Review

Pharmacological approaches in either intermittent or permanent hypoxia: A tale of two exposures

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ABSTRACT

Hypoxia induces several responses at cardiovascular, pulmonary and reproductive levels, which may lead to chronic diseases. This is relevant in human populations exposed to high altitude (HA), in either chronic continuous (permanent inhabitants) or intermittent fashion (HA workers, tourists and mountaineers). In Chile, it is estimated that 1.000.000 people live at highlands and more than 55.000 work in HA shifts.

Initial responses to hypoxia are compensatory and induce activation of cardioprotective mechanisms, such as those seen under intermittent hypobaric (IH) hypoxia, events that could mediate preconditioning. However, whenever hypoxia is prolonged, the chronic activation of cellular responses induces long-lasting modifications that may result in acclimatization or produce maladaptive changes with increase in cardiovascular risk.

HA exposure during pregnancy induces hypoxia and oxidative stress, which in turn may promote cellular responses and epigenetic modifications resulting in severe impairment in growth and development. Sadly, this condition is accompanied with an increased fetal and neonatal morbi-mortality. Further, developmental hypoxia may program cardio-pulmonary circulations later in postnatal life, ending in vascular structural and functional alterations with augmented risk on pulmonary and cardiovascular failure.

Additionally, permanent HA inhabitants have augmented risk and prevalence of chronic hypoxic pulmonary hypertension, right ventricular hypertrophy and cardiopulmonary remodeling. Similar responses are seen in adults that are intermittently exposed to chronic hypoxia (CH) such as shift workers in HA areas. The mechanisms involved determining the immediate, short and long-lasting effects are still unclear. For several years, the study of the responses to hypoxic insults and pharmacological targets has been the motivation of our group. This review describes some of the mechanisms underlying hypoxic responses and potential therapeutic approaches with antioxidants such as melatonin, ascorbate, omega 3 (Ω3) or compounds that increase the nitric oxide (NO) bioavailability.

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Contents

1. Introduction

Hypoxia is defined as a deficient oxygen (O_2) supply for the physiological demands of a tissue, organ or organism. This is a restrictive condition frequently faced by environmental hypoxia as seen in the highlands at any stage of life. Hypoxia associated with reduced inspiratory oxygen pressure determines an pathophysiological response in living organisms. The lower partial pressure produces a decrease in the O_2 supply in the blood and tissues with various detrimental effects [1]. According United Nation Environment Programs (UNEP) report, approximately 12% of the human population lives in mountain regions, being a relevant public heath problem [2]. Further, in 1998 there were an estimated 140 million people permanently living above 2500 m worldwide and 35 million living in the Andean Mountains [3]. Considering the South American rate of population increase in the last 15 years, the total population living in the Andean cord should be around 41 million inhabitants. Indeed, there are others forms of hypobaric hypoxia such as acute (tourism people) to intermittent CH (ICH) (working shift, e.g., miners, observatory workers, and armed forces). This type of exposure to hypoxia determines intercalated periods of stay at HA with periods at sea level. The duration of these shifts as short as one day to several days [4]. Most of the different models of HA hypoxia are characterized by marked cardiovascular and pulmonary effect to offset a global decrease in tissue O_2 supply.

Currently, living at HA is considered a health risk during development and adulthood [5,6] and it should be taken as a public health issue. Hence, establishing the impact of hypobaric O_2 restriction during prenatal and postnatal life would, represent a substantial advantage in understanding the role of hypoxia in determining cardiovascular diseases at HA populations. The latter shall help to introduce new pharmacologic approaches for hypoxia-related pathologic conditions.

For this reason, we and others scientists in Chile have developed lines of research related to the pathophysiological response in HA environments and studying potential pharmacological targets.

The International Center for Andean Studies (INCAS) was established 16 years ago, as an autonomous branch of the University of Chile, conceived as a place in which research on HA environment can be developed [7]. Most of our lines of research have been developed in this research station based at 3600 m in the Andean altiplano. Other experiments have been developed in hypobaric chambers located at near sea level. Here, we discuss some of the studies held by our group, the effects of intermittent and chronic exposure to hypobaric hypoxia and some potential pharmacological approaches.

