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Hydrogen sulfide (H_2S) - therapeutic relevance in rehabilitation and balneotherapy Systematic literature review and meta-analysis based on the PRISMA paradigm

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Abstract

Background. An active molecule in sulfurous mineral - therapeutic waters and also in sapropelic mud is H_2S, a hormetic gaseous molecule that can actively penetrate the skin. While high levels of H_2S are extremely toxic, low levels are tolerated and have potential cytoprotective effects, with anti-inflammatory and antioxidant applications.

Objective. This systematic review aims to rigorously select related articles and identify within their content the main possible uses of hydrogen sulfide from balneary sources and to explain its physiological mechanisms and therapeutic properties.

Methods. To elaborate our systematic review, we have searched for relevant *open access* articles in 6 international databases: Cochrane[1], Elsevier[1], NCBI/PubMed[1], NCBI/PMC[1], PEDro[1], and ISI Web of Knowledge/Science[1], published from January 2016 until July 2021. The contextually quested keywords combinations/ syntaxes used are specified on this page. The eligible articles were analyzed in detail regarding pathologies addressed by hydrogen sulfide. All articles with any design (reviews, randomized controlled trials, non-randomized controlled trials, case-control studies, cross-sectional studies), if eligible according to the above-mentioned selection methodology, containing in the title the selected combinations, were included in the analysis. Articles were excluded in the second phase if they did not reach the relevance criterion.

Results. Our search identified, first, **291** articles. After eliminating the duplicates and non-ISI articles, remained **121** papers. In the second phase, we applied a PEDro selection filter, resulting in **108** articles that passed the relevance criterion and were included in this systematic review.

Conclusions. H_2S biology and medical relevance are not fully understood and used adequately for sanogenic or medical purposes. More research is needed to fully understand the mechanisms and importance of this therapeutic gase. The link between balneotherapy and medical rehabilitation regarding the usage of hydrogen sulfide emphasises the unity for this medical speciality.

Keywords: *Hydrogen sulfide / H_2S **AND** balneotherapy / inhalations / mud / Rheumatoid arthritis / neuroprotection / neurodegenerative disorders / Stroke / Parkinson / Alzheimer / Huntington / vascular dementia / Immune system / Diabetes / Cancer / Cardioprotection / Asthma / Alergic Rhinitis / COPD / COVID-19 / Burns / Analgesic effect / Clinical trials*

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1. INTRODUCTION

Early life forms experienced and had to survive in an environment containing highly reactive chemicals such as NO, CO, and hydrogen sulfide (H_2S). During evolution, early life forms learned to tolerate these compounds and include them as essential molecules in their metabolism and signaling mechanisms.

Increasing evidence suggests that mammalian homeostasis strongly depends on a mutualistic relationship with gut bacteria. Interestingly, it has been found that metabolic and cardiovascular diseases, including hypertension, are associated with gut microbiota dysbiosis. Hydrogen sulfide (H2S) regulates the functions of biological systems, including the circulatory system. H2S released by bacteria in the colon may also contribute to the control of arterial blood pressure. Incidentally, sulfate-reducing bacteria are ubiquitous in the mammalian colon, and H2S is just one among many molecules produced by the gut flora, and gut-bacteria-derived molecules, such as H2S, have an important role in circulatory system homeostasis (1).

H_2S is, on the one hand, an endogenous gas signal molecule, which plays a vital role in various systems and diseases. H_2S is mainly produced in our body by three enzymes, including Cystathionine γ-lyase (CSE), Cystathionine β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3MST) together with cysteine aminotransferase (2). H2S is also produced nonenzymatically from glucose, glutathione, thiosulfate, and sulfur-containing proteins and by the bacterial reduction of sulfur in the intestinal tract. H_2S is, on the other hand, an exogenous therapeutic gas, found in combination with carbon dioxide in sulfurous waters, with medicinal properties well known and appreciated in balneotherapy, and also in therapeutic muds (3).

Hydrogen sulfide (H_2S) naturally occurs in volcanic gases, natural gas, and some therapeutic waters (4). Also, H_2S is produced when bacteria break down organic matter in the absence of oxygen. H_2S is a flammable and colorless gas with a characteristic odor of rotten eggs. H_2S intoxication has been observed mainly in industrial environments. Workers may be exposed to H_2S in agriculture and industries, such as oil and wastewater treatment. H_2S is a toxic gas to humans, and acute exposure to large amounts of H_2S (> 500 ppm) can lead to death. The first biological experiment reported to study the effect of H_2S in animals was published in 1908 and described the lethal impacts of H_2S gas when absorbed through the skin or administered directly into the stomach or rectum. In the human oral microbiota, it has been found that H_2S is responsible for oral halitosis and is related to periodontal inflammation. In the intestinal microbiota (5), H_2S is a component of flatus (6).

The discovery of gaseous signaling molecules such as H_2S, NO, and CO has added a new era in biomedical science, as these molecules are of great importance in mammalian physiology. They have been called "gas transmitters" because they are produced internally or synthesized (endogenously) in the body or are received from the atmosphere and transmit chemical signals promoting or inducing various physiological changes inside the mammalian body. Gas transmitters are permeable through the cell membranes, but their functions inside the body depend on their concentration.

Like other gas transmitters, the physiological role of H_2S has been overlooked or ignored due to its toxicity. The latest gas signaling molecule discovered after NO and CO has an enormous pathophysiological significance in various diseases and conditions. Due to its weak intermolecular strength, it is a sulfur analog of water and exists in gaseous form. It is synthesized both enzymatically and non-enzymatically within mammalian tissue, but the non-enzymatic pathway is of a more negligible significance.

Cystathionine β-synthase (CBS) and Cystathionine γ-lyase (CSE) are two enzymes responsible for H_2S biosynthesis from L-cysteine. Sulfurtransferase 3-mercaptopyruvate (3MST) is another enzyme that can generate H_2S via cys-catabolism. CSE and CBS are located in the cell's cytoplasm, but 3MST is partially expressed in the mitochondria and cytoplasm. A recent study showed that H_2S could be produced from D-cysteine by the enzyme D-amino acid oxidase (DAO). Non-enzymatically, H_2S can be produced from thiosulfate and glucose (by glycolysis) or phosphogluconate by NADPH oxidase (7).

Although H_2S has beneficial roles in various hematological diseases, urological diseases, cardiovascular function, and oxidative stress, the effects of H_2S in the CNS have attracted much more attention in recent years (8).

In vivo, H2S can exert a dual effect on cell bioenergetics, at lower concentrations stimulating via sulfide - quinone oxidoreductase mitochondrial respiration and thus ATP synthesis or causing a reversible inhibition of cytochrome c oxidase at higher concentrations (9). H2S has been shown to regulate all significant aspects of mitochondrial dynamics: mitochondrial fusion, mitochondrial fission, mitochondrial macroautophagy /mitophagy, and mitochondrial biogenesis). The majority of the published studies indicate that H2S (especially in lower concentrations) tends to stabilize and preserve mitochondria, and, in many cases, can also stimulate mitochondrial biogenesis (10). The intricate interplay between H2S and O2 has been extensively investigated. As O2 facilitates both the chemical and enzymatic oxidative decomposition of H2S into persulfides and polysulfides, at low O2 tension higher stability of H2S is expected. Furthermore, hypoxic/ischemic conditions have been reported to enhance H2S synthesis, through

upregulation or stimulation of the sulfide-synthesizing enzymes, accumulation of CBS in mitochondria, likely augmenting the H2S mitochondrial levels, and release of CO-mediated inhibition of CBS and CSE. Hypoxia is thus expected to increase H2S bioavailability, a condition that can have opposite physiological consequences. Indeed, while H2S is protective against ischemic injuries, the enhanced biosynthesis and chemical stability of H2S, combined with the reduced content in mitochondria, may increase the risk of H2S toxicity in hypoxic cells (9).

Tissue development and repair have long been associated with sulfur metabolism (11). Since the first reports illustrating the expression of H2S-producing enzymes in mammalian systems, there has been tremendous growth of preclinical studies investigating the protective effects of H2S modulation, by way of donors or overexpression /inhibition of its synthesizing enzymes, against cardiovascular diseases. H2S possesses infarct-sparing effects following myocardial I/R injury, but also it exerts anti-apoptotic, anti-oxidant, anti-inflammatory, and pro-angiogenic benefits under various cardiac stressors (12).

The H2S functions in the secretion of corticotrophin-releasing hormone from serotonergic neurons and the relaxation of smooth muscle. H2S shields neurons and cardiac muscles from oxidative stresses and helps to maintain insulin secretion. H2S has diverse physiologic functions such as relaxing blood vessels, lowering blood pressure, antiapoptosis, anti-inflammation, and anti-oxidative stress. Among these functions, the role of H2S in antioxidative stress has been one of the main focuses over years (13).

