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Compound Danshen Dripping Pill inhibits high altitude-induced hypoxic damage by suppressing oxidative stress and inflammatory responses

Yunhui Hu[a]*, Jia Sun[a]* ![ORCID], Tongxing Wang[a], Hairong Wang[a], Chunlai Zhao[a], Wenjia Wang[a], Kaijing Yan[a,b,c], Xijun Yan[a,b,c] and He Sun[a,b,c]

[a]GeneNet Pharmaceuticals Co. Ltd, Tianjin, P.R. China; [b]The State Key Laboratory of Core Technology in Innovative Chinese Medicine, Tasly Academy, Tasly Holding Group Co., Ltd, Tianjin, China; [c]Tasly Pharmaceutical Group Co., Ltd, Tianjin, China

ABSTRACT

Context: Previous studies indicate that compound Danshen Dripping Pill (CDDP) improves the adaptation to high-altitude exposure. However, its mechanism of action is not clear.
Objective: To explore the protective effect of CDDP on hypobaric hypoxia (HH) and its possible mechanism.
Materials and methods: A meta-analysis of 1051 human volunteers was performed to evaluate the effectiveness of CDDP at high altitudes. Male Sprague-Dawley rats were randomized into 5 groups ($n = 6$): control at normal pressure, model, CDDP-170 mg/kg, CDDP-340 mg/kg and acetazolamide groups. HH was simulated at an altitude of 5500 m for 24 h. Animal blood was collected for arterial blood-gas analysis and cytokines detection and their organs were harvested for pathological examination. Expression levels of AQP1, NF-κB and Nrf2 were determined by immunohistochemical staining.
Results: The meta-analysis data indicated that the ratio between the combined RR of the total effective rate and the 95% CI was 0.23 (0.06, 0.91), the SMD and 95% CI of SO_2 was 0.37 (0.12, 0.62). Pre-treatment of CDDP protected rats from HH-induced pulmonary edoema and heart injury, left-shifted oxygen-dissociation curve and decreased P50 (30.25 ± 3.72 vs. 37.23 ± 4.30). Mechanistically, CDDP alleviated HH-reinforced ROS by improving SOD and GPX1 while inhibiting pro-inflammatory cytokines and NF-κB expression. CDDP also decreased HH-evoked D-dimer, erythrocyte aggregation and blood hemorheology, promoting AQP1 and Nrf2 expression.
Discussion and conclusions: Pre-treatment with CDDP could prevent HH-induced tissue damage, oxidative stress and inflammatory response. Suppressed NF-κB and up-regulated Nrf2 might play significant roles in the mechanism of CDDP.

Introduction

Acute high-altitude hypoxia affects the blood flow and the efficiency of oxygen utilization, causing multi-organ injury and ultimately leading to life-threatening high-altitude cerebral edoema (HACE) or high-altitude pulmonary edoema (HAPE) (Imray et al. 2010). Decreased barometric pressure and subsequent reduction in available oxygen are the primary causal factors in these medical conditions (Clarke 2006). Acetazolamide, dexamethasone and montelukast are widely used to prevent acute altitude sickness. However, they produce a variety of adverse effects, including headache, sensory abnormalities, cardiopalmus osteoporosis and increased risk of infection (Fagenholz et al. 2007; Nieto Estrada et al. 2017). Thus, there is an increasing need for the development of alternative therapies to treat and prevent these conditions.

Hypoxia rapidly increases the levels of free radicals in cells and whole-body hypoxia overwhelms the reserves of scavengers, leading to the accumulation of reactive oxygen species (ROS), the release of inflammatory factors and injury of red blood cells (RBCs), vascular endothelial cells (VECs) and other tissues, such as lung, brain and heart (Lisk et al. 2013). Therefore, it is important to identify effective antioxidant and anti-inflammatory drugs for the prevention of hypoxia-related disorders. Some studies indicate that antioxidant supplementation diminishes acute mountain sickness (AMS) incidence (Bailey and Davies 2001; Fan et al. 2013; Zhang et al. 2020), suggesting that antioxidants could be used as a protective measure against acute hypoxia injury.

Compound Danshen Dripping Pill (CDDP, T89, Dantonic®), which is a traditional Chinese medicine produced by combining traditional Chinese and modern medical technologies, contains *Saviae miltiorrhizae* Bunge (Lamiaceae), *Panax notoginseng* Burkill (Araliaceae) and borneol (Guo et al. 2016). Many studies have indicated the antioxidant and anti-inflammatory properties of CDDP in the cardiovascular system, and it has been widely used for the prevention and treatment of acute myocardial ischaemia and other cardiovascular diseases for over 25 years (Yao et al. 2015; Liang et al. 2018; Yao et al. 2019). Clinical studies indicate that CDDP can also improve oxygen saturation

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CONTACT He Sun ✉ henry@tasly.com; Yunhui Hu ✉ tsl-huyunhui@tasly.com 🖷 GeneNet Pharmaceuticals Co. Ltd, Tianjin, P.R. China
*Contributed equally.

ⓘ Supplemental data for this article can be accessed here.

(SO$_2$) and prevent or relieve AMS-related symptoms as well as hypoxia-induced tissue damage (Li, Li, et al. 2020; Li, Guo, et al. 2020). However, other CDDP protective mechanisms under acute high-altitude hypoxia remain elusive.

In the present study, we performed a meta-analysis of clinical studies in order to evaluate the efficacy of CDDP in maintaining SO$_2$ and relieving high-altitude hypoxia-related symptoms. Furthermore, using a rat model of high-altitude hypoxia we demonstrate that pre-treatment with CDDP prevents hypobaric hypoxia-induced tissue damage, oxidative stress and inflammatory response and offer data suggesting that this effect is due to suppression of NF-κB and up-regulation of AQP1 and Nrf2.

Materials and methods

Meta-analysis of randomized controlled trials

Data from randomized controlled trials reporting the use of CDDP for prevention or treatment of hypobaric hypoxia were obtained from China National Knowledge Infrastructure (CNKI) and PubMed databases. The search terms were 'Danshen Dropping Pills', 'Compound Danshen Dripping Pills', 'Fufang Danshen Diwan', 'T89', 'Dantonic' and 'Cardiotonic Pills'. As of April 30, 2021, six studies (Zhang et al. 2008; Tian et al. 2012; Liu and Zhang 2017; Feng 2019; Li, Li, et al. 2020; Li, Guo, et al. 2020) involving 1051 patients were obtained.

