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Potential Candidate Molecules of Past and Present for Combating High Altitude Hypoxia Induced Maladies

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ABSTRACT

Hypobaric hypoxia occurs at high altitudes where barometric pressure is low causing insufficient supply of oxygen leading to many high-altitude illnesses like Acute Mountain Sickness (AMS), High Altitude Pulmonary Edema (HAPE), High Altitude Cerebral Edema (HACE) etc. Medications have been applied to treat and prevent injuries caused by HBH, showing anti-inflammatory, anti-edemagenic, and antioxidant properties. AMS symptoms, such as headache, nausea, weariness, usually go away in 1-2 days. HACE causes brain swelling, elevated intracranial pressure, resulting in confusion, stupor, ataxia, and death. Acetazolamide, dexamethasone, nifedipine are the drugs used for treatment acting on carbonic anhydrase enzyme, calcium channels. Acetazolamide increases arterial partial pressure of oxygen. Nifedipine relaxes vascular smooth muscles and increases blood flow. Some drugs cause side effects also like dizziness, diuresis, nausea, malaise, etc. Hence, a new drug search is needed to find more targeted and fewer side effects for faster relief and better health at high altitudes.

Keywords: Hypobaric Hypoxia; Pathophysiology; Targets; Drugs

1. INTRODUCTION

Oxygen is an essential element of life for aerobic organisms. The survival and proper functioning of the body depend on the amount of oxygen received by our body's tissues and organs. Oxygen serves as the last electron acceptor in the respiratory electron transport chain of the mitochondria, helping in the generation of ATP molecules, which are crucial for cellular viability. A high consistent proportion of ATP/ADP ratio is maintained in cells under normoxia conditions. A decreased oxygen supply leads to hypoxia, affecting cell viability[1,2]. At high altitudes (HA), the barometric pressure is low, which in turn decreases the partial pressure of oxygen. This condition is termed as hypobaric hypoxia (HBH). which causes severe dysfunction in the body. Exposure to HA (approx. 3000–5000 m) changes physiological parameters[1,2]. Acclimatization to HA is necessary to avoid illnesses caused by HBH, but if an individual fails to acclimatize, they may suffer from disorders such as Acute Mountain Sickness (AMS), Pulmonary Hypertension (PH), Right Ventricular Hypertrophy (RVH), HA Cerebral Edema (HACE) and HA Pulmonary Edema (HAPE). PH is linked to an increase in thickness of the vessel wall of muscular arteries and a decline in the peripheral arterioles number. Prolonged exposure to HA has been reported to decrease working memory[3]. A reduction in the partial pressure of oxygen at high altitudes causes an imbalance in lung fluid clearance and leads to acute lung injury. The upregulation of ET-1/2/3 and with its receptors, and the downregulation of Na+ / K+ ATPase during hypoxia exposure disrupts pulmonary fluid clearance by increasing the permeability. This, in turn, leads to HAPE[4].

The hypoxia response is mainly of two types acute and chronic. The acute response to hypoxia causes significant disturbances in the ions' homeostasis. During HBH, the concentration of intracellular Ca2+ increases which is responsible for changes in the mitochondrial metabolism and the activation of proteases and lipases. CaM kinase II is believed to link acute hypoxia to chronic hypoxia. The binding of Ca2+ to calmodulin activates CaM kinase II, that phosphorylates p300, the coactivator of the Hypoxia Inducible Factor-1 (HIF-1) complex and activates HIF-1[5].

HIF is a transcriptional regulator with three members: HIF-1, HIF-2, and HIF-3.HIF-1 is ubiquitously expressed, whereas HIF-2 is expressed in the kidney, heart, lungs, small intestine and endothelial cells[6]. HIFs regulate transcription in chronic hypoxia, consisting of HIF-1 complex consisting of a HIF-1α subunit and HIF-1β[7]. HIF-1 induces the genes engaged in angiogenesis, including VEGF receptors FLT-1, transforming growth factor-β3 (TGF- β3), VEGF, and angiopoietins[8].

