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## Bosentan Reduces Pulmonary Artery Pressure in High Altitude Residents

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### Abstract

Kojonazarov, Baktybek, Jainagul Isakova, Baktybek Imanov, Nurmira Sovkhozova, Talantbek Sooronbaev, Takeshi Ishizaki, and Almaz A. Aldashev. Bosentan reduces pulmonary artery pressure in high altitude residents. *High Alt Med Biol.* 13:217–223, 2012.—Endothelin-1 (ET-1) plays a critical role in the regulation of pulmonary vascular tone. The aim of this study was to investigate the role of ET-1 in the pathogenesis of high altitude pulmonary arterial hypertension (HAPH). **Methods:** Pulmonary artery pressure (PAP) was measured by echocardiography in permanent residents of the Kyrgyz Republic (3200–4000 m above sea level) both before and 3 h after a single oral dose of ET receptor antagonist, bosentan (125 mg). Plasma ET-1 levels were measured by ELISA assay. Genomic DNA was extracted from peripheral blood samples and the frequency of -3a and -4a alleles of the ET-1 gene determined by PCR. **Results:** Plasma ET-1 in HAPH highlanders was significantly higher than in healthy subjects ( $7.05 \pm 2.35$  vs.  $4.65 \pm 1.65$  pg/ml,  $p < 0.002$ ). After the treatment with 125 mg bosentan, systolic PAP decreased from  $46 \pm 1.9$  to  $37 \pm 2.2$  mm Hg ( $p < 0.01$ ), and pulmonary artery acceleration time (PAAT) increased from  $0.086 \pm 0.001$  to  $0.098 \pm 0.001$  sec ( $p < 0.001$ ). The frequency of the -4a allele was significantly higher in HAPH patients compared to healthy highlanders (0.43 vs. 0.3,  $\chi^2 = 4.3$ ,  $p = 0.03$ ). **Conclusion:** Increased ET-1 levels play an important role in development of HAPH.

**Key Words:** highlanders, pulmonary hypertension, endothelin-1, bosentan.

### Introduction

AN ESTIMATED 4%–6% of Kyrgyz residents of the Tien-Shan and Pamir Mountains (3000–3500 m above sea level) in the Kyrgyz Republic develop high-altitude pulmonary hypertension (HAPH) with right ventricular failure (Mirrakhimov and Winslow, 1996). HAPH is characterized by increased pulmonary artery pressure (PAP) and pulmonary vascular remodeling involving all elements of the vessel wall, including endothelial and smooth muscle cells and fibroblasts (Arias-Stella and Saldana, 1963; Aldashev, 2000; Leon-Velarde et al., 2005).

A number of factors are known to influence the development of HAPH and the large interindividual variability in the magnitude of PAP response to hypoxia has been demonstrated by several studies (Westcott et al., 1955; Groves et al.,

1993; Aldashev et al., 2002). Moreover, the genetic susceptibility to high altitude-related illness is suggested (Weir et al., 1974; Morrell et al., 1999; Aldashev et al., 2002; Droma et al., 2002; Droma et al., 2006).

The prevailing view is that imbalances of endogenous vasodilator and vasoconstrictor factors contribute to pulmonary vascular vasoconstriction and structural remodeling (Humbert et al., 2004). Endothelin-1 (ET-1) is one of the most potent vasoconstrictors produced by vascular tissue (Yanagisawa and Masaki, 1989). Several studies have demonstrated elevated ET-1 levels in experimental hypoxic pulmonary hypertension (PH) (Shirakami et al., 1991; Li et al., 1994; Yang et al., 1997) and in patients with idiopathic pulmonary arterial hypertension (PAH) and PH associated with other diseases (Stewart et al., 1991; Giaid et al., 1993; MacLean, 1998). Moreover, the plasma level of ET-1 correlates

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with right atrial pressure, pulmonary vascular resistance, and survival of patients with PAH (Nootens et al., 1995; Cacoub et al., 1997).