Two reviewers independently, and in duplicate, evaluated the risk of bias of the eligible studies according to the assessment tool of the Cochrane Handbook for Systematic Reviews of Interventions 4.2.2 (Higgins and Green 2011). The criteria included: (1) The sequence generation; (2) The allocation concealment; (3) The blinding of participants and personnel; (4) The blinding of outcome assessments; (5) Incomplete outcome data; (6) Selective reporting; and (7) Other sources of bias. A meta-analysis was conducted using Review Manager 5.3 (Cochrane; www.cochrane.org/). The risk ratio (RR) with a 95% CI was used for the improvement of symptoms, while the standardized mean difference (SMD) with a 95% CI was adopted for the change of SO$_2$.

Chemicals and reagents

CDDP was obtained from Tasly Pharmaceutical Co., Ltd. (Tianjin, China). Acetazolamide (ACTZ) was purchased from Shanghai Yuanye Biotechnology (Shanghai, China). Rabbit anti-AQP1 (A15030), NF-κB (A11201) and Nrf2 (A0674) polyclonal antibodies were purchased from ABclonal Biotechnology Co., Ltd (Wuhan, Hubei, China).

Animals, treatments and sample preparation

Male Sprague-Dawley rats were obtained from SBF Biotechnology (Beijing, China). Rats were distributed randomly in 5 groups (6 rats per group) and received treatment by intragastric administration at 10 mL/kg once a day for 3 d. Treatments consist of 0.5% sodium carboxymethylcellulose (CMC), CDDP (at 170 and 340 mg/kg, in 0.5% CMC) and 50 mg/kg ACTZ in 0.5% CMC. Rats in the control group (0.5% CMC) were left at atmospheric pressure, whereas animals in all other treatments, including a model group treated with only 0.5% CMC, were introduced in a hypobaric chamber (QTK-LP; IVD Biotechnology, Henan, China) for 24 h in order to simulate an altitude of 5500 m.

Rats were then anesthetized, blood collected from the abdominal aorta, sacrificed and their organs harvested. A summary of the experimental strategy is shown in Figure 1. Animals were housed under specific pathogen-free conditions with free access to water and food at the animal centre of Tianjin Pharmaceutical Research Institute in China. All animal experiments complied with the Guidance of the protocol IACUC-2020042603 approved by the Institutional Animal Care and Use Committee (IACUC) of Tianjin Pharmaceutical Research Institute in China.

Arterial blood-gas analysis and fitting of the oxygen-dissociation curve

Rat arterial blood (1 mL) was used for blood-gas analyses. The partial pressure of oxygen (PO$_2$), SO$_2$, and pH were detected at 37 °C by microelectrodes in a blood gas analyzer (iSTAT-300; Abbott Laboratories, Chicago, IL, USA). The oxygen-dissociation curve and the pressure of oxygen when SO$_2$ level reaches 50% (P50) was calculated according to the Hill equation (Balaban et al. 2013):

$$SO_2 = K \times PO_2{}^n / (1 + K \times PO_2) \quad (a)$$
$$K \times PO_2{}^n = SO_2 / (100 - SO_2) \quad (b)$$
$$\text{Log}_{10}\ SO_2 / (100 - SO_2) = \log_{10}\ K + n\ \log_{10} PO_2 \quad (c)$$

In Equation (a), K is the oxygen dissociation constant, and n is the Hill coefficient. We converted Equation (a) into Equation (b) mathematically. After taking the logarithm on both sides of Equation (b), we obtained Equation (c) for a linear equation in the form of $Y = aX + b$. When we made $Y = \log_{10} SO_2/(100 - SO_2)$ and $Y = \log_{10} PO_2$, the slope 'a' became the 'n' value of the Hill equation, and b the logarithmic value of k. We calculated n and K values using the data for PO$_2$ and SO$_2$ of each group. After the regression, and making PO$_2$ range from 0 to 100, the oxygen-dissociation curve was fitted and P50 was calculated.

Measurement of antioxidant status and inflammatory cytokines

To evaluate the antioxidant status, levels of ROS, TAOC, SOD and GPX1 were measured using commercial assay kits. Rat heart and lung tissues were weighed and converted to a cell suspension using a 300-mesh sieve. From each rat, whole blood (1 mL) was taken for a ROS test along with heart cells/lung cells following the manufacturer's instructions of a ROS assay kit (E004-1-1; Jiancheng Bioengineering Institute, Nanjing, China). Plasma was obtained by removing blood cells through centrifugation at 1800 g for 15 min at room temperature. TAOC was measured according to the instructions of a TAOC Assay Kit (A015; Jiancheng Bioengineering Institute, Nanjing, China). The concentrations of SOD, GPX1, NT-proBNP and inflammatory cytokines IL-1, IL-6, TNF-α, ICAM1, MMP9 were determined using commercial ELISA kits (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions (Supplementary Table S1), Samples from every rat were detected separately.