2. METHOD

Literature Search Strategy. To elaborate our systhematic review, we have searched for relevant *open access* articles, in 6 international databases: Cochrane[1], Elsevier[2], NCBI/PubMed[3], NCBI/PMC[4], PEDro[5] , and ISI Web of Knowledge/Science[6], published from January 2016 until July 2021. The contextually quested keywords combinations/ syntaxes used were *Hydrogen sulfide / H_2S* ***AND*** *balneotherapy / inhalations / mud / Rheumatoid arthritis / neuroprotection / neurodegenerative disorders / Stroke / Parkinson / Alzheimer / Huntington / vascular dementia / Immune system / Diabetes / Cancer / Cardioprotection / Asthma / Alergic Rhinitis / COPD / COVID-19 / Burns / Analgesic effect / Clinical trials*. The eligible articles were analyzed in detail regarding pathologies addressed by hydrogen sulfide.

[1] https://www.cochrane.org/
[2] https://www.elsevier.com/
[3] https://pubmed.ncbi.nlm.nih.gov/
[4] https://www.ncbi.nlm.nih.gov/pmc/
[5] https://pedro.org.au/
[6] http://apps.webofknowledge.com/WOS_GeneralSearch

Inclusion and Exclusion Criteria

All articles with any design (reviews, randomized controlled trials, non-randomized controlled trials, case-control studies, cross-sectional studies), if eligible according to the above-mentioned selection methodology, containing in the title the selected combinations, were included in the analysis. Articles were excluded in the second phase if they did not reach the relevance criterion.

3. RESULTS

Our search identified, first, **291** articles. After eliminating the duplicates and non-ISI articles, remained **121** papers. In the second phase, we applied a PEDro selection filter, resulting in **108** articles that passed the relevance criterion and were included in this systematic review.

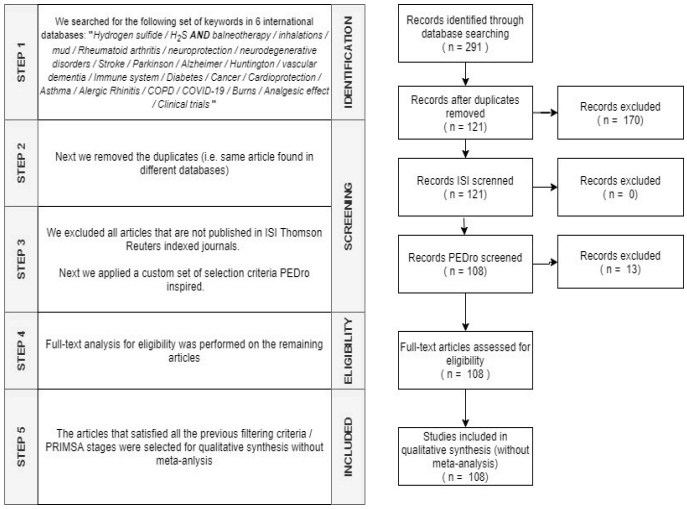

Fig. 1 PRISMA inspired flow diagram for articles search

4. A timeline of hydrogen sulfide research

The H_2S history – as an environmental toxin – dates back to 1700, with the observations of the Italian physician Bernardino Ramazzini (1633–1714), whose book ‘‘De Morbis Artificum Diatriba” is the reason why he is widely considered the founding father of the occupational medicine. Ramazzini predicted that this gas may damage the lungs (14). As a pure chemical species, the discovery of H_2S (1775) is credited to Swedish-German chemist Carl Wilhelm Scheele (1742– 1786), who produced it through the reaction of an acid with metal (poly)sulfides or by heating sulfur in hydrogen gas. In 1776, the chemical composition of the gas was determined by the French chemist Claude Louis Berthollet (1789).

The first biological experiment that directly investigated the effect of pure H_2S in animals was published in 1803 by French anatomist Francois Chaussier (1746–1828). He described the lethal effects of H_2S gas when the gas was absorbed through the skin. Subsequent studies used injections of H_2S-saturated solutions and reported respiratory effects (excitation, deep breathing), convulsive movements, and, at high doses, asphyxia and death. Over the subsequent 150 years, generations of toxicological researchers have investigated the

toxicological effects of H_2S in various species including humans. The results of these studies are now summarized in comprehensive monographs, including limits and methods of its detection, occupational exposure limits, and the multiple biological effects of H_2S on various cells and organs (14).

H_2S, at low concentrations, has an entirely different pharmacological profile than what was previously characterized in the toxicological literature (where, typically, high concentrations were used to demonstrate adverse/ noxious effects).

With the use of pharmacological inhibitors, as well as mice lacking various H_2S-producing enzymes, and by using transient and stable silencing of H_2S-producing enzymes, the functional role of endogenously produced H_2S began to emerge in more detail (as opposed to testing the effect of low concentrations of exogenously applied H_2S on biological systems). There were also significant advances made in the field of H_2S-mediated signaling, including the concept of sulfhydration (post-translational modification of protein cysteine residues, with functional consequences), which is prominently induced by polysulfides. Another area within the growing field of H_2S biology relates to the interactions of H_2S with various oxygen-and nitrogen-derived species, which includes functional interactions as well as the recognition of the biological roles of various hybrid S/N species. The state-of-the-art of H_2S biology can be overviewed in general reviews and monographs as well as in specialized review articles focusing on the roles of H_2S in the regulation of the nervous system, cardiovascular system, gastrointestinal system, renal system, metabolic and mitochondrial aspects, cellular signaling, and the biochemistry of the various H_2S-producing enzymes. Separate articles focus on the details of the chemical properties and reactivity of H_2S and its functional interactions with other reactive species including oxygen-derived oxidants and free radicals and NO. Other review articles focus on pharmacological donors and inhibitors of H_2S biosynthesis, and the therapeutic aspects and translational potential of H_2S biology (14).

5. Chemical and Physical Properties of H_2S

Aqueous species elements can be quantified by chemical speciation analysis or estimated by speciation modeling based on chemical thermodynamics (15). Although H_2S exists as a gaseous molecule under ambient temperature and pressure, it can easily dissolve in water with a solubility of around 80 mM at 37 °C. The half-life of H2S in air varies from 12 to 37 h (16). Upon dissolution, H_2S promptly reaches the equilibrium of H_2S /HS^-/S^{2-} species, which is different from the other two gaseous transmitters, respectively, NO and CO. Based on its PKa value, it is estimated that there will be 14% of H_2S, 86% of HS^-, and a negligible level of S^{2-} in physiological solution (pH 7.4, 140 mM NaCl, 37 °C).

Moreover, H_2S gas is highly lipophilic, which allows its free penetration into biological membranes of all types and becomes biologically active. Sulfur is a versatile atom with multiple oxidation states ranging from −2 to +6. In H_2S, the oxidation state of sulfur is −2; therefore, it can act as a reductant and only be oxidized. In line with this, accumulative evidence has suggested that H_2S actively takes part in three reactions in mammalian systems, including interaction with the reactive oxygen species (ROS) or reactive nitrogen species (RNS) species; reduction of the protein irons, namely, the metal center on proteins; transformation of protein R-SH group into R-SSH group, a process called S-sulfhydration (17).

6. H_2S toxic effects

There are more than 300 years since the first description of H_2S as a poisonous molecule. Among the more recent studies, the work of Attene-Ramos should be mentioned, who demonstrated the genotoxic effect of high doses of H_2S, Nicholson, Khan, and later Dorman and colleagues have directly demonstrated the inhibition of cytochrome c oxidase activity ex vivo in tissues after H_2S exposure of experimental animals and implicating these effects in the disruption of respiratory and mitochondrial functions in the mammalian brain (and other tissues) after H_2S exposure in vivo. H_2S has wide-ranging effects on gene expression (associated with a variety of biological processes including cell cycle regulation, cellular division, DNA metabolism and repair, protein kinase regulation, cytoskeletal organization, and biogenesis). It is currently accepted that H_2S exerts its toxicological actions on the molecular level primarily through the inhibition of mitochondrial respiration via inhibition of mitochondrial Complex IV (cytochrome c oxidase). Via this action, the consumption of O2 is inhibited, and electron transport and ATP generation are blocked (14).

7. The physiological role of hydrogen sulfide

Under physiological conditions (aqueous solutions at pH 7.4), one-third of H_2S is un-dissociated and two-thirds is hydrolyzed to sulfide and hydrosulfide ions in the following reaction: $H_2S \leftrightarrow H^+ + HS^- \leftrightarrow 2H^+ + S^{2-}$. In mammalian tissues and blood, the concentration of H_2S is 1–160 μM, under physiological conditions. Higher concentrations of H_2S are present in the brain (50–160 μM) and blood (10–100 μM) (18).