2. Intermittent CH: high-altitudes shifts

In Chile due to job-related conditions, such as the mining industry, astronomers, army forces, border control staff, academics, and rural health officers, about 55,000 workers are subjected to an intermittent and chronic exposure to hypobaric hypoxia (above 3500 m) [8,9]. This population should grow as new mining and observatory projects are due to commence. Therefore, an increasing amount of people will be exposed to intermittent CH (ICH) [8,9,10]. Shift workers at HA must adjust to the requirements of hypobaric hypoxia for some days and then return to sea level, where, they lose some of the acclimatization to hypoxia, depending on the time in normoxia [9]. In this context, the adaptive or detrimental responses can be generally predicted by the frequency, severity, and duration of IH. Here, the two possible paradigms derived from IH in human and experimental animal approaches will be discussed.

2.1. Cardiovascular response & treatment

Cardiovascular manifestations in response to a decrease in tissue O_2 supply, including polycythemia and an increase sympathetic tone, determines the response to hypoxia. In a similar way to CH, there is a hypoxic pulmonary vasoconstriction, which leads to high pulmonary arterial pressure and the probable right ventricular hypertrophy, if the hypoxic exposure is too prolonged [11]. Currently, our understanding of the pathophysiological mechanisms linking IH and cardiovascular function is limited by the diversity of hypoxic phenomenon in human subjects and the multiple comorbidities, including obesity, insulin resistance and previous cardiac alterations [9,12]. Moreover, animals subjected to various acute IH become more resistant to tissue injury in subsequent exposures to severe hypoxic exposures [13]. This preconditioning effect has been demonstrated for example in mice treated with brief episodes of IH that survive longer when exposed to severe hypoxia [14]. Further, IH appears to provide a beneficial effect on coronary artery ligation-induced myocardial infarction by reducing the infarct size, myocardial fibrosis, and improve contractile dysfunction [15,16]. Interestingly, these effects involve the activation of pathways similar to those described in models of cardiac ischemic preconditioning [17]. It has been described antioxidant and anti-inflammatory mechanisms triggered in a short-term manner [18] and other related with genomic response [19]. Experimental approaches have shown that ICH attenuates the hypoxic damage in heart rat [16,20]. This cardioprotective mechanism was showed as an improvement in LV function associated with the higher antioxidant enzymes activity [21]. In a recent work, we showed that ICH (hypoxia-normoxia: 4×4 days) reduced myocardial injury, lipid peroxidation and IL-1 β levels [16]. These results are in agreement with other works that show an improvement of mitochondrial function and a reduced ROS-derived damage in rats exposed to ICH [22]. Understanding the ICH mechanisms, which can exert protective effects, may lead us to know relevant information for developing potential therapeutic approaches and a better management in clinical contexts [23]. A number of studies have been conducted using ICH-treated animal models to explore the effects of antioxidants, but the outcomes of these studies are controversial. Using an animal model of ICH in rats, it has been demonstrated that the use of allopurinol, a free radical scavenger was associated with a reduction in oxidative stress, myocardial dysfunction and apoptosis [24,25]. Also, It could be useful in the clinical treatment of chronic heart failure in humans as it is the only drug that has been proven able to lower O_2 consumption of the dysfunctioning myocardium [26]. In contrast, Kolár et al. demonstrated that the

pretreatment with *N*-acetylcysteine, an antioxidant and precursor of reduced glutathione (GSH), completely prevented the development of cardioprotection in rats [27]. This evidence implies the dual paradigm, adaptive and maladaptive, of reactive oxygen species (ROS) in ICH. The suppression of ROS production prior to hypoxia impairs the preconditioning responses, but on the other hand, it can show positive effects on cardiac function. In this regard, Inamoto et al. [28] confirmed that pitavastatin preserves the left ventricular (LV) myocardium structure in mice exposed to ICH, mediated by its antioxidant and anti-inflammatory effect. Recently, administration of atorvastatin following ICH significantly ameliorated the cardiac hypertrophy, due to attenuation of pro-inflammatory signaling pathways [29].