Red blood cell (RBC) aggregation index and hemorheology

Rheology tests were undertaken by using blood specimens to ascertain the viscosity of whole blood under a 'high' (1/200), 'medium' (1/60) and 'low' (1/10) shear rate using an automatic blood rheometer (LBY-6COMPACT, Precil Instrument Co., Ltd,
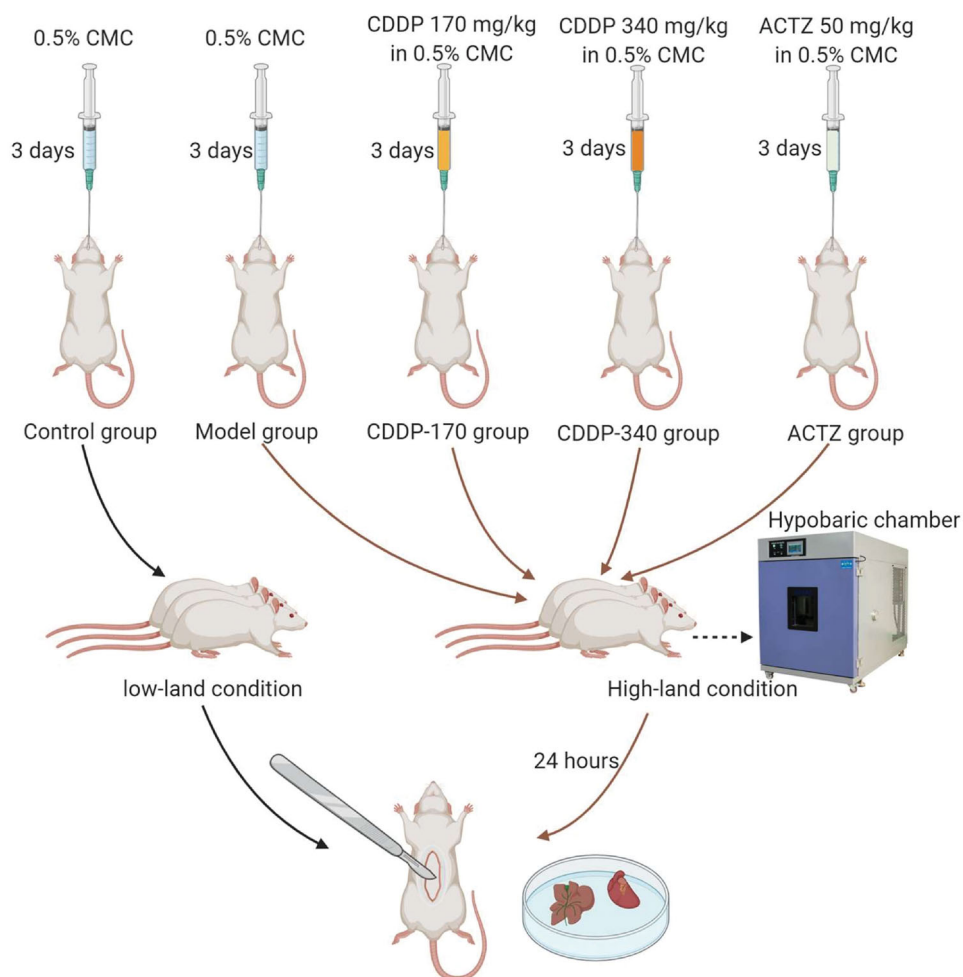
Figure 1. Workflow for a hypobaric hypoxic model establishment and experimental design of treatment for each group.

Beijing, China). The RBC aggregation index was calculated from the ratio of low shear to high shear viscosities. The concentration of D-Dimer was determined using a commercial assay kit according to the manufacturer's instruction (E029-1-1; Jiancheng Bioengineering Institute, Nanjing, China).

Haematoxylin and eosin (H&E) staining and immunohistochemical (IHC) detection

Rat heart and lung tissues were fixed in formalin and stained with H&E using standard histological procedures. Expression of AQP1, NF-κB and Nrf2 in these two organs was detected by standard IHC staining essentially as described (Hu et al. 2018). Three randomly selected fields were photographed with a Nikon Eclipse Ti-SR microscope (Nikon, Japan).

Statistical analyses

Values are expressed as mean ± SEM. All experiments were repeated at least 3 times independently. Initially, all data were conducted the normal distribution analysis with GraphPad Prism. The data followed normal distribution were then analyzed by a one-way ANOVA with Tukey post-test by SPSS software

(SPSS Inc., Chicago, IL, USA). Significant difference was considered if p-value < 0.05 ($n \geq 3$).

Results

CDDP attenuates hypobaric hypoxia symptoms and increases SO_2

Five trials were selected for the evaluation of CDDP effectiveness in alleviating hypobaric hypoxia symptoms. The meta-analysis indicated that the ratio between the combined RR of the total effective rate and the 95% CI was 0.23 (0.06, 0.91) (Figure 2(A)), suggesting that CDDP can significantly alleviate the symptoms of hypobaric hypoxia.

The changes of SO_2 due to CDDP treatment were reported in six trials. The meta-analysis indicated that the SMD and 95% CI of SO_2 was 0.37 (0.12, 0.62) (Figure 2(B)), suggesting that CDDP can significantly improve SO_2.

CDDP alleviates tissue damage and improves tissue oxygenation in rats exposed to acute hypobaric hypoxia

In order to investigate the function of CDDP in tissue damage caused by hypobaric hypoxia, we used a hypobaric chamber to
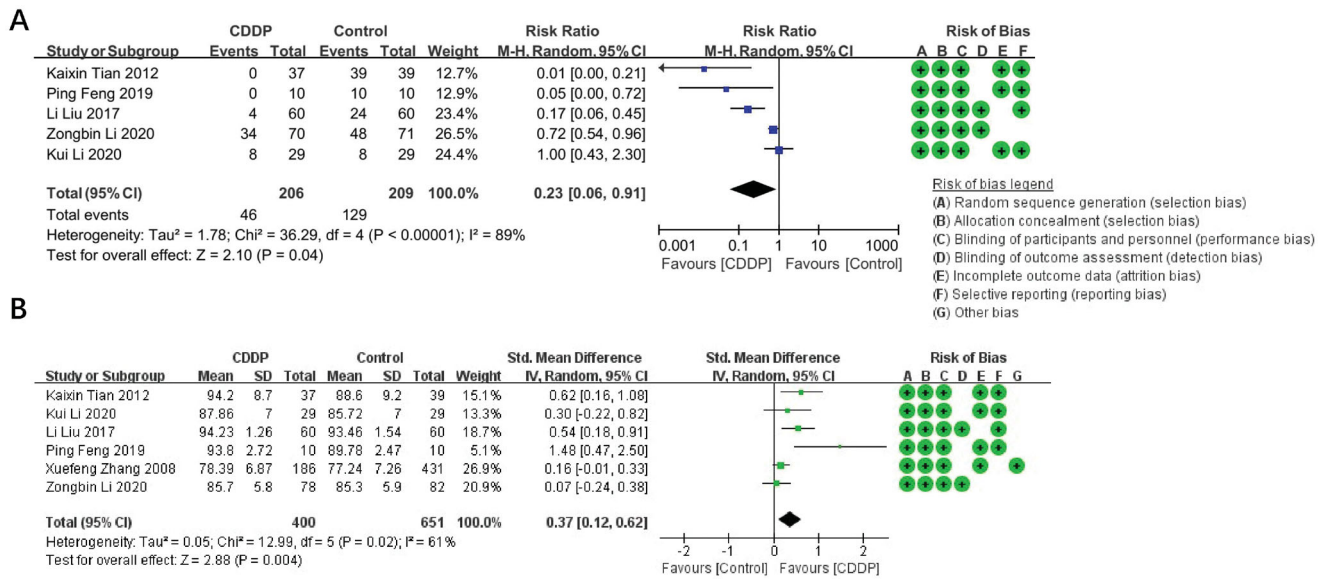
A