Through the participation of H_2S in many physiological and pathological processes, including its role in regulating inflammatory processes, hypoxia, cell proliferation, apoptosis, neuromodulation, and cardioprotection, H_2S is now accepted as a player - as a gas transmitter (gas signaling compound) that is equally important as nitric oxide and carbon monoxide in mammals and as a signaling molecule as necessary as hydrogen peroxide (H2O2) in plants.

Hydrogen sulfide (H2S) has emerged as a critical mediator of multiple physiological processes in

mammalian systems. The pathways involved in the production, consumption, and mechanism of action of H2S appear to be sensitive to alterations in the cellular redox state and O2 tension. Indeed, the catabolism of H2S through a putative oxidation pathway, the sulfide quinone oxidoreductase system, is highly dependent on O2 tension. Dysregulation of H2S homeostasis has also been implicated in numerous pathological conditions and diseases (19).

8. Measurement methods of H_2S

Reliable bioanalytical methods for highly specific and sensitive detection of H2S are key for the investigation of H2S functions in biological systems. Over the past few decades, several techniques have been reported for H2S detection in bulk solutions. Of various approaches, optical detection using H2S responsive sensors has been recognized as one of the most promising technology due to its inherent advantages, such as high sensitivity and simplicity, rapidity, and efficiency. The H2S responsive sensors are normally designed based on different sensing mechanisms, such as thiolysis of dinitrophenyl ether, reaction with azide, nucleophilic addition with formaldehyde groups, and displacement of metal ions from luminescent dyes. Under these reactions, several sensors have been developed for sensitive and selective H2S detection and H2S visualization in live cells and organisms. The majority of the reported H2S sensors are designed based on the "OFF-ON" changes of luminescent signals (20).

H_2S in aqueous solution can be found in the form of hydrogen sulfide gas (H_2S) or one of its dissociated forms, hydrosulfide anions (HS^-) and sulfide anions (S^{2-}), although at physiological pH, S^{2-} is found only in a negligible concentration. In addition, H_2S can bind to some biological matrices (proteins, glutathione, etc.) and can dissociate in response to a physiological stimulus in free H_2S. Moreover, the HS and H_2S anion have a high tendency to oxidize, especially in the presence of metal ions and oxygen in aqueous solutions (21).

The first H_2S quantifier was published in 1949 and included spectrophotometric determination using the methylene blue method, but the sensitivity was very low. In 1969, an improved method based on the methylene blue method using n,n-dimethyl-β-phenylenediamine sulfate was described, which increased the sensitivity of the method by 10%, with concentration limits of 1 to 1000 μM. However, the main disadvantages of this method for biological sample measurements are low sensitivity, overestimation of H_2S below acidic pH, and interference due to turbidity of biological samples. Monobromobiman (MBB) derivative: this is another method widely used in biological samples to measure H_2S concentration. In this method, H_2S is derivatized into a sulfide-dibiman product, which HPLC can subsequently measure due to its fluorescence. MBB sensitivity reaches the nanomolar range, but the weaknesses of this method are the instability of the standards and the need for a complete pH control when comparing the samples. Another advantage of this method is that it allows the detection and quantification of all three biological forms of sulfur: free hydrogen sulfide, labile acid sulfur, and bound sulfan sulfur. An improved MBB-based method was developed that included 36S-labeled sulfide-dibiman as an internal standard and measured the derivatized products by liquid chromatography-mass spectrometry.

9. H_2S Mechanisms of Action

There is an important effect of H2S binding to target proteins such as cytochrome c oxidase, hemoglobin, and myoglobin, among others. It has, however, become widely accepted that a huge number of the processes controlled by H2S are caused by a posttranslational modification of cysteine residues called persulfidation. Protein persulfidation is an oxidative posttranslational modification of cysteine residues caused by H2S, in which a thiol group (–SH) is transformed to a persulfide group (–SSH). Sulfane sulfur species, persulfides, and polysulfides are more nucleophilic than H_2S (22).

It is well established that H2S regulates different physiological processes in cells directly or by crosstalk with other signaling molecules. There is clear evidence of crosstalk of H2S and NO in the literature. In mammals, both gasotransmitters interact with each other to modulate the cardiovascular system by regulating angiogenesis and endothelium-dependent vasorelaxation, and to modulate Alzheimer's disease by regulating pathways involved in the central nervous system. Furthermore, inhibition of NO generation by H2S has been extensively studied, but there is also evidence that NO can activate the production of H2S in endothelial cells. However, NO can bind to cystathionine β-synthase (CBS), which is responsible for H2S biosynthesis and can impede its enzymatic activity, showing the complexity of the crosstalk between these two gasotransmitters.

Carbon monoxide is another important gasotransmitter in animals; carbon monoxide is generated from oxidative degradation of heme by the enzyme heme oxygenase. CO may inhibit CBS activity and therefore may modulate H2S biosynthesis. Exogenous H_2S also upregulates the CO/heme oxygenase system in the pulmonary arteries of hypoxic rats and stimulates heme oxygenase levels.

H2S is a regulator of glucose homeostasis and plays an important role in the metabolism of hormones, such as insulin and glucagon. It has been demonstrated that β cells of the pancreas can produce high levels of H2S, predominantly by cystathionine γ-lyase (CSE), which blocks glucose-stimulated insulin secretion. This effect is caused by increased endoplasmic reticulum stress, leading to apoptosis of β cells, which drives the reduction in insulin secretion. Some other studies revealed the importance of H2S in the modulation of estrogen receptor

expression and its anti-proliferative effect on vascular smooth muscle cell growth and proliferation. Further research concluded that the antiatherosclerotic effect of estrogen is mediated by CSE-generated H2S and that H2S production in the liver and vascular tissues is enhanced by estrogen via its stimulatory effect on CSE activity. H2S signaling was also linked with the regulation of two endocrine hormones associated with longevity control, growth hormone and thyroid hormone.

10. Hydrogen sulfide (H2S) role in Balneotherapy

Balneotherapy (Latin balneum, bath (23)) has been defined as the use of natural mineral waters (24), natural peloids and mud, and natural sources of different gases (CO2, H2S, and Rn) for medical purposes such as prevention, treatment, and rehabilitation (25)

Among the inorganic molecules, which generally constitute the mineral waters, sulfur has currently been recognized as a crucial element with a wide range of functions, mainly when it was found in the form of hydrogen sulfide (H2S). Sulfurous waters (26) contain at least 1 mg of H2S, HS, S, or thiosulphates per liter or sulfur colloidal complexes, in the form of simple or mixed sulfurous waters (alkaline earth, carbonated, sodium chlorinated). H2S represents the main active molecule of sulfurous mineral-medicinal waters (27). It has been also shown by in vitro and in vivo studies that there is a positive action of mineral waters on the oxidant/antioxidant system. Many authors investigated the possible activity of different hydrogen sulfide (H2S) donors in normal or osteoarthritic (28) chondrocytes stimulated by proinflammatory stimuli such as Interleukin-1b or lipopolysaccharide. The incubation with exogenous H2S sources inhibits the release of Nitric oxide, Prostaglandin E2, Metalloproteases, Tumor necrosis Factor-a, Interleukin-6, Interleukin-8 and decreases the Nuclear Factor-kB activation (29). It is well known that sulfurous water baths were used by ancient civilizations and were known to have healing effects against particular diseases. H2S has been recognized as having anti-inflammatory, anti-bacterial, vasodilator, and anti-fungal properties owing to its sulfur content. Sulphur (S), Hydrogen Sulphide (H2S), Sulphate (SO_4^{2-}) are involved in cartilage, hair/nails formation, enzyme activity in redox processes, and cellular respiration. SO42− is essential to several metabolic and cellular processes, particularly in fetal growth and development (30).

Sapropel's complex chemical and biological structure explains its multifunctional effect on the body (31). Baths stimulate redox reactions, which is indicated by an increase in the hemoglobin level and erythrocyte count. observed also in patients with rheumatoid arthritis (32) receiving artificial sulfur and hydrogen sulfide baths (33). The potential of sulfurous thermal water (STWs) to directly regulate bone cells function and to use STWs in complementary medicine for the treatment of bone-wasting diseases is a new terrain of investigation. A growing body of evidence shows that H2S has therapeutic potential in age-associated diseases. Exogenous H2S stimulation induces osteogenic differentiation. H2S donors can be used, similarly to other osteoinductive molecules, to increase the osteogenicity of bone implants, and most importantly that therapeutic intraperitoneal administration of H2S prevents the onset of bone loss in the mouse model of osteoporosis (34). Hydrogen sulfide (H2S) seems to affect cells involved in degenerative joint diseases, it was the objective of this study to investigate the effects of exogenous H2S on fibroblast-like synoviocytes, which are key players in OA pathogenesis being capable of producing pro-inflammatory cytokines and matrix-degrading enzymes (35).

