuate myocardial edema and improve cardiac function.

Materials and Methods

Reagents

Acetazolamide was purchased from Sigma-Aldrich (St. Louis, MO, USA). The rabbit anti-*AQP1* antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Ethics Statements

Male C57BL/6 mice, weighing 20-25 g and 8-10 weeks old, were obtained from the Animal Experiment Center of Nanjing Medical University. Animal experiments were performed in accordance with the Guide for Laboratory Animals published by the NIH. All research mice were fed with standard mouse food and water, and were kept under 12:12 hour light/dark cycles at 22°C. This study was approved by the Animal Ethics Committee of Hebei Medical University Animal Center.

Construction of the Animal Model of Aortic Constriction

All the mice were anesthetized by isoflurane, and were then intubated and ventilated. A midline incision was made at the sternum. After opening the mediastinal space, the aortic arch was blunt dissected at the base of heart. A blunt 27-G injection needle (OD 0.4 mm) was placed parallel to aorta between the left carotid and the right innominate arteries. Then, the needle and the aortic arch were tied together by using a 7-0 suture. After removing the needle, a 60-70% constriction with an outer aortic diameter of 0.4 mm was created. Sham mice underwent the same surgical procedure, and the 7-0 suture was placed in the same position without ligation. After 2 weeks' operation, all mice were sacrificed and the hearts were harvested. The ratio of heart weight/body weight (HW/BW) was calculated. The heart tissues were frozen in liquid nitrogen and then stored at -70°C. The experiment was divided into four groups: (1) the sham group; (2) the sham + acetazolamide group: mice were orally gavaged with acetazolamide (20 mg/kg/day) after sham operation; (3) the TAC group; (4) the TAC + acetazolamide group: mice were orally gavaged with acetazolamide (20 mg/kg/day) after TAC. Totally 10 mice were included in each group (n=10).

Echocardiography

Two weeks' after the operation, all experimental mice were anesthetized by isoflurane. Subsequently, the cardiac function was detected by a rodent animal ultrasonic instrument (Vevo 2100, VisualSonics, Toronto, Canada). The interventricular septum diameter (IVS), left ventricular (LV), posterior wall thickness (LVPW), LV end diastolic diameter (LVEDD), LV end systolic diameter (LVESD), LV ejection fraction (LVEF), and fractional shortening (FS) were calculated.

Determination of Myocardial Water Content

Heart samples were weighed and dried in a thermostat oven at 80°C for 48 h. The myocardial water content was calculated according to the following formula: myocardial water content (%) = [(wet weight - dry weight) / wet weight] × 100%

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA in myocardial tissues was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Complementary deoxyribose nucleic acid (cDNA) synthesis was performed by using the reverse transcription (RT) reagent kit according to the instructions (TaKaRa Biotechnology, Tokyo, Japan). Real-time PCR reaction system was performed by Eppendorf Mastercycler ep realplex. Primers (5'-3') used in this study were as follows: *AQP1* (forward) TGCGTTCTGGCCACCACTGAC, (reverse) GATGTCGTCAGCATCCAGGTC. *GAPDH* (forward) CTGGAGAAACCTGCCAAGTA, (reverse) TGTTGCTGTAGCCGTATTCA.

Western Blotting

Total proteins were isolated from heart tissues by using a protein extraction kit (Keygen Biotechnology, Nanjing, China). Gel electrophoresis was performed to separate proteins with different molecular weight. Then, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were incubated with anti-*AQP1* at 4°C overnight. After incubation with primary an-

