Nitric oxide (NO) may act as either a pro-oxidant or an antioxidant in biological systems. Previous work has found inhalation of NO improved survival in a high altitude rat model. NO donor isosorbide mononitrate derivatives might have a protective effect against hypoxia. We synthesized a series of isosorbide mononitrate derivative compounds to test their anti-hypoxia activities. Normobaric hypoxia and hypobaric hypoxia models were used to study the protective role of NO donor in mice. The results showed isosorbide mononitrate derivatives had protective effects in hypoxia mice. Among those compounds, acetyl ferulic isosorbide mononitrate (AFIM) was the most effective. It prolonged the survival time during the normobaric hypoxia test. It decreased malondialdehyde (MDA) and H$_2$O$_2$ in hypobaric hypoxia mice. The antioxidant activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) remained in normal ranges in the AFIM group. As a sign of mitochondrial dysfunction, the activities of ATPase were down regulated in mice under hypobaric hypoxia conditions. AFIM also protected ATPase activities. The protective effects of AFIM might come from a sustained NO supply and the release of acetyl ferulic acid with anti-oxidant activity.

Key words altitude; hypoxia; nitric oxide; acetyl ferulic isosorbide mononitrate

More than 140 million persons lives at above 2500 m (the conventional definition of high altitude as that where arterial O$_2$ saturation (SaO$_2$) measurably begins to fall). Barometric pressure falls with increasing altitude and consequently there is a reduction in the partial pressure of oxygen resulting in a hypoxic challenge to any individual ascending to altitude. With the ever increasing number of people ascending to high altitudes, medicines to deal with potential problems are becoming increasingly relevant to non-specialists, including general practitioners.

A spectrum of high altitude illnesses can occur when the hypoxic stress outstrips the subject’s ability to acclimatize. Acute mountain sickness (AMS) and high-altitude cerebral edema (HACE) strike people who travel too fast to high altitudes that lie beyond their current level of acclimatization. AMS is a condition affecting otherwise healthy individuals on going rapidly to altitude. A spectrum of symptoms related to acute mountain sickness may develop at altitudes below 3000 m: commonly reported symptoms are nausea, vomiting, headache, dizziness, fatigue, and sleep disturbance. Acute hypoxia induces pulmonary vascular permeability and contributes to forms of noncardiogenic pulmonary edema such as high altitude pulmonary edema and acute respiratory distress syndrome. AMS can sharply limit recreation and work at high altitude, especially in the first few days following arrival at a new, higher altitude, and if AMS worsens and HACE develops, the risk of fatality is significant. Although slow ascent to altitude remained the most important measure to prevent acute mountain sickness, evidence from the literature on high altitude suggested that drugs could complement gradual ascent in preventing acute mountain sickness. Treatment typically occurred on the mountainside, but seeking to prevent acute mountain sickness begins before the ascent to high altitude (generally >3000 m). More and more studies were concerned on the prophylaxis and therapy of AMS.

Nitric oxide (NO) is a gaseous signaling molecule that participates in a large variety of physiological functions and may have a role in the pathology of altitude illnesses, such as acute mountain sickness (AMS). It plays an important role in people’s adaptation to high altitude hypoxia. Erzurum et al. found Tibetans had 10-fold-higher circulating concentrations of bioactive nitric oxide products in comparison to sea level controls, which suggested that NO production was increased and that metabolic pathways controlling formation of NO products were regulated differently among Tibetans. Those findings shifted attention from the traditional focus on pulmonary and hematological systems to vascular factors contributing to adaptation to high-altitude hypoxia. Hoit et al. found that NO in the lung might play a key beneficial role in allowing Tibetans at 4200 m to compensate for ambient hypoxia with higher pulmonary blood flow and O$_2$ delivery without the consequences of higher pulmonary arterial pressure. Macinnis et al. found that subjects who developed AMS had a significantly lower mean exhaled NO at baseline compared to resistant subjects. Respiratory NO synthesis was suggested to represent a protective mechanism against hypoxic pulmonary hypertension.