The acute increase in PAP seen in mountaineers on exposure to hypoxia has been reported to be closely related to elevated plasma ET-1 levels (Goerre et al., 1995). Furthermore, it has been demonstrated subjects prone to high altitude pulmonary edema (HAPE) had higher ET-1 plasma levels than the subjects resistance to this condition, and ET-1 levels closely related to PAP measured at high altitude (Sartori et al., 1999). Also, Droma and co-authors published a case study in which elevated plasma ET-1 in patient with acute HAPE occurred at 3180 m altitude returned to normal levels after oxygen inhalation at 660 m altitude (Droma et al., 1996).

The ET-1 gene located on chromosome 6p24–23, consists of 5 exons and encodes pre-pro-ET, which is cleaved into ET-1 (Yanagisawa 1989; Xu et al., 1994). Numerous studies suggested that single nucleotide polymorphisms in the ET-1 gene contribute to development of cardiovascular disorders and are associated with high level of plasma ET-1 (Asai et al., 2001; Tanaka et al., 2004; Dong et al., 2004). Also Rajput and co-authors investigated four ET-1 variants in Himalayan natives and found that in subjects with mutant heterozygous genotype 3a/4a, ET-1 plasma level was significantly higher than in highlanders with homozygous 3/3a genotype. However, the association between ET-1 gene polymorphism and development of HAPH is still to be elucidated.

Additionally, dual ET receptor antagonist, bosentan effectively attenuates hypoxic pulmonary vasoconstriction in healthy subjects (Modesti et al., 2006; Pham et al., 2010). However, it has been reported that bosentan improves aerobic exercise capacity in a hypoxic environment (Faoro et al., 2009), while another report demonstrated that bosentan does not reduce resting or exercise systolic PAP or improve exercise capacity (Sehult et al., 2009). The efficacy of ET-1 inhibition in patients with HAPH remains to be demonstrated.

The main aim of this study was to investigate the acute effect of 125 mg of bosentan on PAP and the potential role of endothelin-1 gene polymorphism in patients with HAPH.

## Methods

The ethics committee of the Institute of Molecular Biology and Medicine and National Center of Cardiology and Internal Medicine (Bishkek, Kyrgyzstan) approved the study and followed international guidelines for medical research on human subjects. All subjects included in this study gave informed consent in a written form.

### Patients

138 residents of villages 2500–3800 m above sea level in the Tien-Shan Mountains of the Kyrgyz Republic with symptoms of dyspnea or exercise limitation as recorded at the local high altitude clinics were admitted to the National Center of Cardiology and Internal Medicine (Bishkek, 760 m above sea level) in a period from 2006 to 2008. All subjects underwent health screening by history, physical examination, ECG, echocardiography, spirometry, blood pressure measurement, and biochemical analysis for liver and kidney function for excluding coexisting diseases that might influence PAP.

### Right heart catheterization

Right heart catheterization was performed within 1 week of descent to Bishkek, as described previously (Aldashev et al., 2005; Kojonazarov et al., 2007). Briefly, a Swan-Ganz catheter (Baxter Healthcare, Compton, UK) introduced via an internal jugular vein, and after 30 min of rest, the mean PAP and pulmonary artery wedge pressure were measured during end-expiration. Arterial oxygen saturation, ECG, and blood pressure were monitored throughout the experiment. During the catheterization study, blood samples for DNA analysis were taken. HAPH was defined according to the consensus recommendation (Leon-Velarde et al., 2005). Eighty-eight subjects were found to have HAPH and 50 had normal pressures.

### Acute bosentan study protocol

An acute bosentan study was performed during expedition to high altitude. Fifteen nonsmoker patients with HAPH, underwent echocardiography examinations on 2 occasions, 1 day apart, at a clinic in their home village (3200–3800 m). On the first day, Doppler-echocardiography were performed before and 30 min after 100% oxygen. On the second day, echocardiographic measurements were performed before and 3 hours after a single oral dose of bosentan (125 mg). All subjects then breathed 100% oxygen for 30 min, and echocardiography was repeated. SaO<sub>2</sub>, HR, and systemic blood pressure (BP) were monitored continuously during echocardiography.

