

ALDH2 improved irbesartan treatment efficacy among rats with hypertension

Weizhong Huangfu¹, Fuhou Chang³, Minjie Wang² and Ruiying Gao^{1*}

¹Department of Gerontology, The Affiliated Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia, China

²Basic Medical College, Inner Mongolia Medical University, Hohhot, Inner Mongolia, China

³College of Pharmacy, Inner Mongolia Medical University, Hohhot, Inner Mongolia, China

Abstract: Hypertension is a common cardiovascular disease in clinical scenario. The level of leptin changes with the development of hypertension and is regulated by Aldehyde dehydrogenase2 (ALDH2). Our study explored the relationship between irbesartan treatment and ALDH2. Spontaneously hypertensive rats were treated with irbesartan solution and ALDH2 over expression adenovirus vector for experimental group, and the equivalent amount of spontaneously hypertensive rats was treated with irbesartan solution and null adenovirus vector for control group. Sham group included spontaneously hypertensive rats treated with saline solution and null adenovirus vector. Pathological change of cardiac muscle tissue was observed with microscope. N-terminal Pro-brain natriuretic peptide, blood pressure, left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS) and renal function were assessed to determine cardiovascular function. Expression of serum leptin and mRNA of leptin were examined, respectively. ALDH2 was confirmed by western blot examination. Statistical Analysis was performed to determine correlation. Compared with sham group, ALDH2 were decreased significantly in control group. Remarkable pathological changes of cardiovascular and renal injury were observed in control group rats, including increased NT-proBNP, renal interstitial fibrosis and aberrant hypertension. Compared with control group, experimental group had lower levels of blood pressure and NT-proBNP, but higher level of Glomerular Filtration Rate (GFR). Moreover, irbesartan -treated rats had significantly higher levels of leptin, suggesting irbesartan treatment ameliorated symptoms of hypertension. Expression of serum leptin had a negative correlation with mRNA of leptin ($P < 0.05$). Moreover, compared with control group, ALDH2 over expression significantly improved irbesartan treatment, verified by hypertension related index. Decreased ALDH2 expression were correlated with progression of hypertension. Rats with Hypertension indeed benefited from irbesartan treatment. ALDH2 elevated the drug susceptibility of irbesartan treatment for hypertension via regulating serum leptin, and improved efficacy of irbesartan treatment on hypertension.

Keywords: Hypertension, leptin, irbesartan, ALDH2.

INTRODUCTION

Incidence of cardiovascular disease increases gradually with the increment of hypertension (Yuan *et al.*, 2019). Irbesartan treatment was proved with efficacy on hypertension in clinical scenario. However, clinical trial showed that remarkable difference was observed on efficacy of irbesartan treatment in hypertension patients, suggesting that some factors influenced drug sensitivity of irbesartan. Previous study showed that ALDH2, widely expressed in human and various animals, was associated with protection against hypertension (Chen *et al.*, 2018). As a polypeptide hormone, leptin is the stimulating signal of feeding center, and was under regulation of ALDH2 (Ma *et al.*, 2017). Previous study indicated that leptin could influence progress of chronic hypertension and resulted in weight-decrease via crosstalk between irbesartan treatment and ALDH2 (Zhang *et al.*, 2018).

Drug sensitivity is a novel research hotspot in the field of cardiovascular diseases, which is caused by nonpathogenic microorganism. Drug sensitivity always

*Corresponding author: e-mail: gaoruiyingdr@163.com

results from expressions of different factors with multiple complications and clinical examination can determine increased levels of specific proteins induced by hypertension (Fan *et al.*, 2013). In addition, increasing renal levels of ALDH2 are common in chronic hypertension patients with anti-hypertension therapy, suggesting ALDH2 was involved in increased drug sensitivity (Matouk *et al.*, 2008). However, there is no report between ALDH2 expression and irbesartan treatment in hypertension.

We hypothesized irbesartan treatment could influence chronic hypertension via ALDH2 related drug sensitivity pathways and leptin was the possible downstream target. In summary, our study explored relationship between ALDH2 expression and irbesartan treatment with animal model of chronic hypertension.

