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Effect of acetazolamide on cytokines in rats exposed to high altitude

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ABSTRACT

Acute mountain sickness (AMS) is a dangerous hypoxic illness that can affect humans who rapidly reach a high altitude above 2500 m. In the study, we investigated the changes of cytokines induced by plateau, and the acetazolamide (ACZ) influenced the cytokines in rats exposed to high altitude. Wistar rats were divided into low altitude (Control), high altitude (HA), and high altitude + ACZ (22.33 mg/kg, Bid) (HA + ACZ) group. The rats were acute exposed to high altitude at 4300 m for 3 days. The HA + ACZ group were given ACZ by intragastric administration. The placebo was equal volume saline. The results showed that hypoxia caused the heart, liver and lung damage, compared with the control group. Supplementation with ACZ significantly alleviated hypoxia-caused damage to the main organs. Compared with the HA group, the biochemical and blood gas indicators of the HA + ACZ group showed no difference, while some cytokines have significantly changed, such as activin A, intercellular adhesion molecule-1 (ICAM-1, CD54), interleukin-1α,2 (IL-1α,2), L-selectin, monocyte chemotactic factor (MCP-1), CC chemokines (MIP-3α) and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1). Then, the significant difference pro-inflammatory cytokines in protein array were chosen for further research. The protein and mRNA content of pro-inflammatory cytokines MCP-1, interleukin-1β (IL-1β), tumor necrosis factor (TNF-α), interferon-γ (IFN-γ) in rat lung were detected. The results demonstrated that the high altitude affected the body's physiological and biochemical parameters, but, ACZ did not change those parameters of the hypoxia rats. This study found that ACZ could decrease the content of pro-inflammatory cytokines, such as MCP-1, IL-1β, TNF-α and IFN-γ in rat lungs, and, the lung injury in the HA + ACZ group reduced. The mechanism that ACZ protected hypoxia rats might be related to changes in cytokine content. The reducing of the pro-inflammatory cytokines in rat lung might be other reason to explain ACZ against the acute mountain sickness.

1. Introduction

More and more people living at or near sea level spend their spare time with activities such as hiking, skiing, or climbing at higher altitude (2500 m above sea level) [1]. However, the non-altitude acclimatized individuals ascending to altitudes often occurs acute high altitude illness, such as acute mountain sickness (AMS), high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE). It can be indicated that the incidence of AMS depend on individual susceptibility, speed of ascent, and maximum height reached above 2500 m. The exposure to hypobaric hypoxia causes severe damage to different organs. It's a challenge for people residing in or visiting high altitudes.

Lots of studies support that environmental conditions (hypoxia, temperature, etc.) may influence the functional behavior of immunocompetent cells [2–4]. Although several efforts have been devoted to understand the effects of high altitudes on respiratory, musculoskeletal, and cardiovascular systems, the impact of the immune system to high altitude is less known. Specific parameters, including cytokine expression and lymphocyte subsets which were induced by hormone production and strenuous exertion had been

Abbreviations: ACZ, acetazolamide; AMS, acute mountain sickness; HAPE, high altitude pulmonary edema; HACE, high altitude cerebral edema; TP, total protein; ALB, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TBIL, total bilirubin; DBIL, direct bilirubin; CRE, creatinine; UA, Uric acid; pCO_2, pressure of carbon dioxide; BB, buffer base; BE, base access; AB, actual bicarbonate; SBC, standard bicarbonate; pO_2, pressure of oxygen; sO_2, oxygen saturation; Hb, Hemoglobin; Lac, Lactic acid.

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considered [5,6]. And the acute hypobaric hypoxia is known to influence cytokine levels [7–10].

Acetazolamide (ACZ) is considered for increasing pulmonary ventilation by enhanced periodic breathing, and promoting renal elimination of bicarbonate by inducing a metabolic acidosis. The resulting stimulation of ventilation increase arterial oxygen saturation, but enhances the altitude-induced hyperventilation and hypocapnia, which may reduce cerebral blood flow and oxygenation [11–13]. ACZ is the only medication approved by the US Food and Drug Administration (FDA) for AMS prevention [14]. Most studies investigated that ACZ had prophylactic effects when it was ingested at high altitude or the day before arrival [15].

The mechanism of high altitude related disease is still unknown. It seems that the hypoxia is the key problem. The high altitude damage is not just a simple physiological and pathological changes. Many studies showed that hypoxia cause systemic inflammatory response, which was the major factor causing high altitude multiple organ dysfunction syndromes [16–26]. Therefore, we screened for the major cytokines by Rat Cytokine Antibody Array technique and studied the influence of rat acute exposure to high altitude and administration ACZ. The mechanism of ACZ on changing cytokine content was discussed. The reducing of the pro-inflammatory cytokines in rat lung might explain ACZ against the acute mountain sickness in the paper.

