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Review article

Hydrogen sulphide: a new member of Gasotransmitters, an overview article

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ABSTRACT

Hydrogen sulphide commonly known to mankind by its foul smell of rotten egg. This gas is endogenously produced in mammalian cells and in humans by the action of Cystathionine β-synthase and cystathioninase enzyme action through trans sulfuration pathway in Methionine metabolism. The principal source of this pollutant gas is by refinery gases, kraft pulp, paper manufacturing, or during degradation of sulphur containing compounds etc. It is known as potent toxic, asphixiant, poisonous gas that inhibits the cytochrome oxidase of the electron transport chain and thus blocks the cellular respiration. Thereby, it mimics the action of the hydrogen cyanide and carbon monoxide. The less concentration of this gas produced in the gut environment by the anaerobes by the action of specific enzyme system, which is detoxified in the body in to water soluble thiosulfate and excreted in urine. Recently, this pollutant gas has been shown to be an important gaseous transmitter modulating many physiological and pathological processes. Small amount of hydrogen sulfide synthesis takes place in the human body and where it acts a signaling molecule gasotransmitter. It is released from endothelial lining of blood vessels, such gassy stuff regulates particularly decrease in blood pressure by relaxation of smooth muscles of blood vessels thorough K ATP channel opening . Unlike nitric oxide, dopamine, acetyl choline, it does not require receptors mediation, but easily diffusible across membrane due to its water solubility. Any mechanism that regulates the increase formation of this gas favors as therapeutic agent in hypertensive subjects, in this overview article considerable efforts are made to present the various biological functions of the hydrogen sulphide.

I. Introduction

Hydrogen sulphide (H_2S) is a colorless gas commonly known to mankind by its foul smell of rotten egg [1] and odor of flatulence. This inflammable gas was first discovered in 1977 by Carl Wilhelm Scheele during his experimentation of distilling potassium ferrous sulfide with a mineral acid concentrated sulphuric acid. He felt an unpleasant odor of characteristic stinking type that was characterized, and regarded as sulfuretted hydrogen or hydrogen sulphide.[2] The synonyms of hydrogen sulphide are known as hydrosulfuric acid, hydrogen sulfuric acid, sulfureted hydrogen, hepatic gas, stink damp, sulfur hydride, sulfurated hydrogen, dihydrogen monosulfide, dihydrogen sulfide, and sewer gas.[3]

This sweetish H_2S [4] gas is heavier than air, therefore its accumulation generally occurs at the bottom zone and may travel along the ground in poorly ventilated space. The molecular weight of this gas stuff is 34.08 grams/molecule, structurally similar to water, however, differs with replacement of oxygen by sulfur atom. Its structural formula illustrated as H-S-H. Hydrogen sulfide may evaporate easily from water, depending on temperature and pH and thus prevents the bio-concentration. In general, Low pH and high temperature is favorable for evaporation[3]. The solubility of this gas when dissolved in water is about 4,13 gm / Litre at 20°C. H_2S is weak acid dissociates in to proton (H+) and hydro sulphide anion (HS-) with pKa1 value is 6.89. The temperature and pH influences the dissociation of hydrogen sulphide. At 2[illegible]C, in.

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solution state with pH 7.4, H_2S exists one third as H_2S and two-thirds as HS-, with traces of sulphide ion (S2-) due to the high pKa2 11.96. However, it is not true when temperature reaches 37°C because pKa1 at 37°C is 6.755, rather than 7.04 for standard conditions at 20°C. Accordingly, in saline at 37°C and pH 7.4, less than one-fifth of H2S exists as the un dissociated form (H2S)[5,6,7] As described by various authors, the three forms of species of hydrogen sulphide are un dissociated H2S, HS- and S2- and these were collectively named as total hydrogen sulphide, however in this article the usage of the terminology restricted to hydrogen sulphide only.

Generally, H_2S reacts with metal cation and produce metal sulfides often have a dark color such as Ag2S (tarnish formed on silver), CdS (cadmium sulphide) etc. This property is use full in separating metal ions from aqueous solution. H_2S on burning with high temperature produce sulfur dioxide (SO_2) possess a characteristic smell of burnt match stick, It burns in air with a blue flame.[4] The mixture of air and hydrogen sulphide in closed system is explosive.

H_2S is a byproduct of many industrial operations during coking and the hydrosulfuration of crude oil and of coal.[8] The natural production of hydrogen sulphide occurs by hydrolysis of mineral sulfides by massive volcanic eruptions, crude petroleum, natural gas, and hot springs, and also by natural human activity. Natural sources include non specific and anaerobic bacterial reduction of sulfates and sulfur containing organic compounds. It is also found in groundwater, released from stagnant or polluted waters and manure or coal pits. Hydrogen sulfide is also produced as a decomposition product of xanthates used in the mining industry during the contact with water. Accidental release or improper disposal of materials resulting from these processes may result in hydrogen sulfide emissions to air.

The principal source of hydrogen sulfide as by-product during recovery from the purification of natural and refinery gases, Kraft pulp, paper manufacturing and carbon disulfide production etc. During the degradation of sulfur containing amino acids in the colon, this odor of flatulence contains H_2S.Similarly, bad breath produced in the mouth also due to this gas release by the bacterial degradation on the residual food particles present in the teeth. Ozone or manganese dioxide used as clearing agent of hydrogen sulphide by oxidizing toxic gas in to non toxic sulfates.