MATERIALS AND METHODS

Experimental animals

20 male Wistar rats and 60 spontaneously hypertensive rats (SHR) were purchased from Laboratory Animal

Center of CAMS (Chinese Academy of Medical Sciences), and bred in SPF environment for laboratory animal. All rats were bred with chow fat and drinking water to adapt the SPF environment. All experimental animals and related procedure were registered and approved by local animal ethics committee (DWSL20150347A).

Drugs and reagents

Irbesartan was purchased from Boster Company (Wuhan, China). Leptin kit was purchased from Zhong Shan Biotechnology Company (Beijing, China). PCR primers were synthesized by Boya Company (Shanghai, China)

Experimental approach

Establishment of chronic hypertension model

Sham group: 20 male wild type rats were treated with saline solution for 30 days (intragastric administration, irbesartan 25mg/kg, dissolved in normal saline) and null adenovirus vector. **SHR group:** 20 spontaneously hypertensive male rats were treated with saline solution for 30 days and null adenovirus vector.

Experimental group: 20 spontaneously hypertensive male rats were treated with irbesartan solution for 30 days (intragastric administration, irbesartan 25mg/kg, dissolved in normal saline) and ALDH2 over expression adenovirus vector.

Control group: 20 spontaneously hypertensive male rats were treated with irbesartan solution for 30 days (intragastric administration, irbesartan 25mg/kg, dissolved in normal saline) and null adenovirus vector. Equivalent fasting bloods were extracted from all experimental animals at the same time for examination.

Sample collection

Fix the head of rats and press bilateral cervical parts gently with left thumb and index finger. The blood was derived from post-ocular venous plex with capillary tube. Collect blood sample into EP tubes and perform cold preservation.

Cardiovasculars were harvested from experimental animals and rinsed with 0.9% sodium chloride injection at 4[°]C. Cut cardiovascular tissues into 0.5cm×0.5cm tissue blocks. Half of blocks were formalin fixed and the others were stored at -80[°]C.

Pathological examination

Formalin fixed tissue blocks were dehydrated with routine protocols, and Paraffin embedding were performed before serial section. HE staining were performed for microscopic observation.

Isolation and culture of adipocytes

Subcutaneous fatty tissues of rats were harvested in a sterile environment. Cut fatty tissue into pieces and digest

cells with routine protocols. Discard supernatant and resuspended adipocytes into DMEM with the cell amount of 10⁴/ml. Culture adipocytes at 5% CO₂, 37[°]C.

Examination of factors correlated with renal function

Extract 2ml fasting blood for examination of RBC and HGB. Biochemical analyzer was used to examine BUN, Scr and GFR.

Enzyme linked immunosorbent assay

Enzyme linked immunosorbent assay was performed to examine levels of leptin, TNF- α and CRP with routine protocols. Light absorption values were assessed at 450nm.

Real-time fluorescent quantitative PCR

200ng total RNA was extracted for PCR examination. Primers of leptin and GAPDH were showed in table 1. PCR reaction conditions were as follows: 95[°]C, 30s; 95[°]C, 5 s; 60[°]C, 30s; 40 cycles.

Genes		T(°)
Leptin	5'-CACCATGAAGCCTAC	60
	CTGTGTTTCC-3'	
	5'-TTAAACCATTCGGCAGC	
Gapdh	GCGG-3'	60
	5'-GCCAAGGTCATCCATGACAA	
	CTTTGG-3'	
	5'-GCCTGCTTACCACCTTCTTG	
	ATGTC-3'	

STATISTICAL ANALYSIS

SPSS17.0 software was used for data processing. Measurement data are normal distribution to $X \pm S$. χ^2 test was performed for statistical significance. P value < 0.05 was considered to be statistically significant.

RESULTS

Spontaneously hypertensive model were verified by cut off value

To determine whether spontaneously hypertension were successfully induced, blood pressure value were measured in four groups. The cut off value of systolic pressure is 150mmHg and as showed in table 1, the spontaneously hypertensive rats indeed had higher systolic pressure, which is over 150 mmHg.

ALDH2 improved efficacy of irbesartan treatment on pathological changes in renal injury of chronic hypertension

Compared with sham group, SHR group had lower level of ALDH2. In addition, ALDH2 expression was significantly after irbesartan treatment or over expression of ALDH2 (fig. 1A, P<0.05). Moreover, compared with control group, experimental group had lower level of hypertension-induced renal injury, such as patchy atrophy of nephrons and cystic dilatation of renal tubule (fig. 1B).