Over the last decade, many measures for the treatment and prevention of injuries caused by HBH at high

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altitudes have been applied. Many therapeutic drugs and formulations are now available that directly or indirectly act on targets and regulate them to ease the HBH-induced pathophysiology. Many of the drugs have shown anti-inflammatory, anti-edema genic, and antioxidant properties that reduce stress. Therefore, here in the paper, we will discuss the drugs being used against the targets involved in HBH-induced maladies.

2. HYPOBARIC-HYPOXIA INDUCED ILLNESSES

HBH is responsible for organ injuries in both acute and chronic state. Duration of exposure to high-altitude determines the extent of injuries, and symptoms a person has to endure.

2.1 AMS

The individual's climbing pace and elevation significantly affect the severity of the pathology. During the first days of high-altitude exposure, the symptoms of AMS likeheadache, nausea, fatigue or weakness, and light-headedness may limit a person's ability to move around and perform daily activities[9]. The symptoms of AMS usually go away in 1-2 days and are not life-threatening. If untreated, extremely severe AMS can develop into HACE, that is marked by brain edemaand elevated intracranial pressure and can result in drowsiness, confusion, stupor, ataxia, and eventually death[10]. Acute hypobaric hypoxia exposure causes oxidative stress, which harms many body organs, including the heart, lungs, and brain.

2.2 HACE

The most severe form of high-altitude disease, known as high altitude cerebral edema (HACE)thought to exist is a rare but potentially lethal neurologic disorder whose risk factors are rapid climb, poor acclimatisation, severe elevation, physical exertion, and a prior record of HAI. HACE is defined by elevated intracranial pressure related to cytotoxic and vasogenic edema[11]. The current notions in AMS, HACE, and HAPE state that hypoxia causes significant vasodilation that results in higher capillary pressure and leakage. Cerebral edema is caused by the neurohormonal response's release of reactive cytokines and free radicals, which further compromise the blood-brain barrier[12]. According to another view, cerebral edema is caused by free radicals created during hypoxia that inhibit the Na+/K+ ATPase pumps[13].

2.3 HAPE

HAPE is a fatal, noncardiogenic pulmonary edema caused by hypoxia at altitudes above 3,000 m in non-acclimatised individuals. HAPE is a clinical condition marked by tiredness, breathlessness, and an exertional dry cough which becomes worse with activity. It may potentilly cause cyanosis, dyspnea during rest, rales, and a 50 % risk of mortality if left without treatment[14].

3. DRUG MOLECULES AND TARGETS

Till date, many drug molecules have been formulated to accord protection against HBH induced illnesses. The molecules are formulated to act on different targets located at different organ sites for amelioration of illnesses. But not all drug candidates are potent to use without causing any side-effects.

3.1 Flavonoids from Plant Sources as Neuroprotectants

Naringenin (NGEN) and Quercetin (QUR)are flavonoid compounds derived from plant sources. Citrus fruits contain naringin in (5, 7, 4-trihydroxy flavanone), a flavanone[15] which has powerful anti-oxidant and neuroprotective