Recently, this pollutant gas has been shown to be an important gaseous transmitter modulating many physiological and pathological processes. Small amount of hydrogen sulfide synthesis takes place in the human body and where it acts a signaling molecule gasotransmitter. It is released from endothelial lining of blood vessels such gassy stuff regulates particularly decrease in blood pressure by relaxation of smooth muscles of blood vessels. Unlike nitric oxide, dopamine, acetyl choline, does not require receptors mediation, but easily diffusible across membrane due to its water solubility. Any mechanism that regulates the increase formation of this gas favors as therapeutic agent in hypertensive subjects, Dietary intake of allium vegetables such as garlic, onion contains organic sulphur compounds, they are diallyl disulfide, diallyl trisulfide and S-allyl cysteine residues. These can be seen in garlic in more amounts which generate increase in the H_2S production that intern results vasodilation.

2.0 Hydrogen sulphide toxicity

H_2S is known as potent neurotoxic, asphixiant, poisonous gas from almost few centuries. Therefore, 'Hydrogen suphide poisoning' is a serious issue in the community health; its production is widespread from environmental, industrial and occupational human activities. It is broad spectrum poisonous gas affects different systems; most predominantly nervous system is highly vulnerable for toxicity. H2S is not mutagenic or teratogenic.[9]

The less concentration of H_2S formation takes place in the human body particularly in the gut environment. The accumulation of gas is prevented due to presence of specific enzyme system in the body that detoxify the gas by oxidation to harmless sulfate.[10] But once the threshold level is achieved, oxidative enzymes inactivated and thus exhibit toxic effects.[11] The normal concentration of hydrogen sulfide in natural air at ambient temperature is 0.0001-0.0002 ppm. The nose knows this gas by virtue of its rotten egg odor at the level of 0.02-0.1ppm. When the gas concentration increases 0.2-0.3 ppm in breathing air results symptoms of headache, nausea, and sleep disturbances because it cross the human threshold value 0.05ppm and develops intolerance.[12] H_2S in body fluids dissociated to proton and hydrosulfide HS- anion, the latter binds to methemoglobin to form sulfmethemoglobin which is similar to cyanomethemoglobin.[13]

At about, 10-20ppm level of H_2S gas is the boarder line concentration for eye and mucosa membrane irritation. At the concentration of 30-100ppm results the eye irritation followed by eye damage (corneal opacity), at a concentration above 100ppm odor becomes imperceptible because of olfactory fatigue characterized by paralysis of olfactory nerve and also at this level gas impairs the cellular respiration and results respiratory depression as well as cardiac problem.[14]

H_2S toxicity attributed by the following biochemical mechanism, where it is a potent inhibitor of cytochrome oxidase enzyme system of oxidative phosphorylation in electron transport chain in inner mitochondrial membrane where it binds to iron atom of complex-IV and blocks the cellular respiration and reduces the ATP production. This energy depletion affects the functions of nervous system. [15] H_2S intoxication believed, that it inhibits Mono amino oxidase [MAO] also contributing factor for central respiratory failure.[16] Generation of Reactive oxygen species in oxidative stress by H2S inhibitory action on cytochrome P450 dependent mechanism.[17]It also inhibits the metalloproteins such as horse raddish peroxidase, Potato polyphenol oxidase and catalase.[18]

At a concentration of 150 -250ppm, the olfactory nerves is paralyzed after a few inhalation and the sense of smell disappears this observation creates an awareness of danger caused by H_2S toxicity characterized by CNS depression and respiratory paralysis since the route of gas absorption is lungs occur at 320-530ppm. At this concentration H_2S is an extremely hazardous gas and also life threatening.[19]

Inhalation of concentration of 500ppm for thirty minutes produces severe toxicity characterized by headache, dizziness, excitement, gastro enteric disorder, bronchitis etc. The concentration above 600ppm is fatal with in thirty minutes through drastic respiratory paralysis.[20]

Knockdown to fatalities occurs at 530-1000ppm range thus human toxicity values are 200 ppm is severe toxic effect, 600ppm is lethal effect within thirty minutes, with 800 ppm immediately lethal effect reported is called as Lethal concentration with blood concentration of H_2S is 0.092mg/dl.[21]

Thus, exposure of various degree of hydrogen sulfide concentration involves eye irritation shortness of breath, chest tightness, wheezing to unconsciousness and finally leads to death. However, the concentration of 1000ppm causes immediate collapse with loss of breathing even after inhalation where as at 1400ppm in breathing air cause acute intoxication results seventy five percent of the death from the exposed subjects. Such affected people after their demise when checked their blood concentration of thiosulfate contains less than100 mg/dl which is the detoxification product of H2S in the body.

Thus, H_2S toxicity is widespread with broad spectrum; finally H_2S at higher concentration more than 700ppm is potentially toxic to human beings and fatal. As per the available various reports on H_2S toxicity it is known to have two major effects such as local inflammation and irritation to moist membrane including the eye and deeper part of respiratory tract at lower concentration, CNS depression, respiratory paralysis, finally leads to unconsciousness or knockdown. Ultimately results death at higher concentration.[22] Since it is metabolized in the body in to thiosulfate the accumulating effect of H_2S is not observed.[23,24] H_2S toxicity can be detected either by discoloration of copper coins or formation of lead acetate in to lead sulfide on exposure to gas. The affected H_2S toxic subjects needs to be shifted from the toxic zone immediately to highly ventilated area, and also therapy by Hyper Baric Oxygen recommended.[25,26] An antidote sodium nitrite through intravenous therapy and amyl nitrite by inhalation recommended.[27] Intravenous or Intraperitoneal infusion of sodium bicarbonate prevents hyperpnoea, apnea and death in animal models injected with H_2S [28] and also ethanol that lowers the threshold for a person to overcome by H_2S exposure .[29] Bronchospasm is prevented by using bronchodilators.

