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The Possible Role of Inflammation in HAPE: Deterrence by Curcumin Prophylaxis

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Abstract

This review deals with the emerging evidence of an association between oxidative stress and inflammation in high altitude induced pulmonary edema. Emphasis is also given to provide evidence that curcumin - a neutraceutical derived from turmeric is highly potential prophylactic drug in eliminating the fluid influx in to the lungs of rats under hypoxia.

Introduction

Hypoxia induced inflammation and its association with oxidative stress at molecular level:

The term hypoxia induced inflammation is not new to scientific community. There exists a debate that (i) is hypoxia causes inflammation or not? (ii) Is inflammation the root cause of HAPE or is the outcome HAPE? It is not completely explored yet. Based on this aspect the authors have put efforts to explain the putative role of inflammation in hypoxia induced inflammation at high altitude regions. In Latin Inflammation is a protective phenomenon of the body from the invading organisms or it is a response involving immune cells, blood cells and molecular mediators which form the part of the complex biological response of body tissues to harmful stimuli, such as pathogens, pollens, dust, damaged cells, irritants or removal of any unwanted debris from the body. Therefore, in lucid terms, the main function of inflammation is to clear the pathogens from the body by stimulating immune system to eliminate the actual cause of cell injury, invading pathogens and also removing the dead cells, from the original insult followed by initiating the tissue repair.

Inflammation is classified into acute (i) inflammation and (ii) chronic inflammation. Acute inflammation is the foremost response of the body to invading pathogens, dangerous antigens or exposure to any kind of stress. This leads to the increased vascular leakage of plasma proteins from blood into the injured tissues making both systemic vascular and immune cells come into action either to repair or to eradicate the illness. In contrast to this, chronic inflammation involves persistence of inflammation for long duration, which further leads to destruction of the tissue.

High Altitude Pulmonary Edema (HAPE) is a life threatening sort of non cardiogenic pulmonary edema that occurs in un-acclimatised healthy, young people who ascend rapidly to altitude and also engage in several robust physical activities [1-4]. One of the aetiologies of HAPE was found to be the oxidant injury due to disturbances in micro vascular system leading to increased alveolar permeability to plasma proteins [5]. Low availability of oxygen (Hypoxia) at high altitudes has a favourable tendency to increase the Reactive Oxygen Species (ROS) from mitochondria, as a form of NADPH oxidase, xanthine oxidase/reductase and nitric oxide synthase enzymes along with establishing an inflammatory process leading to vasoconstriction, vascular leakage therefore pulmonary edema [6,7].

HAPE is potentially a life threatening condition that is surprisingly common among soldiers, pilgrims, business

people, vacationing glaciers in the Rocky Mountains, Himalayas or some other high altitude regions who have easy, rapid access to moderate-high altitude regions. The most important attribute of HAPE occurrence always depend up factors like (i) the altitude height reached (ii) the speed of travelling and (iii) the mode of ascent. Nonetheless, the incidence of HAPE occurrence is utterly depending upon individual's susceptibility to hypoxic stress at high altitudes. It is noticed that, short or acute exposure to altitudes above 2500 m to 3000 m, HAPE occurs in less than 2 days to 3 days. Even though till today, the exact pathophysiology of HAPE is unknown, but several reports revealed rather paid attention to the putative role of hydrostatic mechanisms involved in causing capillary leak and pulmonary edema. Symptoms vary from person to person; however, some common symptoms include headache, shortness of breath or dyspnoea, incapacitating fatigue, chest tightness, orthopnoea, dry cough due to haemoptysis in an advanced stage of the disease with pink frothy sputum [8]. The characteristic feature of HAPE is said to be excessively increased elevated pulmonary artery pressure before the pulmonary edema takes place [9]. However several researchers have given explanation for the occurrence of HAPE is due to (i) overstated pulmonary vasoconstriction (ii) increased sympathetic tone (iii) enhanced pulmonary capillary pressure (iv) occurrence of irregular pulmonary vasoconstriction which leads to over perfusion of vascular bed at some regions of lung (v) vascular leakage of fluid across pulmonary capillary endothelium causing interstitial and lung edema [2,8,10]. Sikri et al. [11] reported first time that, the subclinical incidence of HAPE observed in 7 subjects out of 109 subjects (which constitute 6.42 %) who stayed 3 days at 3600m height, however, it increased up to 70 % in subjects who ascended to a height of 4500m. Not only at these heights, but HAPE do occur in those individuals who perform heavy exercises even at 2400m also [12]. In support to these findings, George et al. [13] stated that when low landers ascent to high altitudes areas with greater speed and also involved in doing physical exercises are more susceptible to get HAPE. The Chest X-RAY investigation is found to be one of the best investigation methods in identifying the subclinical HAPE [11].