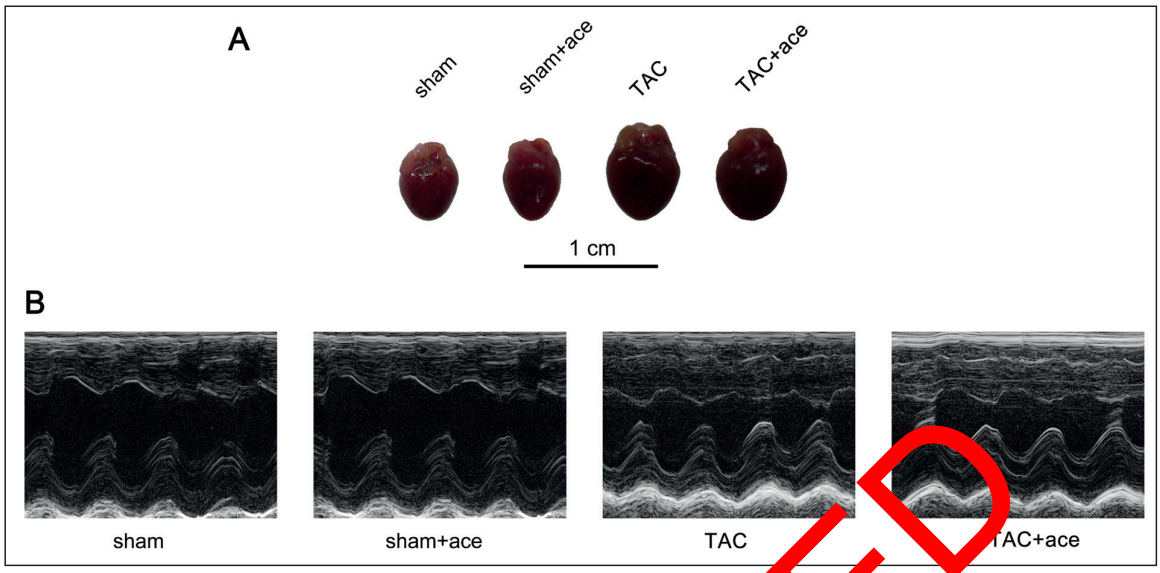

Figure 1. Photographs of the hearts and echocardiograms in each group. *A*, Representative photographs of the hearts. ***B***, Representative images of echocardiograms. Ace: acetazolamide.

tibodies, the membranes were washed with Tris-buffered saline-Tween (TBST) (Beyotime, Shanghai, China). Then, the membranes were incubated with the HRP-conjugated secondary antibody at room temperature for 2 h. Western Blot Detection Kit and ImageJ Software were used to measure the blot signal and density.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA) was used for all statistical analysis. All quantitative data were expressed as mean ± standard deviation. The difference between groups was compared by using One-way ANOVA test, followed by the Post-Hoc Test (Least Significant Difference). $p < 0.05$ was considered statistically significant.

Results

Acetazolamide Attenuated Cardiac Dysfunction Induced by TAC

Representative photographs of the hearts and echocardiograms from each group were shown in Figure 1. IVSD and LVPWD were significantly thicker in the TAC group than those in the sham group, whereas LVEDD was significantly decreased in the TAC group ($p < 0.001$, $p < 0.001$, $p < 0.05$, respectively) (Table I). Meanwhile, LVEF and LVFS of the TAC group

Table I. Cardiac function in each group.

	Sham	Sham + ace	TAC	TAC + ace
IVSD, mm	0.711 ± 0.049	0.713 ± 0.031	1.052 ± 0.096***	1.036 ± 0.125***
IVSS, mm	1.113 ± 0.115	1.137 ± 0.105	1.389 ± 0.093***	1.383 ± 0.156***
LVEDD, mm	3.194 ± 0.159	3.133 ± 0.192	2.888 ± 0.173*	2.872 ± 0.258*
LVESD, mm	1.923 ± 0.155	1.929 ± 0.113	2.088 ± 0.198	2.092 ± 0.103
LVPWD, mm	0.872 ± 0.122	0.892 ± 0.098	1.149 ± 0.118***	1.144 ± 0.133***
LVPWS, mm	1.341 ± 0.172	1.325 ± 0.143	1.358 ± 0.109	1.361 ± 0.122
LVEF, %	72.72 ± 3.841	71.38 ± 3.337	56.80 ± 2.137***	61.57 ± 3.431$^{\#}$
LVFS, %	43.63 ± 1.841	43.30 ± 1.151	29.19 ± 3.251***	35.76 ± 2.471$^{\#}$

IVSD, interventricular septum diastolic dimension; IVSS, interventricular septum systolic dimension; LVEDD, left ventricular (LV) end-diastolic diameter; LVESD, LV end-systolic diameter; LVPWD, LV posterior wall thickness diastole; LVPWS, LV posterior wall thickness systole; LVEF, LV ejection fraction; LVFS, LV fractional shortening; ace, acetazolamide. n=10 mice/group. *$p < 0.05$ and ***$p < 0.001$ versus sham; $^{\#}p < 0.05$ versus TAC.

were significantly decreased than those of the sham group ($p < 0.001$, $p < 0.001$, respectively). By contrast, acetazolamide administration significantly inhibited the reduction of LVEF and LVFS induced by TAC ($p < 0.05$, $p < 0.05$, respectively). However, acetazolamide administration exhibited no effect on IVSD, LVPWD, and LVEDD in TAC mice.