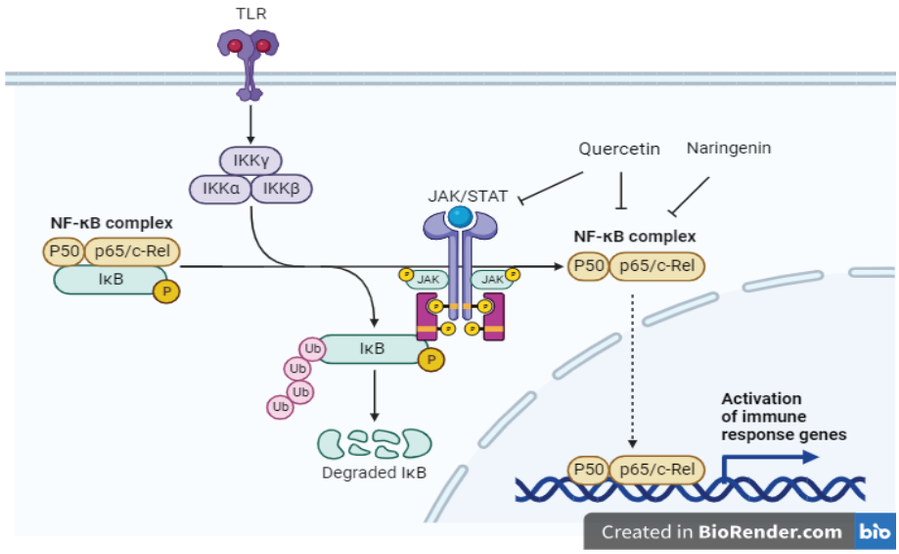

Figure 1. Naringenin and Quercetin Inhibit the NF-kB complex activation. Quercetin inhibits the activation of STAT-1 signalling to prevent the activation of inflammatory cytokines. IKK nuclear factor- κB kinase inhibitor, IκB inhibitor kappa B, NF-κB nuclear factor kappa B, JAK/STAT Janus kinase/signal transducer and activator of transcription, TLR-4 Toll-like receptor-4, p65/c-Rel- An important regulator of inflammation.

properties. These are known to act as anti-inflammatory agents as they can NF-kB activation whereas only Quercetin is able to inhibit the activation of STAT-1 and prevent inflammation as shown in Fig. 1[16]. Quercetin (2- (3, 4-dihydroxyphenyl)-3, 5, 7-tryhydroxy-4H-chromen-4-one) demonstrates anti-inflammatory, anti-cancer and anti-oxidant properties. Its neuroprotective properties in different neuronal cell cultures have also been explored.

Prolonged hypoxia at high altitude triggers neuronal apoptosis in the brain. This causes proteome damage and enhances the accumulation of abnormal proteins. Along with E1, E2, and E3 ligase enzymes, ubiquitin molecules play a critical role in maintenance of cellular balance between functional and unwanted proteins. QUR and NGEN treatment significantly downregulate the accumulation of abnormal proteins, reduce oxidative stress, and increase the level of reduced glutathione. Treatment with QUR and NGEN in hypoxic conditions shows a lesser extent of ubiquitination[17].

3.2 Carbonic Anhydrase (CA) Inhibitors as Cardio Pulmonary and Neuroprotectant

The primary approach for the treatment of AMS and HACE involves oxygenation by using drugs such as CA inhibitors, and reduction of inflammatory and cytokine responses with glucocorticoids or antioxidants.CA inhibitors act by binding to the zinc ion of the enzyme. Acetazolamide and methazolamide are CA inhibitors and are used for the management of altitude-related illnesses. Acetazolamide accelerates acclimatization at high altitude. Acetazolamide increases the poikilocapnic hypoxic ventilatory response and the arterial partial pressure of oxygen[18–20]by inhibiting the renal CA, which generates metabolic acidosis as shown in Fig. 2[21]. A dose of 250 mg/day is effective for the treatment of AMS[22]. Methazolamide possesses lesser affinity towards plasma proteins and diffuses very swiftly intissues[23]. It has fewer side effects, which are anorexia, nausea, malaise, drowsiness, and paresthesia[24]. Dexamethasone, a corticosteroid, is also a CA inhibitor. Dexamethasone is primarily used in the severe cases of AMS, HACE, where the severity of the condition requires heavy-dose steroids. It has anti-inflammatory and anti-edema genic effects. Dexamethasone suppresses inflammatory cytokines and reactive oxygen species (ROS) production. Methazolamide protects against ROS by activating nuclear-related factor-2 (Nrf-2) in the brain, which confers protection against cerebrovascular leakage in rats. Acetazolamide leads to aquaporin (AQP) inhibition, mainly in the AQP-1 and AQP-4 genes and accelerates its ubiquitin-mediated proteasomal degradation. This reduces the vasogenic form of cerebral edema in animals. Acetazolamide increases the level of heat shock protein-70 (HSP-70), which protects from hypoxic cellular stress[21].