2. Material and methods

2.1. Reagents

Acetazolamide (content >98.5%, Hubei Xing Yinhe Chemical Co., CAS: 59-66-5, bulk drug), 0.9% sodium chloride injection (size 250 ml: 2.25 g, Zhejiang country mirror Pharmaceutical Co., Ltd. production batch number: C113012009). According to body surface area conversion method [27], the human dose will be converted into rats' dose, acetazolamide twice daily dosing, the dose is 22.33 mg/kg, and with a 0.9% 0.5 ml of sodium chloride solution preparation, and the drug were continuous administration in twice a day for 3 days, and others were administration with a 0.9% 0.5 ml of sodium chloride only.

2.2. Experimental animals

Twenty-four male Wistar rats were used in the study. They were age two weeks and weight 200 ± 20 g. All Wistar rats used in this experiment were SPF animals and obtained from Shanghai SLAC Laboratory Animal LLC (Shanghai, China, Certificate of Conformity: 2007000524909). They were randomly divided into three groups (n = 8): Low altitude group (called "Control" group, Shanghai, 31°18′23.1″ NW, 121°31′13.8″ EL, 55 m above sea level, 20–22 °C), high altitude group (called "HA" group, Maduo, Qinghai, 35°6′57.3″ NW, 98°51′57.1″ EL, 4300 m above sea level, 18–22 °C), and high altitude + Acetazolamide group (called "HA + ACZ" group, Maduo, Qinghai, 35°6′57.3″ NW, 98°51′57.1″ EL, 4300 m above sea level, 18–22 °C).

2.3. Experimental design

The rats in control group fed at low altitude (Shanghai, 55 m). The high altitude rats were air-shipment from Shanghai Hongqiao Airport to Lanzhou airport, immediately after arrival by incubator truck transport to Maduo County, in the northwest China's Qinghai province, to an altitude of 4300 m. During the motor transport, the rats were housed in air-conditioned room which could put in the car and keep rats living in 18–22 °C, and they were allowed access to food and water ad libitum [28]. When the HA and HA + ACZ group arrived at Maduo County, the high-altitude rats were given ACZ or normal saline by intragastric administration. The control group was given normal saline at the same time. All animals were kept on a 12 h day–night cycle and fed for 3 days. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the Lanzhou Command General Hospital (Certificate of Approval: 201207050016).

2.4. Measurements of plasma biochemical parameters

We collected 1 ml blood from the orbital venous plexus of rat to a centrifuge tube containing with heparin. At room temperature, the blood centrifuged 3000 r min^{-1} for 10 min and the supernatants were detected by automatic biochemical analyzer (BX 3010, Sysmex, Japan). The biochemical indicators included total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), creatinine (CRE), Uric acid (UA) [29–31].

2.5. Measurements of blood gas parameters

After the last administration 1 h, the rats were anesthetized by 10% chloral hydrate and abdominal cavity was dissected. We collected 1 ml abdominal aortic blood to detect by a portable automatic blood gas analyzer (PHOX-plus-L, Nova, USA). The blood gas indicators included blood pH, pressure of carbon dioxide (pCO_2), buffer base (BB), base access (BE), actual bicarbonate (AB), standard bicarbonate (SBC), pressure of oxygen (pO_2), oxygen saturation (sO_2), Hemoglobin (Hb), Lactic acid (Lac) [30,32–34].

2.6. Application of protein chip

We collected 1 ml blood from the orbital venous plexus of rat to a centrifuge tube in each group. The rat serums were got and stored at −80 °C for the protein chip experiment. RayBiotech, Inc, the Protein Array Pioneer Company, provide the technical support. We chose the RayBio® Rat Cytokine Antibody Array G-Series 2 Cat# AAR-CYT-G2-4. It could provide the most rats' cytokines. The numbers of proteins tested were 34. All of the proteins had important physiological role in the body. The antibody array included eight kinds of chemokines, neutrophil chemotactic factor 1 (CINC-1), neutrophil chemotactic factor 2 (CINC-2), neutrophil chemotactic factor 3 (CINC-3), LPS-induced chemokine (LIX), thymus chemokine (thymus CK-1), monocyte chemotactic factor (MCP-1), CC chemokines (MIP-3α), chemokine CX3 (CX3CL1); eight kinds of interleukins, interleukin-1α, 1β, 2,4,8,10,13 (IL-1α, 1β, 2,4,8,10,13), interleukin-1 receptor 6 (IL-1r6); four kinds of growth factors, vascular endothelial growth factor (VEGF), β nerve growth factor (β-NGF), platelet derived growth factor AA (PDGF-AA), activin a (activin a); four kinds of CD signal molecules, intercellular adhesion molecule-1 (ICAM-1, CD54), L-selectin (L-selectin, CD62L), B7-related surface antigen (B7-2, CD86), fatty acid synthase ligand (Fas ligand, CD178); three kinds of inflammatory factor, tumor necrosis factor (TNF-α), granulocyte macrophage colony stimulating factor (GM-CSF), interferon-γ (IFN-γ); seven variety of other factors, prolactin receptor (prolactin R), matrix metalloproteinase-8 (MMP -8), leptin, tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), a combination of advanced glycation end products (RAGE), agrin, ciliary neurotrophic factor (CNTF). The sample cell of antibody array was showed in Fig. 1.