Other pharmacological treatment that has been demonstrates cardioprotective effects are the (3 fatty acids [30]. This protective mechanisms of (3 are supported in various works that show an improvement of heart and vascular parameters, such as endothelial function, LV contractility and diastolic relaxation, regulation of heart and some determinants of cardiac oxygen consumption [31]. Indeed, dietary fish oil induces a form of preconditioning, limiting the hypoxic cardiac injury, arrhythmic events and endows cardioprotection as powerful as ischemic preconditioning [32,33]. In a previous study of our group, we showed that both, ICH and (3 in an independent manner induce functional improvement by antioxidant and anti-inflammatory mechanisms, determining a reduction in infarct size and improvement in LV function [16,34]. Probably, the occurrence of these protective mechanisms is determined by temporal exposure of the oxygenation shifts. Despite all of the above, the clinical trials with antioxidants failed to confirm promising data obtained in a number of animal studies. It is obvious that beneficial consequences of antioxidant supplementation in normal healthy heart cannot be used to predict an outcome in adapted or diseased hearts.

2.2. Reproductive responses & treatment

Hypobaric hypoxia has been known to affect male fertility, in both, humans and animals [35]. Studies on male rats have revealed significant differences in the histology of the testis in comparison to normoxic control animals. Animals exposed to hypobaric hypoxia either chronic or ICH showed a decrease in testicular mass, increment in the interstitial space, reduction of the seminal epithelium, cellular depletion in epithelium and vacuolization of epithelial cells, changes that intensified further with the continuous hypoxic exposure [36,37,38,39]. In addition, other studies have characterized the changes in the amount and the form of spermatozoids present in epididymis (i.e., teratozoospermia), with an 85% of the spermatozoids presenting morphology abnormality [40,41,42]. Therefore, testicular hypoxia can affect fertility. In humans, similar effects have been described [42] and the observed changes induced by CH are reversible provided that the exposed subject stays under normoxic conditions for a minimum of 6 months. After this period, male fertility normalizes, with normal seminal count parameters, motility, form and maturity of spermatozoids [43]. The findings of our group related with the effects of ICH on male fertility has taken us to consider the specific activation of ICH pathways, that in conjunction with other variables such as temperature, vascularization and apoptosis, would be involved in the adverse effects on fertility of individuals intermittently exposed to HA [37,44].

Recent studies reinforce the existence of an increased oxidative metabolism in rat epididymis subjected to ICH [45,46]. Hence, an increased production of ROS gives way to a rise in apoptosis at the germinal cell level, leading to a state of hypospermatogenesis [46,47]. A mature and fertile spermatozoid is characterized by containing its chromatin highly compacted and stable, which protects against damage in DNA [48]. Nevertheless, due to the effects of oxidative stress, the sperm DNA remains susceptible to ROS and oxidant radicals' attack thereby jeopardizing the integrity of the gamete genetic material, and therefore, male fertility [49,50]. A continuous and persistent oxidative DNA damage can lead to altered signal cascades, gene expressions, induce or arrest transcription and cause errors in replication, giving genomic instability whose mutations can lead to spark off carcinogenesis, neurodegenerative disorders, infertility and cardiovascular disease [51]. When considering oxidative stress development in spermatogenic cells and consequently the spermatozoids of workers exposed to HA, it turns out critical when it poses as an imminent risk to the viability and quality of the reproductive cells [9,52]. However, despite of the importance of determining what occurs in the DNA of the reproductive cells under hypobaric hypoxia, there is still scare literature that associates hypobaric hypoxia, oxidative stress, apoptosis and infertility. This is a key step in order to generate potential pharmacological therapies to prevent male infertility. One potential alternative is to incorporate food with high antioxidant levels to the diet in those people exposed to HA, and we have shown that this may have a positive effect in the protection of cells facing oxidative stress [45]. We described, in rats exposed to ICH, that testicles and epididymis shown an increase in lipid peroxidation markers and lower GSH levels compared with normoxic normobaric animals, and that these effects were fully reverted with concomitant administration of ascorbic acid, blueberry extracts or (3 chronic supplementation [44,45,46]. Indeed, these protective effects can be supported by the induction of enzymatic and non-enzymatic antioxidant defense systems. For instance, genomic response would involve the activation of antioxidant transcriptional factors such as Nrf2 [23]. Further, it have been also described that melatonin, another recognized endogenous antioxidant, counteracted the damage of the testis and spermatogenesis in rats subjected to ICH [53]. However, results of our group showed that melatonin had no protective effect in testis and epididymis damaged, despite its amphiphilic nature, and its action of ROS scavenger [54,55]. Further studies are needed to improve our knowledge on the reproductive effects of ICH, not only on the germinal cells, but as well in the short and long-lasting of the offspring. These, may give us the chance to get the best therapeutical approach to improve the fertility affected by hypoxia.