### Echocardiography

Doppler-echocardiography was performed using a portable ultrasound system equipped by a 2.5 MHz probe (SpectraMax, SonoSite, USA). Pulmonary artery systolic pressure (PAPs) and pulmonary artery acceleration time (PAAT) were estimated as previously described (Kitabatake et al., 1983; Yock and Popp, 1984). Briefly, images were obtained from standard parasternal views (long axis and short axis) and an apical four-chamber view. The PAPs was calculated using the modified Bernoulli equation ( $PASP = 4 VTR^2 + \text{right atrial pressure}$ ), where VTR is the tricuspid regurgitation peak velocity recorded from multiple jet windows with continuous-wave Doppler, and right atrial pressure is estimated from the vena cava diameter variations (Yock and Popp, 1984). A pulsed Doppler pulmonary blood flow velocity signal was sampled in the right ventricular outflow tract for the estimation of PAAT, which was defined as the time interval from the onset of forward flow in the pulmonary artery to the peak velocity of this flow as described (Kitabatake et al., 1983). Cardiac output was estimated from left ventricular outflow tract cross-sectional area and pulsed Doppler velocity-time integral measurements. All measurements were performed by an experienced echocardiographer and validated by another independent observer.

### Measurements of plasma ET-1 level

Blood samples were taken from 37 highlanders (17 with HAPH and 20 healthy) at high altitude. The samples were immediately put on ice and then centrifuged at 3000 rpm for 10 min. Plasma samples were then snap-frozen in liquid nitrogen until their return to the laboratory, where ET-1 con-

centrations were measured by ELISA assay (R&D, Germany) according to the supplied protocol.

### ET-1 gene polymorphism

The -3a/-4a polymorphism of ET-1 gene was determined in 138 highlanders by polymerase chain reaction (PCR); the following primers were used 5'-TTGTCTGGGGCTGGAA-TAAAGTGG-3' and 5'-CGAGCGTGCCTACCTGTTTCTG-3'. The products of PCR were digested by Bse II enzyme and separated by 2% agarose DNA electrophoresis as described (Rajput et al., 2006).

### Statistical analysis

All data are given as means±SD. Differences between groups were assessed by unpaired two-sample Student *t* test and analysis of variance, and Student-Newman-Keuls post-hoc test for multiple comparisons with a *p* value≤0.05 regarded as significant. Frequency of genotype or allele of ET-1 was compared by use of the contingency table, and significance of difference was examined by  $\chi^2$  analysis.

## Results

### Subjects

138 residents of villages 2500–3800 m above sea level underwent right heart catheterization and health screening at Bishkek (760 m above sea level). After right heart catheterization, 88 were defined as HAPH patients and 50 as healthy with normal PAP. The baseline characteristics of the HAPH and control groups are shown in Table 1. There were no differences in HAPH and control groups in terms of age, male/female ratio, and spirometrical data except PAP. There were no coexisting diseases other than HAPH in patients group.

### -3a/-4a polymorphism of ET-1 gene and HAPH

The -3a/-4a polymorphism of ET-1 gene polymorphism was investigated in 138 subjects who underwent right heart catheterization. The frequency of homozygous -3a/-3a genotype was higher in healthy highlanders compared to HAPH subjects, and the frequency of heterozygous -3a/-4a was higher in highlanders with HAPH ( $\chi^2 = 11.15$ ;  $p < 0.001$ ). Accordingly the frequency of the mutant -4a allele was significantly higher in highlanders with HAPH compared with healthy controls ( $\chi^2 = 4.3$ ;  $p < 0.03$ , Table 2). The mutant homozygous -4a/-4a genotype was not detected in highlanders

TABLE 2. -3A/-4A POLYMORPHISM OF ET-1 GENE AND HAPH

Groups	Genotypes (number of patients)			Alleles (number of alleles)	
	-3a/-3a	-3a/-4a	-4a/-4a	-3a	-4a
Healthy (n=50)	0.49 (20)	0.42 (30)	0.09 (0)	0.70 (70)	0.30 (30)
HAPH (n=88)	0.32 (13)	0.49 (75)	0.19 (0)*	0.57 (101)	0.43 (75)**

\* $\chi^2 = 11.15$ ;  $p = 0.0008$ , \*\* $\chi^2 = 4.304$ ;  $p = 0.03$ .