Whether supplement of exogenous NO was beneficial or futile remained uncertain. Scherrer et al. found that the inhalation of NO improved arterial oxygenation in high-altitude pulmonary edema, and the beneficial effect might be related to its favorable action on the distribution of blood flow in the lungs. Inhaled NO improved survival in high altitude pulmonary edema (HAPE) rat model and might have a therapeutic role in the management of HAPE. The combined use of inhaled NO and oxygen had additive effects on pulmonary hemodynamic and even greater effects on gas exchange. Whether intravenous administration NO donor compounds
had the same beneficial effects as inhaled NO for circulatory system and nervous system remained unclear. For answer this question we synthesized 6 NO donor compounds as source of exogenous NO and screened their anti-hypoxia activities by the survival time of the mice under normobaric hypoxia. Among those compounds, acetyl ferulic isosorbide mononitrate (AFIM) was the most effective one. For answer this question we synthesized 6 NO donor compounds as source of exogenous NO and screened their anti-hypoxia activities by the survival time of the mice under normobaric hypoxia. Among those compounds, acetyl ferulic isosorbide mononitrate (AFIM) was the most effective one. For answer this question we synthesized 6 NO donor compounds as source of exogenous NO and screened their anti-hypoxia activities by the survival time of the mice under normobaric hypoxia. Among those compounds, acetyl ferulic isosorbide mononitrate (AFIM) was the most effective one. 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to compare the anti-hypoxic activity. As soon as the mouse stopped breathing, the thorax was opened, about 0.5 mL blood sample was withdrawn from heart and 0.4 mL was added to the centrifuge tube citrate-stabilized with 3% sodium citrate. The mixture was centrifuged at 2500 g for 5 min. The plasma was collected and used to determine the concentration of lactic acid and lactate dehydrogenase.

**Lactic Acid (LD), LD Accumulation Rate and LDH Assessment** To assess the LD, LD accumulation rate and LDH, blood samples were collected, centrifuged and kept at −20°C until analyses. Standard techniques using commercialized assay kits according to the manufacturer’s instructions (Nanjing Jiancheng Biotechnology Institute, China) were performed for analysis. LD accumulation rate were calculated as LD/survival time. LD values were expressed as mmol/L. LD accumulation rates were expressed as μmol/L·min. LDH values were expressed as U/L.

**Effect of NO Donors on Heart Rate and Blood Pressure of Rats under Hypobaric Hypoxia Test** We used large low pressure oxygen compartment (Guizhou Fenglei, China) to stimulate high-altitude condition. NO donors and ACZ were administered as mentioned above. Twenty minutes after being administered with NO donors or vehicle by vein, rats except normal control group were put into the hypobaric hypoxia compartment and decompressed at a speed of 100 m/min. At last the simulated altitude of 8000 m was obtained. Rats were adapted to this hypobaric hypoxia environment (8% oxygen and 92% nitrogen, 0.4 MPa) for 12 h and then recovered to altitude of 4500 m (100 m/min, 0.06 MPa). Meanwhile the experimenters entered the large low pressure oxygen compartment (400 g, 60 s) at 4°C in cold buffer, w/v containing 0.01 mol/L Tris–HCl, 0.1 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.01 mol/L sucrose, 0.8% saline. The tubes with homogenate were kept in ice water for 30 min and centrifuged at 4°C (2500 g, 10 min), as recommended in the assay kits. The supernatant was separated, and then stored at −80°C. Supernatant was used for assay of various enzymatic activities. Measurement of protein concentration was estimated using commercial BCA assay kits (Nanjing Jiancheng Institute, China). The activities of SOD, GSH-Px, CAT and ATPase were measured using commercial assay kits (Nanjing Jiancheng Institute, China) according to the manufacturer instructions. Briefly, SOD activities were measured following the reduction of nitrite by a xanthine–xanthine oxidase system which was a superoxide anion generator. The activities were expressed as U/mg protein. GSH-Px activities were assayed by the decrease of the GSH, which can be reflected by the alteration of the absorbance at 412 nm. CAT activities were determined by decrease of H₂O₂ absorption at 405 nm. The activities of SOD, GSH-Px, CAT and ATPase were expressed as U/mg protein, U/g protein, U/mg protein and μmol Pi/mgprot/h, respectively.