3. CH: acclimatization to high altitudes

The etiology of most of the non-communicable diseases has been partially linked with adverse periconceptional and perinatal conditions that are able to increase the risk to develop disease later in life [56,57], theory known as Developmental Origins of Health and Disease. This states that in utero stresses are able to program the fetal and early postnatal developmental pattern, resulting in permanent modifications of the function of cells, organs and systems. These changes are adaptive outcomes to face adverse prenatal and/or neonatal conditions, becoming maladaptive and increasing the risks of developing adult metabolic, cardiovascular and endocrine diseases [57]. One of the perinatal programming conditions is chronic O_2 restriction as seen in highland [5,58]. CH induces pulmonary hypertension of the neonate, which if sustained in time this condition may ends in cardiopulmonary remodeling and right heart failure [5].

Similarly, CH at adulthood may induce marked cardiovascular and pulmonary responses that may end in maladaption [5,59]. The most extreme expression of these responses is the chronic mountain sickness (CMS) syndrome [60], which may end in multisystemic failure, but in between there are several conditions manifesting tachycardia, systemic and pulmonary hypertension,

respiratory distress, cardiopulmonary remodeling, endothelial dysfunction and polycythemia among others [61].

3.1. Perinatal cardiovascular responses & treatment

The South American Andean altitudes (starting from zones of Colombia and Ecuador, through Peru and Bolivia, and finishing to the North zone of Argentina and Chile) have been visited by numerous researchers, which use this place as a natural laboratory to study physiological and pathophysiological phenomena. Some of the outcomes of these works have shown that Andean population hold genetic mix coming from a diverse ancestries, mainly quechuas and aymaras [62], that lived in the altiplano Andino more than 11 thousand years ago and Spanish Conquistadores with less than 500 years from chronic exposition to HA hypoxia [63].

However, when human gestation (a lowland species) takes place in the CH of the Andean altiplano, the result is pulmonary arterial hypertension and vascular dysfunction of the neonate [6]. At low altitudes the prevalence of persistent pulmonary hypertension of the neonate is 1.9 per 1000 live births [64], whereas, above 3000 m this condition is dramatically increased [6] reaching up to 10% of live newborns with respiratory problems. Acute and unsustained fetal hypoxia results in a reversible pulmonary arterial vasoconstriction, but in contrast chronic fetal hypoxia also causes remodeling of the pulmonary vessels, thus augmenting postnatal pulmonary vascular resistance (PVR) and pulmonary hypertension in highlands [65]. Frequently, the changes induced in the pulmonary vessels structure by fetal CH do not spontaneously revert with re-oxygenation and the pulmonary arterial hypertension becomes persistent [5,65,66,67,68]. The neonatal pulmonary hypertension is a pathologic condition with multifactorial etiologies, due to an imbalance between vasodilator and vasoconstrictor mechanisms and maladaptative vascular remodeling of pulmonary arteries. At birth, the pulmonary circulation is quickly and markedly modified, from a high resistance–low blood flow condition to a low resistance-high blood flow state in minutes to hours. The success of this transition is essential for the newborn survival; thus, any fail in this event may induce neonatal pulmonary hypertension or even death [5,65].