3.3 Steroids as Neuroprotectants

Progesterone (PROG) and 5α-and rost-3β,5,6β-triol (TRIOL) reduce brain and acute HH transcriptomic changes. These neuroprotectants help protect the brain by targeting different pathways. HBH activates the NF-κB, Toll-like receptor, and FoxO signalling pathways, which form a positive feedback loop with HIF-1[25]. The inflammatory factor IL1B and the chemokine CXCL1[26,27] are also upregulated and cause vascular impairment and vasogenic edema[28].

PROG enhances erythropoiesis, and TRIOL suppresses glutamate-induced excitotoxicity. HACE treatment groups show significant reduction in brain water content. The blood-brain barrier (BBB) disruption also gets reversed by drug administration. The analysis of both WBC transcriptome, and functional enrichment indicated that HIF-1 signalling is considerably elevated by the differentially expressed genes (DEGs) EPAS1 and EGLN1, which are associated with high-altitude adaptation[29,30]. PROG (82.19 %) or TRIOL (77.54 %) treatment restored the abnormal expression levels of some HH-induced DEGs. Some of the up-regulated DEGs involved in angiogenesis by HH, like ADM, VWF, and VEGFA, were not recovered.

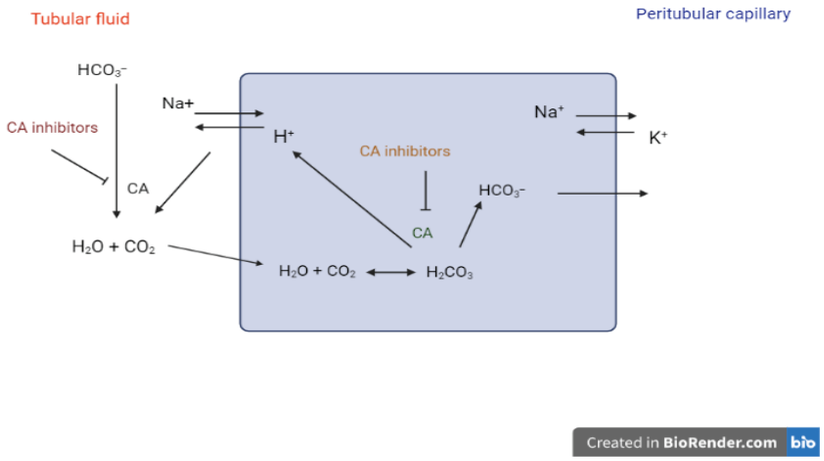

Figure 2. Acetazolamide, Dexamethasone act on CA enzyme in the renal tubules causing the carbonic acid to accumulate in tubular lumen which leads to acidosis.

PROG treatment up-regulates angiogenesis genes, including VEGFA, its receptor FLT1, haemoglobin HBB, and HBA2. TRIOL treatment enriches DEGs in excitatory neuronal signalling pathways, downregulating key genes like NMDA receptors, VGLUT, EAAT, and GRM5[31]. PROG acts as a neuroprotective by modulating GABA a receptor to suppress excitotoxicity[32]. Medroxyprogesterone (MPA), a synthetic analogue of PROG, lacks neuroprotectant properties[32], but treats chronic mountain sickness by stimulating breathing and increasing oxygen levels[33].

3.4 Solnatide Peptide as Lung Protectant

Solnatide is a synthetic peptide and a novel pharmacological agent. Its molecular makeup is similar to that of human tumor necrosis factor's (TNF) lectin-like domain[34], which is a potent drug candidate to treat HAPE. Solnatide has anti-inflammatory properties. Inflammation is an important pathogenic feature of HAPE and it is mainly triggered by ROS released upon hypoxia exposure. Pulmonary application of solnatide results in increases in occludin protein expression in the alveolar walls. This enhances the stability of the alveolar-capillary gas blood barrier and helps in the reduction of extravascular lung water, which improves the functioning of the lungs[35]. By binding directly to the essential alpha-subunit of the lung epithelial sodium channel (ENaC), the absorption of Na^+ ion from the alveolar space across the cell membrane of alveoli is enhanced[36,37]. The administration of solnatide also decreases the IL-1β, IL-6, TNF and IL-8 levels.