3.0 Metabolism of Hydrogen Sulphide

Biosynthesis and Degradation of hydrogen sulphide:

The awareness of Hydrogen sulphide formation in mammalian cells created interest in health science. Which was later on regarded as third member of gasotransmitter family thus the triad Carbon Monoxide (CO), Nitric Oxide (NO), and Hydrogen Sulphide (H_2S) are well known to scientific community. In mammals H_2S is endogenously produced by enzymatic and even some by non enzymatic reactions. H_2S present in micromolar concentration in blood.[30] H_2S production and metabolism are closely regulated in order to meet different cellular metabolic and functional demands.

The Hydrogen sulphide formation takes place either by Methionine metabolism, by cysteine degradation, and also by additional minor pathway as described in figure 1. The key enzymes involved in endogenous synthesis of hydrogen sulphide are Cystathionine β-synthase [CBS E.C. 4.2.1,22] and Cystathionine γ-lyase or Cyatathininase [CSE E.C.4. 4. 1. 1.) H2S synthesized endogenously by an essential aminoacid L-Methionine through trans sulfuration reactions.[31] and the substrate cysteine released from alimentary sources or by tissue protein degradation by CSE action.[32] The expression of these two enzymes is organ specific because CBS is H2S generating enzyme in brain and nervous system and also in liver and in vascular and non vascular smooth muscles. The major amount of H2S production occurred by the action of CSE by desulfhydrase reaction from cysteine.

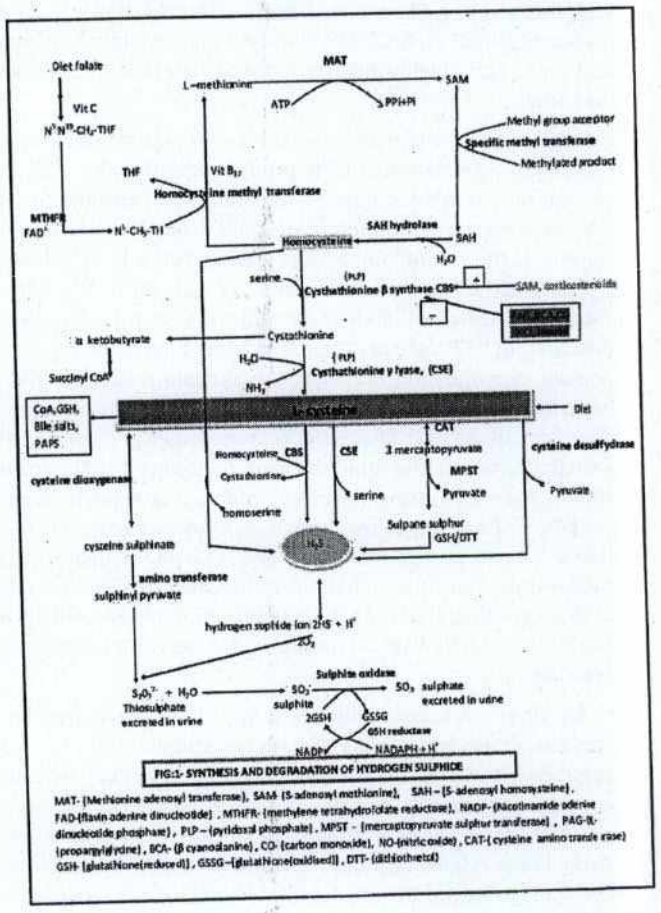

FIG:1- SYNTHESIS AND DEGRADATION OF HYDROGEN SULPHIDE

MAT- (Methionine adenosyl transferase), SAM- (S-adenosyl methionine), SAH – (S-adenosyl homocysteine), FAD-(flavin adenine dinucleotide), MTHFR- (methylene tetrahydrofolate reductase), NADP- (Nicotinamide adenine dinucleotide phosphate), PLP – (pyridoxal phosphate), MPST - (mercaptopyruvate sulphur transferase), PAG-(L-(propargyl)-lysine), BCA- (β cyanoalanine), CO- (carbon monoxide), NO-(nitric oxide), CAT-(cysteine amino trande rase) GSH- (glutathione(reduced)), GSSG –(glutathione(oxidised)), DTT- (dithiothretol)

Deficiency of CSE expression (results endogenous decreased synthesis of H_2S) in the pancreas and adipose tissue responsible for decreased insulin secretion and insulin resistance results metabolic syndrome obesity, hypertension, diabetes.

A transfuration pathway in the brain mediated by CBS and CSE links to GSH homeostasis which greatly contributes to the redox buffering capacity in the brain.[33] The complete proteins provide Methionine in to the body is converted in to S-Adenosyl Methionine catalyzed by Methionine adenosyl transferase using high amount of energy by ATP complete degradation. Subsequently S-Adenosyl Methionine or active Methionine or methyl group donor compound participate in transmethylation reaction and transfer methyl group to methyl group acceptor compounds to form methylated products. This reaction catalyzed by specific methyl transferase. Subsequently, the end product produced is S-adenosyl homocysteine (SAH) which undergoes hydrolysis by SAH hydrolase in to homocysteine.

Under normal metabolic process, the homocysteine is formed in lesser concentration of 13-18µmol/L in serum and 10-15µmol/L in plasma.[34] This homocysteine has two fates where it can be converted back to Methionine to restore the normal cellular concentration of Methionine by homocysteine methyl transferase using coenzyme vitamin B12, N5.N10 methylenne tetra hydrofolate (THF), This reaction is highly significant because it needs various water soluble vitamins such as folate, L- ascorbic acid, FAD, and B12, The unique feature of this reaction is that from the water soluble vitamins the dietary supply of folate, L- ascorbic acid and B12are highly important due to their lack of synthesis in the body.