The pulmonary hypertension is a condition with an elevated pulmonary vascular PVR, as a result of an initial vasoconstriction followed by a progressive vascular remodeling [67,69,70]. Both, vascular vasoconstriction and vascular remodeling end up in a significant reduction of the lumen of the intrapulmonary arteries increasing the PVR [65,67,71]. Chronic pulmonary arterial hypertension often mistaken for right ventricle hypertrophy and associates with marked endothelial dysfunction [67,72,73]. In addition, gestation at HA results in prolonged exposure to CH yielding pulmonary hypertension and elevated vascular remodeling, even after 10 days of re-oxygenation at sea level in a model of newborn lambs [68].

Numerous studies have been carried out to determine the mechanisms involved in the elevated PVR, in the neonate with gestation submitted to CH. In the ovine newborn from HA, the vascular reactivity is elevated compared to sea level counterpart [67,74]. The imbalance in the cGMP signaling, favors the vasoconstriction. In these neonates, the vascular reactivity response to Sildenafil, an inhibitor to phosphodiesterase 5 (PDE5), is greater in the HA newborn than sea level, demonstrating an elevated PDE5 function. Moreover, the soluble guanylyl cyclase (sGC) protein expression is lowered in the HA newborn compared to sea level neonates [74]. This observation is suggestive of a diminished sGC function or an insensitivity of this enzyme to NO, due to oxidation of the iron molecule of the hem prostetic group in the pulmonary arteries of HA newborns. Moreover, the relaxation to sodium nitroprusside, a NO donor, is diminished at HA compared to sea level neonates,

indicating that the cGMP dependent vasodilator machinery is inefficient, maintaining elevated the vascular tone [67,74].

Actually, new pharmacological approaches in whole animal, are necessary to increase the offer of treatments to neonatal pulmonary hypertension, since the only probed therapy is inhaled NO, scarcely available in common hospital or primary care facilities [75]. In the HA newborn, we have demonstrated that acute administration of Sildenafil attenuates the pulmonary vasoconstriction basally and in a superimposed episode of acute hypoxia [75]. Other emerging pharmacological targets are store operated calcium channels. Drugs like 2-aminoethyldiphenylborinate (2-APB), initially described as an inhibitor of inositol trisphosphate dependent calcium channels inhibitor [76], was later described as a store-operated calcium (SOC) channels blocker, limiting the calcium flux to intracellular compartment, attenuating the vasoconstrictor tone [77,78,79]. The acute administration attenuates the pulmonary vasoconstriction in an episode of superimposed hypoxia, and this effect is more marked in HA newborn lambs, where these channels are up-regulated [79]. Adiitionally, up-regulation of SOC in pulmonary artery smooth muscle cells increases proliferation, and pharmacological blockade or knock down of these SOC forming proteins stops this proliferation [80,81,82]. The efficacy treatments based on the blockade of these channels is an unexplored and promising field that awaits demonstration in whole animal models.

Additionally, Fasudil, a Rho-kinase (ROCK) inhibitor, decreases the function of the RhoA/ROCK pathway, decreasing the ratio between phosphorylated and dephosphorylated myosin heavy chain, promoting relaxation and reducing the PAP and PVR in chronically hypoxic highland neonatal lambs. The inhibition of ROCKs by fasudil may offer a possible therapeutic tool for the pulmonary hypertension of the neonate. The inhibition of the functions of PDE5, SOC and ROCK, did not show adverse effects in the cardiovascular and respiratory function of animal model during the studies.