Study or Subgroup	CDDP Events	Total	Control Events	Total	Weight	Risk Ratio M-H. Random, 95% CI
Kaixin Tian 2012	0	37	39	39	12.7%	0.01 [0.00, 0.21]
Ping Feng 2019	0	10	10	10	12.9%	0.05 [0.00, 0.72]
Li Liu 2017	4	60	24	60	23.4%	0.17 [0.06, 0.45]
Zongbin Li 2020	34	70	48	71	26.5%	0.72 [0.54, 0.96]
Kui Li 2020	8	29	8	29	24.4%	1.00 [0.43, 2.30]
Total (95% CI)		206		209	100.0%	0.23 [0.06, 0.91]
Total events	46		129			

Heterogeneity: Tau² = 1.78; Chi² = 36.29, df = 4 (P < 0.00001); I² = 89%
Test for overall effect: Z = 2.10 (P = 0.04)

Risk Ratio M-H. Random, 95% CI — 0.001 0.1 1 10 1000 — Favours [CDDP] Favours [Control]

Risk of Bias A B C D E F

Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

B

Study or Subgroup	CDDP Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Kaixin Tian 2012	94.2	8.7	37	88.6	9.2	39	15.1%	0.62 [0.16, 1.08]
Kui Li 2020	87.86	7	29	85.72	7	29	13.3%	0.30 [-0.22, 0.82]
Li Liu 2017	94.23	1.26	60	93.46	1.54	60	18.7%	0.54 [0.18, 0.91]
Ping Feng 2019	93.8	2.72	10	89.78	2.47	10	5.1%	1.48 [0.47, 2.50]
Xuefeng Zhang 2008	78.39	6.87	186	77.24	7.26	431	26.9%	0.16 [-0.01, 0.33]
Zongbin Li 2020	85.7	5.8	78	85.3	5.9	82	20.9%	0.07 [-0.24, 0.38]
Total (95% CI)			400			651	100.0%	0.37 [0.12, 0.62]

Heterogeneity: Tau² = 0.05; Chi² = 12.99, df = 5 (P = 0.02); I² = 61%
Test for overall effect: Z = 2.88 (P = 0.004)

Std. Mean Difference IV, Random, 95% CI — -2 -1 0 1 2 — Favours [Control] Favours [CDDP]

Risk of Bias A B C D E F G

Figure 2. Effect of CDDP on the alleviation of hypobaric hypoxia symptoms (A) and improvement of SO₂ (B) in people exposed to hypobaric hypoxia and summary of bias risks: the reviewers' judgement of risk for each bias item in each included study.
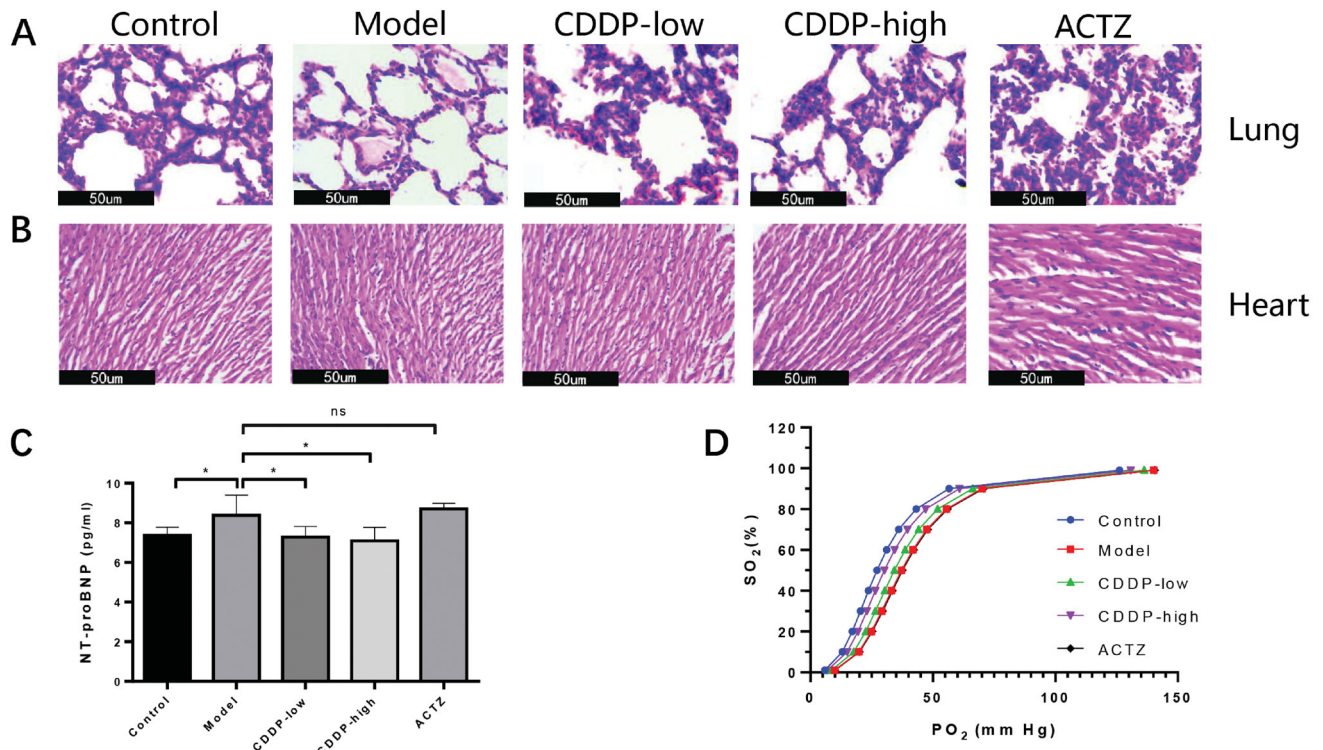