On the other hand, homocysteine undergoes transsulfuration reaction using serine and PLP to produce cystathionine. CBS gene present on the chromosome 21 (position 22.3) encodes for CBS that catalyse transsulfuration reaction from homocysteine to convert in to cystathionine.[35] CBS enzyme is an allosteric enzyme, tetramer, with molecular weight of 63Kd, possess N-terminal heme and catalytic domain and C- terminal regulatory domain.[36] CBS activity regulated by its C-terminal regulatory domain, any mutation occurs at this domain results hereditary hyperhomocysteinemia overexpression of CBS gene observed in down syndrome.[37] Therefore, pharmacological significance is availability of suitable inhibitor for CBS. CBS is a multi enzyme complex contains N-terminal heme domain ,a catalytic domain contains PLP and regulatory C-domain . Allosteric activator is SAM. Heme domain acts as redox sensor [38] a PLP dependent CBS catalyses condensation of homocysteine and serine in to L-serine in to L-cystathionine through transulfuration pathway in normal Methionine catabolism to produce non essential amino acid cysteine.

An alternate reaction catalysed by CBS is condensation of cysteine with homocysteine to form cystathionine and hydrogen sulphide.[39] Cystathionine-γ-lyase (CSE) is a PLP dependent enzyme subsequently converts cystathionine in to L-cysteine and α-ketobutyrate. The latter channeled into TCA cycle as succinyl Co-A. H_2S is a gas signaling molecule endogenously produced from L-cysteine by CBS and CSE.[40,41,42]. In 2002,Wang described that thus produced H_2S in mammalian system is an another gasotransmitter that has few functional similarities with the NO and CO.[43]

Thus produced H_2S travels rapidly through plasma membrane diffused in to the cells without receptor mediation , where H_2S exerts a biological effect range from cytotoxic to cytoprotective functions.[44] The normal H_2S concentration is less than 1.5 micro mole /L in blood.[45] H2S dissociates in to hydrogen sulphide anion and proton through non enzymatic reaction converts in to thiosulphate which then gets converted to sulphite SO2 by thiosulphite reductase (TSR) or thiosulphate sulphurtransferase (TSST) SO2- sulphite gets oxidized to sulphate SO3- by sulphite oxidase (SO) by using reducing substances like GSH/ or DTT. Which is converted in to active sulphate as PAPS used for sulfation of MPS and etc. CBS inhibited by DL-propargyl glycine (PAL) B-cynoalanine (BCA) .[46]

4.0 Absorption and Distribution of Hydrogen sulphide

H_2S present in atmosphere as gaseous state at the concentrations of 0.14 to 0.4 µgm/m3 at ambient temperature and pressure. [47] H_2S is soluble in water and oil and therefore distributed in moist soils. Due to water solubility it can travel from place to place through water. This property helps to determine the H_2S content in water. The ninety percent of total H_2S concentration present in the atmosphere is contributed by natural sources such as anaerobic bacterial reduction of sulfates and their conjugates by stagnant manure, pollutant water, coal pit etc.

The intake of H_2S occurs through inhalation process and also endogenous productions by anaerobic bacteria in human activities. The endogenous productions of H_2S occurs in brain, blood vessels, kidney pancreas etc. Generally, H_2S is absorbed through the route of lungs.[48] , GIT tract and from intact skin due to its lipid solubility, receptor independence and transported across cell membrane by simple diffusion.[49,50]. At physiological pH , H_2S easily dissociated in to hydrogen sulfide anion which is readily absorbable form in to the body.[51] Human data indicated that the tissue distribution of H2S in the blood 0.08-0.2, brain 0.2-1.06, lungs 0.21-0.68, liver 1.3-1.56, spleen 0.32-0.64, kidney 0.47-1.5 microgram/gm of tissue respectively.[52] However, the studies available are enlightening about the absorption and distribution of H_2S in various tissue are based on the animal experimental model since the human studies furnish this information are minimal.

H_2S is catabolised in to sulfate and thiosulfate which is excreted in urine therefore, few authors are at the opinion that thiosulfate in blood and urine serves as a marker for H_2S exposure and its toxicity rather than the measure of true H_2S.

5.0 Quantification of Hydrogen sulphide.

The various well established analytical methods for quantification of hydrogen sulphide and its metabolites are available. The materials used for detection are grouped in to biological samples and environmental samples. The former includes blood, urine, saliva, and muscle tissues etc. where as latter includes the air, water, sludge etc. The approved analytical techniques for quantitative measurement of hydrogen sulphide

are Gas chromatography coupled with Flame Ionization Detection (GC/FID), Gas chromatography coupled with flame photometric detection(GC /FPD), Iodometric titration, Potentiometry with Ion selective Electrodes (ISE), spectrophotometry with a detection limit is 0.2 µg/m3 and High Performance Liquid Chromatography (HPLC) with a detection limit is 10 µg/m3, Iodometric titration with detection limit is 1 µg/m3 etc.

In biological samples, particularly in blood hydrogen sulphide concentration measured by Iodometric method and potentiometric methods.[53] similarly by ISE method.[54] , In blood and urine H_2S estimated by Gas chromatography with electrochemical detection (GC/ECD) method.[55] In urine by Liquid chromatography (LC), In blood and feces by Ion chromatography coupled with electrochemical detection (IC/ECD).[56,57] In breath and saliva by Gas chromatography coupled with Flame Ionization Detection (GC/FID) or spectrophotometry.[58,59] In muscle and tissues like brain, lung, femoral muscle by Gas chromatography coupled with mass spectrometry GC/MS [60] and also Brain, liver and kidney of mouse by Ion interaction reversed phase High Performance Liquid Chromatography (HPLC) etc.[61]

In environmental samples, the commonly employed methods for detection of H_2S are GC/FPD, Gas chromatography coupled with Electron capture detection (GC/ECD), Iodometric methods, Methylene blue colorimetric and spectrophotometric methods, spot methods like using paper impregnated with lead or mercuric chloride, Ion chromatography with conductivity and potentiometric titration with a ISE for sulfide ion.