**Statistical Analysis** All data were expressed as the mean±standard deviation (S.D.) Data was subjected to ANOVA followed by Student–Newman–Keuls tests. p≤0.05 was considered significant.

**RESULTS**

To determine the protective capability of NO donor against hypoxia, we administered mice with three concentrations of AFIM and subjected them to oxidative challenge via normobaric hypoxia and hypobaric hypoxia. Our initial aim was to prove whether NO donor compounds could protect mice against oxidative stress caused by high altitude hypoxia. Our study was to estimate the protective effect of the AFIM in vivo compared to a commonly used anti-hypoxia drug, ACZ, and also to identify an appropriate dose that offers beneficial effects with no toxicity. To test whether AFIM could protect mice through the hypobaric hypoxia progress, we carried out both pathology and biochemistry assay to determine the morphology and physiology change during the test.

**Synthesis of NO Donors** The compound was synthesized according to above method.

Compound A: (1S,4S,5S,8R)-8-Nitroxy-2,6-dioxabicyclo-[3.3.0]octan-4-yl-3-(4-acetoxyphenyl)acyrlate

Yield: 83%; mp: 78–79°C; ¹H-NMR: 7.69 (d, 1H, J=15.6 Hz,
H-C=C), 7.54 (d, 2H, J=7.8 Hz, Ar-H), 7.13 (d, 2H, J=7.8 Hz, Ar-H), 6.36 (d, 1H, J=16.2 Hz, C=C-H), 5.37 (s, 2H, Cy-H), 5.04 (s, 1H, Cy-H), 4.57 (s, 1H, Cy-H), 4.11–4.12 (m, 1H, Cy-H), 4.06–4.07 (m, 2H, Cy-H), 3.94 (m, 1H, Cy-H), 2.33 (s, 3H, CH$_3$); IR (cm$^{-1}$): 2935, 1768, 1713, 1610, 1508, 1276, 1251, 1166, 1098, 1098, 911, 851, 750; electrospray ionization (ESI)-MS [M+H]$^+$: 380.1; Anal. Calcd for C$_{17}$H$_{17}$NO$_3$: C, 53.83; H, 4.52; N, 3.69. Found: C, 53.89; H, 4.43; N, 3.58%.

Compound B: (1S,4S,5S,8R)-8-Nitrooxy-2,6-dioxabicyclo[3.3.0]octan-4-yl-3-(methoxyl-4-acetoxyphenyl)acrylate

Yield: 85%; mp: 162–163°C; $^1$H-NMR: 7.66 (d, 1H, J=16.2 Hz, H-C=C), 7.05–7.13 (m, 3H, Ar-H), 6.37 (d, 1H, J=16.2 Hz, C=C-H), 5.38 (s, 2H, Cy-H), 5.04 (s, 1H, Cy-H), 4.57 (m, 1H, Cy-H), 4.11–4.13 (m, 1H, Cy-H), 4.05–4.07 (m, 2H, Cy-H), 3.94–3.95 (m, 1H, Cy-H), 3.85 (s, 3H, OCH$_3$); IR (cm$^{-1}$): 2930, 1760, 1710, 1635, 1625, 1503, 1424, 1270, 1070, 1040, 915, 855, 750; ESI-MS [M+H]$^+$: 410.1; Anal. Calcd for C$_{17}$H$_{17}$NO$_3$: C, 52.81; H, 4.68; N, 3.42. Found: C, 52.91; H, 4.65; N, 3.35%.