Figure 3. CDDP alleviates hypobaric hypoxia-induced tissue damage and improves tissue oxygenation. HE staining of rat lung (A) and heart (B) sections. Representative pictures from three randomly selected fields are shown. Black bar represents 50 µm. (C) Expression level of blood NT-proBNP. Statistical comparisons were made against the model group. Data indicate the average ± SEM of at least three independent experiments. *$p < 0.05$. (D) Oxygen dissociation curve determined by arterial blood gas analysis and calculated according to the Hill equation. Arterial blood gas values and oxygen-dissociation curve parameters are fully described in Table 1.

simulate high altitude low pressure. Exposure to hypobaric hypoxia for 24 h induced pulmonary edoema and vacuoles, which were alleviated by CDDP or ACTZ (Figure 3(A)). ACTZ is a drug approved by US Food and Drug Administration for AMS and used in our study as a reference control. Although no obvious damage could be observed in H & E staining of heart tissue (Figure 3(B)), expression of N-terminal pro-brain natriuretic peptide (NT-proBNP), an important marker indicating injured myocardium (Hall 2004), increased in the rats exposed to hypobaric hypoxia. However, CDDP administration, but not ACTZ, attenuated its increase significantly (Figure 3(C)).

Oxygen tension at 50% haemoglobin saturation (P50), which reflects the degree of peripheral oxygen offloading and tissue oxygenation, is a parameter that indicates the inverse effect of

Table 1. Arterial blood-gas values and oxygen-dissociation curve parameters.

Experiment groups	PO_2	SO_2	n	logK	P50
Control	87.67 ± 4.84	$97.00 \pm 0.63^*$	$2.99 \pm 0.15^{**}$	$4.29 \pm 0.37^{**}$	$27.19 \pm 3.16^{**}$
Model	73.33 ± 15.91	89.83 ± 5.91	3.479 ± 0.20	-5.45 ± 0.49	37.23 ± 4.30
CDDP-low	90.83 ± 12.07	95.33 ± 3.78	3.33 ± 0.25	-5.11 ± 0.62	34.28 ± 5.51
CDDP-high	$93.67 \pm 6.80^*$	$97.00 \pm 0.89^*$	$3.13 \pm 0.18^{**}$	$4.65 \pm 0.43^{**}$	$30.25 \pm 3.72^{**}$
ACTZ	68.67 ± 33.13	80.33 ± 18.73	3.37 ± 0.15	-5.20 ± 0.35	35.08 ± 3.10

All statistical comparisons were made against the model group. $^*p < 0.05$, $^{**}p < 0.01$
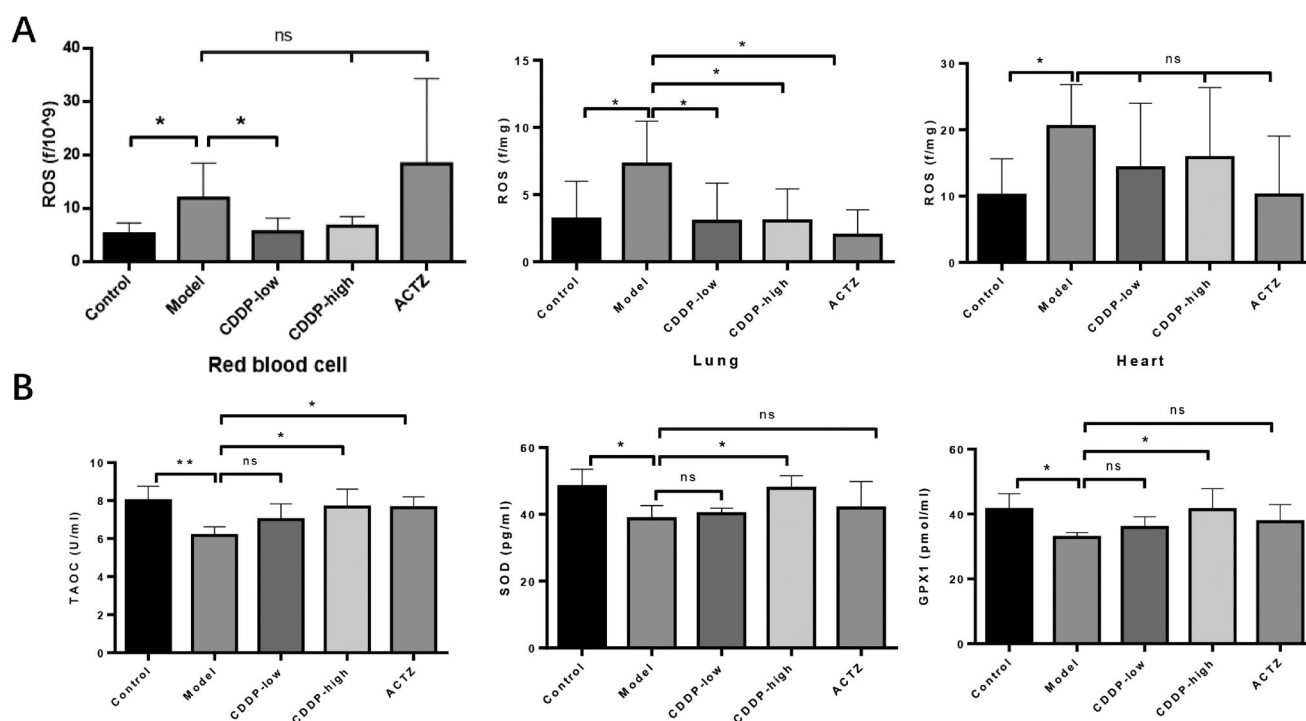
Figure 4. CDDP attenuates hypobaric hypoxia-induced oxidative stress. Blood, lung and heart samples collected from rats have conducted the following assays. (A) ROS levels determined by the assay kit. (B) Serum TAOC, SOD and GPX1 levels detected by the assay kit. Samples from every rat were detected separately. Data indicate the average $\pm$ SEM of at least three independent experiments. $n = 6$ of each group. $^*p < 0.05$, $^{**}p < 0.01$.

the affinity between haemoglobin and oxygen (Mairbäurl and Weber 2012) and is used to measure the development of hypobaric hypoxia symptoms. We observed that hypobaric hypoxia significantly increased P50 values and left-shifted the oxygen-dissociation curve in rats, which were reversed by CDDP in a dose-dependent manner (Figure 3(D) and Table 1), suggesting CDDP facilitates the improvement of tissue oxygenation. Importantly, the ACTZ administration didn't improve P50.