In air, H_2S is detected by iodometric, lead acetate impregnated filter paper, mercuric chloride impregnated filter paper, and silver membrane filters.[62] . In the air such as fuel gas produced in petroleum refineries by iodometric titration with detection limit of 8-740 mg/m3.[63]. Similarly, the air emission from the stationary sources by GC/FPD method.[64]

In water by Colorimetry (by methylene blue method) by spectrophotometry (Gas dialysis automated methylene blue method).[65] There are several other methods used to measurement of H_2S in air such as Ion chromatography with conductivity, [66] spectrophotometry, [67] by photo acoustic spectroscopy [68], GC/ECD by [69] by GC/FPD method etc.[70]

Amongst, these the most commonly used is Gas chromatography with Flame Ionization Detection (GC/FID) method, the nature of samples are air, water sediment and sludge. And also Gas chromatography with electrochemical detection (GC/ECD) with detection limit is 10 µg/m3 as thiosulphate.

Besides, spot evaluation procedure like using paper or tiles impregnated with lead acetate or mercuric chloride with a detection limit 0.7 µg/m3 40 µg/ L or 1.2 µMole/litre in blood. Ion chromatography with conductivity and potentiometric titration with a sulfide ion selective electrode. In Atomic absorbtion spectra the detection limit is 25 µg in waste water or sludge.[71]

In water sample about 0.6pmol/litre detection limit can be measured by GC/FPD method.[72] H_2S detection in air by Gas chromatography /Flame photometric Detection (GC/FDT) with a Detection in air by spectrophotometry at 670nm using methylene blue 8.5-63 µg/m3 .[73,74] QualitativeH_2S detection has been done, based on the blackening of coins, keys, lead paint and paper moistened with lead acetate solution.

The blood sulphide concentration determined by selective electrodes combined with Conway micro diffusion cells. The disadvantage is that only fatal concentration of 1.70-3.75 µg/litre can be detected which higher concentration is. [75]

Biological functions of Hydrogen sulphide

6.1 Action of H_2S on central nervous system

Neuromodulation

H_2S serves as a neuromodulator that potentiates the transmission of nerve pulses in neurons and especially regulated long-term potentiation (LTP) through N-methyl-D-aspartic acid (NMDA) receptor via activation of adenylyl cyclase (AC) and the subsequent cAMP/PKA (cyclic adenosine monophosphate/protein kinase) cascades. H_2S promotes the astrocytic glutamate uptake, which plays an important part in clearing excessive glutamate in synaptic cleft and maintaining normal neurotransmission among neurons.[76]

Neuroprotectant

H_2S has demonstrated neuroprotective actions such as anti-necrotic and anti-apoptotic effects through multiple mechanisms. As H_2S is a well-known endogenous reducing agent that readily reacts with hydrogen peroxide, it is presumed that endogenous H_2S can scavenge oxygen species and thus its role in oxidative stress-induced cell damage is initially explored. H_2S increases the reducing activity in neurons and protect neurons against oxidative damage induced by glutamate, peroxynitrite, hydrogen peroxide or hypochlorate by increasing glutathione(GSH)levels instead of directly functioning as an antioxidant. [77,78,79,80,81] Increase in GSH production involves the stimulatory effects of H_2S on the activity of γ-glutamyl cysteine synthetase (γ-GCS), cystine and cysteine transport as well as glutamate uptake.[76,77,78] Cysteine is a rate-limiting factor in GSH synthesis. Extracellular cysteine is easily oxidized to cystine. The transport of cystine into cells, mainly mediated by cystine/glutamate antiporter, is therefore essential in providing cells with cysteine as substrates for GSH synthesis. The promotion of H_2S on glutamate uptake, which lowers glutamate concentrations in synaptic cleft and in turn stimulates the activity of cystine/glutamate antiporter, enables the transportation of cystine into cells, and eventually results in the elevation of intracellular cysteine. These results provide evidence for the powerful anti-oxidative action of H_2S in CNS, and simultaneously offer evidence for its neuroprotective effects because excitotoxicity mainly derived from excessive accumulation of glutamate in synaptic cleft, which greatly contributes to the development of stroke, traumatic brain injury and neurodegenerative disorders as well.

Inflammatory action in brain-ischemic stroke

H_2S plays important roles in the development of seizures, stroke and Down's syndrome. In addition, in human the deficiency of CBS results in homocysteinuria, with increased plasma levels of homocysteine and methionine but decreased levels of cysteine.(figure 1) High plasma cysteine level is correlated with

administration, by either intraperitoneal route, dose-dependently increased the infarct volume in rats after experimental stroke induced by middle cerebral artery occlusion (MCAO). This cysteine effect was mimicked by NaHS.[83] but reversed by co-administration of the CBS inhibitor.[82] Pre-administration of the inhibitors of CBS and CSE (BCA and PAG) before MCAO reduce the infarct volume. These findings imply that cysteine, most likely via its conversion to H_2S, can influence the outcome of stroke and that abnormal H_2S formation may be involved in the pathogenesis of ischemic stroke.[84]

Anti-inflammatory action -Alzheimer disease

In Alzheimer disease, the brain levels of S-Adenosylmethionine, a CBS activator, are severely decreased.[85]. The total serum level of Homocysteine is accumulated and increased in AD patients.[86] One possible explanation is that the transsulfuration pathway linking Homocysteine and glutathione metabolism, mediated by CBS and CSE, is disrupted. Because CBS is an important biosynthetic source of H2S generation in brain, the dysfunction of the transsulfuration pathway may lead to the reduced production of H2S in Alzheimer's(AD), in addition to GSH.