Compound C: (1S,4S,5S,8R)-8-Nitrooxy-2,6-dioxabicyclo[3.3.0]octan-4-yl-3-(3,4-diaceotoxyphenyl)acrylate

Yield: 86%; mp: 130–131°C; $^1$H-NMR: 7.64 (d, 1H, J=16.2 Hz, H-C=C), 7.40 (d, 1H, J=8.4 Hz, Ar-H), 7.36 (s, 1H, Ar-H), 7.23 (d, 1H, J=8.4 Hz, Ar-H), 6.37 (d, 1H, J=16.2 Hz, C=C-H), 5.37 (s, 2H, Cy-H), 5.03 (s, 1H, Cy-H), 4.56 (s, 1H, Cy-H), 4.10–4.12 (m, 1H, Cy-H), 4.04–4.07 (m, 2H, Cy-H), 3.92–3.94 (m, 1H, Cy-H), 2.31 (s, 3H, CH$_3$); IR (cm$^{-1}$): 2917, 1770, 1718, 1636, 1507, 1424, 1285, 1258, 1170, 1095, 1060, 916, 854, 766; ESI-MS [M+H]$^+$: 438.3; Anal. Calcd for C$_{17}$H$_{17}$NO$_3$: C, 52.18; H, 4.38; N, 3.20. Found: C, 52.26; H, 4.33; N, 3.06%.

Compound D: (1S,4S,5S,8R)-8-Nitrooxy-2,6-dioxabicyclo[3.3.0]octan-4-yl-4-acetoxybenzoate

Yield: 87%; mp: 104–105°C; $^1$H-NMR: 8.05 (d, 2H, J=7.8 Hz, Ar-H), 7.18 (d, 2H, J=8.4 Hz, Ar-H), 5.47 (s, H, Cy-H), 5.38 (s, H, Cy-H), 5.06 (s, 1H, Cy-H), 4.62 (s, 1H, Cy-H), 4.12–4.16 (m, 1H, Cy-H), 4.06–4.10 (m, 2H, Cy-H), 3.94 (m, 1H, Cy-H), 2.33 (s, 3H, CH$_3$); IR (cm$^{-1}$): 2952, 1756, 1714, 1639, 1601, 1504, 1415, 1280, 1220, 1198, 1115, 1015, 920, 864, 766; ESI-MS [M+H]$^+$: 354.0; Anal. Calcd for C$_{17}$H$_{17}$NO$_3$: C, 51.00; H, 4.28; N, 3.96. Found: C, 51.08; H, 4.33; N, 3.92%.

Compound E: (1S,4S,5S,8R)-8-Nitrooxy-2,6-dioxabicyclo[3.3.0]octan-4-yl-3-(3,4-diacetoxyphenyl)acrylate; G: Isosorbide mononitrate. Each group represents the mean ± S.D. *p<0.05 vs. Vehicle, **p<0.01 vs. Vehicle.

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Table 1. Effects of NO Donor Compounds on the Survival Time of Mice under Normobaric Hypoxia Condition ($n=10$)

| Name                  | Dose (mg/kg) | Survival time (min) | Prolonged rate (%) |
|-----------------------|-------------|--------------------|--------------------|
| Vehicle               | —           | 30.1 ± 1.2         | —                  |
| Acetazolamide         | 200         | 38.2 ± 5.1*        | 26.9               |
| A                     | 100         | Death              | —                  |
| B                     | 50          | 35.9 ± 5.1*        | 19.3               |
| C                     | 75          | 47.8 ± 9.8**       | 58.8               |
| D                     | 100         | 49.5 ± 10.5**      | 64.5               |
| E                     | 100         | 39.8 ± 11.0*       | 25.6               |
| F                     | 100         | 37.5 ± 5.4*        | 24.6               |
| G                     | 100         | 34.7 ± 3.2         | 8.3                |
|                     | 40          | 48.6 ± 14.6**      | 61.5               |
|                     | 40          | 44.7 ± 11.5*       | 48.5               |