Several treatment methods are available to treat HAPE viz: (i) The first and foremost method of treating HAPE is stopping further ascent and immediately start descent to lower altitudes (ii) O_2 administration or (iii) keeping the person in a potable hyperbaric chambers which increases the barometric pressure of the chamber giving the appearance of lower altitude environment. In addition to these, there are several pharmacological drugs used to control the HAPE, like nifedipine, salbutamol, tadlafil, Sidenafil or steroids like Dexamethasone etc. [8]. Although these drugs are known to reduce the pulmonary artery pressure which is considered as the primary criteria in prevention of HAPE but however, these drugs also reduce the inflammation too [8,14-17]. In addition to these, another well recognised and accepted drug is Acetazolamide (Diamox) which works as both preventive and curative molecule against Acute Mountain Sickness (AMS) and High Altitude Cerebral Edema (HACE). AMS may or may not develop into HAPE. Diamox is a well known carbonic anhydrase inhibitor, there by creates a mild metabolic acidosis leading to increased ventilation; therefore gained significant importance in preventing AMS and HAPE-like alveolar protein leak in rats exposed to hypobaric hypoxia. This mechanism is most probably occurs via extenuation of Hypoxic Pulmonary Vasoconstriction (HPV) [18].

The currently used several HAPE medications have some limitations in their applications, because of their adverse side effects. For example a calcium channen blocker- nifedipine can drive to low blood pressure (hypotension), reflex tachycardia and peripheral edema. Salmeterol (B_2AR agonist) can lead to tachycardia, hence not advisable to cardiac patients. However, acetazolamide (also known as Diamox) management apart from inhibiting the renal carbonic anhydrase enzyme, but may also causes significant red cell carbonic anhydrase inhibition that also impair the carbon dioxide excretion; and all together these changes might lead to

difficulty in breathing (dyspnoea) hence respiratory failure occurs. Not only this, diamox is even, unlikely to be recommended or safe for the patients with sulpha energy problems [19,20]. Another recommended HAPE medication is Tadalafil (a PDE5 inhibitor), generally shows headache and dyspepsia type of problems as side effects. Similarly, dexamethasone (a steroid used to reduce the vascular leakage and inflammation in lungs and brain of people under hypoxia at high altitude regions) is associated with several side effects like headache, nausea, vomiting, and dizziness, swelling in the extremities, stomach upset, hyperglycemia, restlessness, mood imbalnce, sleep disorders and sometimes even depression. It is a known fact that, once this (Dexamethasone) medication is stopped it certainly affects the person again [3]. None the less there are several other drugs or adjuncts like Ginko biloba or Rhodiola crenulata extracts, intravenous hydralazine , phentolamine or some diuretics etc. are being used at several times in order to prevent the HAPE, even though, neither their molecular mechanism of action is known completely nor showed absolutely awesome results [21,22]. Perhaps at present, this could be the reason these drugs are not being recommended to curb the high altitude problems. The following paragraphs will envisage the details about the different transcriptional factors and their regulatory genes involved in maintaining the homeostasis mechanism at high altitude regions and how curcumin is able to modulate these transcriptional molecules leading to abate the hypoxia induced pulmonary edema.

It is highly reported that Hypoxia Inducible Factor (HIF-1α) and one of its downstream genes Vascular Endothelial Factor (VEGF) play a crucial role in adapting the acclimatisation process via initiating the neovascularisation process in the body upon high altitude exposures. Chao et al. [23] pointed out an association between hypoxia induced oxidative stresses with inflammatory mediators, which together contribute in promoting the HIF-1α stabilization. These studies hypothesised the involvement of inflammation in acclimatisation process at high altitudes. Even after many years of vigorous research, still today the question remains same ie. Is HAPE caused due to inflammation or it occurred once the HAPE developed? In fact, in the scientific community, the question remains that, is the microcirculation changes that observed under hypoxia in rodents counterpart to that of humans at high altitude areas? Although several recently published studies conferred that rats or mice exposed to hypoxia showed enhanced ROS production, reduced antioxidant status, increased leukocyte endothelial interactions due to increase in pro-inflammatory cytokines that augment the vascular permeability in the lungs [7,24]. Therefore, it is doable that, the initiation of inflammatory responses shown by the rodents may help in acclimatisation process and may therefore improve gaseous exchange (O_2 supply) in the tissues, as clinically demonstrated by the presence of inflammatory factors in HAPE prone subjects [23,25].