### Plasma ET-1 level

Blood samples were taken from 37 highlanders (17 with HAPH and 20 healthy) at high altitude, and ET-1 levels were measured by ELISA in Bishkek (760 m). Plasma ET-1 levels in highlanders with HAPH were significantly higher than those seen in healthy control subjects ( $7.07 \pm 2.26$  and  $4.56 \pm 1.66$  pg/ml, respectively,  $p < 0.001$ , Fig. 1A). Subjects expressing the heterozygous -3a/-4a genotype had higher ET-1 concentrations ( $6.16 \pm 0.48$  pg/ml) than subjects with homozygous 3a/3a genotype ( $4.81 \pm 0.57$  pg/ml,  $p < 0.07$ , Fig. 1B).

### Effects of oxygen breathing on pulmonary arterial pressure

On the first day, Doppler-echocardiography was performed before and 30 minutes after 100% oxygen in 15 non-smoker patients with HAPH, at a clinic in their home village (3200–3800 m). Oxygen significantly increased PAAT by  $0.014 \pm 0.002$  sec (95% CI 0.01 to 0.02,  $p < 0.001$ ) and decreased PAPs by  $-8.5 \pm 3.19$  (95% CI -1.7 to -15.2,  $p < 0.01$ ). Whereas CO, systemic BP, and HR did not change significantly during oxygen breathing, SaO<sub>2</sub> markedly increased during oxygen breathing ( $p < 0.001$ ).

### Effects of bosentan on PAP

On the second day, echocardiographic measurements were performed before and 3 hours after a single oral dose of bosentan (125 mg). All 15 subjects then breathed 100% oxygen for 30 min, and echocardiography was repeated. Systolic PAP was significantly reduced by  $-10.6 \pm 4.9$  mm Hg (95% CI -6.5 to -14.7 mmHg,  $p < 0.001$ ) and PAAT increased by  $0.012 \pm 0.002$  sec (95% CI 0.016 to 0.007,  $p < 0.001$ ) after the treatment with bosentan, compared with the baseline value (Table 3). Moreover, the combination of bosentan and oxygen increased PAAT more than bosentan alone ( $0.011 \pm 0.002$  sec, 95% CI 0.006 to -0.017,  $p < 0.001$ ), but had the same decreased effect on the systolic PAP ( $-2.0 \pm 3.1$  mmHg, 95% CI -4.6 to 0.6,  $p < 0.2$ ) (Table 2). CO, HR, and systemic BP were not significantly altered in response to single dose of bosentan compared with baseline.

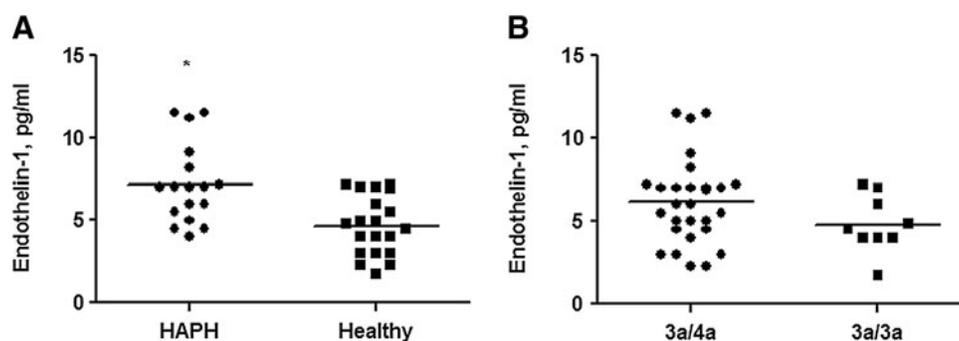
## Discussion

This study found that 1) the -3a/-4a ET-1 gene polymorphism is associated with HAPH in highlanders, 2) plasma ET-1 levels are significantly higher in high altitude residents with HAPH compared with high-altitude healthy controls, and 3) the dual ET receptor antagonist effectively reduced