Healthy cartilage maintenance relies on an equilibrium among the anabolic and catabolic processes in chondrocytes. Hydrogen sulfide (H2S) has shown anti-inflammatory and anti-catabolic properties in several in vitro and in vivo models. H2S in the musculoskeletal system is quite incipient. Yet, it is already accepted that it is present and modulated in the osteoarthritic process and it exerts anti-inflammatory and cytoprotective effects in joint cells at low micromolar doses. H2S is present in the synovial fluid (SF). In patients with rheumatoid arthritis, SF H2S levels were significantly and substantially higher than in paired plasma samples or SF aspirates from OA patients. Additionally, H2S levels in SF and plasma correlated with clinical markers of disease activity and inflammation (36).

Bone tissue is constantly remodeling. It responds to various stimuli, such as physical exercise. H2S can prevent the onset of bone loss in the mouse model of osteoporosis (37).

11. Balneo-rehabilitative role of H_2S in Romania

In our country, sulfurous waters have special healing qualities. One of the most important sources of sulfurous water is found in Dâmboviţa County, in Pucioasa. On the Olt valley, at Calimanesti, Caciulata, the sulfurous waters, through the different concentrations in hydrogen sulfide, are used therapeutically both in the internal and external cure. The localities of Baile Govora and Harghita Bai are also famous, but also Baile Bârzava, Baile Boghiş, Olanesti and those on the sea coast, Venus and Mangalia. In Herculane, a resort arranged and operated for the first time 2000 years ago, it benefits from the sulfurous waters of special quality and the possibility of performing thermal bath cures. Sulfurous waters are indicated both for crenotherapy and in external treatment in the form of baths, inhalations, and vaginal irrigation, due to the effects of H2S, which is reabsorbed through the skin and gastric mucosa, upper airways, bronchopulmonary and vaginal mucosa.

Diseases that can be treated with sulfurous water:

1. Crenotherapy with sulfurous waters, usually mixed, has effects of stimulating gastric secretion as well as intestinal peristalsis, choleretic and cholagogue effects on the liver and bile ducts, antitoxic effects, and lowering blood sugar in diabetics. The mixed carbonated ones also have diuretic effects through the action of CO2. The indications for crenotherapy with low concentration sulfurous waters are hepato-biliary disorders, especially biliary dyskinesias, gallstones, and chronic cholecystitis, urinary tract infections, forms of diabetes, and heavy metal poisoning.
2. Diseases of the musculoskeletal system. These diseases include rheumatic, inflammatory, degenerative, abarticular diseases, post-traumatic sequelae of the hands and feet, etc.
3. Peripheral neurological disorders. Post-traumatic paralysis and parasites of the limbs; polyneuropathy after the acute phase; ponytail syndrome; sequelae after polio with secondary tendon damage, for corrective surgery.
4. Gynecological diseases. Sulfur waters also have an anti-inflammatory (38) and anti-allergic effect in gynecological diseases, favorably influencing the secretory glands of the cervix. It also promotes the restoration of the intravaginal biological component.
5. Respiratory disorders. The treatment for prophylactic purposes is addressed to workers who work with dust, lint, toxic gases, humidity or dry air, temperature variations, etc., and who are prone to inflammatory or allergic bronchopulmonary diseases. Treatment is recommended for convalescents after acute, bacterial and viral pneumonia, simple chronic tracheobronchitis, respiratory neurosis. The sulfurous mineral waters used in inhalation form have an action of wetting the bronchial trajectory, favoring the drainage of the products of bronchial secretion.

12. H_2S role on neurodegenerative disorders

In some brain disorders, endogenous H2S generation is blunted, and H2S deficiency has been implicated in the progression of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and traumatic brain injury (TBI). Although the mechanisms of action of H2S have not been fully characterized, redox regulation has been identified as a critical player in H2S-mediated neuroprotection and toxicity (39).

H2S has a significant neuroprotective role in the central nervous system. H2S provided neuroprotection in TBI via inhibition of autophagy and apoptosis (40). H2S exhibits cytoprotection against injuries in many tissues/organs through its anti-oxidative, anti-inflammatory, and/or anti-apoptotic effects. Neuroprotection of exogenous H2S against cerebral injury is associated with the reduced accumulation of autophagic vacuoles (41).

Important signaling events of H2S in various neuronal cells/cell lines are listed below:

1. Inhibition of monoamine oxidase (via catecholamines)
2. NMDA potentiation (via glutamate)
3. Cystic fibrosis transmembrane conductance regulator (CTFR) channel activation (via chloride channels)
4. KATP and KCa^{2+} channel activation (via potassium channels)
5. Intracellular calcium mobilization, L-type and T-type channel activation (via calcium channels)
6. Suppression of various types of neuronal toxicity (via oxidative stress)
7. Inhibition of p38-MAPK (via mitogen and tyrosine kinase receptors)
8. Stimulation of PKA and elevation of cAMP (via PKA)

AD, a common form of dementia, characterized by memory impairment, personality changes, and various neuropsychiatric symptoms which cause neuronal apoptosis, neuronal inflammation (induced by amyloid-β), and increased oxidative stress.

The level of H2S in the brain of a patient with AD is lower than healthy people of the same age. A recent study revealed that in a rat model of vascular dementia, plasma H2S level was lower and i.p. injection of NaHS (H2S donor) protected neuronal injury and improved behavioral (learning and memory) test results. Another study demonstrated that progression of AD was abrupted after treatment with spa-water rich in H2S content. The role of H2S in the improvement of cognitive functioning, spatial learning and memory, and neuroprotective effects is also providing us hopes against AD.

Research and studies regarding H2S are still in the preliminary stage in the field of biomedical science. Further research also should be focused on their combinatorial effect and signaling pathways regarding their antagonistic effect should be disclosed (8).

13. Hydrogen sulfide and Stroke

Stroke (ischemic or hemorrhagic) is an acute cerebrovascular disease, a leading cause of death and disability worldwide (42). H2S has tissues concentrations of 50-160 mM, much higher than in peripheral blood (0-46 mM), suggesting that H2S may have some effects and potential therapeutic value (43). H2S can facilitate hippocampal long-term potentiation *via* activating the N-methyl-D-aspartic acid (NMDA) receptor. H2S can activate calcium channels to increase calcium influx, whereas decreasing the release of calcium stored in the cells, thus induce the production of calcium waves in primary cultures of astrocytes. This process can be inhibited by a calcium channel antagonist. In addition, H2S has been known as ATP-sensitive potassium (KATP) channel activator in secondary brain injury after stroke or other diseases, H2S could play its neuroprotection through mimicking the KATP channel (42).

Fibrinolytic therapy with recombinant tissue plasminogen activator (tPA) is a globally accepted treatment for ischemic stroke. Unfortunately, the clinical application of tPA remains rather limited due to the enhanced risk of cerebral hemorrhage associated with tPA therapy. H2S-base therapy represents a novel approach to increase the safety of tPA thrombolysis in stroke patients. H2S donor inhibited the Akt-VEGF-MMP9 cascade in both the mouse MCAO model and the endothelial OGD model following tPA treatment (44). Given the ability of GSH to function as a histone modifier, it would be appropriate to consider H2S as an epigenetic modulator albeit in an indirect manner. Concerning oxidative stress, H2S is also mitoprotective as it averts energy failure and upregulates superoxide dismutase (SOD) expression with a reciprocal downregulation of cytochrome oxidase. Paradoxically, both inhibition of H2S production and therapeutic H2S donation seem to substantially influence the disease progression in various animal models of inflammation, reperfusion injury, and circulatory shock.

Treatment with H2S also has been shown to reduce brain injuries and post-ischemic cerebral edema in a dose-dependent manner possibly by blocking apoptosis. (45).

H2S also regulates the cerebral microvascular response to hypoxia. Some reports showed that under normal O2 conditions, CO binds to the heme in CBS and inhibits the activity of producing H2S. Hypoxia induces the production of H2S through the decline of the generation of CO and vasodilation. H2S has been reported to modify specific cysteine residues in proteins through the formation of a polysulfide and persulfide bond and to be involved in the regulation of such activity by sulfhydration of the enzyme (46). H2S has an important role in maintaining the stability of the cerebrovascular environment. The decrease of H2S level leads to endothelial dysfunction and promotes the development of ischemic stroke (47).

14. Hydrogen sulfide and Parkinson disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting over four million people worldwide, of high prevalence among people elder than 65 years of age (48). The brain of a PD patient is featured with the loss of dopamine (DA) - secreting neurons in an area of the midbrain called the substantia nigra, therefore causing bradykinesia, postural instability, resting tremor, and rigidity of patients (49). Organ-protective effects of inhaled H2S have generally been attributed to its ability to induce hypothermia in rodents. Deep hypothermia induced by H2S breathing reduced cerebral-infarct volume in rats after focal ischemia (49). Oxidative damage is considered a primary pathogenic mechanism of nigral dopaminergic cell death in PD (50). Despite decades of efforts, medications to cure or slow the progress of PD are still lacking. Emerging evidence suggests that H2S maybe not only be implicated in the pathogenesis of PD but also protect neuronal loss in various PD models, suggesting that this gaseous molecule may be new hope for the devastating disease (17).