Table 1: Analysis of systolic pressure for four groups of rats

Group	Sham	SHR	Control	Experimental
Systolic pressure (X±S)	127±11mmHg	165±13mmHg	151±14mmHg	142±11mmHg

Table 2: Analysis of multiple factors related to renal function. *P<0.05, versus control group.

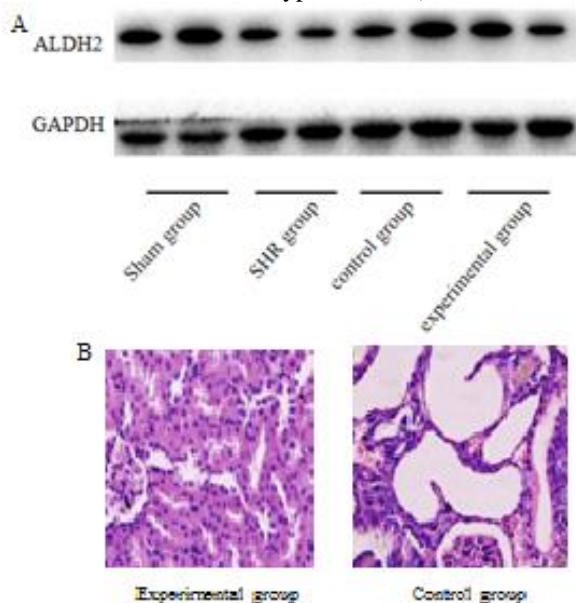
Group	Sample	Systolic Pressure	LVEF	LVFS	NT-pro BNP (pg/ml)	GFR (ml/min)
Experimental	20	163.9±0.4*	73.8±1.4*	72.3±1.7*	201.4±1.5*	118.2±0.9*
Control	20	193.7±8.9	51.4±1.1	54.5±1.2	323.1±1.2	100.2±1.3

Table 3: Analysis of leptin, TNF- α and CRP. *P<0.05, versus control group.

Group	Sample	leptin	TNF- α	ALDH2
Experimental	30	27.7±3.2*	125.2±3.4*	61.7±1.1
Control	30	9.4±1.3	54.1±1.8	143.3±2.8*

Blood pressure dysfunction was alleviated in chronic hypertension under ALDH2 over expression.

Compared with control group, experimental group had lower levels of BUN and Scr, but lower level of GFR, suggesting renal dysfunction was exacerbated in cardiovascular of chronic hypertension (table 2, P<0.05).

**Fig. 1:** (A) Western blot for ALDH2 in four groups (B) Pathological changes in renal tissue of chronic hypertension ($\times 400$)

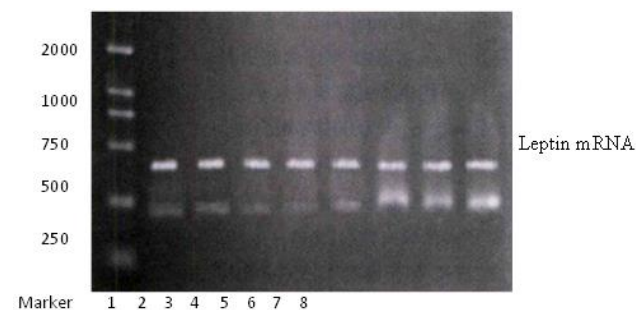
The levels of serum leptin were increased in chronic hypertension but ALDH2 decreased

Compared with control group, adenine-treated group had higher levels of serum leptin and inflammatory microenvironment, verified by increased expression of leptin and TNF- α (table 3, P<0.05), but ALDH2 was decreased.

Expression of leptin mRNA in myocardium

Compared with control group, experimental group had lower levels of leptin mRNA in myocardium (fig. 2).

Correlation analysis showed that the level of serum leptin was negatively correlated with leptin mRNA in adipocytes (table 4, $r=-0.64$, $P=0.01$).