TABLE 1. CHARACTERISTICS OF HIGHLANDERS

	HAPH (n=88)	Healthy (n=50)
Age, year	49.7±14.8	48.8±16.2
Gender (m/f)	69/19	47/3
PAP mean, mm Hg	32.4±6.8	20.5±7.2*
FEV <sub>1</sub> , % predicted	93.0±11.0	92.0±13.0
FVC, % predicted	94.0±15.0	94.0±14.0
Smoking (m/f)	12/0	19/0

Data are presented as mean±SD. PAP mean – mean pulmonary artery pressure, measured by right heart catheterization; FEV<sub>1</sub> – forced expiratory volume in 1 second; FVC – forced vital capacity; \* $p < 0.001$  – HAPH vs. Healthy.



**FIG. 1.** Endothelin-1 plasma level in highlanders. **(A)** ET-1 plasma is significantly higher in highlanders with HAPH,  $*p < 0.001$ , HAPH vs, Healthy. **(B)** Lack of association between ET-1 plasma level and genotype of  $-3a/-4a$  ET-1 gene polymorphism. ET-1 plasma level measured in 37 highlanders.

systolic PAP in HAPH, comparable to that seen with oxygen administration.

Inter-individual susceptibility to HAPH and the response of pulmonary vessels to hypoxic stimuli strongly suggests a genetic trait (Westcott et al., 1951). In previous studies, we found an association between ACE gene polymorphism and development of HAPH (Morrell et al., 1999; Aldashev et al., 2002).

ET-1 is a potent endogenous vasoconstrictor and smooth muscle mitogen (Yanagisawa and Masaki, 1989), and it appears to play an important role in PAH. For example, plasma ET-1 levels are increased in animals with experimental PH (Shirakami et al., 1991; Li et al., 1994; Yang et al., 1997). Additionally, it was demonstrated that hypoxia-induced inhibition of voltage-gated  $K^+$  channel expression may result from increased level of ET-1 secondary to induction of Hif1 $\alpha$  (Whitman et al., 2008). ET-1 levels are increased in subjects who develop HAPE compared with HAPE-resistance subjects and correlates with level of PAP (Sartori et al., 1999). Also, the increase in PAP during exposure to high altitude relates to plasma ET-1 concentration (Goerre et al., 1995). In the ACME-1 study, Modesti and co-authors showed that ET-1 plays a significant role in pulmonary vascular vasoconstriction during adaptation to high altitude (Modesti et al., 2006).

First, we have measured the plasma level of ET-1 in highlanders with and without HAPH. We have found that the level of ET-1 is significantly higher in highlanders with HAPH compared with healthy. We then studied the role of  $3a/4a$  ET-1 gene polymorphism in Kyrgyz highlanders, because it has been shown that  $-3a/-4a$  ET-1 gene polymor-

phism is functional and associated with circulating ET-1 levels. Furthermore, the  $-4a$  allele is associated with higher plasma ET-1 levels in patients with essential hypertension (Tanaka et al., 2004) and chronic heart failure (Vasku et al., 2002). Rajput and co-authors have reported that the  $-3a/-3a$  genotype is associated with lower ET-1 levels than the  $-3a/-4a$  genotype in high altitude natives (Rajput et al., 2006). However the ET-1 gene polymorphism was not investigated in high altitude residents with HAPH. In our study, we found that the frequency of mutant  $-4a$  ET-1 allele was significantly higher in highlanders with HAPH as compared with healthy highlanders. The absence of the  $-4a/-4a$  homozygous genotype in our study population suggests a selection pressure against this genotype, which could be associated with a more severe pulmonary vasoconstriction response to hypoxia and the early onset of a pulmonary hypertensive phenotype. But this requires confirmation in a large cohort study.