Other component of the etiology of pulmonary hypertension is the vascular remodeling. The cellular mechanisms involved in this augmented neonatal vascular remodeling still remain poorly understood. The vascular remodeling involves 3 main mechanisms such as: i) cellular differentiation/proliferation (cell cycle regulation), ii) inflammation and iii) oxidative stress. Increasing evidences suggest that inflammatory mechanisms may be involved in early stages of the development of the pulmonary hypertension [83,84,85]. However, most of this studies came from adult studies and few is known about mechanisms that regulate the neonatal pulmonary inflammation induced by CH.

In physiological levels, ROS are involved in very important cellular processes, such as differentiation, inflammatory responses and proliferation. To attenuate excessive ROS and oxidative damage, the cells have a battery of enzymatic systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [86,87] and non-enzymatic mechanisms (natural redox buffers) such as ascorbate, glutathione, alkaloids, melatonin and carotenoids [88,89]. The imbalance between production and ability to degrade the reactive intermediates result in augmented free radical accumulation, which is known as oxidative stress.

Strong evidence argues that oxidative stress contributes to chronic hypoxic pulmonary vasoconstriction [69,90,91,92]. In hypoxic conditions, one of the main ROS generators is nicotinamide adenine dinucleotide phosphate (NADPH)- oxidase, which is responsible of vascular remodeling and hypertension [91,93]. Under these same conditions, SOD is highly expressed in pulmonary artery [92], presumably in response to high ROS levels. Xanthine oxidase, another ROS-generating enzymatic pathway, induces endothelial dysfunction by elevated superoxide production, contributing to neonatal vascular remodeling [94]. Novel treatments have been proposed to decrease the oxidative stress and
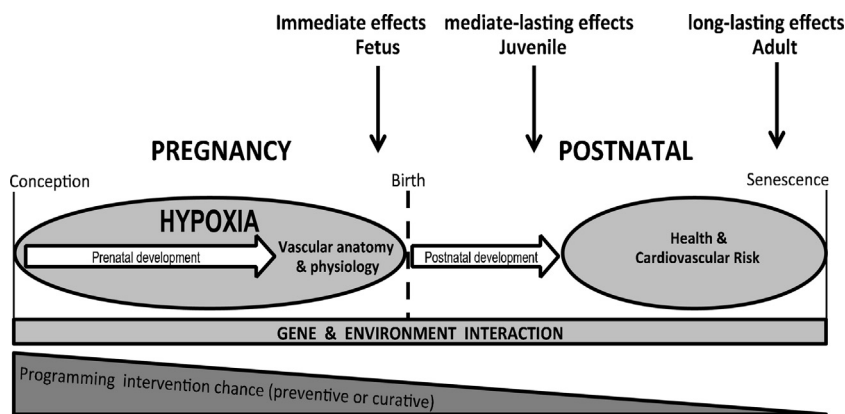
Fig. 1. Chronic hypoxic exposure during life. Diagram showing the potential effects of CH during life, either permanent or intermittent. The more chronic the exposure to hypoxia, the less intervention chance and possibility of an effective treatment.

diminish pulmonary hypertension and cardiovascular remodeling. Recently, we have shown that melatonin increases the enzymatic antioxidants mechanisms and improves pulmonary endothelial function associated with a slight increase of the luminal radius of pulmonary arteries [70]. Interestingly, the same treatment also improved vascular function and decreased oxidative stress in the neonatal brain [95].