2.7. Observation of histomorphology

The heart, liver, and lung of rats were collected and washed with normal saline. We fixed the tissues by 10% formaldehyde.

POS 1	POS 2	POS 3	NEG	Activin A	Agrin	B7-2/CD86	beta-NGF	CINC-1	CINC-2 alpha	CINC-3	CNTF
POS 1	POS 2	POS 3	NEG	Activin A	Agrin	B7-2/CD86	beta-NGF	CINC-1	CINC-2 alpha	CINC-3	CNTF
Fas Ligand	Fractalkine	GM-CSF	ICAM-1	IFN-gamma	IL-1 alpha	IL-1 beta	IL-1 R6	IL-2	IL-4	IL-6	IL-10
Fas Ligand	Fractalkine	GM-CSF	ICAM-1	IFN-gamma	IL-1 alpha	IL-1 beta	IL-1 R6	IL-2	IL-4	IL-6	IL-10
IL-13	Leptin	LIX	L-Selectin	MCP-1	MIP-3 alpha	MMP-8	PDGF-AA	Prolactin R	RAGE	Thymus Chemokine-1	TIMP-1
IL-13	Leptin	LIX	L-Selectin	MCP-1	MIP-3 alpha	MMP-8	PDGF-AA	Prolactin R	RAGE	Thymus Chemokine-1	TIMP-1
TNF-alpha	VEGF	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS 1
TNF-alpha	VEGF	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS 1

Fig. 1. The sample cell of antibody array. Note: POS, the positive control; NEG, the negative control.

Table 1
Primers used for real-time PCR analysis.

Gene	Amplification length (bp)	Tm (°C)	Primer sequences
MCP-1	250	75.1	Forward 5′-CAGCAGCAGGTGTCCCAAAGAA-3′ Reverse 5′-AGTGCTTGAGGTGGTTGTGGAA-3′
TNF-α	239	80.6	Forward 5′-AGAACTCCAGGCGGTGTCTGT-3′ Reverse 5′-CTGCTCCTCTGCTTGGTGGTT-3′
IL-1β	190	76.9	Forward 5′-AATGCCTCGTGCTGTCTGACC-3′ Reverse 5′-AGGGTGGGTGTGCCGTCTTT-3′
IFN-γ	107	73.9	Forward 5′-ACCCACAGATCCAGCACAAAGC-3′ Reverse 5′-CCAGAATCAGCACCGACTCCTT-3′
β-actin	150	77.8	Forward 5′-GGAGATTACTGCCCTGGCTCCTA-3′ Reverse 5′-GACTCATCGTACTCCTGCTTGCTG-3′

The tissues were consecutively cut into 4-μm thick sections that were stained by hematoxylin and eosin (H&E). Pathological changes were observed under the light microscope (Olympus, Japan). The results were made in 200× high power photomicrograph.

2.8. ELISA analysis

A small portion of each rat lung from the left lobe was snap-frozen in liquid nitrogen and stored at −80 °C. The MCP-1, IL-1β, TNF-α and IFN-γ in rat lungs were selected for further research. Lung tissue homogenate was diluted to 2.5‰. Both the concentration of total protein (C_{total}, mg/ml) and cytokines ($C_{cytokine}$, pg/ml) were detected. The experiment in strict accordance with ELISA kit instructions (Boster, Wuhan, China), and the detection wavelength 450 nm. The protein content of MCP-1, IL-1β, TNF-α and IFN-γ was the ratio of $C_{cytokine}$ and C_{total} (pg/mg).

2.9. RNA isolation and real-time PCR

Approximately 50–100 mg of lung tissue was homogenized and total RNA was isolated with the TRIzol reagent (TakaRa, Dalian, China). The quality of the RNA solutions was determined using an ultraviolet light spectrophotometer. The primers for rats MCP-1, IL-1β, TNF-α and IFN-γ and the reaction system were designed by TakaRa Biotechnology Corporation (Dalian, China). The reverse transcription of cDNA was carried out using a PrimeScript™ RT Master Mix kit (TakaRa, China). All the PCR reactions were carried out using an SYBR® Premix Ex Taq kit (TakaRa, China) and the manufacturer's instructions followed. Amplification was performed in the ViiA™ 7 Real Time PCR System (Applied Biosystems, USA). Amplification of predenatured products was conducted at 95 °C for 30 s, followed by 40 cycles at 95 °C for 30 s, 60 °C for 31 s. Fold induction values were calculated according to the cycle threshold numbers between the target gene and the differences between control and treatment groups (Table 1).