The cellular response to hypoxia is not only restricted to HIF-1α expression, but many other transcription factors like (i) Nuclear Factor-κB (NF-κB) and (ii) Nuclear erythroid like factor 2 (Nrf2) have also been identified in response to hypoxia.

NFkB is the master regulator of inflammation and it plays a significant role in HAPE. NF-κB transcriptional factor regulate more than 200 genes which are responsible for both the arms of immunity i.e. innate immunity and adaptive immunity. It is composed of its subunits RelA, RelB, cRel, NF-κB1 (p105/p50) and NF-κB2 (p100/p52) either in heterodimer form or in homodimer form in the cytoplasm in inactive form and are bound to a family of inhibitors called κB (IκBs) [26]. Upon stimulation by different stresses, for (eg. Hypoxia) a number of pathways are being activated that allows NFkB to trans locate itself in to the nucleus, accumulate and ultimately binds with promoter regions of the DNA which further leads to activation of genes involved in induction of pro-inflammatory cytokines (IL-1, IL-6, TNF- α etc) and anti-inflammatory cytokines (IL-10, IL-4, TGF-β etc.), Cell adhesion molecules like ICAM-I, VCAM-I and also P-select in and E-select in etc. It was hypothesised that, the

inflammatory alternation that occurred in pulmonary vasculature perhaps play a significant primary role in developing HAPE [27]. However, this concept was opposed by a recently published study [9]. Swenson et al. [9] have showed that, inflammation is the result of HAPE and it is a secondary concept. However, it is to be noticed that, this study have not measured the cytokine production while developing HAPE in human subjects. It is therefore perceptible that, once HAPE developed, the inflammatory response that occurred due to hypoxia might settle spontaneously and then later, the microcirculation may not respond to the inflammatory molecules. This would possibly explain the fact that production of inflammatory mediators might precede the onset of pulmonary edema because NFkB directly regulate the pro-inflammatory cytokines production. This phenomenon was addressed by Sarada and colleagues [28] that when rats exposed to hypoxia for different durations like 1h, 3h, 6h, 12h and 24 h at 25000 ft at 25^0C, the NFkB protein expression was found to be enhanced as the exposure time increased. These results when correlated with vascular leakage studies, the results revealed that the NFkB was up regulated before the accumulation of fluid in to the lungs of rats, indicating that NFkB has a role to play in vascular leakage in rodents. In support to these findings Eldridge et al [29] noticed that, there was increased E-selection levels from Bronchoalveolar Lavage Fluid (BALF) obtained from subjects who were exposed to an altitude of 3810m. Further, these authors reported that, under cycloergometric exercise, subjects with HAPE at moderate altitudes (2600m to 3600m) showed elevated proteins, alveolar macrophages, lymphocytes, followed by increased pro-inflammatory cytokines like IL-1β, IL-6, IL-8 and TNF-α; but once recovered all these values came back to normal basal level. Kubo et al [27] noticed similar findings in human subjects at such comparable heights. This type of findings were also reported in animals exposed to hypoxia even for several days, where the inflammation got resolved under lower PO_2, but it could not enhance the inflammation further, indicating the acclimatisation of endothelium to hypoxic stress [30]. A part from increased pulmonary artery pressure in the course of development of HAPE, and several studies have reported some other mechanisms like (i) lung inflammation caused by alveolar macrophages due to reduced alveolar oxygen (ii) impairment in ion channel function, (iii) altered tight junction proteins integrity, (iv) surfactant proteins oxidation, which also contribute for fluid build up in lungs (HAPE) [10,31,32]. In accordance with the studies of Madjdpour et al. [31], Chao et al. [24] and Arnada and Tuesta [33] also inferred the importance of a chemokine Monocyte Chemoattractant Protein-1(MCP-1) which is released by hypoxia induced alveolar macrophages contribute in causing micro vascular inflammatory cascade in rats but not by the low systemic tissue PO_2. In addition to these findings substantiated with his studies that, rats exposed to acute hypobaric hypoxia showed increased mucosal barrier injury leading to disturbed gastrointestinal immune cascade because of increased number of natural killer cells (NK cells), toll like receptors (TLR4) followed by elevated pro-inflammatory cytokines (IL-17) [34]. In some other experiments, the production of several Pro-inflammatory cytokines like IL-1, IL-6, TNF-α, MCP-1, TGF-β and INF-γ followed by cell adhesion molecules like ICAM-I and VACM-I in BALF were found in rats when they were either exposed to 9,142 m for 5 h or at 7619 m [33,35] for 48 hr. Similarly in another experiment, Rats exposed to 10% O_2 showed increased NFkB protein levels, mRNA of ICAM-1, VCAM-1, HIF1-α levels along with elevated ROS production by alveolar macrophages [31,36].