3.5 Platelet-activating Factor (PAF) Antagonists as Pulmonary Hypertension Protectant

PAF contributes to the development of chronic PH, and it can be treated with PAF antagonists (thienotriazolodiazepine brotizolam derived synthetic compounds). PAF levels increase in lung lavage fluid[38] and plasma during hypoxia. WEB 2170 (heterodiazepin)[39]and BN 50739 (ginkolide-derived compound)[40] are two specific PAF antagonists. Treatment with these agents reduces hypoxia-induced PH and right-ventricular hypertrophy (RVH)after3 weeks of hypoxic exposure but does not show any effect on cobalt chloride (CoCl2)- induced PH.

3.6 Meldonium as Oxidative Stress and Lung Protectant

Glycolysis is an important pathway, and the enzymes regulating it are controlled by HIF-1α in hypoxia. During hypoxia, there is an increase in glycolysis, which can lead to lung injury. Meldonium, that is a structural analogue of carnitine, can ameliorate cardiovascular diseases. Meldonium pre-treatment significantly decreases glycolysis by promoting aerobic oxidation of pyruvate and reducing lactate concentrations in vitro following hypoxia. Meldonium targets the enzymes of glycolysis, PFKP, PFKL, and PKLR, and regulates their activity. It creates two hydrogen bonds in Asp561 of PFKP and one hydrogen bond in Asp564 of PFKP[41]. PFKs (phospho-fructokinases) are rate-limiting enzymes in glycolysis and increase during hypoxia. PFKP, the platelet type of PFK, regulates glycolysis[42] and has a major role in the progression of pulmonary disease[43].

Under oxidative stress, pre-treatment of meldonium also decreases MDA levels and elevates SOD levels. Moreover, meldonium supplementation also increases Nrf2translocation into the nucleus. Nrf2 promotes the antioxidant response element (ARE) genes transcription for protection of cell from oxidative stress injury[44]. The inverse relationship between glycolysis and Nrf2 helps in preventing glycolysis and stimulating Nrf2 translocation from the cytoplasm to the nucleus[45]. Meldonium pre-treatment also correctly balances mitochondrial homeostasis by significantly reversing the alterations in the protein expression of MFN2, OPA1, DRP1 and FIS1induced by hypoxia and ameliorating lung injury[41].

3.7 EDHB as PHD Inhibitors in HIF Stabilisation

EDHB (ethyl 3,4-dihydroxybenzoate), is a prolyl hydroxylase enzyme inhibitor. Prolyl hydroxylase inhibitors (PHI) interfere with the activity of PHD (prolyl hydroxylase domain) by substituting their co-substrates (2-oxoglutarateand iron) or obstructing their active site directly. Protocatechuic acid ethyl ester, or EDHB is a substrate equivalent of 2-oxoglutarate (2OG) and a competitive PHD inhibitor[46]. PHDs require oxygen besides 2OG, iron (Fe^{2+}), and ascorbate to be functionally active and thus work as oxygen sensors[47,48]. EDHB is known to reduce HACE along with the reduction of NF-κB transcription in the brain. With EDHB supplementation, the pro-inflammatory cytokines TNF-α, interferon-γ, IL-6, and monocyte chemoattractant protein-1 levels increase with a decrease in the anti-inflammatory cytokine interleukin-10. Upregulation of VEGF by EDHB helps to maintain the blood-brain barrier. Hemoxygenase (HO-1) and metallothionein (MT-1), the antioxidant proteins expressed during stress help in the maintenance of cell homeostasis. MTs are cysteine-rich, free-radical scavengers. Hypoxia decreases MT-1 expression. Supplementation with EDHB enhances the HO-1 and MT-1 proteins expression[49].