2.10. Statistical analysis

Data were expressed as the mean ± S.D. Statistical significance was determined using SPSS 13.0 for Windows (SPSS Inc., Chicago, USA). One-way ANOVA was performed for multiple comparisons followed by Fisher LSD post-hoc comparisons. Differences were deemed significant if $P < 0.05$.

3. Results

3.1. Effect of hypoxia and acetazolamide on the Biochemical indicators

Venous blood biochemical parameters were compared and the results were presented at Table 2. Compared with the Control group, the TP, ALB, AST, ALT, ALP, TBIL and DBIL values of the HA

Table 2
The comparison of main biochemical parameters between the experimental groups.

Biochemical parameters	Control	HA	HA + ACZ
TP (g/l)	38.27 ± 3.44	64.82 ± 2.02[a]	69.67 ± 0.76[b]
ALB (g/l)	14.47 ± 0.51	35.42 ± 1.41[a]	34.70 ± 2.56
AST (U/l)	35.67 ± 11.18	107.53 ± 13.55[a]	108.73 ± 1.61
ALT (U/l)	43.17 ± 5.23	60.85 ± 7.18[a]	46.77 ± 14.79
ALP (U/l)	101.14 ± 15.54	169.47 ± 24.07[a]	145.57 ± 10.12[b]
TBIL (μmol/l)	7.80 ± 0.11	23.64 ± 5.63[a]	12.63 ± 1.22[b]
DBIL (μmol/l)	-0.83 ± 0.10	9.86 ± 2.27[a]	5.6 ± 3.37[b]
Urea (mmol/l)	4.74 ± 0.89	2.55 ± 0.16[a]	2.87 ± 0.52
CRE (mg/dl)	39.95 ± 1.81	21.2 ± 2.95[a]	13.00 ± 4.38[b]
UA (μmol/l)	41.63 ± 43.54	48.15 ± 24.96	25.06 ± 21.20

TP: total protein, ALB: albumin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, TBIL: total bilirubin, DBIL: direct bilirubin, CRE: creatinine, UA: uric acid. Each group was expressed as mean ± S.D, n = 8.
[a] $P < 0.01$ compared with Control group.
[b] $P < 0.05$ compared with HA group.

group were significantly increased by 69.38, 144.78, 201.46, 40.95, 67.56, 203.08 and 1287.95%, respectively, while the Urea and CRE were significantly decreased by 46.20 and 46.93%, respectively ($P < 0.01$). The TP level of HA + ACZ group increased by 7.48% compared with the HA group, whereas the ALP, TBIL, DBIL and CRE levels significantly decreased by 14.10, 46.57, 43.20 and 38.68%, respectively.

3.2. Effect of hypoxia and acetazolamide on the blood gas indicators

The results were shown in Table 3. Arterial blood gases and metabolic parameters were analyzed. Compared with the Control group, the pCO_2, BB, BE, AB, SBC, pO_2, and sO_2 of the HA group were significantly decreased by 18.22, 183.87, 222.53, 21.37, 16.03, 32.28 and 3.80%, respectively, while the Hb were significantly increased by 10.88% respectively ($P < 0.01$). Compared with the HA group, the pH, BB, BE, AB and SBC values of the HA + ACZ group were significantly decreased by 3.10, 383.46, 281.61, 29.63 and 32.50%, respectively, while the pCO_2 and pO_2 were significantly increased by 15.09 and 17.81%, respectively ($P < 0.05$).

3.3. Effect of hypoxia and acetazolamide on the cytokines

Cytokines played important roles and they acted as signaling molecules, chemokines, inflammatory mediators, and so on. The results of protein chip showed that both hypoxic environment and acetazolamide made almost all of the cytokine changes (Fig. 2, A_1, A_2, A_3). According to Raybiotech's proposal, we had chosen two-fold difference as a significant change. The cytokines that changed significant were activin A, ICAM-1, IL-1 alpha, IL-2, L-selectin, MCP-1, MIP-3 alpha, TIMP-1 (Fig. 2, B).

3.4. Effect of hypoxia and acetazolamide on the pathological changes of myocardium, liver and lung tissue

3.4.1. Results of hematoxylin and eosin staining in myocardium tissue

The results show significant pathological changes in the myocardial tissue among the groups (Fig. 3). The myocardial structures and nuclear morphology of Control group were normal, and the myofibrils were neatly, as it showed in Fig. 3, C_1. The myocytes of HA group arranged disarray, and the muscle fibers were dissolved (Fig. 3, C_2). In the HA + ACZ group, part of the myocardial fibers was broken, but the majority of cells arranged in neat rows (Fig. 3, C_3).