In view of all these findings in 2008, Sarada and colleagues have addressed the role of inflammation in contributing the hypoxia induced pulmonary edema in rodents exposed to hypoxia. Sarada et al [28] had noticed that, when rats exposed to different hours of hypobaric hypoxia, there was no linear relationship occurred between ROS generation and vascular leakage, although ROS continued to get accumulate even after 24 hrs of hypoxia exposure, in spite, maximum lung water content obtained between 6 hrs to 12 hrs of hypoxia exposure. But surprisingly the NFkB activity was continuously increased

from 1h onwards to 48 h of exposure which, lead to conclude the hypothesis that importance of NFkB role in pulmonary edema formation. These studies preclude that ROS are not solely involved in causing vascular leakage in lungs of rats, but might be involved in initiating the vascular leakage probably via triggering the NFkB up regulation. Beck Schimmer et al. cell lines studies support these findings. Beck Schimmer et al. [37] reported the expression of increased cell adhesion molecules in alveolar epithelial cells at 5 % of hypoxia exposure.

The different results obtained in different studies conducted by researchers related to the putative role of inflammation under hypoxia basically differs because of several reasons, viz. (i) the height at which the animals are exposed (ii) the duration of the exposure (iii) the speed of ascent to reach high altitudes (iv) the differences in barometric pressures (v) Oxygen percentage availability (vi) whether animals are exposed to normobaric hypoxia or hypobaric hypoxia and (vii) further the reoxygenation of the animals- all these conditions are important under animal experimentation.

Hypoxia Inducible Factor -1 α (HIF-1α) is primarily induced in response to Hypoxia, where as nuclear factor erythroid like factor (Nrf2) is induced in response to hypoxia and oxidative stress therefore plays a putative role in maintaining oxidative homeostasis. Nrf2 is generally binds to its negative regulator KEAP Kelch like erythroid cell derived protein with cap"n' collar (CNC) homology -associated protein 1 in the lack of oxygen (hypoxia) or oxidative stress. That means, under normoxia or normal oxygenated conditions binding of Nrf2 to KEAP1 facilitates its ubiquitination and it becomes non-functional. However under oxidative stress, the KEAP1 undergoes oxidation at several key cystene residues leading to alter its confirmation therefore allows Nrf2 to escape from ubiquitination mediated degradation which further leads to translocate itself in to the nucleus and binds to its promoter regions containing Antioxidant Response Element (ARE). This way the Nrf2 gets activated and there by promotes transcription of antioxidant enzymes such as NADPH (Nicotanamide adenine dinucleotide phosphate), quinine dehydrogenase 1(NQO1), heme oxygenase 1(HO-1). These enzymes help in reducing the damaging effects of ROS levels in the cells thereby maintains homeostasis.