3.2. Adult cardiovascular responses & treatment

Humans have been successful adapting to hypobaric CH of highlands in different HA plateau around the world, among them the Tibetan, Andean and the Semien Plateau of Ethiopia. Nowadays more than 35 million people dwells in the Andes' highlands submitted to hypobaric CH with low O_2 partial pressure in the environment [3]. Humans have successfully colonized the Alto Andino around 11 thousand years ago [63] and Aymaras and Quechuas ethnic groups are descendants of these colonizers of the thin air of the Andean mountains, although an important admixture took place between Amerindians and of the Spanish Conquistadores since their arrival 500 years ago. Therefore, the Andean people today has a set of genes coming from his HA adapted and from Europeans ancestors not adapted to HA. The dwelling time in the Andes highlands and some European ancestry may explain a rather lesser adaptation to HAs in the Amerindians in relation to the Tibetans and Sherpas, the two latter denominated the King of the Mountains by Gilbert-Kawai et al. [96]. It is considered that an ethnic group has reached an important adaptation to a new environment when has accomplished the capacity to reproduce and give birth healthy neonates in this new setting. During the colonial time, Father Antonio de Calancha, tells us in his chronicles that it took 53 years after Spaniards arrival to Potosi, at 4067 m above the sea level, to give birth to an alive newborn [97]. It is well known that pregnancy at HA restricts fetal growth, but today in La Paz, Bolivia, at 3700 m, Aymara mothers have heavier neonates than babies born from mothers with European ancestry, independently of their income and nutritional conditions [98]. This is an example how Amerindians are more adapted to live a full life in CH than the acclimatized newcomers with European ancestry.

People chronically dwelling at HA live in a milieu with hypobaric hypoxia with low atmospheric PO_2. Accordingly, they have alveolar hypoxia, hypoxemia, and polycythemia. The low alveolar and blood PO_2 triggers pulmonary vasoconstriction by inhibiting mainly potassium channels of the Kv family, raising the pulmonary artery pressure [99,100], that is one of the health problems at highlands. It is clear the advantage of the pulmonary vasoconstriction in lowlands, that permits to divert pulmonary blood towards

more oxygenated alveoli whenever hypoxia is acute or sectored in the lung. However, at HA there is a generalized vasoconstriction imposing an important afterload to the right ventricle. The degree of the pulmonary vasoconstriction and the increase in pulmonary arterial pressure is variable, for instance mild pulmonary arterial hypertension with increase in right ventricle weight is described in healthy humans at HA [5]. In contrast, severe pulmonary hypertension accompanied with right heart failure can also be the case, particularly in CMS patients [101]. Furthermore, an increase in muscularized pulmonary arterioles is also observed in Peruvian HA natives [102]. Some of treatments utilized in HA pulmonary arterial hypertension are the same used in this syndrome at sea level, such as calcium channel blockers, NO inhalation, prostacyclin analog, endothelin-receptor antagonists and PDE inhibitors. Nevertheless, the usefulness of these treatments for this special form of pulmonary hypertension still requires to be evaluated [103]. Though, in the case of HA pulmonary arterial hypertension, the ideal treatment is to descent to low altitude. However, many patients cannot do this, for economical and family reasons and they have to continue living at HA [103].

A second major health problem that the chronic HA hypoxia elicits in humans is the CMS, first described by the Peruvian physician and professor, Carlos Monge Medrano in an Amerindian [60]. CMS is characterized by marked polycythemia and hypoxemia accompanied with neurological symptoms as headache, somnolence, dizziness, insomnia, cognitive dysfunction, slowed mental function, confusion and impaired memory [103,104]. CMS is described in HA natives or long-term dwellers above 2500 m, and as in HA pulmonary hypertension, the disease is treated by descending to live at lowlands. The main mechanism producing CMS is an important hypoventilation in spite of the marked hypoxemia, showing a loss of the hiperventilatory response to the decrease in the arterial PO_2 [105]. Pulmonary arterial hypertension is also present, however its presence is not necessary for the diagnosis of CMS, since mean pulmonary arterial pressure at rest is usually only mildly elevated in HA Amerindians with or without CMS [106]. The evolution without treatment is fatal mainly as a consequence of right ventricular failure, due to the marked hyperviscosity, hypoxemia and pulmonary arterial hypertension [106]. The most important and reliable treatment is to bring the patient at lowland, which results with a complete reversal of the polycythemia and pulmonary arterial hypertension [106]; nevertheless, unfortunately this is a rather infrequent situation. For those patients that stay in HA, the treatment used for years is the blood-letting, producing temporal improvement along with reductions in hemoglobin concentrations that may persist for some weeks [106]. However, continuous blood-letting may produce iron depletion, which may

Table 1
Pharmacological therapeutic approaches and mechanisms in chronic hypoxic models.