Table 3
The comparison of blood gas parameters between the experimental groups.

Blood gas indicators	Control	HA	HA + ACZ
pH	7.42 ± 0.03	7.41 ± 0.03	7.18 ± 0.09[b]
pCO_2 (mmHg)	41.17 ± 2.38	33.67 ± 1.21[a]	38.75 ± 3.40[b]
BB (mmol/l)	3.10 ± 0.99	−2.60 ± 2.18[a]	−12.57 ± 2.93[b]
BE (mmol/l)	2.53 ± 0.98	−3.1 ± 2.11[a]	−11.83 ± 2.17[b]
AB (mmol/l)	26.48 ± 0.92	20.82 ± 1.87[a]	14.65 ± 1.34[b]
SBC (mmol/l)	26.20 ± 1.09	22.00 ± 1.67[a]	14.85 ± 1.97[b]
pO_2 (mmHg)	103.62 ± 7.54	70.17 ± 4.22[a]	82.67 ± 7.71[b]
sO_2 (%)	97.82 ± 0.96	94.10 ± 0.92[a]	93.88 ± 1.5
Hb (g/dl)	13.33 ± 0.56	14.78 ± 1.86[a]	13.45 ± 1.08

Note: pCO_2, pressure of carbon dioxide; BB, buffer base; BE, base access; AB, actual bicarbonate; SBC, standard bicarbonate; pO_2, pressure of oxygen; sO_2, oxygen saturation; Hb, Hemoglobin. Each group was expressed as mean ± S.D, n = 8.
[a] $P < 0.05$ compared with Control group.
[b] $P < 0.05$ compared with HA group.

3.4.2. Results of hematoxylin and eosin staining of liver tissue

The liver lobules of Control group were integrity and clarity, the liver cells arranged neatly (Fig. 4, D_1). Compared with Control, the rats in HA and HA + ACZ group revealed liver injury and inflammation, had much inflammatory cell infiltration and edema (Fig. 4, D_2 and D_3).

3.4.3. Results of hematoxylin and eosin staining in lung alveoli

The results showed significant pathological changes in the alveoli among the groups (Fig. 5). Compared with the Control group, the alveolar wall of HA group became hyperemic, edematous and incrassate; the alveolar epithelium became hyperplastic and neutrophilic granulocyte infiltrates were presented (Fig. 5, E_2). In the HA + ACZ group, the alveolar wall was hyperemic, edematous and incrassate; the alveolar epithelium was hyperplastic (Fig. 5, E_{32}). Compared with the HA group, the damage of the acetazolamide group was reduced.

3.5. Effect of hypoxia and acetazolamide on the protein expression of MCP-1, IL-1β, TNF-α and IFN-γ in the rat lung

The content of MCP-1, IL-1β, TNF-α and IFN-γ were significantly increased by HA group in rat lung, and were 51.01, 30.77, 12.64, 34.52% higher in the HA group when compared with the Control group. The content of MCP-1, IL-1β, TNF-α and IFN-γ in HA + ACZ group were significantly decreased 29.32, 38.04, 15.17, 21.79% when compared with the HA group (Table 4).

3.6. Effect of hypoxia and acetazolamide on the mRNA expression of MCP-1, IL-1β, TNF-α and IFN-γ in the rat lung

The mRNA expression of MCP-1, IL-1β, TNF-α and IFN-γ in the HA group were increased significantly by high altitude, and were 5.23, 1.66, 1.75, 2.57 higher than the Control group, respectively (Fig. 6). The mRNA expression of MCP-1, IL-1β, TNF-α and IFN-γ in the HA + ACZ group were decreased significantly by Acetazolamide, and were 0.27, 0.35, 0.47, 0.48 lower, when compared with the HA group, respectively (Fig. 6).

4. Discussion

Biochemical analysis was the method to evaluate the changes of multiple metabolites which were distributed in the blood. The results from biochemical analysis showed that acute exposure to high-altitude environment might cause a significant increase in TP, AST, ALP, TBIL and other biochemical indicators. Wenbin Li et al. [31] found that the UREA and ALP of acute exposure rats were significantly increased. According to the biochemical results, we