A: (1S,4S,5S,8R)-8-Nitrooxy-2,6-dioxabicyclo[3.3.0]octan-4-yl-3-(4-acetoxyphenyl)acrylate; B: (1S,4S,5S,8R)-8-Nitrooxy-2,6-dioxabicyclo[3.3.0]octan-4-yl-3-(3-acetoxyphenyl)acrylate; C: (1S,4S,5S,8R)-8-Nitrooxy-2,6-dioxabicyclo[3.3.0]octan-4-yl-3-(3,4-diacetoxyphenyl)acrylate; D: (1S,4S,5S,8R)-8-Nitrooxy-2,6-dioxabicyclo[3.3.0]octan-4-yl-4-acetoxybenzoate; E: (1S,4S,5S,8R)-8-Nitrooxy-2,6-dioxabicyclo[3.3.0]octan-4-yl-3-(3-methoxyl-4-acetoxyphenyl)acrylate; F: (1S,4S,5S,8R)-8-Nitrooxy-2,6-dioxabicyclo[3.3.0]octan-4-yl-3-(3-methoxyl-4-triaceotoxybenzoate; G: Isosorbide mononitrate. Each group represents the mean ± S.D. *p<0.05 vs. Vehicle, **p<0.01 vs. Vehicle.
AFIM significantly decreased the lactic acid accumulation rate comparing with vehicle31 (Fig. 3). The decrease rates of lactic acid accumulation rate were 13.6% (low dose), 61.2% (middle dose), 35.2% (high dose) and 17.9% (positive control) compare with model, respectively.

**Activity of Lactate Dehydrogenase** The activity of lactate dehydrogenase was coincidence with the trend of lactic acid accumulation rate. The treatment with the AFIM signifi-
cantly decreased the lactate dehydrogenase activity comparing with model (Fig. 3).

**MDA** The level of malonaldehyde was coincidence with the trend of lactic acid accumulation rate. The treatment with the AFIM significantly decreased the MDA comparing with model (Fig. 4).

**AFIM Directly Degrade Hypoxia-Induced H$_2$O$_2$ Production in Hypobaric Hypoxia Mice** We determined production of H$_2$O$_2$ as an indication of ROS formation in mice brains and hearts after 6h hypobaric hypoxia test. Hypoxia induced oxidative stress stimulated cerebrum and myocardium to increase H$_2$O$_2$ production compared to normal control. Treatment with AFIM significantly decreased H$_2$O$_2$ production in myocardium in hypobaric hypoxia mice model (Fig. 4). The degrading rate of H$_2$O$_2$ in AFIM 100 mg/kg group was 29.1% in mice myocardium compared with vehicle group.

**Effect of the AFIM on the Activities of SOD, GSH-Px, CAT and ATP in Hypoxic Mice** As the biomarker of the antioxidant defenses, the activity of SOD in cerebrum and myocardium, GSH and CAT in liver was measured (Fig. 5). The activities of SOD were conspicuous decreased in cerebrum and myocardium in vehicle. Activity of GSH-Px was also descent significantly. AFIM protected SOD activity in mice cerebrum compared with the vehicle group. There is no reduced GSH deficiency observed throughout the trial period at high doses of AFIM and ACZ compared with the vehicle group. The activity of CAT was increased compared with the control group. It was not descent but ascent, which probable because of the positive feedback regulation of H$_2$O$_2$. These results may indirectly indicate that the antioxidant enzymes in AFIM group had good antioxidant activities even after 12h hypobaric hypoxia test.

**Reduction of Cerebrum and Myocardium ATP Activities in Hypoxic Mice** Mice cerebrum and myocardium ATP activities were examined using a luciferase assay kit. The biochemical activities of Na$^+$-K$^+$-ATPase, Mg$^{2+}$-ATPase and Ca$^{2+}$-ATPase in mice cerebrum and myocardium were significantly lower in vehicle group after 12h hypoxia treatment compared with normal control group. On the contrary AFIM lessened the decrement of three kinds of ATPase in hypoxic mice cerebrum and myocardium compare to vehicle group. The data showed that AFIM had a dose-dependent effect (Fig. 6).