Thus, these data indicate that CDDP alleviates tissue damage and improves tissue oxygenation in rats exposed to acute hypobaric hypoxia.

CDDP antagonizes oxidative stress in rats during hypobaric hypoxia

In order to elucidate the mechanism underlying CDDP-mediated protective role in hypobaric hypoxic tissue damage, we explored the effects of CDDP on oxidative stress, which has been confirmed as responsible for the development of high altitude-triggered tissue damage (Dosek et al. 2007). Exposure to hypobaric hypoxia triggered the production of ROS relatively to the control group, which was inhibited following CDDP treatment in lung and red blood cells (high dose group), but not the heart. ACTZ treatment did not inhibit ROS formation in RBCs or the heart but did so in the lung (Figure 4(A)). We further investigated the

effect of CDDP treatment on the antioxidant status measured in plasma. ACTZ restored TAOC, but not SOD or GPX1 levels, but high dose CDDP treatment restored the levels of the three markers (Figure 4(B)). Thus, CDDP inhibits oxidative stress in rats during hypobaric hypoxia.

Treatment with CDDP blunts hypobaric hypoxia-evoked inflammatory response and thromboembolic risk in rats

In order to elucidate the mechanisms by which CDDP protects rats against hypobaric hypoxia, we explored CDDP effects on the inflammatory response. Inflammatory response, with increased inflammatory cytokine levels, is a critical cause of tissue damage under various adverse conditions including hypobaric hypoxia exposure (Wang et al. 2018). Increased TNF-α serum levels were detected in hypobaric hypoxia-exposed rats, but this elevation was blocked by CDDP, although not by ACTZ, pre-treatment (Figure 5(A)). CDDP also suppressed the increase in pro-inflammatory cytokines IL-1, IL-6, ICAM1 and MMP9 in rat serum upon hypobaric hypoxia condition. Importantly, ACTZ only suppressed IL-1 increase, but not IL-6, ICAM1 or MMP9 (Figure 5(A)). This indicates that CDDP inhibits the inflammatory response.

Some studies show evidence of thrombosis and procoagulatory activity under hypobaric hypoxia (Mannucci et al. 2002;
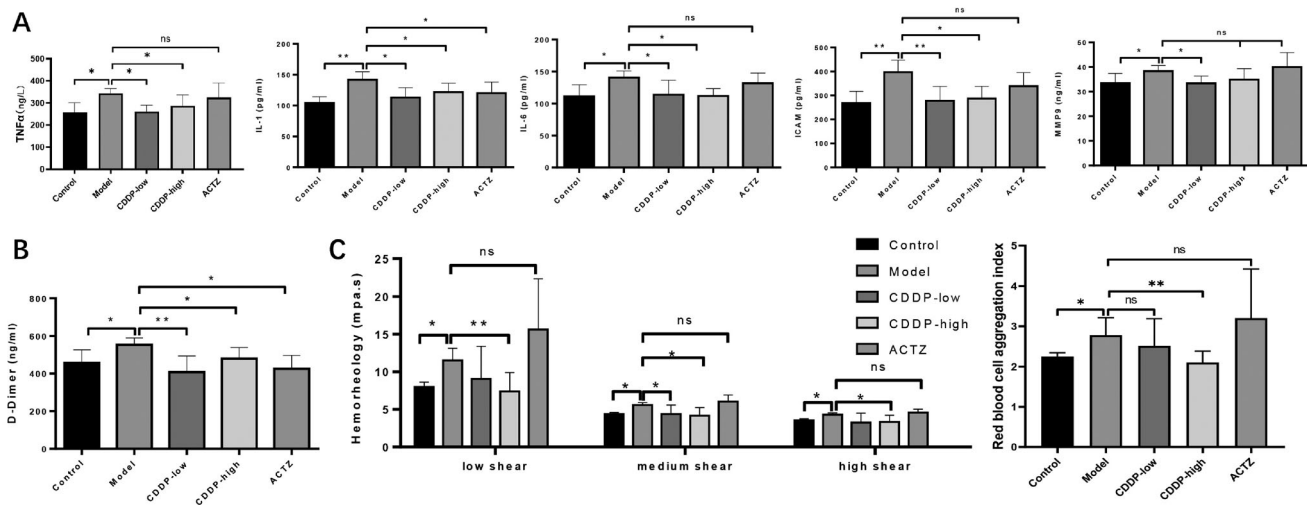
Figure 5. CDDP alleviates inflammation and blood viscosity in hypobaric hypoxia-stimulated rats. Determination of inflammatory cytokines concentration by ELISA (A) and D-Dimer levels determined by assay kit (B). (C) Blood hemorheology determined using a blood rheometer (left panel); Red blood cell aggregation index was calculated according to blood hemorheology (right panel). Samples from every rat were detected separately. Data indicate the average ± SEM of at least three independent experiments. $n = 6$ of each group. $*p < 0.05$, $**p < 0.01$.

Torgovicky et al. 2005). Elevated D-dimer, RBCs aggregation and rheology parameters have been demonstrated to be involved in thrombosis in various disease models including hypobaric hypoxia exposure (Barshtein et al. 2007; Guilbert et al. 2017). In order to explore the effect of CDDP pre-treatment on these parameters, we measured D-dimer concentration, erythrocyte aggregation and blood hemorheology in our rat hypobaric pressure model system. Results indicate that both CDDP and ACTZ restored the elevation of D-dimer levels due to hypobaric hypoxia, but only high dose CDDP pre-treatment reduced the increase in hypobaric hypoxia-triggered RBC aggregation to levels found in the control group maintained at normal pressure (Figure 5(B)). Importantly, CDDP, but not ACTZ, pre-treatment abolished the increase in RCB aggregation due to hypobaric hypoxia (Figure 5(C)). Thus, pre-treatment with CDDP in this rat model of hypobaric hypoxia reduces the risk of thrombosis and severity of hypoxia.

