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ROLE OF CARBONIC ANHYDRASE IN HYPOXIC CONDITIONS: FROM MOLECULAR MECHANISMS TO THERAPEUTIC INTERVENTIONS.

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Abstract: Hypoxia, characterized by insufficient supply of oxygen that triggers cascade of physiological responses from the individual level down to the regulation and function of the cell nucleus. Prolonged periods of low oxygen tension are a core feature of several disease states, with traditional treatment options limited to supportive measures and oxygen therapy. However, the emergence of carbonic anhydrase inhibitors presents a promising breakthrough in managing certain hypoxic conditions. Carbonic anhydrase inhibitors exhibit the capacity to modulate acid–base balance addressing metabolic alkalosis, and enhancing ventilation, thereby influencing respiratory responses. Although carbonic anhydrase inhibitors are not first line of treatment for hypoxia, recent literature shows the promising impact of carbonic anhydrase inhibitors in hypoxic conditions such as hypobaric hypoxia, hypoxic tumors, cerebral hypoxia, and coronary obstructive pulmonary disease. These findings suggest a shift from conventional approaches to a targeted pharmacological strategy for treatment and prophylaxis of such hypoxic conditions. In this review, we will highlight the latest insights into how low oxygen levels affect the body at a molecular level in common and significant diseases such as being at high altitudes (hypobaric hypoxia), tumors with insufficient oxygen (hypoxic tumors), low oxygen in the brain (cerebral hypoxia), and reduced oxygen levels in individuals with chronic obstructive pulmonary disease (COPD). Furthermore, we will explore how well carbonic anhydrase inhibitors work in addressing and managing these conditions.

Index Terms **-** Hypoxia, Carbonic anhydrase inhibitors, Acetazolamide, Hypobaric hypoxia

1. INTRODUCTION

Hypoxia is a condition characterized by an insufficient supply of oxygen (O_2) to the body's tissues, leading to an imbalance between oxygen supply and cellular consumption. This deficiency compromises the ability of cells to maintain optimal function (Ward et al. 2011; Wilson & Shapiro 2001).When the supply of oxygen is diminished or disturbed, organisms employ diverse adaptive mechanisms to support cell survival under hypoxic conditions. Generally, this hypoxic response subsides upon the restoration of oxygen levels. However, complications can arise when hypoxic stress persists, leading to chronic hypoxia, or when there is a recurring pattern of normal oxygen levels followed by hypoxic episodes, known as intermittent hypoxia. Extended or intermittent hypoxic conditions trigger a cascade of gene expression, forming a hypoxia-mediated gene regulatory network. This network results in alterations in cellular function and behavior. Consequently, irreversible processes may occur, leading to physiological disorders or even pathological consequences (Chen et al. 2020).Hypoxic effects are broadly categorized into two types, immediate effects and delayed effects, each manifesting distinct physiological consequences (Table 1). Barcroft's classification system further classifies hypoxia into four types based on specific physiological parameters (Kane et al. 2020). These parameters include the utilization of oxygen by cells, oxygen tension in arterial blood, the velocity of blood flow, and the oxygen-carrying capacity of blood (Table 2). By considering these factors, Barcroft's classification provides a comprehensive framework for understanding different manifestations of hypoxia and modifying treatment approaches accordingly.

Treatment options for hypoxic conditions have traditionally been limited to supportive measures such as oxygen therapy, which involves the administration of pure or mixed oxygen (Choudhury 2018), as well as conventional approaches like symptomatic treatment using bronchodilators and anti-inflammatory medications to improve airflow and reduce airway inflammation. In severe cases, mechanical ventilation may be necessary to ensure sufficient oxygen supply.

Carbonic anhydrase inhibitors (CAIs) belong to diuretic class of medications, primarily used to manage and treat glaucoma, idiopathic intracranial hypertension, altitude sickness, congestive heart failure, and epilepsy. The diuretic properties of these inhibitors not only impact the acid–base balance but also exhibit the capacity to stimulate respiration, thereby contributing to the management of certain hypoxic conditions. Although CAIs may not be the first line of treatment and are not universally applicable across all hypoxic scenarios, recent literature highlights their impact on hypobaric hypoxia, hypoxic tumors, cerebral hypoxia, and chronic obstructive pulmonary disease (COPD). This review focuses on CAIs delving into their classification, mechanisms of action, and their pivotal role in managing hypoxic conditions such as hypobaric hypoxia, hypoxic tumors, cerebral hypoxia, and chronic obstructive pulmonary disease (COPD) related hypoxemia.

Table 1. Immediate and delayed effects of hypoxia

A. Immediate Effects	
Blood	Hypoxia triggers the release of erythropoietin from the kidneys. In response erythropoietin increases the generation of red blood cells, this raises the blood's ability to carry oxygen (Scholz et al. 1990).
Cardiovascular system	Initially, reflex activation of the cardiac and vasomotor centers raises blood pressure, cardiac output, heart rate and contraction force of heart. Later, there is a decline in cardiac output and blood pressure along with a drop in heart rate and heart contraction force (Heinonen et al. 2016).
Respiration	Chemoreceptor response causes a spike in breathing rate, which in turn causes a significant amount of carbon dioxide to be expelled from the body and causes alkalemia. Subsequently, breathing becomes shallow and sporadic, and respiratory center failure ultimately results in a significant reduction in breathing force and rate (Brinkman et al. 2023).
Digestive system	Hypoxia is linked to nausea, vomiting, and appetite loss (Kozak et al. 2006).
Kidneys	Hypoxia increases erythropoietin secretion from the juxtaglomerular apparatus. In addition, alkaline urine is secreted (Wojan et al. 2021).
Central nervous system	The symptoms of minor hypoxia resemble those of alcohol intoxication like depression, apathy and a general loss of self-control. Individuals may exhibit talkativeness, irritability, and rudeness, along with behaviours like shouting, singing, or crying. Disorientation, impaired memory, and a diminished ability to make judgments become apparent. Hypoxia also induces weakness, muscle fatigue, and lack of coordination (Wang et al. 2022). In cases of severe hypoxia, a sudden

	unconsciousness can occur. Without immediate intervention, this may progress to coma, ultimately leading to fatality.
B. Delayed effects	
The symptoms related to hypoxia vary based on the duration and severity of exposure. Individuals may experience heightened irritability, accompanied by symptoms commonly associated with mountain sickness, including nausea, vomiting, feelings of depression, as well as sensations of weakness and fatigue.	