We went on to confirm that the plasma ET-1 level is related to  $3a/4a$  ET-1 gene polymorphism in Kyrgyz highlanders. In our study, we found that in highlanders with  $3a/4a$  genotype the ET-1 concentration is relatively, but not significantly higher than in subjects with homozygous  $3a/3a$  genotype. Unfortunately, a small number of patients was entered in this study. However, the changes in plasma ET-1 concentration do not necessarily reflect changes in local tissue, because (as has been shown) ET-1 is mainly a paracrine mediator and only 20% of produced ET-1 is released into the blood stream (Wagner et al., 1992). Nonetheless, additional study on a large cohort is required.

**TABLE 3.** EFFECTS OF BOSENTAN AND OXYGEN ON PAP IN HIGHLANDERS

Parameters	1 <sup>st</sup> day (n = 15)		2 <sup>nd</sup> day (n = 15)		
	Baseline	Oxygen	Baseline	Bosentan	Bosentan + Oxygen
PAAT, sec	0.086 ± 0.003	0.102 ± 0.008*	0.086 ± 0.003	0.098 ± 0.003*	0.110 ± 0.005*†
PAP <sub>syst</sub> , mm Hg	46.3 ± 6.2	38.0 ± 7.7*	46.4 ± 6.2	37.0 ± 7.2*	33.0 ± 5.8*
CO, l/min	5.9 ± 1.1	6.1 ± 1.7	5.9 ± 1.0	6.0 ± 1.5	5.9 ± 1.0
BP systolic, mm Hg	129.0 ± 17.1	125.0 ± 10.1	127.5 ± 14.6	126 ± 13.7	123 ± 9.7
BP diastolic, mm Hg	82.0 ± 7.4	79 ± 6.2	80.0 ± 1.6	78 ± 5.7	77 ± 4.7
HR, bpm	83.0 ± 14.6	79.0 ± 11.6	80.0 ± 13.1	83.0 ± 13.5	81 ± 10.5
SaO <sub>2</sub> , %	88.4 ± 2.3	97.0 ± 2.1*	88 ± 2.1	89 ± 1.6	96 ± 0.8*

Data are presented as mean ± SD. PAAT – pulmonary artery acceleration time, PAP<sub>syst</sub> – pulmonary artery systolic pressure, CO – cardiac output, BP – blood pressure, HR – heart rate, bpm – beats per minute, \* $p < 0.05$  – vs. baseline; † $p < 0.05$  – vs. bosentan.

The ideal treatment of HAPH is migration to low altitude (Leon-Velarde et al., 2005), but due to economic reasons it is almost impossible or not applicable. Alternative ways such as oxygen supplementation have been used, but it has some limitations. It was shown by Groves and co-authors in Operation Everest II study that in healthy volunteers acute oxygen breathing at simulated altitude 7620 m lowered mean PAP, but not calculated pulmonary vascular resistance (PVR), due to CO also decreased (Groves et al., 1987). However, several studies demonstrated that oxygen decreases PAP in highlanders and reduces the increase in PAP during exercise (Grover et al., 1966; Penalzoza and Sime, 1971). In our study, we demonstrated that oxygen markedly reduced systolic PAP, improved PAAT, and did not change CO. In our study, oxygen supplementation blunted hypoxic pulmonary vasoconstriction in high altitude residents, but did not reverse PAP till normal value, due to the presence of remodeling of the pulmonary vessels, as was shown previously (Aldashev et al., 2002).