Role of Curcumin in Prevention of HAPE:

High altitude where harsh environmental factors prevails (such as, hypoxia -low O_2 availability, low barometric pressure, high wind velocity, increased UV radiation exposure, temperature variations etc) overwhelms a number of high altitude melodies like Acute mountain sickness, High altitude pulmonary and cerebral edema, sleeping sickness, etc. So at these conditions body produces more ROS, lowers antioxidants and increases inflammation with various manifestations. Manifestation of these conditions (hypoxia induced oxidative stress) manyatimes matches the conditions like ischemia/reperfusion type of insult. The acclimatisation or the adaptive processes to this oxidative stress induced inflammation takes pretty long time to recover, however is also individual specific. On the other hand physical exercise or activities involving physical strength further execrates the extent of fatigue due to increased oxidative challenge. Therefore, special attention is required to curtail the degree of oxidative stress followed by attenuation of inflammation before it happens by prior treatment with phytochemical molecules which might provide better adaptation to such environmental habitats. Therefore, it is necessary to identify a suitable prophylactic agent which can prevent or reduce onset of hybobaric hypoxia induced oxidative stress and inflammation which further leads to reduce the high altitude pulmonary edema (HAPE). One such exemplified molecule identified is curcumin. Curcumin is a bright yellow compound derived from turmeric which is used to treat number of inflammatory diseases.

Curcumin -a natural phyto-constituent obtained from turmeric (*Curcumin Longa* Linn) - is popularly used as dietary and medicinal moiety in India and South East Asian countries. Curcumin is sold as an herbal supplement, used as cosmetics ingredient, food flavouring agents etc. It is also excessively

used as food colouring agent and also in preparation of household food items in India. Curcumin is a well known potent antioxidant [38]. Curcumin structure contains an unsaturated aliphatic chain with two aromatic rings and the electrons in curcumin are found to be highly conjugated. Such type of structures is generally good antioxidants as they form stable radicals after receiving electrons [39]. Curcumin is also a well known potent anti-inflammatory molecule. By administering the curcumin (50mg/kg BW) to rats one hour prior to hypoxia exposure for 6h, Sarada and colleagues (2008, 2014) observed a reduction in lungs ROS and MDA levels compared to control ($P<0.001$) and this reduction might be due to well known curcumin's scavenging activity as an anti-oxidant agent. They further revealed that, reduction in reduced glutathione (GSH) levels in lungs of rats under hypoxia was found to be increased up on curcumin supplementation. However, the same curcumin dose administered to rats under normoxia conditions did not show any changes in lung glutathione levels indicating that the observed increase in GSH levels under curcumin administration is mainly to handle the hypoxia induced oxidative stress which is an adaptive phenomenon at high altitude regions. A characteristic feature of curcumin is its ability to alter several cell signalling pathways which perhaps leads to increase in antioxidant glutathione levels in stressful conditions. Prophylaxis with curcumin enhanced the levels of Lung GSH, GPx, and SOD levels, stabilised the HIF1 α expression along with down regulating one of its downstream gene VEGF expressions in lungs of rats under hypoxia compared to control [7]. This might leads to maintain the cellular homeostasis to counteract the oxidative stress under hypoxic conditions at high altitude regions. One of the reasons for accumulation of fluid at interstitial and also in the non-vasoconstrictor lung areas as suggested by Oelz et al. [40] was mainly, due to the occurrence of uneven pulmonary vasoconstriction that enhances the filtration rate leading to fluid influx (lung edema). In relation to these findings another interesting observation was revealed by Sarada et.al. [7,28] that lung injury measured in terms of LDH release in to BALF (LDH is an indicator of tissue injury) was noticed from 1h of hypoxia exposure to 48 hrs of continuous hypoxia exposure. These results point out that inflammation takes place before the fluid get accumulates into the lungs and therefore aids in augmenting the vascular leakage. Further the appearance of albumin (protein) influx in to the lungs in one hour of hypoxia exposure onwards gives enough evidence, that inflammation contribute in causing edema development in the lungs of the animals [7,28]. The studies pointed out that, when these rats were prior administered with curcumin and then exposed to hypoxia showed significantly reduced the levels of albumin in BAL fluid compared to control animals. By these observations Sarada and colleagues reported that, enhanced levels of LDH activity confirm the cellular injury in lungs of rats under hypoxia where as curcumin prophylaxis reduces the LDH levels.