Table 2. Barcroft's classification of hypoxia

Hypoxia	Impairment	Example
Hypoxemic Hypoxia	Gas transport across the alveoli decreases leading to decrease in partial pressure of the oxygen.	Hypobaric Hypoxia (High altitude hypoxia) Obstructive Lungs disease (chronic obstructive pulmonary disease, asthma) Restrictive lung disease (e.g. fibrosis) Pulmonary edema ,Acute respiratory distress syndrome
Anemic hypoxia	Oxygen-carrying capacity of the blood is reduced, typically due to a decrease in the concentration of haemoglobin or a decrease in the ability of haemoglobin to bind oxygen.	Low haemoglobin concentration (e.g. iron deficiency, folate deficiency) Genetic hemoglobinopathies (e.g. thalassemia)
Circulatory/Stagnant hypoxia	Reduced blood flow to the tissues, leading to inadequate oxygen delivery.	Hypovolemic Cardiogenic shock Distributive shock (e.g. septic, anaphylactic) Obstructive shock (e.g. cardiac tamponade, tension pneumothorax, massive pulmonary embolism)
Histotoxic hypoxia	Cells are unable to utilize the oxygen delivered to them,	Cellular dysfunction

	despite normal oxygen availability and adequate oxygen transport in the blood.	Cyanide poisoning Carbon monoxide poisoning

2. CARBONIC ANHYDRASE

Carbonic anhydrases (CAs) constitute a super family of metalloenzymes (Chiaramonte et al. 2018), that are distributed across prokaryotes and eukaryotes. These genes are encoded by four distinct, evolutionarily unrelated gene families:α-CAs, which are found in vertebrates, bacteria, algae, and the cytoplasm of green plants; β-CAs, which are primarily found in bacteria and algae; the chloroplasts of both mono- and dicotyledons; γ-CAs, which are primarily found in Achaea and some bacteria; and δ-CAs, which have been found in certain marine diatoms (Supuran et al. 2004). This enzyme is found in many body tissues, including the pancreas, red blood cells (RBCs), lungs, gastric mucosa, renal cortex, and central nervous system (CNS). It is also found in many tissues related to the eyes, including the retinal pigment epithelium, Muller cells, pigmented and nonpigmented ciliary process epithelium, corneal endothelium, and nonpigmented iris epithelium (Stamper et al. 2009).

The catalytic activity of most CAs is attributed to the presence of Zn (II) ions in their active sites (Supuran 2007).These enzymes aid in the inter-conversion of carbon dioxide (CO_2) and bicarbonate ions (HCO_3^-), which is a basic physiological reaction. As a result, they are vital to critical physiological functions such as respiration and the movement of CO_2/$HCO_3^-$from metabolizing tissues to the lungs. Furthermore, CAs supports the regulation of biosynthetic reactions such as ureagenesis, lipogenesis, and gluconeogenesis, as well as the release of electrolytes in many tissues and organs and the maintenance of pH and CO_2 homeostasis. In addition, they play a role in calcification, tumorigenicity, bone resorption, and a number of other physiological or pathological processes. Carbonic anhydrases catalyze the following reaction (Supuran 2008):

$$CO_2 + H_2O \overset{\text{Carbonic anhydrase}}{\rightleftharpoons} HCO_3^- + H^+$$

The inhibition and activation of CAs are well known processes: Activators anchor to the entrance of the active site cavity and are involved in proton shuttling processes between the environment and the water molecules bound by the metal ions, which increases the formation of the catalytically active species of the enzyme, the metal hydroxide. However, classical inhibitors typically bind to the metal center (Supuran 2016). In this review, we will primarily focus on carbonic anhydrase inhibitors, exploring their mechanism of action and pharmacological effects.

2.1.Carbonic Anhydrase Inhibitors

CAIs are a class of drugs that inhibit the action of enzymes called carbonic anhydrases. Its inhibition has been exploited clinically for decades for various classes of diuretics, systemically acting anti-glaucoma agents, anti-obesity, analgesic, and antitumor agents/diagnostic tools (Table 3) (Supuran 2010). These inhibitors interact with the Zn^{2+} ion of the enzyme through two primary mechanisms: either by adding to the metal coordination sphere to form a trigonal-bipyramidal species or by replacing the non-protein zinc ligand to form a tetrahedral adduct (Supuran 2008). CAIs are divided into two categories based on these interactions (Figure 1). The first category comprises unsubstituted sulfonamides and their derivatives commonly referred to as organic inhibitors. Compounds within this group have sulfonamide-based (SO_2NH_2) zinc-binding groups (ZBGs) or their bioisosteres, such as sulfamates and sulfamides, are known to typically suppress CA. By dislodging zinc-bound water/hydroxide ions, sulfonamides bind in a tetrahedral geometry and interact directly with the catalytic zinc in its deprotonated state, hence inhibiting CA activity. The second category encompasses metal-complexing anions, known as inorganic inhibitors. Anions within this category can bind as trigonal-bipyramidal adducts or in the tetrahedral geometry of the metal ion. Sulfonamides and their isosteres, such as sulfamates and sulfamides, represent the primary class of CAIs (Figure 2). These compounds bind to metal ions within the active site of the enzyme and have been extensively used for decades as diuretics, antiglaucoma agents, antiepileptic drugs, and treatments for altitude sickness. Despite their longstanding clinical utility, the exploration of CAIs has extended to other compound families, with a particular focus on natural products as a source.

Numerous CAIs from diverse families have been identified, each with distinct mechanisms of action compared to sulfonamides. For instance, phenols, polyamines, certain carboxylates, and sulfocoumarins have been found to attach to zinc-coordinated water molecules within the enzyme's active site rather than directly binding to the metal center. Additionally, coumarins and some lactones have been identified as prodrug inhibitors that bind in a hydrolyzed form at the entrance of the active site cavity (Supuran 2013).