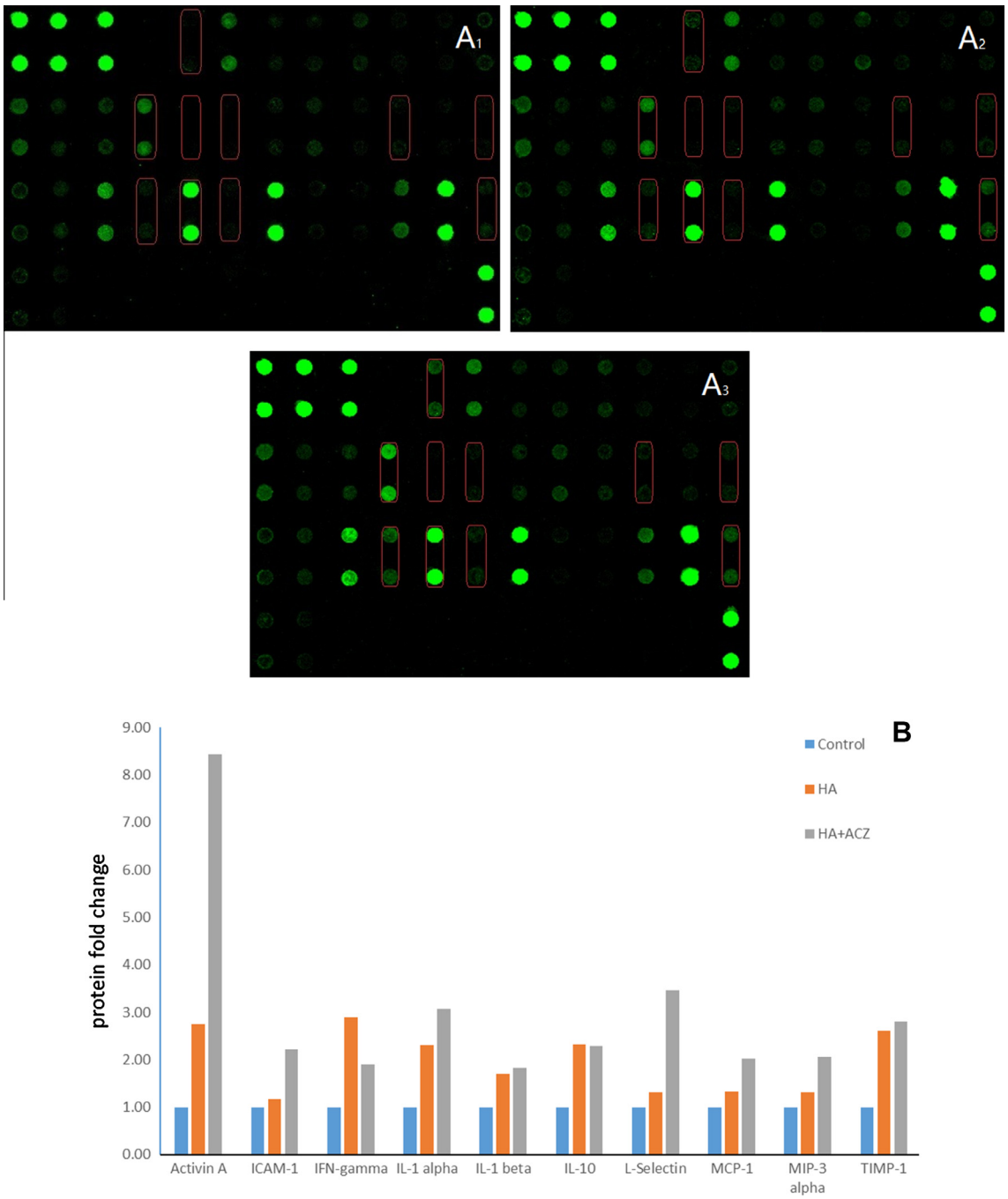

Fig. 2. Hypoxia and acetazolamide are caused by a variety of inflammatory cytokines. Note: A_1: the microarray results of Control group. A_2: the microarray results of HA group. A_3: the microarray results of HA + ACZ group. B: significantly different cytokines, and 2-fold higher than the control group.

indicated that acute hypoxia would affect the heart and liver function in rats, it could accelerate tissue and organ damage. Pathology results also showed that the heart, liver and lung tissue of the HA group were significant damaged after acute exposure. In high altitude, acetazolamide had little effect on rat biochemical indicators, only TBIL, DBIL and CRE index lower than the HA group.

Arterial blood gas is an important indicator to evaluate body acid-base balance. From the blood gas results, we can see that both the HA and HA + ACZ have changed greatly. In the HA group, pH unchanged, BB, BE, AB, SBC, pO_2 and sO_2 indicators significantly decreased, the rats appeared compensatory respiratory alkalosis with metabolic acidosis. The morphology results showed that the lungs of hypoxia rats were seriously damaged. Comprehensive blood gas results, the HA group rats had respiratory dysfunction and meionectic blood. Compared with the HA group, the HA + ACZ group aggravated acidosis obvious, but respiratory function improved, the pO_2 increased 17.81%.