In addition to these observations, Sarada et al. reported that, curcumin significantly reduced the up regulation of IKKαβ resulting into prevention of degradation of IKBα [7]. These findings infer that curcumin administration under hypoxic conditions can attenuate the NFkB degradation at its upstream path in the cell's cytoplasm. Apparently, this prevents the IKB α phosphorylation and degradation there by its activation and translocation of the NFkB in to the nucleus is stopped. Therefore further transcription of pro-inflammatory cytokine production is stopped. This process is very congenial to cell to survive due to reduction in pro-inflammatory cytokines (IL-1, IL-2, IL-6 and TNF α) and cell adhesion molecules (ICAM-1, VCAM- 1, E-Selectin and P-Selectin).

One of the cytokins released by activation of NFkB is IL-18 (belongs to IL-1 cytokine family) earlier referred as interferon √ inducing factor is a potent pro-inflammatory cytokine [41]. This cytokine is very much essential for facilitating the nutrophil dependent injuries via inhibition of anti-inflammatory cytokine production during hypoxia and reperfusion injury. In relation to these findings Kim et. al. [42] stated that, hypoxia induces production of IL-18. IL-18 then

triggers the induction of HIF1-α via activity of Rac1 dependent NFkB pathway. All these studies provide enough evidence that, increased levels of IL-18 production under hypoxic stress arbitrates the induction of increased HIF1-α expression under hypoxia which provide an association link between inflammation and edema formation.

Recent research had revealed that curcumin prophylaxis under hypoxia led to decreased expression of MURF-1, ubiquitinated protein and μ-calpain, with degradation of myofibrillar proteins followed by increased total skeleton muscle turnover, therefore leading to enhanced physical performance in rats [43]. These studies point outs that, curcumin supplementation has benefits of modulating the overactive pathways to basal levels without completely blocking the pathways. The enormous therapeutic properties of curcumin is well evidenced in several *in vitro* and *in vivo* studies and even in several human clinical studies and all these research studies revealed its promise in curing several inflammatory diseases.

In general in normoxic conditions also, paracellular leakage of fluid into alveoli can occur in rare instances. The fluid thus leaked into alveoli is pumped back to the interstitial space by the Na+ gradient generated combined action of basolaterally located Na+/K+-ATPase and apically located ENaC. Exaggerated pulmonary hypertension is not the single most factor that is contributing HAPE, however other mechanisms like trans-epithelial sodium and water transport in the lung also play major role in fluid accumulation in lungs at high altitude have shown that hypoxia inhibits the Na+/K+-ATPase activity as well as decreases its copy number in the plasma membrane of alveolar epithelium under hypoxia [44,45]. This process is mediated by the increased generation of ROS followed by increased lipid peroxidation. The increased oxidative stress further up regulated the transcription factor NFκB, thereby switching on to an intracellular pro-inflammatory signalling pathway, triggering decrease in copy number and activity of α1-Na+/K+-ATPase and α- ENaC proteins expression. A novel finding was reported by these authors was that even though α1-Na+/K+-ATPase stopped functioning from 6 h of hypoxia exposure but α- ENaC was functional up to 24h of hypoxic exposure in rats. This indicate that under hypoxia, fluid keep on getting accumulated in the alveoli and could not be cleared duo to failure in the α1-Na+/K+-ATPase activity at the basolateral side of the lung epithelium leading to pulmonary edema. However, rats prior treated with curcumin (50mg/Kg BW) and exposed to hypoxia significantly activated the α1-Na+/K+-ATPase activity and its copy number along with increased α- ENaC proteins expression leading to enhanced alveolar fluid clearance [45]. These authors further reported that, Curcumin being a powerful antioxidant and anti-inflammatory molecule is capable of providing a reduced oxidative stress environment in A549 cells as well as in rat lungs thereby capping the activation of NF-κB and inflammation induced by hypoxia which was considered to be contributor of reduced alveolar fluid clearance. These studies indicate the importance of curcumin supplementation in facilitating the alveolar fluid clearance during hypoxia exposure by improving the clearance capacity of lung to remove the accumulated fluid by increasing the α-ENaC, α1-Na+/K+-ATPase proteins expression and activity.

It is quite often noticed in HAPE was the pulmonary surfactant oxidation that leads to alveolar collapse. As reported by Haagsman [46] that, exposure to hypoxia has been reported to be associated with increased pulmonary surfactant's oxidation leading to changes in structural integrity, lungs collapse and edema formation. Mathew and Sarada et al. [44] have reported that prophylactic administration of curcumin (both *in-vitro* and *in-vivo*) reduced the oxidative stress by enhancing the expression of Phase II antioxidant enzymes mediated through Nrf2 and HIF-1α pathway leading to balanced expression of pulmonary surfactant homeostasis there by reinstated the survival signaling under hypoxia.