Table 3.Pharmacological action of carbonic anhydrase inhibitors

Pharmacological action	Mechanism	Drug	Reference
Diuretics	CAIs inhibit the enzyme, leading to inhibition in the resorption of bicarbonate by tubular cells. This, in turn, causes the retention of bicarbonate in the tubular lumen.	Acetazolamide Methazolamide	(Leaf and Goldfarb 2007)
Glaucoma and associated eye disorders	CAIs inhibit ciliary-course enzyme which is the sulfonamide vulnerable Carbonic anhydrase isozyme II (CA II) ,reduces the amount of bicarbonate and aqueous humor excretion, resulting in a 25–30% drop in intraocular pressure.	Dozolamide Brinzolamide Methazolamide	(Stoner et al. 2022)
Rhematoid arthritis and osteoporosis	CA II is the high activity isozyme and present only in osteoclasts of the bone. Due to inhibition of CA II acid formation by osteoclasts is inhibited.	Acetazolamide	(Gay 1991).
Obesity	CAs participates in various steps of de novo lipogenesis, both in the mitochondria and the cytosol of cells. CAIs inhibit CA isoforms involved in lipogenesis, hence producing anti-obesity effect.	Topiramate , Zonisamide	(De Simone and Supuran 2007).
Epilepsy	By inhibiting ion channels and disrupting the CO_2 equilibrium, CAIs may reduce seizures.	Topiramate Zonisamide Acetazolamide Methazolamide	(Aggarwal et al. 2013; Ciccone et al. 2021)
Neuropathic pain	CAIs exhibit analgesic effects by reducing the bicarbonate-dependent depolarization of Gamma-aminobutyric acid-A receptors.	Acetazolamide	(Carta et al. 2015)

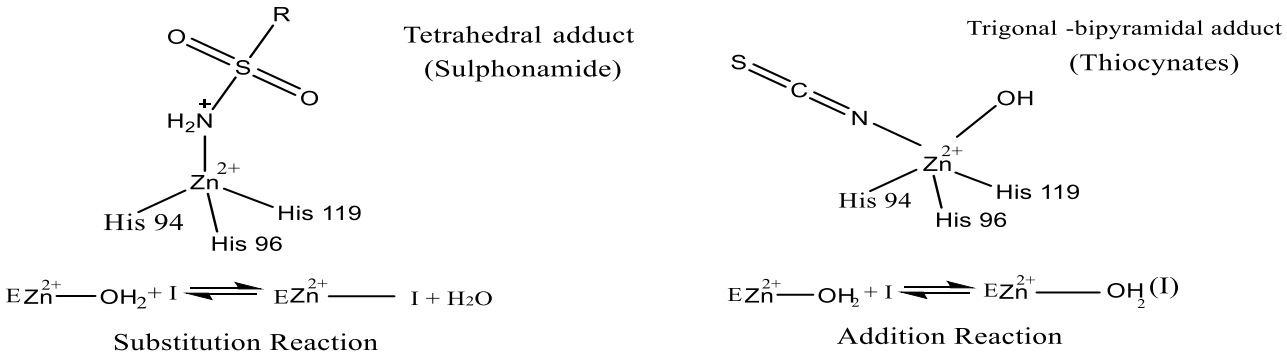

Fig.1 Carbonic anhydrase inhibition mechanism of organic (sulfonamide) and inorganic (anion) inhibitors.