First of all, H_2S is shown to scavenge the cytotoxic lipid oxidation product 4-hydroxynonenal.[87] , which is markedly increased in brains of severe AD patients. Secondly, H_2S was shown to ameliorate β-amyloid-induced damage in PC12 cell culture line through reducing the loss of mitochondrial membrane potential and attenuating the increase of intracellular reactive oxygen species.[88] Thirdly, H_2S-releasing compounds are capable of attenuating neuroinflammation, a contributing factor implicated in AD pathogenesis.[89,90]

In down's syndrome, H_2S hypothesis' (less H_2S) has been proposed to be responsible for the mental retardation due to neuronal damage via cytochrome-c oxidase inhibition and NMDA receptor [35,91,92]

6.2. Cardiovascular actions of H_2S

H_2S, a vasodilator and regulator of blood pressure, is involved in the pathogenesis of hypertension. Therefore, it is most likely that H_2S derived from both smooth muscle cell and endothelium participates in regulation of vascular tone. H_2S is recently referred to as a new endothelium derived relaxing factor (EDRF) inducing the release of NO (nitric oxide), activation of KATP channels and stimulation of Cl-/HCO3- exchanger.[30,93] H_2S could negatively modulate β-adrenoceptors function via inhibiting adenyl cyclase activity .[94] It is known to inhibit pulmonary carbonic anhydrase enzyme.

A single bolus injection of NaHS at 0.1-10 μmol/kg, one day before myocardial infarction could produce a strong infarct-limiting effect lasting at least three days. Administration of sodium hydrogen sulphide (NaHS) 1 μmol/kg/day for 3 consecutive days after myocardial infarction significantly reduced the infarct size; however, the cardio protection was lower than that offered by H_2S pretreatment.

Cardio protective activity have been attributed to

→ an ability of the compound to induce myocardial preconditioning because of opening of mitochondrial K_{ATP} channels responsible for preconditioning. [95,96,97,98]
→ KATP-channel activation can also induce local coronary vasodilatation [30].
→ ability of sulphide to reduce neutrophil adhesion which is a KATP-channel-dependent response.[99]
→ Beneficial outcome of ischemia reperfusion may relate to its ability to react and neutralize cytotoxic reactive species, such as peroxynitrite, as this reactive nitrogen species plays an important pathogenic role in myocardial infarction. [80,96]

6.3. Gastrointestinal tract

H_2S produced by colonic bacteria in gastrointestinal tract was the thought to be the source of H_2S in flatus. It can also be enzymatically produced in gastrointestinal tissues, H_2S may also serve as an important signaling molecule to regulate the physiological and pathological functions of gastrointestinal tract. H_2S was reported to induce intestinal epithelial cell proliferation and initiate epithelial hyperplasia, commonly seen in ulcerative colitis (UC) and colorectal cancer.[100]

The expression of both CSE and CBS leads increased H_2S in gastric tissues after serosal application of acetic acid. [101] Treatment with an H_2S donor - Lawesson's Reagent (clear mixture of anisole with phosphorus pentasulfide) promoted the ulcer healing. The favorable effect of H_2S on ulcer healing was not dependent on the opening of K_{ATP} channels. The beneficial effects of H_2S on ulcer healing offer a new treatment modalities for patients suffering from NSAIDs induced gastric ulcer. The healing of ulcers was promoted in rats co-administered with diclofenac and H2S donor, Lawesson's reagent. Hence, H_2S releasing NSAIDs would be of tremendous value for this kind of patients.

Additionally sulphide donors stimulate gastric ulcer healing in rodent models [101,102]

Other models in which H_2S has demonstrated efficacy include a rodent experimental model of colitis associated colorectal distension [103] and a non-steroidal anti-inflammatory agent induced gastropathy model. [99]. The gastroprotective effects are mediated, at least in part, by the vasodilatory effect of H_2S.

6.4.Endocrine actions of H^2S

In Diabetes mellitus, it was demonstrated for the first time that H_2S formation, as well as CSE and CBS mRNA were increased in pancreas and liver in streptozotocin induced diabetic rats, implying that H_2S may play a part in the etiology or development of diabetes.[104]. H_2S reduced insulin secretion from HIT-T15 cell culture line via activating K_{ATP} channels indicating role in the pathogenesis of diabetes.[105] H_2S induced apoptosis of insulin-secreting beta cells by enhancing ER stress via p38 MAPK (Mitogen activated protein kinase) activation.[106] Overproduction of H_2S in pancreatic islet was associated with reduced insulin release in Zucker diabetic rats.[107]. Endogenous H2S derived from adipose tissues may inhibit the basal and insulin-stimulated glucose uptake via PI3K (phosphoinositide 3-kinases) but not K_{ATP} channels. Thus, it is proposed that H2S might be a novel insulin resistance regulator.[108]

H_2S open KATP channels located on beta cells of pancreas leading to loss of positive ions and leading to hyperpolarization and decrease in insulin secretion. L-cysteine mediates inhibitory action on insulin secretion through H_2S.