The presently clinically used drug for treating Parkinson's disease is L-DOPA (51), which only ameliorates the symptoms but could not reverse the process of DA neuronal degeneration (52). A reduction in endogenous H2S in the striatum appears to be responsible for the onset of Parkinson's disease and that exogenous H2S treatment results in attenuation of DA neuronal degeneration in a mouse model of Parkinson's disease. These findings suggest a neuroprotective effect of H2S during the neural injury of Parkinson's disease. Hydrogen sulfide appears to confer cytoprotection via multiple mechanisms including antioxidant and antiapoptotic effects. H2S may upregulate endogenous antioxidants through a nuclear-factor-E2–related factor-2 (Nrf2)-dependent signaling pathway. Nrf2 regulates gene expression of several antioxidant proteins and phase II detoxification enzymes (for example, glutathione S-transferase (GST). Whether H2S protects neurons by upregulating Nrf2-dependent antioxidant mechanisms remains to be determined. The role of the Rho/ROCK/LIMK pathway in neurodegeneration has been acknowledged in recent studies. Activated ROCK2 is significantly inhibited by H2S. It has been well established that microglial ROCK leads to phagocytosis of dopaminergic neurons. In addition, CaMKK-beta-dependent AMPK activation causes NaHS suppression of neuroinflammation (48).

Adult neurogenesis is one method to combat the loss of DA neurons in the adult midbrain, which may prevent cell death and relieve dyskinesia in PD. Previous studies have focused on DA neurons and how to delay neuronal degeneration by treatment with H2S. However, it remains unclear whether H2S therapy could induce neurogenesis in adults to alleviate the progression of PD (53).

15. Hydrogen sulfide and Alzheimer disease

Alzheimer's disease (AD), the most common cause of dementia and neurodegeneration in the elderly, is characterized by deterioration of memory and executive and motor functions (54). Neuropathologic hallmarks of AD include neurofibrillary tangles (NFTs), paired helical filaments, and amyloid plaques. Mutations in the microtubule-associated protein Tau, a major component of the NFTs, cause its hyperphosphorylation in AD. Signaling by the gaseous molecule hydrogen sulfide (H2S) is dysregulated during aging. H2S signals via a posttranslational modification termed sulfhydration / persulfidation, which participates in diverse cellular processes. Cystathionine γ-lyase (CSE), binds wild-type Tau, which enhances its catalytic activity. By contrast, CSE fails to bind Tau P301L, a mutant that is present in the 3xTg-AD mouse model of AD (55).

Hydrogen sulfide plays an important role in vasorelaxation, inflammation, cell angiogenesis, hippocampal memory formation, and cellular bioenergetics. In addition, it was observed that H2S levels were decreased, and Hcy levels were elevated in AD brains. Likewise, it was noted that the physiological concentration of H2S specifically enhances the NMDA receptor-mediated response and facilitates hippocampal long-term potentiation (LTP), which is extensively associated with proper learning and memory function.

Matrix metalloproteinases 9 (MMP9) role is well known in the regulation of synapse function and plasticity, its association with Hcy-induced functional remodeling of synapses remains uncertain. Administration of Hcy into the mice brain affects memory function and H2S seems to protect against Hcy-induced neurotoxicity, but the underlying pathology was unclear. The therapeutic potential of H2S on synaptic remodeling. Additionally, these data may be critical to better understand the role of MMPs in neurovascular injury in hyperhomocysteinemia. However, more detailed studies using this model may be warranted to more closely simulate the clinical scenario in cerebrovascular diseases such as Alzheimer's and stroke (56).

The level of H2S is decreased in AD patients and that this change in the H2S levels may be related to the severity of AD (57). The emerging mitochondrial roles of H2S include antioxidant, antiapoptotic, and anti-inflammatory effects. H2S modulates Aβ-induced damage attenuating the increase in intracellular ROS levels. H2S may attenuate spatial memory impairment and neuroinflammation within the hippocampus of AD model mice. With regard to the regulatory role of H2S in cellular bioenergy metabolism, recent studies have shown that H2S can serve as a physiological electron donor and as an inorganic energy source inmammalian cells. H2S on mitochondrial dysfunction in AD models are significant (54).

16. Hydrogen sulfide and Huntington disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by the expansion of CAG repeats in the gene huntingtin, htt, present on chromosome 4. About 1 in 7500 individuals are affected by HD worldwide, and no satisfactory cure exists so far. HD primarily affects the corpus striatum of the brain and manifests as abnormal involuntary movements, motor and cognitive deficits. Once symptoms appear, median survival is about 18 years. The number of CAG repeats in mutant htt (mHtt) inversely correlates with the age of onset of disease. Translation of the CAG repeats results in an abnormally long polyglutamine repeat at the N-terminal end of Htt. mHtt is proteolytically cleaved and the N-terminal fragments aggregate to form inclusion bodies, a characteristic feature of the disease, although its role in disease progression is debated. Immunostaining with antibodies against mHtt led to the discovery of intranuclear inclusions in mouse models and patient samples, a hallmark of the disease. mHtt impacts multiple cellular processes ranging from transcriptional and translational regulation, mitochondrial function, DNA replication and repair to nucleocytoplasmic transport which leads to neurotoxicity (58).

H_2S signals *via* sulfhydration, wherein it mediates the conversion of −SH groups of reactive cysteine residues on target proteins to −SSH or persulfide groups. H_2S is synthesized by cystathionine gamma lyase (CSE), cystathionine beta synthase (CBS) and 3-mercaptopyruvate sulfurtransferase of the reverse transsulfuration pathway. Aberrant H_2S metabolism is involved in the progressive neurodegeneration seen in Huntington's disease (HD). The neuronal redox effector functions of H_2S are differentially modulated by the action of mHtt on CSE and CBS thereby affecting disease progression in HD by variable regulatory mechanisms.

17. Hydrogen sulfide and vascular dementia

Epidemiological studies show that vascular dementia (VD) is the second commonest cause of dementia after Alzheimer's disease (AD), and results from ischemic, ischemic-hypoxic, or hemorrhagic brain lesions.

In CNS, studies suggest that Notch signaling regulates neuronal synaptic plasticity, and therefore learning and memory. Interestingly, Notch signaling pathway has crosstalk with other signaling pathways, such as Akt, Wnt, and NF-κB pathways. Studies report that GSK-3β modulates Notch signaling. The production of H2S is high in the brain, considered as a neuromodulator. A previous study demonstrate that H2S facilitates the induction of hippocampal LTP by enhancing the activity of NMDARs in neurons. H2S improves LTP in hippocampus and inhibits the neuronal death induced by bilateral common carotid arteries ligation. H2S attenuates neuronal injury induced by VD via inhibiting apoptosis (59).

18. Hydrogen sulfide and the Immune system

Concerning cell-mediated immunity, balneotherapy applications can improve the survival capacity and activity of neutrophils. Sulphurous mineral water favours the short-term survival of neutrophils, speeding up the resolution of infections and preventing further inflammation. Studies conducted in patients with osteoarthritis who underwent hydrotherapy or mud-bath therapy showed that, after the treatment, neutrophils' circulating and functional capacity increased, reflecting a greater defence capacity against pathogens and thus a potential lower susceptibility to infections. Balneotherapy may also contribute to increase cortisol levels in healthy and subhealthy people. Changes in cortisol levels suggest that mineral baths may modulate the activity ofthe hypothalamic-pituitary-adrenal axis, inducing a transitorily but significant rise in ACTH production (60).

One important property of H2S is its bell-shaped (or biphasic/bimodal) pharmacological mode of action: in many conditions, low concentrations of H2S can exert physiological, regulatory or modulatory effects, and act as cytoprotective, antioxidant and anti-inflammatory agents. Neutrophils are the most abundant leukocytes in the blood and constitute the first line of immune defense against numerous infectious pathogens (bacteria, fungi, protozoa). They are traditionally viewed primarily as phagocytic cells and are among the first to migrate to infected sites for pathogen killing and debris removal. Neutrophil-mediated killing of invading pathogens involves several different strategies: engulfment of microbes, secretion of anti-microbials and formation of neutrophil extracellular traps (NETs). The inhibitory effect of endogenous and exogenous H2S on neutrophil adhesion has been confirmed in several studies. monocytes/macrophages express all 3 major H2S-producing enzymes, with perhaps CSE and 3- MST playing the major roles. The role of H2S in the regulation of macrophage chemotaxis is complex. The fact that H2S affects multiple signalling pathways and immunological and inflammatory mechanisms can be a strength from a translational standpoint: simultaneously downregulating multiple interacting pathways of inflammation (61) or cell death may more effective than targeting a single pathway that could be bypassed or replaced by various compensatory mechanisms (62).