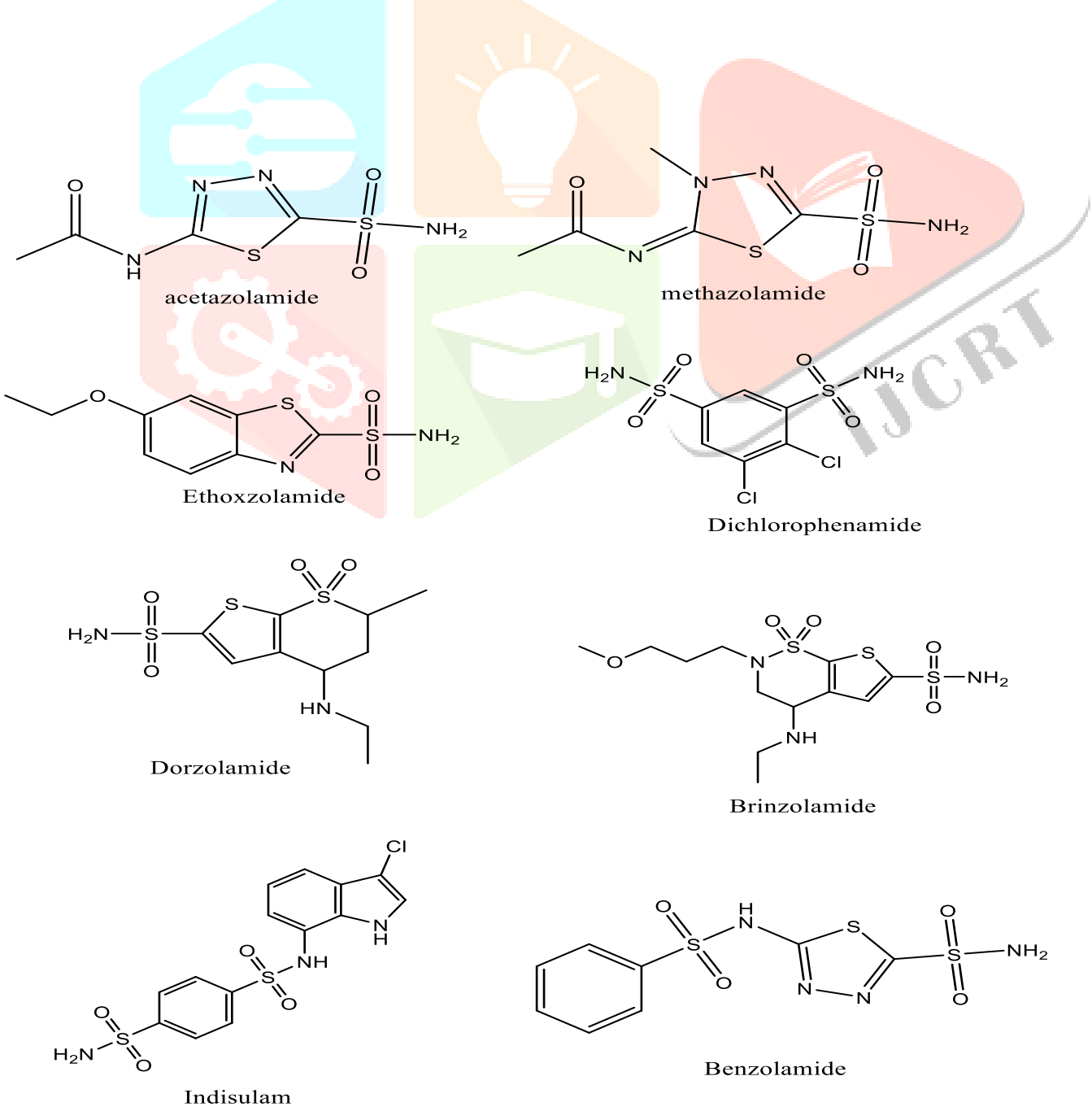

Fig.2 Molecular structure of carbonic anhydrase inhibitors bearing sulfonamide moieties that is in clinical use

3. CARBONIC ANHYDRASE INHIBITORS IN THE TREATMENT AND MANAGEMENT OF DIFFERENT HYPOXIC CONDITIONS

CAIs are employed in the treatment and management of certain hypoxic conditions due to their ability to modulate key physiological processes associated with oxygen transport and acid–base balance. This review will primarily concentrate on the role of carbonic anhydrase inhibitors in addressing four specific hypoxic conditions, namely, hypobaric hypoxia, hypoxic tumors, cerebral hypoxia and hypoxemia related to COPD.

3.1 Carbonic anhydrase inhibitors in the treatment of hypobaric hypoxia

Hypobaric hypoxia refers to the state in which a reduced oxygen concentration is experienced at high altitudes, where the atmospheric partial pressure of oxygen decreases in correlation with the decline in barometric pressure (Brown and Grocott 2013). This condition significantly influences the physiological processes of individuals residing at high altitudes and those who travel to such locations (Hackett and Roach 2001). At high altitudes, the partial pressure of oxygen diminishes, resulting in reduced oxygen availability in the atmosphere (Peacock 1998). This can give rise to a spectrum of pathophysiological symptoms, such as ischemia; altitude sickness, including acute mountain sickness; high-altitude pulmonary edema; high-altitude cerebral edema; mental dysfunction; and memory deficit, insomnia, dizziness, nausea, irritation, motor impairment, inflammation, and lung disorders. Deterioration of cerebral functions may be observed during the initial phases of exposure to high altitude. Pathophysiological effects (Chawla & Saxena 2014; Savioli et al. 2022) of high altitude and different illnesses related to high altitude (Peacock 1998) are listed in the table. (Table 4, 5).

Table 4. Physiological effects of high altitude on different body parts

Body Parts	Effect of high altitude
Cerebral	Increased blood flow
Respiratory	Hypoxic ventilation Response. Increased Alveolar oxygen Increase oxygen delivery Increased pulmonary vasoconstriction Alkalosis
Peripheral chemoreception (Carotid body)	Senses low arterial pO_2 and raised PCO_2
Kidneys	Increased Diuresis Increased Bicarbonate excretion Increased erythropoietin secretion

	Compensation for respiratory alkalosis
Heart	Increased cardiac output Increased pulse rate, velocity of blood flow Decreased oxygen demand
Blood	Increase in haematocrit, haemoglobin and RBC number Decreased Plasma volume

Table 5. Different illnesses related to high-altitude hypoxia

Conditions	**Presentation**	**Pathophysiology**
Acute Mountain Sickness	Headache, Nausea, Vomiting, Anorexia, and Insomnia	Imbalance between the cerebral vasoconstriction from hypocarbia and cerebral vasodilatation from hypoxia
High-altitude Cerebral Edema	Impaired judgment, disorientation, and gait disturbances	Changes in cerebral vascular tone
High-altitude Pulmonary Edema	Non-productive cough, progressively worsening dyspnea, fatigue, and headaches, along with other nonspecific symptoms commonly observed in AMS. Tachypnea, tachycardia, and pulmonary rales are often present, with the development of cyanosis being a common occurrence.	Blood vessels in the lungs constrict, increasing pressure. As a result, fluid starts to leak into the air sacs from the lung tissues and blood vessels.
High-altitude Retinal Haemorrhage	Painless diminution of vision, on fundus examination of the eyes multiple flame-shaped superficial haemorrhages were observed, extending throughout all four quadrants up to the mid-periphery (Bhende et al. 2013).	Insufficient oxygen at high altitudes induces compensatory mechanisms in the retinal vasculature, including auto regulation in response to hypoxia and an elevation in venous pressure resulting from increased intracranial pressure.

CAIs prove valuable in managing hypobaric hypoxia, specifically in the prevention of altitude sickness and the management of pulmonary and cerebral edema by following mechanism.

- Prevention of Altitude Sickness:

  In preventing Altitude Sickness, CAIs employ various mechanisms, including the inhibition of carbonic anhydrases (CAs) in renal proximal and distal tubules, resulting in urinary bicarbonate loss and the induction of mild metabolic acidosis (Swenson 1998). This response mimics the body's natural adaptation to high altitudes, where the kidneys reduce bicarbonate reabsorption to counteract respiratory alkalosis. CAIs also induce diuresis and natriuresis, aiding fluid balance and preventing fluid retention, which are crucial for the prevention and treatment of acute mountain sickness (AMS) (Basnyat et al. 2011). In addition, CAIs inhibit vascular endothelial cell CAs, particularly CA IV and XII, leading to slight CO2 retention, which, combined with renal metabolic acidosis, stimulates ventilation, countering the effects of increased ventilation at high altitudes (Swenson and Hughes 1993). CAIs enhance the hypercapnic ventilatory response, alleviate respiratory alkalosis, stimulate central chemoreceptor, and increase respiratory drive, improving oxygenation and reducing AMS symptoms (Kiwull-Schöne et al. 2001) (Bashir et al. 1990). Furthermore, CAIs mitigate periodic breathing while sleeping at high altitudes, enhancing sleep quality and contributing to the alleviation of AMS symptoms (Masuyama et al. 1989; Wickramasinghe and Anholm 1999).