6.5.Reproductive system

Human penile tissue expresses both CBS and CSE, and can efficiently convert L-cysteine to H_2S [109]. Both NaHS and L-cysteine cause a concentration-dependent relaxation of strips of human corpus cavernosum. L-cysteine-induced relaxation could be inhibited by CBS inhibitor, aminooxyacetic acid (AOAA). Similar to NO, H_2S also promotes penile erection in rats. H_2S relaxed uterine smooth muscle in vitro.[110] Sidhu and his colleagues demonstrated that L-cysteine and NaHS inhibited spontaneous contractility of isolated pregnant rat uterine strips in vitro.[111]

6.6.Anti-inflammatory actions of H_2S

H_2S can also induce an upregulation of anti-inflammatory and cytoprotective genes including haem oxygenase 1 (HO1, also known as HMOX1) in pulmonary smooth muscle cells in vivo, in macrophages in vitro and in rat nasal tissues in vivo.[112,113]

By upregulating HO1, H_2S can trigger the production of CO , an another gasotransmitter with well-documented cytoprotective and anti-inflammatory effects, including inhibition of the nuclear factor-κB (NFκB) pathway and downregulation of inducible NO synthase (iNOS) expression and NO production by inflammatory stimuli.[112,114]

6.7 Potential mechanisms of protective effects of H_2S

Sodium hydrogen sulphide inhibits the adhesion of neutrophils to endothelial cells. Hydrogen sulphide (H_2S) inhibits aspirin-induced leukocyte adherence in mesenteric venules through activation of ATP-activated potassium (KATP) channels. Sodium sulphide (Na2S) dose dependently suppressed leukocyte adherence induced by intragastric aspirin (50 mg/ kg). The inhibition of aspirin induced adherence by Na2S was abolished by pretreatment with glibenclamide (10 mg per kg), implicating the role of KATP-channel activation.

H_2S modulates inflammatory processes at the leukocyte–endothelial interface. Under normal conditions, H_2S is synthesized in blood vessels primarily by cystathionine-γ-lyase (CSE), which is expressed in endothelial cells and smooth-muscle cells. H_2S tonically down regulates leukocyte adherence via activation of KATP on leukocytes and the endothelium. When endogenous H_2S synthesis is inhibited by BCA, leukocyte rolling and adherence to the vascular endothelium increase, probably due in part to elevated expression of adhesion molecules on leukocytes (CD11/CD18) and endothelial cells (P-selectin). Marked increases in endothelial permeability, resulting in oedema formation, also occur when H_2S synthesis is suppressed.[43]

6.8.Inflammatory actions of H_2S

Another area in which H_2S has been shown to exert protective effects is in animal models of inflammation. Carrageenan induced paw edema and air pouch inflammation are simple test systems that are commonly used to explore the anti-inflammatory effects of experimental compounds. H_2S has been effective in reducing paw edema and inflammatory cell infiltration in these models. [115,116] The anti-inflammatory effect of H_2S was attenuated by pretreatment with glibenclamide, thereby pointing to the involvement of K_{ATP} channels.[115.116] It is possible that endogenous sulphide at low concentration has anti-inflammatory and high local concentrations lead to inflammation.

6.9.Anti-oxidant action of H_2S

At micromolar concentrations, H_2S demonstrated the cytoprotective effects such as antinecrotic or antiapoptotic actions.[79.80,117,118] They are related to its ability to neutralize a variety of reactive species including oxyradicals and peroxynitrite, hypochlorous acid and homocysteine.[79,80,117]

Low levels of H_2S can up regulate endogenous antioxidant systems. Higher (millimolar) H_2S exposure tends to be cytotoxic to the cells; this is due to free radical and oxidant generation, calcium mobilization, glutathione depletion, intracellular iron release, as well as induction of mitochondrial cell death pathways. [119]

6.10 Angiogenesis/wound healing

Hydrogen sulphide may have therapeutic potential in the angiogenesis/wound healing area.

In invitro studies sulphide induces angiogenesis in chicken chorioallantoid membranes, stimulates endothelial cell proliferation, migration and tube formation.[120,121]

6. 11. Antinociceptive effects

The effects of H_2S also include antinociceptive effects, which may be, at least in part, mediated by the ability of H_2S to downregulate c-fos expression in spinal-cord neurons, but may also involve effects on NO production and/or the K_{ATP}-channel pathway.[122] H_2S can inhibit the adhesion and activation of neutrophil granulocytes, and also has a slight inhibitory effect on platelet aggregation. [99,123]

6.12 Suspended animation

The discovery that H_2S can induce suspended animation began with experiments showing that hibernation-like states can be induced on demand in animals that do not naturally hibernate. H_2S, similar to CO, acts as an inhibitor of cytochrome-c oxidase. When mice were placed in an atmosphere containing relatively low concentrations of H_2S gas (20–80 ppm), a dose-dependent reduction in the core body temperature and metabolic rate of the animals were observed.[124] The animals ceased all movement and appeared to lose consciousness. The metabolic rate continued to decrease, as measured by their carbon dioxide, ultimately falling to tenfold. Their breathing rate slowed from 120 breaths per minute to fewer than 10. When the chamber of the animals was cooled, body temperature reached as low as 15oC. These findings support the hypothesis that H_2S can induce a suspended-animation-like state in a mammal that does not normally hibernate. [124,125]

H_2S induces cardiovascular responses that are consistent with the physiology of hibernation. Although 6 hours of 80 ppm H_2S in the breathing air of the mice induced a decrease in heart rate from over 600 beats per minute to 130 beats per minute, and decreased the body temperature from 38oC to 30oC. The mean arterial blood pressure and stroke volume remained unchanged.[126] In principle, hibernation is known to confer a cytoprotective phenotype, that is, tissues from hibernating animals are remarkably resistant to various hypoxic and ischaemic insults. [125] Inhibition of cellular respiration by inhibiting cytochrome-c oxidase, a key factor in regulation of cellular respiration and cellular oxygen consumption which has pharmacological role and induction of suspended animation.[127,128,129]

The effects of sulphide may also be explored in the context of preservation of transplantable organs. Organs from hibernating animals are protected against the injury associated with storage and transplantation.[130] The possibility that addition of H_2S to storage fluid of transplantable organs may extend their storability is an attractive hypothesis. Thus, H_2S or H_2S-donor compounds exert beneficial effects in multiple models of disease.