19. Hydrogen sulfide and Diabetes

Diabetes mellitus is one of the most prevalent chronic diseases worldwide (63). Emerging data suggest that H2S improves diabetic endothelial dysfunction, nephropathy, retinopathy, and cardiomyopathy. Some recent studies indicate that H2S is cytoprotective during myocardial ischemia-reperfusion injury in the setting of diabetes by alleviating oxidative stress (64). H2S inhibits insulin secretion by activating ATP-sensitive K+channels. H2S promotes blood flow by dilating blood vessels and inhibiting platelet aggregation. H2S has been reported to have either pro- or antiapoptotic effects on β cells. Studies on the animal models suggest that excess of H2S in pancreatic islets may contribute to both type 1 and type 2 diabetes. H2S has also been demonstrated to regulate insulin sensitivity. In the liver, H2S stimulates gluconeogenesis and glycogenolysis and inhibits glucose utilization and glycogen storage. H2S may also regulate adipose tissue lipolysis, adipokine production and inflammation; the processes important for local and systemic insulin sensitivity (65). The plasma H2S levels are significantly reduced in type 2 diabetes patients, particularly in those with a history of cardiovascular disease. The plasma H2S levels in patients with type 2 diabetes may reflect the HbA1c levels (66).

Diabetes leads to microvascular and macrovascular complications, and diabetes-associated cognitive decline is one of the central nervous system complications, which induces advanced brain ageing, cognitive impairment and an increased risk of dementia. Exogenous H2S could supplement endogenous loss of H2S and improve spatial learning and memory abilities, and the underlying mechanism identified in these studies is the Akt-GSK-3β signalling pathway (67).

H2S levels in patients with diabetes. H2S reduction is related to diabetes-induced bone marrow cell (BMC) dysfunction and impaired ischemic tissue repair. Restoration of H2S production in diabetes mice improves reparative property of BMCs (68).

H2S is generated in pancreatic β-cells as well as in insulin target tissues including the liver, adipose tissue and skeletal muscles where it may control insulin secretion and insulin resistance. H2S protects pancreatic β-cells against apoptosis induced oxidative stress. H2S may act as an “intrinsic brake” against glucose-induced apoptotic death in β-cells. Exogenous H2S has also been shown to protect β-cells from apoptosis induced by hydrogen peroxide, fatty acids, and cytokines; and through phosphorylation and activation of Akt signaling, promote cell proliferation and survival. H2S inhibites the renin-angiotensin system in the diabetic kidney and attenuated high glucose induced mesangial cell proliferation by suppression of the MAPK signaling pathway (69).

Several studies reported that H2S directly regulated insulin sensitivity in adipocytes. H2S improves high glucose-induced insulin resistance. H2S mediates the effect of L-cysteine on PIP3 and glucose uptake. H2S activates PI3K but inhibites PTEN. Adipogenesis participates in obesity and insulin resistance regulation. H2S promotes adipogenesis in adipocytes Skeletal muscle serves regulates systemic glucose metabolism. CBS and CSE are abundant in human skeletal muscles similar to their expressions in human kidney and liver. H2S might promote glucose uptake in the muscle through sensitizing the IR-PI3K-Akt signaling pathway, thus ameliorating insulin resistance.

H2S regulates circadian clock in the skeletal muscle. Disruption of core clock genes (CCG) contributes to the progression of metabolic disorders. H2S synthesis enzymes (CBS, CSE and 3-MST), antioxidant genes, CCG and clock-controlled genes were all decreased in muscle of HFD-induced mice, but markers of oxidative stress and expression of oxidative stress related genes were increased. Application of H2S donor NaHS or GSH precursor improved expression of CCG. H2S may be an important endogenous regulator of the circadian clock and metabolic disorder (70).

Skeletal muscle is now considered to be an endocrine organ, synthesizing and secreting a variety of bioactive molecules including inflammatory cytokines, growth factors, adipokines, carnitine, and more recently

hydrogen sulfide. H2S appears to provide protection against low oxygen and nutrient supply as well as ischemic injury in multiple organs including skeletal muscles. (71).

Diabetic macroangiopathy can cause cerebro-cardiovascular diseases and constitutes one of the major causes of death in patients with diabetes. H2S has many beneficial roles in protecting against diabetes-induced alterations in kidney, heart and blood vessels, such as diabetic renal fibrosis, palmitic acid-induced myocardial injury and nephropathy, proliferation of VSMCs and decreased thickness of VSM. H2S can also regulate autophagy in liver cells, H9c2 cells and colon epithelial cells. However, whether H2S protects VSMCs in DM by regulating autophagy is unknown (72).

Mitochondria, the powerhouse of the cell, participate in multifaceted regulatory pathways in normal and pathophysiological conditions. H2S is a regulator of the Ca^{2+} channel under different physiological conditions. It modulates Ca^{2+} concentration. H2S inhibits Ca^{2+}-induced MPTP opening in adult and old rat heart. H2S is a strong antioxidant that protects the tissue against pathological matrix turnover, in part, by scavenging ROS. H2S is known to inhibit the L-type Ca^{2+} channel. High Ca^{2+} activates CypD, a gatekeeper protein of MPTP, thus facilitating molecular exchange between matrix and cytoplasm causing oxidative outburst and cell death (73).

20. Hydrogen sulfide and Cardioprotection

Hydrogen sulfide, an endogenous signaling molecule, plays an important role in the physiology and pathophysiology of the cardiovascular system (74). It may be worth mentioning that the roots of the cardiovascular protective effect of H2S can be traced back to a parallel line of studies that began in the 50s, focusing on balneotherapy (beneficial effects of H2S-containing spring baths). The first long-acting H2S donor, synthesized and characterized by Philip Moore's group in Singapore, was designated as GYY4137; this compound was found to exert beneficial blood pressure and vascular effects in the initial report, and was subsequently used in many dozens of follow-up studies and showed therapeutic benefit in many systems.

Hydrogen sulfide (H2S) has been demonstrated to reduce myocardial infarction and cardiac ischaemia–reperfusion (I/R) injury. H2S- mediated cardioprotection has been reported to involve activation of Nrf2, increased glutathione levels, and activation of endothelial nitric oxide synthase (eNOS). However, the precise details of the mechanism by which H2S activates these pathways are poorly understood. Furthermore the observation that H2S can elicit protective effects when administered on reperfusion suggests a role for a rapid signalling mechanism. H2S has also been recently suggested to signal via protein S-sulfhydration (SSH), another important redox-based post-translational modification on protein cysteine residues. H2S signalling has also been reported to alter NO bioavailability and to alter eNOS activity. Indeed the protective effects of H2S against I/R are lost in eNOS phosphomutant mice. Furthermore, the H2S activation of eNOS is reported to be mediated by SSH of eNOS. Taken together, these studies suggest that H2S-induced protection is NO signalling dependent (75).

21. Hydrogen sulfide and Cancer

In various forms of systemic inflammation, as well as several forms of cancer (76), as breast (77), gastric (78), colon (79), prostate (80) or colorectal cancer (81) - circulating H2S levels are increased due to the upregulation of H2S-producing enzymes. Pharmacological inhibition of H2S biosynthesis shows therapeutic potential.

Recent studies indicate that H2S has both pro-cancer and anti-cancer effects. Indeed, different types of cancer utilize different H2S-associated pathways, and the final effect on cell survival or cell death appear to be dose- and tumour cell type-dependent (82).

In the field of antitumor research, endogenous H2S induces angiogenesis, accelerates the cell cycle and inhibits apoptosis, which results in promoting oncogenesis even- tually. Interestingly, high concentrations of exogenous H2S liberated from donors suppress the growth of various tumors via inducing cellular acidification and modulating several signaling pathways involved in cell cycle regulation, proliferation, apoptosis and metastasis. The selective release of certain concentrations of H2S from H2S donors in the target has been considered as a tumor therapy strategy (83).

It has been shown that endogenous production of H2S is generally low, making it difficult to elucidate the precise biological functions. Chemical compounds that could degrade in response to a specific trigger to release H2S, termed H2S donors, include a number of delivery systems and functional groups, some of which mimic the controlled endogenous production in response to biologically specific and relevant conditions. Hyaluronic acid (HA) is a non-sulfated glycosaminoglycan composed of two disaccharide units, D-glucuronic acid and N-acetyl-D-glucosamine, linked via β-1,3- and β-1,4-glycosidic bonds. HA has been considered one of the best biopolymers in terms of safety issues and widely used for many biomedical applications including tissue engineering and drug delivery. H2S donors could suppress the growth of human cancer cells by inhibiting the PI3K/AKT/mTOR and RAS/RAF/MEK/ ERK signaling pathways (84). H2S has been shown to inhibit cell survival of androgen-independent, androgen-dependent, and antiandrogen-resistant prostate cancer cells through different mechanisms. Various H2S-releasing compounds, including sulfide salts, diallyl disulfide, diallyl trisulfide, sulforaphane, and other polysulfides, also have been

shown to inhibit prostate cancer growth and metastasis (80).