Pharmacological therapy	Hypoxic model	Mechanisms	Results [Ref.]
Allopurinol	ICH; fetal hypoxia	Free radical scavenger; inhibition ROS production	↓Myocardial dysfunction [24,25] ↓peripheral vasoconstriction [109]
N-acetylcysteine	ICH; fetal hypoxia	Free radical scavenger; GSH precursor	↓ICH- induced cardioprotection [27]; ↓cardiac fibrosis [110]
Statins	ICH; fetal hypoxia	↓superoxide production; ↓TGF-β levels; ↑NO bioavailability	↓LV myocardium remodeling [28,29]; ↓ fetal metabolic responses [111,112]
Omega 3	ICH; CH	↓NF- κB activity; ↑antioxidant response	↓Infarct size, ↑LV function [16,34]; ↓testicular oxidative damage [44]; ↓Hypoxic pulmonary hypertension [113]
Ascorbate (vitamin C)	ICH; Fetal hypoxia; CH	Free radical scavenger; ↑NO bioavailability	Preserve sperm count [45]; ↓cardiac and peripheral vascular dysfunction [114]
Melatonin	Fetal & neonatal hypoxia	↑antioxidant response; ↑NO bioavailability	↓PVR [70] ↓fetal metabolic responses [115,116]
Sildenafil	Fetal & neonatal hypoxia	↑cGMP concentration ↑vasodilator pathway	Prevented the PAP increase during hypoxia [74]
2-APB	Fetal & neonatal hypoxiaCMS	↓Store Operated Calcium Entry ↓PVR in response to hypoxia	↓PVR [79]
Acetazolamide		↑pulmonary ventilation & ↑in blood oxygenation	↓polycythemia [107,108] ↑pulmonary vasodilation [108]

ICH: Intermittent chronic hipoxia; GSH: reduced glutathione; TGF-β: transforming growth factor-β; NO: nitric oxide; NF- κB: nuclear factor kappaB; LV: left ventricular; CH: CH; 2-APB; 2-aminoethyldiphenylborinate; PAP: pulmonary arterial pressure; CMS: chronic mountain sickness; PVR: pulmonary vascular resistance.

augment the hypoxic pulmonary vasoconstriction and then aggravating of pulmonary arterial hypertension in CMS patients [106].

Finally, Richalet et al. [107] studied the use of acetazolamide as a potential pharmacological treatment of CMS. Acetazolamide decreases erythropoietin production by an improvement in blood oxygenation. They showed in randomized controlled trial of acetazolamide in Cerro de Pasco at 4350, that the drug decreased the hematocrit, serum erythropoietin and increased ventilation and oxygenation. These results were corroborated in a 6-month randomized controlled trial, in 55 patients with CMS. Moreover, pulmonary vasodilation was showed by Echo Doppler study [108]. This is an example that old drugs can learn new tricks. The summary of the potential effects of CH during life, either permanent or intermittent is shown in Fig. 1.

4. Conclusions & perspectives

The Andean mountains are a natural laboratory to study hypobaric hypoxia. Several types of exposures as well as ranges of adaptation and responses make this area a rich field of potential research. Being able to predict, prevent and treat hypobaric hypoxia induced-health problemsshould be a public health issue, particularly in the Andean mountains and other mountainous regions around the world.

Pharmacological strategies have been proved to be reproducible in preclinical studies, across a range of studies with in vitro, ex vivo and in vivo experimental models (Table 1). Still, new pharmacological strategies should be further tested with experimentally induced pathologies that mimic clinical settings (age, co-morbidities, and specific organ functions). Further, once using the drug in clinical trials, there is a need to evaluate the possible interferences with the strategy to confirm whether the intervention is truly effective in highlander population.

Conflict of interest

The authors declare that there are no conflict of interests.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phrs.2015.07.011

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