- High-altitude pulmonary edema (HAPE):

  CAIs have been found to have a preventive effect on the development of HAPE. Its use has been associated with reduced pulmonary artery pressure and improved oxygenation, likely due to its impact on respiratory drive and the reduction of periodic breathing. Additionally, the diuretic effect of a drug can contribute to a reduction in pulmonary capillary pressure. The role of CAIs in preventing HAPE has been observed in various studies, and CAIs is often recommended as a prophylactic measure for individuals at risk of developing HAPE during rapid ascent to high altitudes.

- High-altitude cerebral edema (HACE):

  The application of CAIs has been considered for both prophylaxis and treatment. The proposed mechanism involves a reduction in cerebral blood flow (CBF) and the prevention of cerebral vasodilatation. By inhibiting carbonic anhydrase, CAIs may contribute to a decrease in cerebrospinal fluid (CSF) production, thereby lowering intracranial pressure. Although the role of CAIs in managing HACE has not been firmly established, as has its role in addressing AMS and HAPE, CAIs are frequently used for both the prevention and treatment of HACE.

  CAIs also impact the CNS by diminishing CSF production through the inhibition of choroid plexus carbonic anhydrase. This action has the potential to reduce intracranial pressure (Salvaggio et al. 1998; Kennealy et al. 1980).

3.2 Carbonic anhydrase inhibitors in the treatment and imaging of hypoxic tumors

Tumor cells exhibit distinct features that distinguish them from normal cells, and two fundamental characteristics, namely, low oxygenation and high acidity, are collectively known as the Warburg effect (WARBURG O 1956). Approximately 50% of locally advanced solid tumors exhibit hypoxia as a characteristic feature, irrespective of their size and histology. Tumor hypoxia is a condition that has significant clinical implications because it impacts patient survival, disease development, and response to therapy. Therefore, identifying hypoxic areas inside tumors is critical for stratifying patients according to the appropriate treatment and predicting how the disease will progress (Pastorekova et al. 2008).Hypoxia within tumors can vary in severity, duration (acute to chronic), and pattern (intermittent to persistent), triggering diverse cellular responses that contribute to aggressive tumor phenotypes (Harris 2002; Gillies et al. 2018). These alterations are mostly regulated at the molecular level by hypoxia-inducible factor (HIF).

In solid tumors, there is rapid proliferation and division of cells that disrupts the microcirculation both spatially and via flow cytometry, ultimately resulting in tissue hypoxia. The transcription factor hypoxia-inducible factor-1α (HIF-1α) is activated as a result of these processes, triggering a cascade of adaptive cellular responses. The accumulation of HIF-1αtriggers the transcription of downstream effectors, such as erythropoietin, glycolytic enzymes, carbonic anhydrase enzyme IX (CAIX), and vascular endothelial growth factor (Kaluz et al. 2009). The tumor microenvironment significantly influences the response to cancer treatment. Tumor hypoxia is associated with increased malignancy, poor prognosis, and resistance to radiotherapy and chemotherapy. Extensive research has focused on understanding cellular responses to oxygen deprivation (Keith et al. 2011). Abnormalities in the tumor microenvironment, such as reduced oxygen levels, disturbed pH balance, and unregulated glucose metabolism, are potential targets for developing antitumor drugs that can selectively target tumor cells without affecting normal cells.

Hypoxic conditions within tumors trigger adaptive responses, including the up regulation of carbonic anhydrase enzymes, particularly the CAIX isoform, which are crucial for maintaining pH balance and supporting tumor survival under low-oxygen conditions. Inhibition of CAs effectively interferes with these processes, leading to several key anti-tumorigenic effects (Figure 3). Several factors make CAIX a promising prospective target for inhibition. CAIX is preferentially expressed in the cell membrane of tumor cells and has an extracellular domain, making it prone to antibody and small molecule inhibitor targeting (Parkkila et al. 2000). Targeting the tumor-associated isoform IX is considered a potential approach for the development of new cancer therapeutics

against hypoxic tumors. This metalloprotein, which functions as a survival factor and a facilitator of tumor growth, is regarded as a crucial element of the physiology of cancer (Fang

et al. 2008). Therefore, CA IX has been confirmed tobe a target for imaging and treating hypoxia malignancies and metastasizing hypoxic cancers based on its catalytic activity.

- CAIs in the treatment of hypoxic tumors–

  CAIs have emerged as promising therapeutic agents for the treatment of hypoxic tumors, offering multifaceted mechanisms to hinder tumor progression. First, CAIs disrupt the pH balance crucial for cellular processes, inducing cellular stress and compromising the survival of tumor cells, ultimately leading to their death (Svastová et al. 2004). Second, CAIs play a pivotal role in inhibiting tumor angiogenesis, a process vital for sustained tumor growth. By suppressing carbonic anhydrase activity, CAIs hinder the release of proangiogenic factors, thereby impeding the formation of new blood vessels and reducing the tumor's ability to establish a network for blood supply. Additionally, CAIs contribute to diminishing the metastatic potential of tumors by neutralizing acidic conditions within the tumor microenvironment (Gieling et al. 2012). This action impedes enzymatic processes involved in extracellular matrix degradation and cell motility, thus reducing the likelihood of tumor invasion and metastasis. Furthermore, CAIs can sensitize hypoxic tumor cells to conventional cancer therapies such as chemotherapy and radiotherapy by modulating the tumor microenvironment and normalizing the acidic pH. This sensitization enhances the efficacy of these treatments, overcoming the resistance often encountered in hypoxic tumor cells (Andreucci et al. 2019). Overall, CAIs constitute a comprehensive approach for treating hypoxic tumors through their influence on survival, angiogenesis, metastasis, and sensitivity to conventional therapies.