Identification of CDDP-protected targets against hypobaric hypoxia-induced tissue injury

In order to shed some further light on the possible mechanisms for CDDP protection against hypobaric hypoxia in our rat model, we determined the expression of several markers by IHC. AQP1 has a protective role by attenuating tissue edoema and inflammation (Dong et al. 2012; Ding et al. 2013). Nrf2 is a key transcription factor in anti-oxidative stress (Lisk et al. 2013). NF-κB is a heterodimeric protein composed of members of the Rel family of transcription factors and can induce a series of pro-inflammatory cytokines, including TNF-α, IL-1, IL-6 and MMP9 (Hsieh et al. 2014; Pan et al. 2020). Our results show that CDDP pre-treatment increases the levels of AQP1 and Nrf2 in both pulmonary and heart tissues (Figure 6). Importantly, and corroborating the anti-inflammatory action of CDDP demonstrated above (Figure 5(A)), NF- κB levels did not increase due to hypobaric hypoxia in the heart and lungs of rats pre-treated with CDDP (Figure 6). Thus, the anti-inflammatory action of CDDP can be attributed to its NF-κB inhibitory capacity.

Discussion

Hypobaric hypoxia, which causes insufficient oxygen availability, leads to multi-organ injury and irreversible tissue damage. Mechanistically, this has been attributed to the large number of oxygen radicals produced by hypobaric hypoxia (Dosek et al. 2007). In clinical trials, CDDP has been confirmed to maintain SO_2 and to decrease the incidence of AMS (Li et al. 2020). In the present study, the beneficial efficacy of CDDP in improving the adaptation to high altitude exposure was further evaluated through meta-analysis and the anti-hypoxic effects of CDDP pre-treatment were confirmed in a rat model of hypobaric hypoxia.

CDDP has been approved by the China Food and Drug Administration for the treatment of coronary heart disease (CHD) for more than 25 years. Numerous studies have demonstrated the therapeutic properties of CDDP through promoting blood circulation, reducing blood viscosity, inhibiting leukocyte adhesion and inflammation, protecting endothelial cells and optimizing myocardial energy metabolism to ameliorate angina symptoms caused by myocardial ischaemia (hypoxia) (Liang et al. 2018; Yao et al. 2019). Oxidative stress and inflammation caused by the imbalance between demand and supply of oxygen are two common pathophysiological processes shared by myocardial ischaemia and hypobaric hypoxia. This suggests that the protective effects of CDDP against acute high-altitude hypoxia and CHD might share common regulatory mechanisms. Our meta-analysis results indicate a beneficial effect of CDDP in alleviating hypobaric hypoxia symptoms. The incidence of hypobaric hypoxia-related syndromes was reduced, whilst SO_2 increased significantly in people treated with CDDP. As hypoxia is believed to be the main factor in AMS occurrence and development (Roach and Hackett 2001; Li et al. 2018), we propose that SO_2 recovery is one of the main contributors to reducing acute high-altitude hypoxia. However, the number of studies used for the analysis is relatively small and many suffer from high heterogeneity. In addition, all the studies used Chinese participants and it is thus unclear whether these results can be extrapolated to other ethnicities. Thus, our conclusions should be treated with a certain amount of caution until larger studies are performed.

In the present study, an acute hypobaric hypoxia model was successfully established, including SO_2 dropping and antioxidant
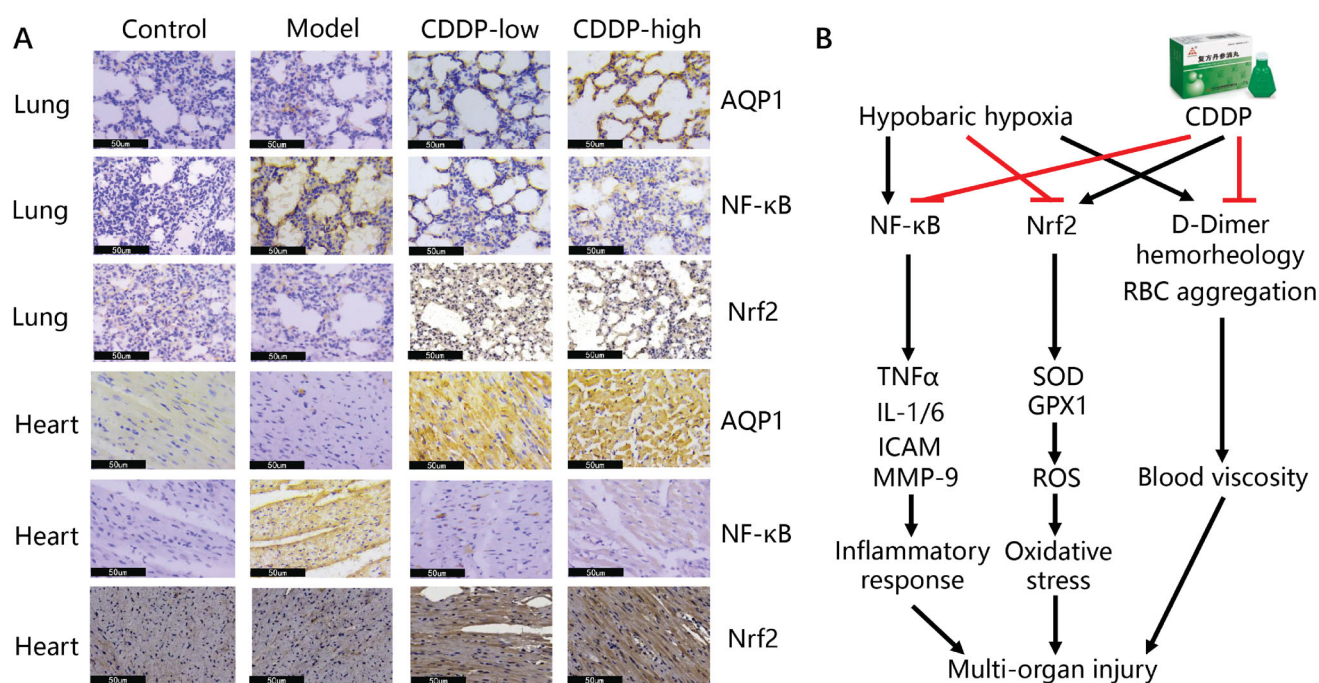
Figure 6. (A) Identification of the CDDP-protected targets against hypobaric hypoxia. Lung and heart samples collected from rats were fixed in formalin, embedded in paraffin, and sectioned for IHC staining using the corresponding antibodies. Representative pictures from three randomly selected fields are shown. Black bar represents 50 μm. (B) Mode of action of CDDP in treating hypobaric hypoxia.