While most studies of H2S and cancer have focused on colonic cancer, there is considerable evidence that H2S-based chemoprevention will be effective for a broader range of tumours, including melanoma (85).

The idea of killing cancers via intracellular acidification is not new. In fact, there are many compounds designed, and are already in clinical trials, to target carbonic anhydrases, ion exchangers or proton pumps. Yet, many are hampered by lack of selectivity for cancer cells, thence giving undesired side effects and enhanced toxicity. Unlike the inhibitors in clinical development that solely targets tumor pH regulatory protein(s), H2S is therefore, the key component that potentiates the action of metformin and simvastatin. Of note, this combinatorial regime possesses therapeutic value, for H2S effect has been demonstrated to be specific to cancer cells (86).

In some pathological process, as cancer and glycometabolic malfunction, different studies show that H2S may exert different or even opposite actions. Reduced endogenous H2S has been linked to the cause of many diseases or their stage-dependent injury. Therefore, to enhance endogenous H2S content is of great pharmacological significance with the use of H2S releasing/stimulating reagents. It is gratifying to see the development of H2S releasing reagents has made great progress in recent years (87).

The fact that the mechanism of H2S-mediated acceleration of cancer metastasis is unknown hampers the development of anti-metastasis therapies. CD36 functioned as a H2S-targeted receptor. The use of neutralizing antibodies or inhibitors to block CD36 could accomplish an almost complete inhibition of metastasis in immunodeficient orthotopic mouse models of oral squamous cell carcinoma (88), with no side effects (89).

22. Hydrogen sulfide and Asthma

The respiratory mucosal epithelium is the first internal line of defense by acting as amajor physical barrier between internal and external environments. H2S-related enzymes are expressed in the human lungs. It is now acknowledged that H2S is required for the development of lung vasculature and alveolarisation, and in other lung functions, including airway tone and pulmonary circulation. H2S appears to be involved in various processes namely airway mucolytic activity, oxidative stress, inflammatory state, cell proliferation, and apoptosis. Both endogenous and exogenous H2S show positive effects upon the respiratory tract by modulating the mucolytic activity. This appears to result from the interactions between H2S and the disulfide bonds of mucins, resulting in breakage of the latter, which allows the mucus to become less viscous. The production of endogenous H2S induces the opening of K+ATP and the activation of the cAMP pathway. Additionally, exogenous H2S inhibits Na+/K+-ATPase and calcium-sensitive potassium channels in human bronchiolar epithelia, thereby triggering electrolyte absorption. This leads to an increase in mucociliary clearance, and therefore the elimination of foreign microorganisms can be more effective. Inhibition of transepithelial sodium absorption (via inhibition of Na+/K+- ATPase) is favored under acute hypoxia, in order to avoid H2S degradation, as well as exogenous H2S exposure. Additionally, an amelioration of mucociliary function was observed with inhalation of exogenous H2S, as confirmed by a substantial reduction of mean mucociliary transport time in patients with chronic rhinosinusitis. H2S antioxidant features seem to involve both an indirect action and an induction of endogenous antioxidant defenses. For instance, by stimulating cysteine and cysteine transporter activity, H2S induces an augmentation of substrate levels that are necessary for GSH production. Unlike large size antioxidants, H2S can easily cross both plasma and mitochondrial membranes. This allows H2S to more promptly reach its biological targets, and therefore it is considered to be more effective at diminishing cellular oxidative stresses, and at increasing antioxidant defenses. Antioxidant effects of sulphurous thermal waters (STWs), an exogenous H2S source, provide protection against oxidative DNA damage.

The anti-inflammatory properties of H2S can in part be explained by its potent reducing, antioxidant, and scavenging features. Nevertheless, there are controversial results concerning H2S properties, since it appears to exert both pro- and anti- inflammatory effects. Some authors have shown an in vitro promotion of granulocyte survival via H2S-induced inhibition of caspase-3-cleavage and p38 phosphorylation and in an oxidative stress environment, activated neutrophils seem to be able to convert H2S into sulfite, which is associated with inflammation. Moreover, there are studies suggesting that this gaseous transmitter is involved in GSH depletion and ROS formation, and consequently induction of mitochondrial cell death pathways. Meanwhile, others have suggested the participation of H2S in some key anti-inflammatory pathways, such as: (i) suppression of leukocyte adherence and migration, mediated by K+ATP activation in endothelial cells and leukocytes, (ii) inhibition of oxidized low-density lipoprotein-induced macrophage inflammation via NF-κB suppression, leading to a reduction of several pro-inflammatory cytokines (e.g., IL-1β, IL-6, and IL-8), and (iii) reduction of neutrophil toxic effects by inhibiting myeloperoxidase activity. However, these discrepancies may be related to differences in H2S concentration. In fact, beneficial effects of H2S generally prevail at lower concentrations, whereas deleterious effects are observed at higher levels.

Since H2S inhibits the activity of phosphodiesterase, cGMP net levels increase. Therefore, H2S might eventually act as a good auxiliary agent in the treatment of pulmonary hypertension (90).

23. Hydrogen sulfide and Alergic Rhinitis

Allergic Rhinitis AR is a chronic inflammatory disease that affects the nasal airways, which become inflamed and engorged after exposure to an allergen to which patients are sensitized (91). H2S can be found in human nasal mucosa as well as in the plasma of healthy subjects. This may be partially explained by the presence of CBS and CSE in human nasal epithelium. While CBS is mainly distributed in the superficial epithelium and submucosal glands, CSE is exclusively localized in vascular endothelium and surrounding smooth muscles. In the nasal and sinus mucosa, the amount of H2S was shown to be increased in mild and moderate/severe persistent AR. In this context, there is an enhancement of human H2S-synthesized enzymes, mRNA and protein levels, which consequently leads to a significant increment of H2S levels in human airway. This may indicate a compensatory mechanism to attempt to revert the pro-inflammatory state. Although there are few data specifically regarding STWs-treatment of AR, an amelioration in AR patients suffering from allergen-specific non-seasonal rhinitis when treated with a S-based compound water was observed, and this was associated with a reduction in total IgE and an increase in IgA serum levels. In accordance with this, it was suggested that STWs may exert an immunomodulatory activity by inducing an increase in IgA levels in nasal mucus. Another study also showed significant amelioration when AR patients were treated with STWs, namely in terms of a significant decrease in nasal flow resistance and nasal mucociliary transport time in 84% of subjects. Likewise, a significant reduction of IgE and an increase in IgA levels as well as an improvement of subjective symptomatology assessment scale were also observed. Data from these studies suggest a compensatory mechanism in order to reduce the presence of pro-inflammatory mediators and an improvement of the inflammatory state. However, clearly more thorough studies on the immunomodulatory and anti- inflammatory effects of STWs treatments in AR patients (as well as in patients with chronic rhinosinusitis) are needed. Due to common immunopathophysiology, there is a close relationship between AR and asthma ("single airways concept"). In fact, AR is regarded as a risk factor for the development of asthma. Thus, although few studies have been carried out with STWs treatment in patients with asthma, we shall now analyse this context (90).

24. Hydrogen sulfide and COPD

Chronic Obstructive Pulmonary Disease COPD is an airways disease caused by significant exposure to noxious particles or gases (92). H2S metabolism may be altered in the lung tissue of COPD patients, just as it is in smokers. H2S levels in COPD subjects vary longitudinally, with higher levels being more frequently seen in a stable state than in acute exacerbations of COPD. Serum H2S levels are significantly decreased in COPD subjects with very symptomatic exacerbations induced by bacteria and viruses, compared to control subjects (93). H2S synthesis is induced in order to counter the infections- mediated exacerbations. Currently, the most frequent therapies used to treat lung viral and bacterial infections are antiviral and antibiotics. However, over time, antibiotic-resistant bacteria may be increasingly observed. To overcome these problems, some studies focused on the clinical efficacy of therapeutic S-based compounds (H2S donors, STWs).