- CAIs for imaging hypoxic tumor-

  CAIs exhibit unique capabilities by selectively binding to enzymes within hypoxic regions of tumors, suggesting that they can be utilized for imaging in combination with treating hypoxic tumors (Tafreshi et.al 2014). Through conjugation with various imaging agents, such as positron emission tomography (PET) or near-infrared fluorescence (NIRF) probes, CAIs enable specific targeting and visualization of hypoxic tumor areas. This targeted imaging approach allows for precise assessment of hypoxic regions in tumors, aiding in the selection of suitable treatment strategies and monitoring therapeutic response. In 2009, the first in vivo data on the sulfonamide class of inhibitors were published. This inhibitor was fluorescently tagged and was previously found to be a powerful CAIX inhibitor. Research findings indicate that the attachment of fluorescent sulfonamides to CAIX relies on the production and activity of the enzymatic complex. This process is limited to hypoxic environments and results in the reversal of tumor acidification. The accumulation of fluorescent sulfonamide in animal models of transplanted colorectal cancer was dependent on tumor oxygenation, specifically in hypoxic regions. This accumulation was reversible upon re-oxygenation, suggesting that imaging of hypoxic regions in solid tumors via molecular markers of CAIX is valuable (Supuran and Winum 2015) Notably, various

fluorescein-labeled sulfonamides have been developed for the detection of CAIX both in vitro and in superficial tumors in vivo.

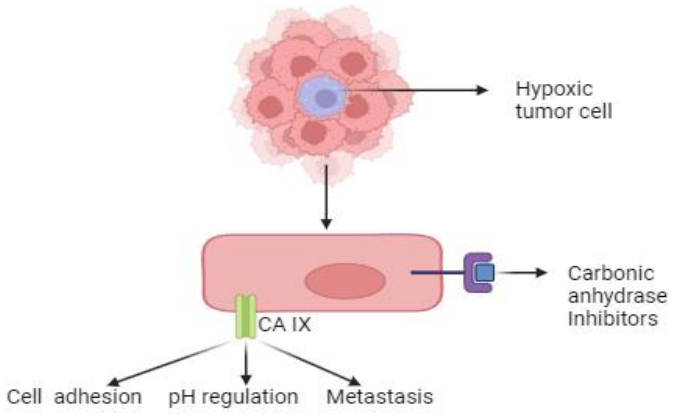

Fig.3 Targeting carbonic anhydrase IX with carbonic anhydrase inhibitors

3.3 *Carbonic anhydrase inhibitors in cerebral hypoxia*

Cerebral hypoxia is a neurological condition that occurs when the brain receives insufficient oxygen despite adequate blood flow. The brain detects a lack of oxygen in the blood, prompting an increase in CBF, resulting in a reversible alteration of brain functions. This response leads to elevated cerebral blood flow, predominantly causing a temporary breakdown of neuronal functions (Miyamoto and Auer 2000).Hypoxia triggers accelerated anaerobic glycolysis and lactic acidosis early in the process, occurring even before ATP depletion. Injury associated with cerebral hypoxia is exacerbated when the energy supply to a neuron falls below a critical level. Additionally, oxygen free radicals and hydrogen peroxides are generated during the reperfusion process, contributing to further cell damage (Chen et al. 2018). In most cases, impaired pulmonary or placental gas exchange causes perinatal cerebral hypoxia, which can result in systemic hypoxia or anoxia, with or without concomitant hypercapnia (asphyxia). The causes of acute reductions in brain oxygen concentrations following CBF interruption are multifactorial. Individuals are particularly vulnerable to common ischemic stroke if they have a history of cardiac arrest, shock, carotid blockage, or specific hereditary variables (Mukandala et al. 2016). Various conditions, such as severe hypotension, exposure to hypobaric settings, irreversible carbon monoxide binding to hemoglobin, hemoglobin deficiency in anemia, and increased oxygen limitation in asphyxia, can result in cerebral hypoxia. Inattentiveness, poor judgment, memory loss, and reduced motor coordination are some of the signs of mild cerebral hypoxia. Because brain cells are extremely sensitive to oxygen deprivation, they can start to die five minutes after the oxygen supply is stopped. Prolonged hypoxia can lead to coma, seizures, and ultimately, brain death.

The pathological sequence resulting from cerebral hypoxia is illustrated in (Figure 4). A decrease in cerebral oxygen (and glucose) availability promptly triggers a decrease in ATP

content, which is vital for the ATP-dependent ionic exchanger and normal metabolism of the membrane. Consequently, there is a substantial influx of sodium, causing cell swelling due to the influx of water along with sodium and chloride. Disruption of calcium and potassium homeostasis, involving calcium influx and potassium efflux, alters the membrane potential, inducing cell depolarization and the release of neurotransmitters (catecholamine, serotonin, acetylcholine, and endogenous excitatory amino acids). Excitatory amino acids are more concentrated in extracellular and synaptic spaces due to equilibrium between increased synaptic release and decreased cellular absorption. Notably, glutamate can activate many pathways that result in hazardous calcium influx into neurons. First, glutamate can activate calcium channels associated with N-methyl-D-aspartate (NMDA) receptors; second, glutamate can induce membrane depolarization and activate voltage-gated calcium channels. The third pathway involves the membrane Na+/Ca2+ exchanger, which is typically responsible for extruding calcium but may operate in reverse under conditions of elevated cytosolic sodium. Ultimately, calcium enters the cell through nonspecific membrane leakage associated with acute glutamate-induced excitotoxic swelling. This calcium- and glutamate-induced neurotoxicity leads to cell loss and is frequently linked to NMDA receptor hyperexcitation, resulting in memory impairment (Weinachter et al. 1990).