3.8 Nanocurcumin as Anti-inflammatory and Mitochondrial Protector

Nanocurcumin is a nanotized curcumin that has antioxidant and anti-inflammatory properties. Upon administration, nanotized curcumin imparts therapeutic effects. The treatment of nanocurcumin restores the levels of cytokines and regulates ET-1/2/3, its receptors, and Na+/K+ ATPase expression, which prevents HAPE. Nanocurcumin reduces the phosphorylation and activation of Akt/Erk signalling and protects from lung injury. Nanocurmin treatment also maintains the production of ROS and VEGF to prevent lung injury[4]. The levels of TNFα, IL-6, and TGF-β also decrease significantly after treatment. In cardiomyocytes, nanocurcumin treatment inhibits hypertrophy and preserves mitochondrial efficiency and stability under hypoxia by inhibiting p53 translocation to the mitochondria and re-establishing the levels of p-300 HAT, HDAC activities, and GATA-4[50]. In H9c2

cells, nanocurcumin down regulates the expression of atrial natriuretic factor, caspase-3/-7 stimulation, and oxidative stress to alleviate hypertrophy[51]. Nanocurcumin supplementation also prevents cardiac damage by reducing HH-induced apoptosis and RVH by modify ingcardiac cGMP/cGK-1 signalling and regulating CaM kinase II, intracellular calcium levels[52].NCF supplementation improves skeletal muscle acclimatization by alleviating oxidative damage, changing the calpain activity, and modulation of the NF-κB signalling pathway[53].

3.9 Nifedipine as Pulmonary Hypertension and HAPE Protectant

Nifedipine, a blocker of calcium channels, is an ideal medication for treating the radiographically positive patients with HAPE. It selectively blocks calcium ion channels and relaxes vascular smooth muscles. and increases inferior vena cava blood flow and pulmonary artery blood flow. Nifedipine lowers pulmonary artery pressure and improves oxygenation, which reduces the alveolar-arterial oxygen gradient and helps in the clearance of alveolar edema. Prophylactic administration of nifedipine at 20 mg every 8 hours prevents HAPE and helps the rapid ascend to high altitude without developing HAPE. It is also helpful in rescuing patients with HAPE when descent or evacuation is not possible and oxygen availability is also difficult[54].Patients who take Nifedipine for the first time may have few side effects such as dizziness, head swelling, gastrointestinal discomfort, nausea, vomiting and blurred vision.

4. CONCLUSION

High-altitude acclimatization is crucial for survival, and failure to do so leads to the development of maladies like HAPE, HACE, etc. Pharmacological and non-pharmacological interventions are an escape from HH-induced maladies at high altitudes. Many drugs are in use for treating these maladies, which are mainly based on the clinical symptoms of the patients. Sometimes these drugs also have serious side effects on patients. Lack of specificities and a general mode of action may not be suggestive for treating the more severe cases of maladies developed at high altitudes, as mentioned elsewhere. Hence, the constant search for new drugs with a more specific mode of action and faster relief from an ailment is the need of the hour. This will further aid in restoring health with little or no side effects.

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Table 1. List of drugs and their targets in Hypobaric Hypoxia-induced illnesses

Drug	Illness targeted	Molecular targets	Side effects	References
Naringenin	HACE	NF-KB		17
Quercetin	HACE	NF-KB		17
Acetazolamide	AMS, Sleep disturbances	CA enzyme	Diuresis, nausea, malaise	55
Dexamethasone	AMS, HAPE, HACE	CA enzyme	Psychiatric alterations, Hyperglycemia	55
Sildenafil	HAPE, PH, RVH	PDE-5 Inhibitor (NO signaling)	Dizziness, Hypotension, Headache	56
Tadalafil and Tempol	HAPE, PH	PDE-5 Inhibitor (NO signaling)	Dizziness, Headache	57
Salmeterol (beta-adrenergic agonist)	HAPE	beta-adrenergic receptor	Tremor, Hypokalemia, Tachycardia	58
Meldonium	Oxidative stress, lung injury	PFK (platelet-type phosphofructokinase)		41
GP-14 (Gypenoside)	HACE	NF-KB		59
Solnatide	HAPE	Epithelial Sodium Channel		35
WEB 2170 and BN 50739	PH, RVH	PAF (Platelet Activating Factor)		60
EDHB (ethyl 3,4-dihydroxybenzoate)	HACE	PHI (prolyl hydroxylase inhibitor)		49

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