defense system dysfunction. In line with previous clinical studies, pre-treatment with CDDP helped maintain SO_2/PO_2, and left-shifted the oxygen-dissociation curve, indicating CDDP increased the oxygen affinity of haemoglobin which would augment arterial SO_2 during hypoxia. We found that hypobaric hypoxia decreases oxygen affinity, leading to increased ROS accumulation and inflammatory factors generation (Ciaccio et al. 2017; Pajuelo Reguera et al. 2020). Importantly, pre-treatment with CDDP exerted strong antioxidant capacity through promoting TAOC and expressions of SOD and GPX1, two important antioxidant enzymes to scavenge ROS in the whole body (Wei et al. 2001). In addition, CDDP counteracted the enhanced inflammation in acute hypobaric hypoxic rats by muting pro-inflammatory cytokines MMP-9, ICAM1, IL-1, IL-6 and TNFα levels. CDDP administration has been demonstrated to ameliorate inflammation and oxidative stress in several myocardial ischaemia animal models (Wei et al. 2013; Han et al. 2017), diabetic mice (Zheng et al. 2011; Zhou et al. 2015; Lu et al. 2016), as well as in the serum of patients who suffered by acute myocardial infarction (A et al. 2020), suggesting that the antioxidant and anti-inflammation capacities of CDDP open a wide window of therapeutic interventions.

It has been previously reported that coagulation parameters and blood rheology change significantly during hypobaric hypoxia (Reinhart et al. 1991; Toff et al. 2006; Jacqueline et al. 2010), in line with results of epidemiological studies indicating that high altitude poses a risk for developing thrombosis (Yanamandra et al. 2020). Previous research has indicated that *Salvia miltiorrhiza* and pseudo-ginseng (*Panax notoginseng*), the two main ingredients of CDDP, are effective in promoting blood circulation and dispersing stasis. Moreover, pharmacological studies have demonstrated that they reduce blood viscosity, accelerate erythrocyte flow, inhibit platelet adhesion and aggregation, and regulate both internal and external blood coagulation (Han et al. 2017; Li et al. 2018; Jia et al. 2019; Ren et al. 2019).

In agreement with this large body of evidence, and using a rat hypobaric hypoxia model system, we find that exposure to hypobaric hypoxia causes elevated D-Dimer, erythrocyte aggregation index and parameters of blood rheology, which decreased to a normal level after CDDP treatment, especially in the high dose group. This suggests that the role of CDDP modulating coagulation parameters and blood rheology is not limited to cardiovascular disease, but operates also in acute high-altitude hypoxia.

Regarding the mechanism of CDDP's protective role in hypobaric hypoxia, we suggest that inhibition of NF-κB and increase in AQP1 and Nrf2 may play important roles. AQP1 provides the principal route for osmotically driven water transport across the epithelial and endothelial barriers and plays an important role in edoema formation during hypoxia (Li et al. 2015; Tan et al. 2020). In addition, AQP1 reduces pulmonary and myocardial edoema to protect lung and cardiac function (Li et al. 2011; Li et al. 2015). Moreover, dysregulated NF-κB and Nrf2 pathways caused by hypoxia have also been demonstrated to induce oxidative stress and inflammatory response (Tripathi et al. 2019; Xiang et al. 2020). Therefore, inhibiting NF-κB or activating Nrf2 alleviates high altitude-induced tissue damage via suppressing oxidative stress and inflammatory response (Lisk et al. 2013; Gong et al. 2018; Pan et al. 2020). Results from our study using a rat model of hypobaric hypoxia indicate that CDDP exhibits wide synergistic effects against hypobaric hypoxia via multiple targets and highlights the holistic approach of traditional Chinese medicine.

ACTZ is the only drug approved by US Food and Drug Administration for AMS, although it has several side effects such as drowsiness, loss of appetite, nausea, vomiting, and diarrhoea (Collier et al. 2016; Harrison et al. 2016; Ono et al. 2017). In the present study, ACTZ was used as a positive control to compare the effects of CDDP, but ACTZ was not as effective as CDDP. In line with these results, ACTZ neither degrades hypoxia-induced H_2O_2, even with 200 mg/kg treatment in rats (Fan et al.

2013) nor activates Nrf2 both *in vitro* or *in vivo* (Lisk et al. 2013). Other studies also reported that oxygen saturation does not change following ACTZ treatment both in rats (Wang et al. 2016) and healthy volunteers (Hackett et al. 1985). It has been indicated that the mechanism by which ACTZ protects from hypoxia may be related to the reduction of several pro-inflammatory cytokines (Wang et al. 2016), which is consistent with our experimental results, suggesting that ACTZ and CDDP mechanisms of action may not be identical. Our data indicate that CDDP has a stronger antioxidant efficacy than ACTZ and offers a strong rationale for promoting CDDP use for people under the harsh environment of high-altitude hypoxia. However, we have used only one animal model and set of conditions not leading to severe pulmonary edoema, brain edoema, myocardial damage or death caused by hypobaric hypoxia. Therefore, further investigations focussing on the protective effects and related mechanisms of CDDP on HAPE, HACE or mortality are required.

Conclusions

Pre-treatment with CDDP prevents hypobaric hypoxia-induced tissue damage, oxidative stress and inflammatory response in a rat model of high-altitude hypoxia. Suppressed NF-κB and up-regulated Nrf2 may play a role in CDDP mechanisms of action.

ORCID

Jia Sun http://orcid.org/0000-0002-6532-962X

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