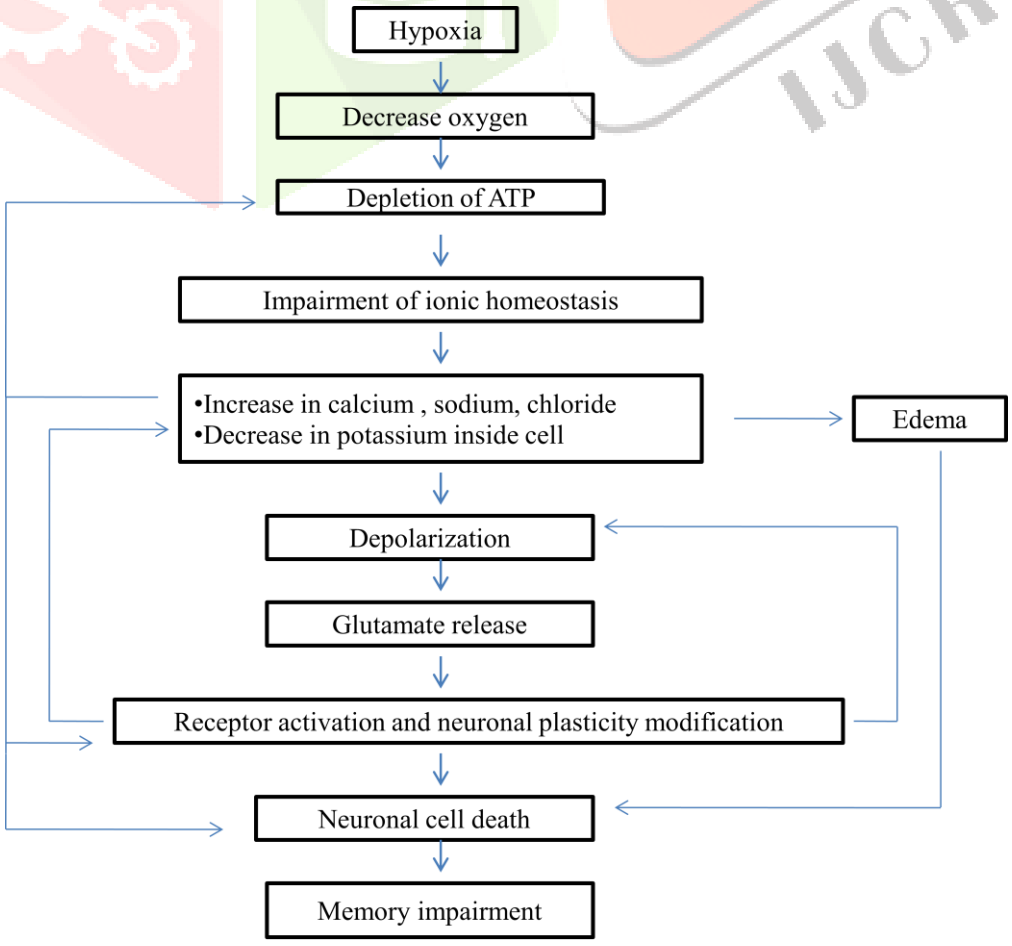

Fig.4 Time-dependent cascade of molecular events resulting from cerebral hypoxia

The CNS of mammals contains the highest number of CA isoforms (9 isoforms). Among these nine isoforms, CAII is one of the most abundant isoforms, followed by isoforms I, VB, VII, VIII, X, XI, XII, and XIV (Supuran 2018). Because of the diverse expression patterns of CA isoforms in the brain, CAIs can be investigated for therapeutic applications in various pathological conditions of the CNS. Understanding the link between brain hypoxia and carbonic anhydrase is pivotal in several therapeutic contexts. Studies have indicated that mice lacking CAII exhibit increased resistance to hypoxia-induced neuronal damage, and blocking CA results in reduced neuronal cell death by stabilizing the pH (Kniep et al. 2006). Furthermore, hypoxic conditions induce the overexpression of two CA isoforms, IX and XII, through HIF (Wykoff et al. 2000). These findings collectively suggest the potential relevance of CAs in brain hypoxia, with CA inhibition contributing to management of cerebral hypoxia by protective effect on cerebral ischemia and cerebral edema (Pettersen et al. 2015).

- The protective effects of CAIs on cerebral ischemia—

  Reducing hydrogen ions and preserving pH homeostasis are likely the major ways that CAIs protect the brain during hypoxia. The extracellular and intracellular pH is normally maintained at 7.3 and 7.0, respectively, under normal physiological conditions (Nedergaard et al. 1991). Carbonic anhydrases are highly expressed by glial cells, particularly astrocytes (Svichar et al. 2006; Tong et al. 2000), and they help transform CO_2 from neurons into protons and bicarbonate. Following their extrusion from the glial cell, these products are transported via monocarboxylate transporters and a Na^+/HCO_3^- co-transporter (Deitmer et al. 2019) (Bélanger and Magistretti 2009). Astrocytes are important for maintaining the pH balance of the brain. Carbonic anhydrase is an essential extracellular enzyme that recycles CO_2 into bicarbonate and protons to buffer the extracellular pH (Tong et al. 2000).Cerebral hypoxia induces tissue acidosis, and a low pH increases the vulnerability of glia to injury caused by oxygen–glucose deprivation (Giffard et al. 1990). The presence of nonspecific CAIs reduces the intracellular lactate-induced acidification of astrocytes (Svichar et al. 2006). The release of neurotransmitters can be affected by variations in the intracellular pH, which increases the sensitivity of neurons to pH fluctuations. A reduced pH causes brain synaptosomes to release more dopamine (Cannizzaro et al. 2003; Pittaluga et al. 2005), noradrenaline, and serotonin (Pittaluga et al. 2005). Glutamate release is initiated by glial acidosis (Beppu et al. 2014), and early excitotoxic neuronal death in brain hypoxia depends on the persistent activation of NMDA-type glutamate receptors (Somjen 2001). CAIs play a protective role during cerebral hypoxia by preventing glutamate-induced early excitotoxic damage by restoring the H+ concentration during ischemia and lowering excitatory amino acid efflux (Bulli et al. 2021).