Taken into account that ET-1 plays an important role in high altitude-related illness, we decided to investigate the effect of dual ET receptors blocker on PAP in highlanders with HAPH. Bosentan is known to reduce PAP, and improve exercise capacity and quality of life in patients with PAH (Channick et al., 2001; Rubin et al., 2002). Modesti and co-authors demonstrated that bosentan effectively attenuates hypoxic pulmonary vasoconstriction in healthy subjects during acclimatization to high altitude, but was also associated with reduction of urinary volume and free water clearance (Modesti et al., 2006). In another study, a single dose of bosentan significantly blunted the hypoxia-induced increase in PASP at rest, but not during exercise (Pham et al., 2010). In line with our study, authors demonstrated that bosentan had no effect on CO, oxygen saturation, and systemic arterial pressure. Additionally, Faoro and co-authors showed that a single oral dose of bosentan improves aerobic exercise capacity and attenuates hypoxic pulmonary vasoconstriction (Faoro et al., 2009). Also, Olfert and co-authors demonstrated that bosentan improves arterial oxygenation during short-term exposure to acute hypoxia (Olfert et al., 2011). However, Seheult and colleagues (2009) recently published data where they initiated treatment with bosentan in healthy nonacclimatized volunteers 5 days before ascent and continued for 2 days at altitude (Seheult et al., 2009) and demonstrated that bosentan does not reduce resting or exercise systolic PAP or improve exercise capacity. Moreover, in their hands, bosentan was associated with lower hemoglobin oxygen saturation during exercise at altitude. The authors suppose that it might be due to bosentan's fluid retention effect on the kidney, and that administration before ascent to altitude may result in more pulmonary alveolar fluid or interstitial pulmonary edema and worsen ventilation-perfusion relationships.

All of these studies investigated the effectiveness of bosentan in healthy human subjects during short-term acclimatization to high altitude or simulated hypoxic challenge, but the acute effects of ET-1 inhibition in patients with chronic HAPH until now was not studied. In our study, we demonstrated that bosentan is as effective as oxygen in acute reduction of PAP at high altitude. The combination of bosentan and oxygen breathing led to further improvement in PAAT, but not systolic PAP, compared to bosentan alone. We know at this point that PAAT correlates well with both sPAP and mPAP in patients with congenital heart disease (Kosturakis et al., 1984) or HAPH (Kojonazarov et al., 2007); however,

PAAT has not become as widely used as the tricuspid regurgitation method. Moreover, the measurement of the maximal velocity of the tricuspid regurgitant jet is more reliable and accurate than PAAT, especially in assessing the changes after therapeutic interventions (Chow et al., 1988). Nonetheless, the noninvasive measurement of PAP is less precise than cardiac catheterization, which remains the gold standard. But there are practical problems with using this technique at altitude.

Summarizing all our findings, we would like to state that ET-1 contributes to the development of HAPH and 3a/4a ET-1 gene polymorphism associated with high PAP in high altitude residents. Our direct measurements of PAP pressure were performed at low altitude, which might be one of the limiting factors in our study. The descent to low altitude reverses hypoxia-induced pulmonary vascular vasoconstriction, but also remodels the pulmonary vessels. It has been demonstrated that PAP reversed to normal values in high altitude natives after their staying 2 years at sea level. However, despite the normal PAP after 2 years at sea level, the pulmonary pressure response to exercise was similar to that observed at high altitude (Sime et al., 1971; Penalzoza et al., 2007). Additionally, Grover et al. (1966) reported a case in which high mean PAP decreased substantially 11 months after residence at sea level. Moreover, it was demonstrated that PAP in patients with chronic mountain sickness time-dependently reduced after descending to sea level, due to suppression of hypoxic vasoconstriction and progressive reduction of polycythemia (Penalzoza and Sime, 1971). These observations may suggest that reversing the pulmonary arterial pressure of high altitude natives to normal levels requires a prolonged stay at sea level because of the slow involution of the anatomical changes of pulmonary vessels (Penalzoza et al., 2007). Nevertheless, taken into account all of these issues, probably it would be more suitable to measure PAP at high altitude as it may increase the number of subjects with HAPH.

We have also observed for the first time that a single dose of bosentan effectively decreased PAP in highlanders, substantiating the important role of ET-1 in the development of HAPH. However, the low number of patients involved in the acute bosentan study can be considered as a limitation. Nonetheless, an additional study of the long-term effect of ET receptor blockade on pulmonary hypertension and renal function in a larger cohort of highlanders with HAPH is warranted.

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### Author Disclosure Statement

No competing financial interests exist.

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