**DISCUSSION**

Previous work had found inhalation of NO improved survival in the rat model of HAPE, improved arterial oxygenation in high-altitude pulmonary edema, and may have a therapeutic role in the management of HAPE. The combined using of inhaled NO and oxygen had additive effects on pulmonary hemodynamic and even greater effects on gas exchange. The beneficial effect may be related to its favorable action on the distribution of blood flow in the lungs. Augmented endogenous NO production, especially at higher altitudes, accounted for the low pulmonary vascular tone observed in high-altitude adapted yaks. In our current work we found the administration of NO donor compounds prolonged the survival time of mice under normobaric hypoxia. NO donors also maintained the heart rate, SBP, MAP and DBP at normal level. The protective results may come from the protective effects for brain and hearts. The toxicity of compound A may
come from the parent structure coumaric acid (LD₅₀ 657 mg/kg, intraperitoneally (i.p.)) while other NO donors showed good safety.32)

During reoxygenation, ROS production markedly accelerates, causing further suppression of electron transport, which promotes greater ROS production, etc., in a vicious cycle. NO inhibits this scenario, potentially contributing to its cardio protective effects when administered at the time of reoxygenation in intact heart. 33) An efficient free radical scavenger need not only prevent the initiation of protein damage (by scavenging the initial attacking radicals), but also be effective interception of protein radicals once they are formed. The latter is a challenging problem due to the high concentration of potential radical targets present in biological fluids and cells. Previous research found that long-lived radicals such as nitroxides (RR\textsuperscript{MN}NO\textsuperscript{.}) and NO might be efficient scavengers of protein-derived radicals. 34) For aim to discover new long half-life NO species, we synthesized series of isosorbide mononitrate derivants as NO donor compounds and tested their anti-hypoxia activities through the survival time of the mice under normobaric hypoxia. Among those compounds, AFIM was the most effective one (Table 1). Also isosorbide mononitrate showed good anti-hypoxia activities during hypoxia test, but the side effect was significant when the dose was higher than 40 mg/kg.

Unlike other radical these isosorbide mononitrate derivants NO donor compounds were stable in biological fluids and have long half-life which meet the requirement for competitive reaction as a free radical scavenger. They might be efficient scavengers of protein-derived radicals, as the reactions with radical should have low energy barriers, being radical–radical reactions, and have rate constants near the diffusion limit.35) NO donors’ protective effects may come from two aspects. One was from the higher blood flow and circulating NO products offset high-altitude hypoxia, the other was from their clearance for radical molecules as stable free radical scavengers. As AFIM was the most effective one during the normobaric hypoxia test, it was consisted of isosorbide mononitrate residues and acetyl ferulic acid residues. Ferulic acid exerts a neuroprotective effect in cerebral ischemia through its anti-oxidant and anti-inflammation activity.35,36) Its protective effect might come from the sustained NO supplying and the anti-oxidant activity of acetyl ferulic acid.

The enhanced radical scavenging capacities of the novel nitronyl nitroxides may be potential drug leads against the deleterious action of reactive oxygen species (ROS)/reactive nitrogen species (RNS).25) Providing exogenous nitric oxide dramatically prevents H$_2$O$_2$-mediated endothelial injury, likely by reducing iron-mediated oxidant generation and subsequent lipid peroxidation.37) The mechanism of the protected effect may be explained by high affinity binding of NO to Fe$^{2+}$, limiting Fe$^{2+}$ oxidation by Fenton reaction. It may seem surprising that NO protects cells exposed not only to exogenous H$_2$O$_2$,32) but also to enzymatic systems generating O$_2^-$,37,38) since NO can react with O$_2^-$ to generate peroxynitrite radicals. AFIM has been previously shown to confer improved resistance against ischemic/reperfused injury39); we wished to verify that it could also protect the antioxidase activity in hypobaric hypoxia mice. This study showed AFIM prolonged the mice survival time during normobaric hypoxia test, decrease MDA and H$_2$O$_2$ in brain and heart of the mice under hypobaric hypoxia test. AFIM also maintained the activities.