More and more studies reported that cytokines play an important role in the development of the high altitude disease and the research mostly focused in inflammatory cytokines and hypoxia-inducible factors [34,35]. But, the complex natural environment had a great impact on the body. Acute exposure to high altitude, to explore the formation of hypoxic injury, was a unique way. It's characterized by more realistic representation of high-altitude environment. The results showed by the protein chip, TIMP-1 and other cytokines might be involved in the development of high

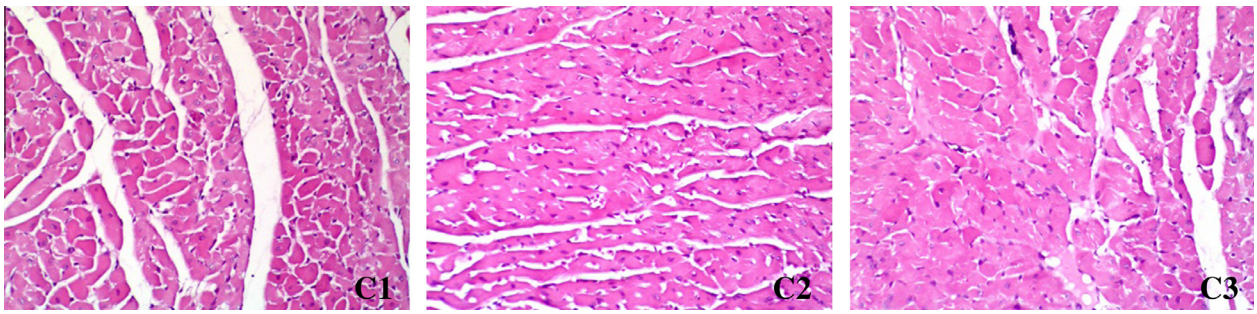

Fig. 3. Hematoxylin and eosin staining showing myocardial tissue of the experimental rat (magnification, 200×). Note: C_1: the Control group; C_2: the HA group; C_3: the HA + ACZ group.

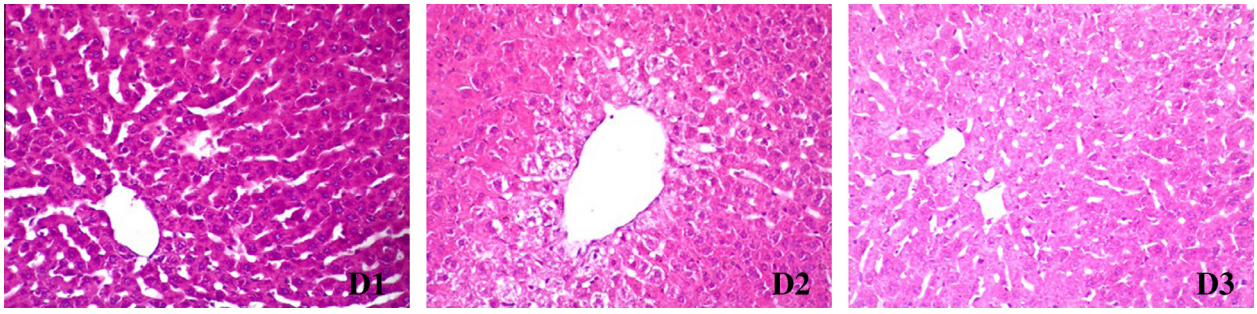

Fig. 4. Hematoxylin and eosin staining showing liver tissue of the experimental rat (magnification, 200×). Note: D_1: the Control group; D_2: the HA group; D_3: the HA + ACZ group.

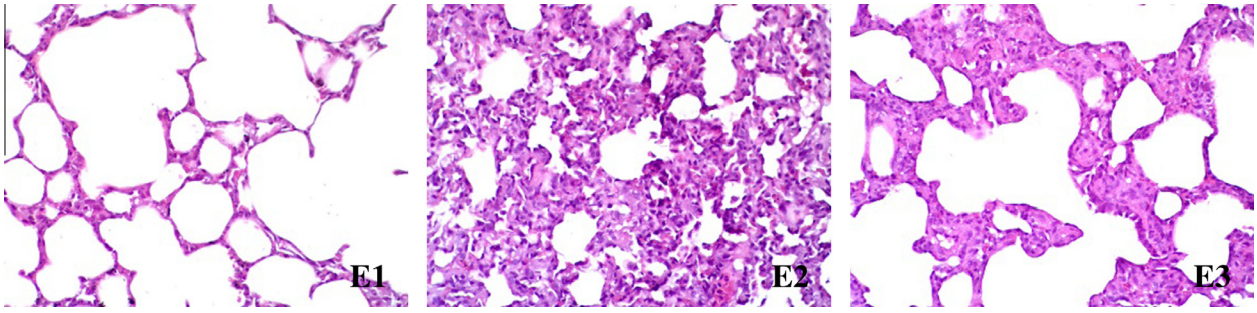

Fig. 5. Hematoxylin and eosin staining showing lung alveoli of the experimental rat (magnification, 200×). Note: E_1: the Control group; E_2: the HA group; E_3: the HA + ACZ group.