- Protective Mechanisms of CAIs in Cerebral Edema–

  Cerebral hypoxia can result in an increase in cerebral blood flow, potentially leading to elevated intracranial pressure (ICP). Within the brain, three sites of CA may contribute to

protective mechanisms: chemoreceptors, the cerebral vasculature, and the choroid plexus. CSF secretion is controlled by the choroid plexus, and a decrease in CSF production may lower intracranial pressure. The ICP is determined by the volume of water in the blood, the volume of interstitial fluid, the volume of intracellular fluid, and the volume of CSF. Under normal conditions, these volumes maintain the ICP at levels less than 20 mmHg. However, any increase in one or more of these cerebral volumes has the potential to quickly increase the ICP, which could impede blood flow, result in discomfort, and affect neurological function (Swenson 2014).By blocking the location of CAs in the choroid plexus, CAIs decrease the amount of CSF produced, which helps to lessen cerebral edema.

3.4 Carbonic anhydrase inhibitors in chronic obstructive pulmonary disease-related hypoxemia

The most common feature of COPD is irreversible pulmonary impairment. Macrophages, cytotoxic T cells, neutrophils, and inflammatory mediators produce structural alterations in the parenchyma and small air passages, which ultimately lead to obstruction of the bronchioles (Kent et al. 2011; Bales and Timpe 2004). COPD is one of the major causes of morbidity and disability worldwide (Murray and Lopez 1997). As the disease worsens pulmonary function declines, and the risk of hypoxemia and alveolar hypoxia increases (Rabe et al. 2007). The extra pulmonary co-morbidities and maladaptive mechanisms that define COPD are significantly influenced by tissue hypoxia. The hypoxemia associated with COPD contributes to a decrease in quality of life, a decrease in exercise tolerance, skeletal muscle dysfunction, and ultimately a greater risk of death (Kim et al. 2008). A ventilation/perfusion (V/Q) mismatch, which arises from emphysematous deterioration of the pulmonary capillary bed and increasing airflow limitation, is the main cause of hypoxemia in individuals with COPD (Gibson 2008).Hypoxemia and a decrease in gas exchange are frequently linked to COPD flare-ups. Notably, the primary factor affecting these alterations seems to be an increasing imbalance in ventilation/perfusion (Barberà et al. 1997) .

Hypoxemia resulting from chronic obstructive pulmonary disease (COPD) can also lead to various consequential health issues. Pulmonary hypertension is a significant outcome (Chaouat et al. 2008), particularly in moderate to severe cases of COPD, where alveolar hypoxia plays a crucial role in its development (Chaouat et al. 2005). Anatomically, factors such as thromboembolic disease, pulmonary vascular remodeling, and emphysematous obliteration of the pulmonary capillary bed contribute to pulmonary hypertension. Luminal constriction, characterized by intimal thickening and arteriolar muscularization, is observed in the pulmonary vasculature of COPD patients with concurrent pulmonary hypertension (Elwing & Panos 2008). Additionally, COPD contributes to secondary polycythemia, and hypoxemia-induced activation of HIF-1, a master regulator of oxygen homeostasis, plays a significant role in its development (Semenza 2009). Another consequence of COPD-related hypoxemia is systemic inflammation, a common feature in individuals with chronic illnesses, in which elevated levels of inflammatory markers circulate in comparison to those in good health (Gan

et al. 2004). Furthermore, neurocognitive dysfunction is prevalent in COPD patients, and its occurrence tends to increase with worsening gas exchange. This dysfunction is linked to systemic inflammation and oxidative stress, leading to direct neuronal damage and the depletion of neurotransmitters due to dysfunction of oxygen-dependent enzymes (Dodd et al. 2010). Overall, the consequences of hypoxemia in COPD patients extend beyond respiratory complications, impacting vascular, hematological, inflammatory, and neurological aspects of health.

CAIs, such as acetazolamide, play a significant role in addressing hypoxemia in COPD patients. These inhibitors induce mild metabolic acidosis and stimulate breathing, making them valuable in the management of respiratory conditions (Jones and Greenstone 2001). In COPD, where impaired lung function is a key characteristic, CAIs have been utilized as respiratory stimulants. The inhibitory action of these compounds on carbonic anhydrase, particularly in the kidneys, vascular endothelium, red cells, lung, diaphragm, CNS, and chemoreceptors, contributes to their multifaceted effects. Renal CA inhibition leads to urinary bicarbonate excretion, generating metabolic acidosis that stimulates ventilation and corrects primary metabolic alkalosis (Adamson and Swenson 2017). The use of CAIs in COPD treatment is rooted in their historical application as respiratory stimulants, especially in addressing conditions such as AMS. These inhibitors, by depressing proximal tubular HCO_3^- reabsorption and enhancing distal tubular H^+ secretion, induce alkaline diuresis. This process results in a reduction in serum bicarbonate levels and a compensatory decrease in arterial pH. The ventilator response, driven by metabolic acidosis, helps lowering arterial carbon dioxide tension (Pa, CO2), improving oxygenation. With their ability to stimulate ventilation and correct metabolic alkalosis, CAIs can be particularly beneficial in COPD patients facing hypoxemia. In situations where metabolic alkalosis is induced by factors such as diuretics or corticosteroids, which may depress ventilation, CAIs can help enhance respiratory drive. Additionally, mild diuresis induced by CAIs can improve cardiac function and gas exchange in congestive heart failure patients and in patients infected with COPD (Swenson 1998).

4. CONCLUSION

CAIs play pivotal roles in hypoxic conditions by serving as respiratory stimulants, addressing metabolic alkalosis, and enhancing ventilation. These multifaceted effects collectively contribute to improved oxygenation and respiratory function in individuals facing hypoxia. The impact of CAIs extends across various physiological systems, including erythropoiesis, blood viscosity, pulmonary circulation, ventilation, and cardiac function; therefore, the use of CAIs emerges as a compelling therapeutic approach for hypoxic conditions from a physiopathological perspective.

Key Points

- Hypoxia is a frequently encountered disorder in hospital settings and is characterized by insufficient oxygen supply.

- Treatment options have traditionally been limited to supportive measures and oxygen therapy.
- Carbonic anhydrase inhibitors are a class of drugs that can stimulate ventilation and correct metabolic alkalosis, thereby influencing respiratory responses.
- Carbonic anhydrase inhibitors can improve gas exchange in conditions such as COPD, hypobaric hypoxia, cerebral hypoxia and hypoxic tumors.

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