All the biological functions of H_2S are mediated by *inhibition of cellular metabolism (in the lethal hypoxia model) ,vasodilatation, KATP-channel activation, up regulation of anti-inflammatory genes (for example, HO1) and down regulation of inflammatory genes (COX2, FOS, IL1).*

7.0 PHARMACOLOGICAL H_2S DONORS

There are several agents that either directly release H2S (NaHS, Na2S, Lawesson's reagent, GYY4137112) or as a precursor for endogenous H_2S synthesis (N-acetylcysteine, L-cysteine).

Pharmacologically useful donors should ideally be soluble in aqueous media, non-toxic, not metabolized quickly, and should release H_2S in vivo slowly over a period of time.

Sodium hydrogen sulphide (NaHS) is a known donor, but it releases H_2S too quickly and is not useful for many studies leading to fall in the blood pressure and is short acting. a water-soluble compound GYY4137 has been synthesized which is a slow releasing H_2S compound with vasodilatator and antihypertensive activity. [131]

8.0 DRUG RELEASING H_2S

Sodium sulphide (IK-1001) has been tested as a parenteral injectable formulation in myocardial infarction, cardiopulmonary bypass surgery, thoracoabdominal aortic aneurysm surgery, liver ischemia and reperfusion, organ storage, transplantation, and acute lung injury.[132,133]

Synthesis of known drugs which has a hydrogen sulphide releasing moiety

Recently it has been shown that the H2S pathway is involved in the erectile function in humans.[134] A H2S donating derivative of sildenafil, ACS6 relaxed cavernosal smooth muscle equipotently to sildenafil citrate. The formation of superoxide and expression of PDE5(phosphodiesterase-5) were reduced by ACS- sildenafil citrate, and NaHS. ACS6 was more active than sildenafil as shown in table 1.[135]

Table 1. H_2S releasing compounds in various phase of drug development

Compound	Characteristics	Potential applications	Development stage
ACS-15	hydrogen sulphide-releasing diclofenac derivative	Arthritis	Preclinical
ATB-429	Hydrogen sulphide-releasing mesalamine derivative, with antinociceptive and anti-inflammatory effects	Inflammatory bowel	Preclinical
ATB-346	Hydrogen sulphide-releasing NSAIDs	Acute and chronic joint pain	Preclinical
ATB-284	NA	Irritable bowel syndrome	Preclinical
IK-1001	Injectable	Suspended animation and multiple hypoxic /ischemic condition	Phase I

H_2S donors reduced the severity of NSAID-induced damage in the rat stomach. 121. Moreover, NSAIDs decreased endogenous H_2S synthesis.[136] ATB 337, a H_2S releasing derivative of diclofenac, exhibits enhanced anti-inflammatory effects as compared to diclofenac. [101]

A new compound, ACS15 has been synthesized and tested.[137] While diclofenac dose-dependently damages the stomach, ACS15 displays significantly reduced GI toxicity. [138,139] Diclofenac, but not ACS15, elevates gastric granulocyte infiltration and expression of TNF-α, lymphocyte function-associated antigen 1, and intercellular adhesion molecule1. ACS15 inhibited COX-(cyclo-oxygenase) and COX-2 activity as effectively as diclofenac and did not induce leukocyte adherence as diclofenac.

ATB-429 is a derivative of mesalamine, a drug commonly used in the treatment of inflammatory bowel disease (IBD). [136,103] In animal models of CD and UC, and it has been shown to be significantly more effective than mesalamine and in treatment of visceral pain associated with IBD(irritable bowel syndrome). [136,103]

ATB-346 is a derivative of naproxen, used as pain relief in osteoarthritis. However, like other NSAIDS, naproxen carries a significant risk of serious gastrointestinal bleeding.[140] In preclinical studies, ATB-346 exhibits increased effectiveness over its parent drug and a remarkable reduction in the gastrointestinal and cardiovascular toxicity. The drug at the present stage is in preclinical effectiveness and toxicology studies.[140]

9.0 Conclusion

Hydrogen sulphide is a gas produced from exogenously and endogenously. The human exposure to this gas occurs via inhalation process due to its rapid absorption through non receptor mediated mechanism in lungs. This on entry in to circulation dissociates in to hydrogen sulphide anion and proton. Hydrogen sulphide interacts with haem compounds and

metabolised to sulfate similarly hydrogen sulphide anion interact with methemoglobin forming sulfmethemoglobin or methylation during detoxication process.

The toxicity of H2S results the inhibition of cytochrome oxidase a component of electron transport chain in cellular respiration that affects nervous and cardiac tissues function because these tissues on high oxygen demand. As a result of that central Nervous system, cardiac, and respiratory systems gets arrested. Hydrogen sulphide performs several diversified functions in the human body they are ocular effects, respiratory effects, neurological effects, cardiovascular effects, metabolic effects, reproductive effects, cancer etc. To understand the concentration of hydrogen sulphide in the environmental and biological samples few well established quantification methods employed they are GC/FPD, GC/ECD, spectrophotometric, HPLC spot methods etc.

Every biological functions of Hydrogen sulphide belongs to gasotransmitter action through vaso dialation thereby it is regarded as a new gasotransmitter which mimics the other member of gasotransmitter carbon monoxide and nitric oxide. The therapeutic benefit of hydrogen sulphide is seen at lower concentration and toxicity at higher concentration. Therefore it is regarded as double edged sword.

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