Table 4
The protein expression of MCP-1, IL-1β, TNF-α and IFN-γ in the rat lung.

Cytokines	Control (pg/mg)	HA (pg/mg)	HA + ACZ (pg/mg)
MCP-1	236.48 ± 21.38	357.11 ± 14.77**	252.42 ± 22.66##
TNF-α	762.83 ± 18.59	997.52 ± 24.74**	618.04 ± 26.59##
IL-1β	842.46 ± 38.30	948.91 ± 23.68**	804.93 ± 22.19##
IFN-γ	314.50 ± 12.52	423.06 ± 16.12**	330.86 ± 13.87##

Note: Each group was expressed as mean ± S.D, n = 8.
** $P < 0.01$ compared with Control group.
$P < 0.01$ compared with HA group.

altitude disease. The cytokines could be classified into inflammatory factor, chemotactic factor and vascular endothelial-related factors. MCP-1 played an important role in the formation of hypoxic systemic inflammation, IL-1α, IL-2 could be directly involved in the inflammatory response [35]. Hypoxia can induce the body to produce a lot of TNF and IL-1β, but no significant differences found in this study [36]. MIP-1α regulated by both LPS and inflammatory cytokines, ICAM could promote adhesion of inflammatory sites. L-selectin, also known as CD62L, was a cell adhesion molecule found on lymphocytes and the pre-implantation embryo [37]. It belonged to the selectin family of proteins, which recognized sialylated carbohydrate groups. It had not been reported associated with hypoxia inflammation. TIMP-1 as a biomarker of lung cancer, often accompanied by microcirculation hypoxia of lung tissue [38]. The results of the study indicated that systemic inflammation caused by high altitude might have something in common on the microcirculation hypoxia disorders. The results might be different with the Chao's report [39].

The gene expression and protein content of the pro-inflammatory were detected. Most of them were increased by high altitude. However, the HA + ACZ group was decreased. The results showed that the formation of hypoxia injury might be associated with inflammation, acetazolamide had anti-inflammatory effects. The relationship between high-altitude hypoxia and inflammation also required in-depth exploration. Molecular mechanisms of high altitude diseases were still unclear. High altitude had comparatively large effect to the physiological and biochemical indicators of rats. The changes of HA + ACZ group on biochemical and blood

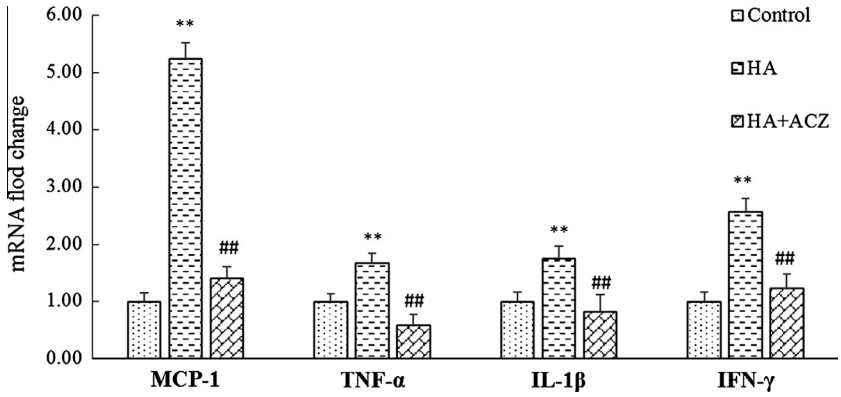

Fig. 6. The mRNA expression of MCP-1, IL-1β, TNF-α and IFN-γ in the rat lung. Note: Each group was expressed as mean ± S.D, n = 8. $^{**}P < 0.01$ compared with Control group, $^{\#\#}P < 0.01$ compared with HA group.

gas indicators were limited. However, cytokines, such as activin A, ICAM-1, IL-1 alpha, IL-2, L-selectin, MCP-1, MIP-3 alpha and TIMP-1, were significantly changed, which involved in the development of inflammation, might be associated with the body's stress and mechanism of ACZ. This study found that ACZ could decrease the content of MCP-1, IL-1β, TNF-α, IFN-γ in rat lungs, and the lung injury in the HA + ACZ group reduced. The mechanism of ACZ might be related to changes in cytokine content. The reducing of the pro-inflammatory cytokines in rat lung might be other reason to explain ACZ against AMS. Our results indicated that the exposure to high altitude and the consequent hypoxia were able to alter a number of cellular and functional immunologic parameters.

Author contributions

C.W., R.W., Z.P.J. designed research, C.W., R.W., Y.H.S., R.T., W.Q. L. did the experimental research.

C.W., R.W. wrote the manuscript.

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