**Fig. 2:** Expression of Leptin mRNA (Experimental group: 1-4; control group: 5-8)

DISCUSSION

Chronic hypertension can be caused by various types of cardiovascular diseases in clinical scenario. Renal dysfunction is always gradually exacerbated in patients with cardiovascular diseases and toxic metabolites retention results in irreversible decline of renal function with progressive symptoms of the whole body system (Miranda *et al.*, 2017, Yan *et al.*, 2016, Fan *et al.*, 2013). Latest studies indicated that patients with chronic hypertension were physique emaciated due to anorexia and malnutrition, which could be induced by hypertension and not be totally cured after dialysis therapy (Bell *et al.*, 2015). Leptin is widely expressed and distributed in human and various animals, which is encoded by OB gene and under synergistic regulation of hypothalamic ghrelin receptor and feeding center (Movassagh *et al.*, 2015). Leptin not only regulates fat metabolism of peripheral tissues, but also is involved in multiple physiological processes, including insulin secretion and body immunity (Cong *et al.*, 2014). In addition, current studies suggested that leptin was associated with levels of ALDH2 expression and related drug microenvironment

(Ma *et al.*, 2015). We also revealed the relationship between leptin and ALDH2 expression in chronic hypertension.

Our study established chronic hypertension model of rats through intragastric administering irbesartan treatment. Cardiovascular index from chronic hypertension rats had significant increases in hypertension and NT-pro BNP, suggesting chronic hypertension model was successful. Moreover, renal function was impaired in chronic hypertension model of rats, suggesting chronic renal injury is indeed complicated with hypertension in our study, verified by increased levels decreased GFR and increased level of leptin. Furthermore, analysis demonstrated positive correlation between serum leptin and ADLH2 expression. Irreversible impaired renal function is always accompanied with retention of toxic metabolites, while ALDH2 over expression could improved of irbesartan treatment on hypertension-induced renal injury. Moreover, such hypertension disorders caused irreversible increase of leptin (Sheng *et al.*, 2012). As the only organ with expression of leptin receptors, kidney excretes more than 80% of leptin, and glomerular filtration rate directly influence metabolism of leptin (Zhang *et al.*, 2015). GFR decrease could possibly induce hypertension in patients with cardiovascular diseases (Zenker *et al.*, 2016).

Recent studies proved that irbesartan treatment positive response in chronic hypertension directly verified by cardiovascular and renal improvement (Trojer *et al.*, 2017). Moreover, such therapy response and decreased levels of inflammatory factors TNF- α could triggered by irbesartan treatment (Frazier-Wood *et al.*, 2014). It is reported that, as an important cardio protection factor, ALDH2 was a promising indicator for hypertension prognosis. The percentage of ALDH2 decrease accounts for more than 75% in the patients with chronic hypertension (Hernández *et al.*, 2015). In addition, some studies showed that ALDH2 was also an independent predictor of anti-hypertensive therapy and associated with leptin (Lavigne *et al.*, 2015). Animal experiments showed that ALDH2 increased the level of leptin and increased drug sensitivity for anti-hypertension therapy (Balassiano *et al.*, 2017). Our study demonstrated that increased level of serum leptin was indeed associated with impaired renal function and decreased ALDH2, verified by decreased GFR. What's more, ALDH2 over expression significantly improved efficacy of irbesartan treatment. These findings suggested that ALDH2 elevated the drug susceptibility of irbesartan treatment for hypertension via regulating serum leptin.

Intriguingly, our further PCR results showed that expression of leptin mRNA decreased in rats of chronic hypertension. The level of serum leptin was negatively correlated with the expression of leptin mRNA. One possible explanation for this phenomenon was that

metabolism of leptin was slowed down due to impaired renal function, which caused high level of serum leptin, while hypertension triggered some feedback regulation to inhibit further synthesis and release of leptin mRNA. Previous studies indicated that serum leptin was regulated by renal clearance and increased serum leptin reversely inhibited further synthesis of leptin (Ma *et al.*, 2017, Chen *et al.*, 2018). Our study indeed found similar phenomenon, and elucidated serum leptin still increased in chronic hypertension despite of reducing synthesis of leptin mRNA.

CONCLUSION

Decreased ALDH2 expressions were correlated with progression of hypertension. Rats with hypertension indeed benefited from irbesartan treatment. ALDH2 elevated the drug susceptibility of irbesartan treatment for hypertension via regulating serum leptin, and improved efficacy of irbesartan treatment on hypertension.

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