Acetazolamide Reduced the TAC-Induced Increase in the Ratio of HW/BW and Myocardial Water Content

As shown in Figure 2A, the HW/BW ratio of the TAC group was significantly increased when compared with the sham group ($p < 0.001$), while acetazolamide administration reduced the HW/BW ratio in contrast to the TAC group ($p < 0.05$). The myocardial water content was significantly increased in the TAC group than that of the sham group ($p < 0.001$). Meanwhile, acetazolamide administration reduced myocardial water content in contrast to the TAC group ($p < 0.01$) (Figure 2B).

Acetazolamide Inhibited the TAC-Induced Increase in the mRNA and Protein Expression of AQP1

As shown in Figure 3, the mRNA and protein expression of *AQP1* were both significantly increased in the TAC group than those of the sham group ($p < 0.001$, $p < 0.001$, respectively). Moreover, acetazolamide administration reduced the mRNA and protein expression of *AQP1* in contrast to the TAC group ($p < 0.01$, $p < 0.01$, respectively).

Discussion

In the present study, we mainly focused on the effect of *AQP1* in cardiac hypertrophy and edema. Our results showed that significant cardiac hypertrophy and edema were occurred in mice after 2 weeks' TAC, which were confirmed by echocardiography and myocardial water content. The mRNA and protein expression of *AQP1* in myocardium were markedly enhanced in TAC mice. Acetazolamide, an *AQP1* channel inhibitor, could significantly inhibit *AQP1* expression in hypertrophic myocardium. Although acetazolamide might not reverse myocardial hypertrophy, it could attenuate myocardial edema and improve cardiac function.

AQPs are transmembrane channel proteins that may promote transmembrane transport of water molecules rapidly and maintain the balance of osmotic pressure both inside and outside of the cells[7,8]. *AQPs* have been confirmed to play an important role in the pathophysiological process of cerebral edema[9], pulmonary edema[10], myocardial edema[6], and tumor growth and metastasis[1]. Currently totally 13 members of the *AQPs* family (*AQP* 0-12) have been found in mammals. *AQP* 1, 4, 7, and 11 have been detected in human and mouse myocardium. Studies have shown that *AQP1* protein is also expressed in the myocardium of human, rat and mouse[12]. In heart, *AQP1* is mainly located in capillary endothelial cells and cardiomyocytes[4,5]. By the construction of a *AQP1* knockout mice model, Tanya et al[12] have found that the water permeability of cardiomyocyte is significantly reduced in *AQP1* deficient mice. Ding et al[6] have reported that *AQP1* is closely associated with cardiac edema, and the

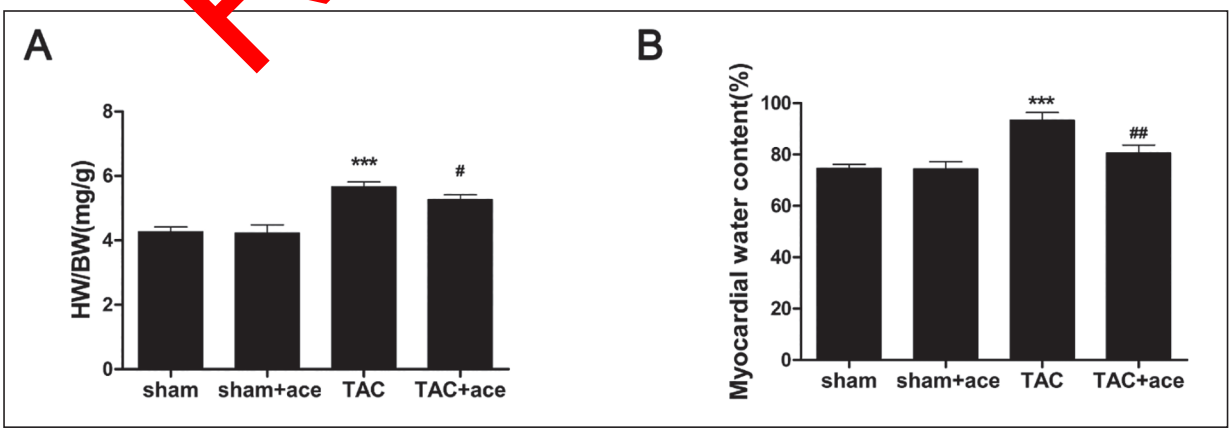

Figure 2. The HW/BW ratio and myocardial water content in each group. ***A,*** HW/BW ratio. ***B,*** The myocardial water content. Ace: acetazolamide; HW: heart weight; BW: body weight. ***$p < 0.001$ *vs.* sham; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* TAC.