Disrupted/ impaired alveolar epithelial barrier made up of Tight Junction (TJ) proteins is also known to cause fluid accumulation in lungs in HAPE patients. It has been reported that the tight junction complex is mainly formed through

interactions between several integral proteins viz. claudin, occludin, Junctional Adhesion Molecule (JAM) and peripheral proteins like zonular occludins (such as ZO-1, ZO-2 and ZO-3) along with other junctional associated proteins [47]. Several authors have speculated that TJ protein integrity is severely affected by NFkB induced inflammation to certain account [48-50]. In support to these studies several clinical and experimental studies have also evidence that pro-inflammatory cytokines like TNF-α and IL-6 plays a critical role in lung injury which further causes nutrophils and macrophages migration to lungs leading to loss in TJ proteins integrity [48-51]. It seems quite obvious that, recruitment of leukocytes trigger signal transduction cascades that leads to disorganization of TJ and alveolar epithelial barrier breakdown [52]. However Mathew et al., [45] 2020 experiments supports the hypothesis that hypoxia induced lung oxidative stress and increased NF-κB activation leads to increased pro-inflammatory cytokines (↑TNF-α, IL-2, IL-6 and γ-IFN) followed by decreased anti-inflammatory cytokines (↓TGF-β, IL4 and IL-10) which then altered the epithelial permeability by impairing TJ organization i.e. decreased tight junction protein expression levels of ZO-1, claudin-4, occludin and increased expression levels of claudin-5 and JAM-C. It is palpable that, in these situations, preventing the activation of inflammatory cascade offers a great remedy and might rescue from alveolar tight junction disruption. Therefore these authors used curcum prophylaxis, which modulated these TJ proteins disorganisation and restored normal configuration of TJ proteins in hypoxia exposed animals compared to control. However, in order to strengthen their findings these authors further explored and reported that, A549 cells transfected with siRNAp65 and/treated with MG132, though showed reduced permeability of fluorescent molecule (Dextran - FITC) in these cells under hypoxic stress leading to slight increase in alveolar epithelial barrier integrity (measured in terms of paracellular transport) but perceived due to increased NFkB activation (inhibited by treating the A549 cells with either siRNA or MG 132 (10 μM)) which were found to be not as equivalence as with that of curcumin treated cells. Even though, these two NF-κB blockers (siRNAp65 and MG132) were incapable to scavenge the free radicals produced under hypoxia, hence little activation of NF-κB might have occurred, but certainly showed a significant reduction in the dextran permeability compared to hypoxia exposed animals. There is a possibility of loss of tight junction proteins unity may also occurs in lung epithelium by NFkB inhibitors in the absence of an inflammatory insult [49]. It appears that complete or global attenuation of NF-κB is detrimental and therefore some expression of NF-κB is required even under stressful environments in order to maintain the immune functions. At the same time, HIF-1α stabilisation has also been accredited to propagate anti-inflammatory responses while down regulating the pro-inflammatory responses [53]. A reporter gene assay study conducted by Mathew et al. [54] had demonstrated that HIF-1α was stabilized by curcumin administration. But silencing of NF-κB p65 with MG132 failed to stabilize HIF-1α under hypoxia as compared to untreated hypoxia exposed A549 cells. HIF-1α gene has a NF-κB binding site at its proximal promoter region [55,56]. More recently, it is evedenced that, NF-κB binds to the HIF-1α promoter under hypoxic conditions [57]. It appears that, down regulating the NF-κB activity leads to reduced expression of HIF-1α under hypoxic conditions. This explains that, the expression of NF-κB must be down-regulated to certain extent under hypoxic conditions, for controlling the inflammation and also at the same time, the HIF-1α expression must be stabilized to maintain the oxygen homeostasis and consequently acclimatization in order to sustain the hypobaric hypoxia insult. The most important conjecture reported by Mathew et al. [54] that, even though curcumin was able to attenuate NF-κB protein expression, it stabilized HIF-1α as well. That means, HIF-1α stabilisation under hypoxia is not due to the attenuation of NFkB by curcumin, but might be via some other mechanism used by curcumin which is not explained in this study. Along these observations, curcumin prophylaxis was

found to appreciably augment the RBC and Hb count there by PaO_2 and $PaCO_2$ levels were maintained more or less similar to that of control. These two major pathways indeed play a crucial role in controlling the oxygen homeostasis on one hand and inflammation on the other hand pointing that a cross talk take places between these two most important transcriptional molecules under hypoxia.