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**Fig. 5.** Effects of AFIM on SOD in Mice Cerebrum and Myocardium, GSH-Px, CAT in Liver, under Hypobaric Hypoxia Condition (n=10)

A: SOD activity in cerebrum; B: SOD activity in myocardium; C: GSH-Px activity in liver; D: CAT activity in liver. *p<0.05 vs. Control. **p<0.01 vs. Control. *p<0.05 vs. Vehicle. **p<0.01 vs. Vehicle.


of SOD, GSH-Px and CAT within a normal range.

As a major oxidation product of membrane peroxidized polyunsaturated fatty acids MDA is an indicator for oxidative stress.40) MDA content level in hypobaric hypoxia mice changed significantly, which indicated that lipid peroxidation occurred in early hypoxia stage. AFIM significantly decreased MDA in mice cerebrum and myocardium in hypobaric hypoxia group. Comparing to normal control, hypoxia-induced oxidative stress stimulated $H_2O_2$ production especially in myocardium. Treatment with AFIM could significantly decrease $H_2O_2$ in myocardium in hypobaric hypoxia mice model. Human antioxidant defense system is equipped with enzymatic scavengers, such as SOD, GSH-Px and CAT, hydrophilic scavengers, e.g. glutathione and lipophilic radical-scavengers, such as tocopherols.41) The enzymatic activities of these antioxidant enzymes were critical for the clearance of free radical. It may be beneficial if antioxidant activity could be maintained or increased. The finding that AFIM could protect mice myocardium against hypoxia-induced $H_2O_2$ could have resulted from the direct decomposition of $H_2O_2$ or from an increase in the activities of $H_2O_2$-degrading enzymes. We found that the activities of SOD and GSH-Px were down-regulated after hypobaric hypoxia treatment, which resulted in the decrease of superoxide anion-scavenging activity decrease and oxidative damage to the organism. This might partially explain the increase of $H_2O_2$ and MDA in myocardium as there were not sufficient cellular antioxidants. Interestingly the activities of SOD, GSH-Px and CAT showed different trends during the hypoxia.

Fig. 6. Effects of AFIM on ATPase Activity in Mice Cerebrum and Myocardium under Hypobaric Hypoxia Condition ($n=10$)

A: $K^+Na^+-ATPase$ activity in mice cerebrum; B: $K^+Na^+-ATPase$ activity in mice myocardium; C: $Mg^{2+}-ATPase$ activity in mice cerebrum; D: $Mg^{2+}-ATPase$ activity in mice myocardium; E: $Ca^{2+}-ATPase$ activity in mice myocardium; F: $Ca^{2+}-ATPase$ activity in mice myocardium. *$p<0.05$ vs. Control, **$p<0.01$ vs. Control, *$p<0.05$ vs. Vehicle. **$p<0.01$ vs. Vehicle.
progress in current study. Unlike SOD and GSH-Px, the activity of CAT was up-regulated. This may attributed to the back donation of increased H$_2$O$_2$ in myocardium. As a sign of mitochondrial dysfunction the activities of ATPase were down regulated. AFIM protected mitochondria in hypobaric hypoxia treatment by scavenging free radical.

CONCLUSION

In conclusion, we found that mice administrated with AFIM could survive longer than ACZ group. We also found that AFIM was more effective to protect antioxidant than ACZ in hypobaric hypoxia mice model. Intravenous administration NO donor compounds had the same beneficial effects as inhaled NO for circulatory system and nervous system. Our result suggested that NO donor administration might be a potential therapeutic way for prophylaxis and therapy of acute high-altitude sickness.

Acknowledgments The authors wish to acknowledge support from the Chinese National Natural Science Foundation Grant Number 81402848 and the Gansu Natural Science Foundation Grant Number 145RJDA331.

Conflict of Interest The authors declare no conflict of interest.

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