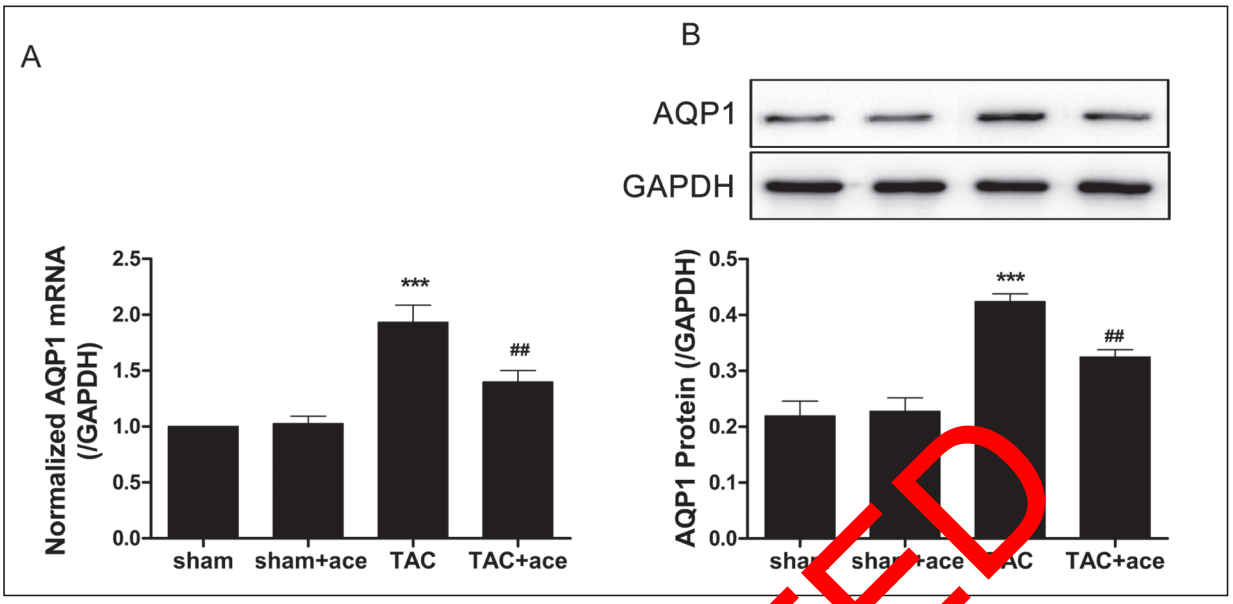

Figure 3. The mRNA and protein expression of *AQP1* in each group. ***A,*** The mRNA expression of *AQP1*. ***B,*** The protein expression of *AQP1*. Ace: acetazolamide. ***$p < 0.001$ *vs.* sham; ##$p < 0.01$ *vs.* TAC.

expression of *AQP1* is significantly increased in the myocardium after cardiopulmonary bypass. In addition, Lin et al[13] have demonstrated that the mRNA and protein expression of *AQP1* are also increased in the myocardium after ischemia/reperfusion injury. In the present work, we found that the HW/BW ratio and the myocardial water content were significantly increased in TAC mice. Meanwhile, the mRNA and protein expression of *AQP1* were also markedly increased in the TAC group, indicating that myocardial edema induced by TAC was also closely related to the increased expression of *AQP1*.

Although previous studies have confirmed that high expression of *AQP1* is closely correlated with the occurrence of myocardial edema[11]. However, whether *AQP1* can be used as a therapeutic target for myocardial edema remains unclear. Ding et al[6] have found that $HgCl_2$ can attenuate cardiac edema, which has no effect on the mRNA and protein expression of *AQP1*. However, Lin et al[13] have reported that acetazolamide may enhance the cardio-protective effect of remifentanil in myocardial ischemia/reperfusion rats through inhibiting the mRNA and protein expression of *AQP1*. In this study, our results demonstrated that acetazolamide could significantly attenuate myocardial edema and improve cardiac function via inhibiting the expression of *AQP1* in hypertrophic myocardium. However, acetazolamide could not reverse myocardial hypertrophy.

Conclusions

Increased expression of *AQP1* in myocardium was closely related to myocardial edema after aortic constriction. Inhibiting the expression of *AQP1* could attenuate myocardial edema and improve cardiac function. Therefore, the targeted therapy of *AQP1* might be a potential treatment strategy for myocardial edema. The limitation of the current study was the lack of research on the cellular mechanism. Thus, future researches are still needed to investigate the potential therapeutic target of *AQP1* in cardiomyocytes *in vitro*.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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