Nowadays apprehension is that, because of its low absorption and faster elimination with the low bioavailability of curcumin normally found in rodents and humans may not be sufficient to reach different organs of the body in appropriate quantities in order to have an anticipated biological effect. However, in order to address this, Pawar et al. [58] had conducted experiments on distribution of curcumin in different tissues of human subjects. Their studies confirmed an appreciable amount of curcumin levels in different tissue of human subjects as oral administration of curcumin was found to be absorbed rapidly and also distributed quickly into the tissues, hence resulting into undetectable or sometimes very low levels in plasma, indicating the proper utilization of curcumin at different tissue levels. Pharmacokinetic modelling of the plasma concentration - time profile after oral administration disclosed the nature of curcumin function in the body ie. Curcumin follows two compartment model systems with first order absorption, lag time followed by first order elimination [58]. These authors have provided insight into the therapeutic efficacy of curcumin despite being undetectable in human plasma. Mishra et al. [59] checked the stability of curcumin in rat's plasma and tissues first time using High Performance Thin Layer Chromatography (HPTLC) method and observed that the maximum amount of curcumin (50 mg/kg BW) in its native form was maintained up to one hour after administration compared to different hours tested. These authors reported that, curcumin distribution majorly occurred in plasma, kidney, liver, heart, lung, muscle and brain up to one hour and began to undergo biotransformation thereafter. So, several cell line studies, animal and human clinical studies have all established curcumin's importance in preventive as well as therapeutic potentials in several diseases [60]. Earlier, in relation to these findings Cheng et al. [61] reported that no treatment related toxicity was observed up on oral feeding of curcumin up to 8g/day in human subjects. However, more than this dose, the large amount of the curcumin was not accepted by the patients. All these studies infer that, it is not only the oxidative stress but also inflammation also plays a putative role in causing fluid influx into lungs. These findings imply that the curcumin supplementation to rats prior to hypoxia exposure is more beneficial and effective in model of HAPE in animals. However, this mechanism might protect the humans from HAPE which requires further studies.

The active phytomolecule Curcumin extracted from *C. Longa* used as an excellent anti-inflammatory moiety in traditional medicines practiced in India and several other South East Asian countries therefore considered as safe. In several cancer studies, the pharmacokinetics of curcumin and its bioavailability had been comprehensively explored in human subjects with safe use [61,62]. Curcumin is considered as safe molecule under the category of Generally Recognised as Safe (GRAS) by Food and Drug Administration (FDA). All these experiments prove the potential substantiation of curcumin use in clinical applications. Based on these studies, it is inferred that curcumin having a multi facet role as an excellent moiety might prevent high altitude illness faced by number of Soldiers deployed at extreme altitudes, sports persons, trekkers, mountaineers of sojourns, high altitude training areas, business people visiting to high altitude areas.

Conclusion

The above discussion infers that, hypoxia induced increased oxidative stress and inflammation leads to fluid accumulation in the lungs. These intensive effects could be effectively prevented by prophylaxis with curcumin. The functional changes that brought by curcumin prophylaxis under hypoxia, might be due to attenuation of NF-κB there by reduction in inflammation, stabilising the over expression of HIF-1α there by maintaining the oxygen homeostasis along with stabilisation of Nrf2 levels which enhances the anti-oxidant

status leading to control the fluid accumulation in lungs, hence, facilitates the body to adjust to the high altitude regions. Therefore, this review abridges the potential prophylactic use of curcumin in reducing the incidence of hypobaric hypoxia induced pulmonary edema in rats. Since the intricate factors are many in case of HAPE, it is obvious that a drug that can protect the organism at different cellular and tissue levels offers a positive prophylaxis and potential moiety to fill this lacuna as a prophylactic drug that can regulate the alveolar fluid influx under hypoxic conditions. It is essential and warranted to evaluate the safety and tolerability studies of curcumin administration against HAPE in human subjects at high altitude areas.

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