Despite certain contradictions founded in the previous studies, all indicated significant changes of H2S and H2S-synthesized enzymes levels in COPD subjects. These H2S metabolism alterations seems to contribute, at least in part, to exacerbations and worsening of this respiratory disease state, affecting general lung function. A significant reduction ofoxidative stress and an amelioration of symptoms in subjects suffering frommoderate to severe COPD were observed after a 12-day inhalation with STWs and 1 month after the end of the treatment. Similar beneficial effects have been observed in patients with chronic rhinosinusitis (6). Further studies focusing on mechanisms underlying such STWs-driven improvement in COPD patients are warranted. In summary, in spite of generally effective drug-based treatments for most cases of AR, bronchial asthma, and COPD, some patients still show a sub-optimal response to such treatments. Furthermore, over time, long-term high-dose therapy may be associated with the development of some adverse effects. Thus, additional thermal spa complementary therapeutic tool mainly for subjects whose symptoms are not adequately controlled with the usual drug-based therapeutic approach seems to be a good option. With supplementary STWs treatment it may be possible to regain symptom control and eventually reduce baseline drug therapy. Another aspect which must be borne in mind is that with STWs treatment at Spas, additional psychological components may also contribute toward clinical improvement. These components include leisure time, opportunity for relaxation, being aware of regular clinical monitoring, and various cultural aspects, all ofwhich may play a part in Spa treatment-associated final results (90).

25. Hydrogen sulfide and COVID-19

The COVID-19 pandemic caused by SARS-Cov-2 demands rapid, safe and effective therapeutic options. The "cytokine storm" syndrome, with subsequent acute lung injury (and other systemic disorders), is a frequent feature of many infectious diseases, including those caused by many viral pathogens. A cytokine storm is also

observed in the most serious cases of COVID-19. H2S is a reducing agent and thus can be oxidized by several circulating oxidants. However, one of the main catabolic pathways for H2S operates in the mitochondria, leading to the formation of thiosulfate. In the case of COVID-19 patients, there is a high incidence of coagulative disorders, generating a disseminated intravascular coagulation (DIC), which is lethal in severe conditions. The possibility of modulating PLA formation by H2S is therefore of therapeutic interest, as PLAs promote both vascular inflammation and coagulation. H2S promoted anti-inflammatory effects through epigenetic alterations.

The effect of H2S on viral infections represents a promising research field, which is presently still poorly investigated. Many pulmonary viral infections increase ROS levels and impair antioxidant enzymes, including those associated with the Nrf2–ARE pathway, with consequent reduction of antioxidant response. H2S-releasing agents evoke significant protective effects against oxidative stress in the host cells, which are mediated by H2S and/or related sulfane sulfur through well-clarified mechanisms, such as increased nuclear translocation of Nrf2 and inhibition of NADPH oxidase enzymes. Besides the triggering of the "antioxidant machinery" directly attributable to H2S, other "indirect" effects are also involved in antioxidant activity of H2S. Very recently, GSH was also proposed as a potentially useful agent against SARS-CoV-2, since high-throughput artificial intelligence-based binding affinity prediction indicated a possible GSH interaction with ACE2 or transmembrane serine protease 2 (TMPRSS2), two human proteins closely involved in the process of viral adhesion and entry into the host cell (94).

26. Hydrogen sulfide and Burns

The known role of H2S in inflammation and sepsis underline the role of H2S in burns. H2S has a key role in inducing edema, which is a cardinal sign of inflammation. Understanding the role of H2S in sepsis and shock is particularly important due to the high mortality associated with both conditions. From current studies, we can postulate that H2S would act as a pro-inflammatory agent in an early burn via a substance P-mediated increase in permeability and edema. H2S seems to have a conflicting role in sepsis, and the underlying mechanism needs to be studied in various animal models at different intervals of sepsis development. H2S plays a novel role in burns, and it appears that the modulation of H2S administration may have a therapeutic role. However, the role of H2S is complex, and the effect of H2S on the inflammatory response varies at different time points following the injury. Studies need to focus on delineating the pathways and mechanisms by which H2S induces and inhibits inflammation. The optimal concentration at which H2S inhibits inflammation also needs to be determined. Studying the role of H2S at each stage will enable us to capture a complete picture of the exact role of H2S following a burn. H2S appears to have two very different roles: as a mediator of inflammation and as an inhibitor of inflammation. Severe burns are characterized by an inflammatory response that can progress to a systemic response and lead to sepsis, shock and multiorgan failure. Therefore, identifying agents that can reduce the duration and complications of burns is of great interest. It appears that H2S acts to promote inflammation in the initial period post-injury, but at later stages, H2S reduces inflammation and improves wound healing. This biphasic action of H2S in burns is a novel concept, and although this action has not yet been extensively studied, it is promising due to the potential therapeutic role of H2S. The development of H2S as a therapeutic agent is currently still in the early stages; however, the potential of H2S for treating burns is significant (18).

27. Hydrogen sulfide and Skin diseases

Although sulfur-rich thermal waters have ancestrally been used in the context of dermatological conditions (95), a global mapping of the molecular effects exerted by H2S on human keratinocytes is still lacking. Most of the scientific literature on H_2S effects on cell viability, proliferation, activation, cytokine secretion and adhesion. As far as skin is concerned, sulfurs are able to penetrate the skin and a sulfur-rich balneotherapy (96), known to be effective in the treatment of psoriasis (97), may be useful also in other T-cell-mediated autoimmune diseases of the skin. Sulfur has a long history of topical use: hydrogen sulfide is present in a variety of skin care products, including soaps and cleansers, and is used to reduce dandruff and decrease scalp scaling and dryness. It has recognized fungicidal, bactericidal and antipruritic properties, and therefore it is used for the therapy of mite infestations in animals. Sulfur was even proposed as an antidote for acute exposure to radioactive material. Indeed, it has been demonstrated that sulfurs favor wound healing, acts as a keratolytic agent and can induce various histological modifications in the skin (including hyperkeratosis, acanthosis, and dilation of dermal vessels) (98).

Vascular endothelial growth factor (VEGF) is an important mediator involved in inflammatory processes associated with aberrant angiogenesis. In skin inflammation, epidermal cells are important VEGF-producing cells which probably contribute to the pathogenesis of diverse epidermal diseases through their chemotactic activity on inflammatory cells and through their angiogenic effect. H2S significantly increases NO, in a manner Akt-dependent, in keratinocyte cells. H2S as both NO donor and anti-VEGF, may be a promising therapeutic for chronic inflammatory disorders of the skin, i.e. psoriasis (99).

A recent study showed that 10-100 μM NaHS and the overexpression of CSE could promote cell proliferation and melanin synthesis by increasing the production of melanogenic enzymes in primary human epidermal melanocytes. Therefore, pharmacologic regulation of H2S may be a novel strategy for skin disorders caused by the loss of melanocytes and dysregulation of oxidative stress. Abnormal H2S metabolism is associated with the pathogenesis of fibrosis, causing damage to the structure and function of tissues and organs. Several in vivo and in vitro studies have shown that both endogenous H2S levels and the expression of H2S-related enzymes in plasma are significantly decreased in fibrotic diseases, but supplementation with exogenous H2S could alleviate the severity of fibrosis. H2S could restore a normal morphologic phenotype in Werner syndrome fibroblasts by attenuating oxidative damage and modulating the mTOR pathway. Beneficial effects of H2S were demonstrated on systemic sclerosis associated skin and lung fibrosis (100).

H2S contained in sulfur-rich spa waters is a bio-active component able to modify the immunological status of non-diseased human keratinocytes (101). Further studies are needed to establish causal links between the above-identified biological effects and the previously reported medical benefits afforded by sulfur-rich spa waters in conditions such as psoriasis, eczema or wound healing (102).

28. H_2S role on Gynecological diseases

Since H2S has a tocolytic action, is produced from all reproductive tissues studied so far and the fact that they all express CBS and CSE, it does seem that H2S has a functional role in the female reproductive system.

For example, H2S could be involved in maintaining uterine quiescence during pregnancy, and there are some parallels with NO in this action. It is possible that upregulation of CBS and CSE in pregnancy causes local production of H2S in the myometrium and the placenta, which would inhibit uterine contractions. In rats it has been shown that externally administered H2S prolonged the duration of labor. H2S donors have been shown to inhibit spontaneous uterine contractions in rats in vitro.So far, there are no data on the effects of H2S on human myometrium. It is now well established that H2S can act as a vasodilator and is involved in the regulation of blood pressure, at least in the mouse and rat. However, H2S can also cause vasoconstriction. These apparently contradictory effects of H2S appear to be related to its concentration; generally H2S causes vasoconstriction at low concentrations and vasodilation at high concentrations. H2S could be involved in the local control of blood flow, particularly in the placenta; however, there are currently no published studies on human placental blood vessels. We have shown that H2S production from human placental homogenates was significantly greater in a low-oxygen environment compared with a normal-oxygen environment, suggesting that under hypoxic conditions in vivo, increased H2S release may cause local vasodilation. The CO/heme oxygenase system has been shown to function in a similar way. As pre-eclampsia is thought to involve placental ischemia, it does raise the fascinating possibility of whether a lack of H2S production locally would cause a failure of reflex vasodilation. It has been suggested that the H2S system may act as a back up to the NO system in vasodilation, with failure of both systems leading to disease. Olson et al. have proposed that H2S is part of an oxygen sensing system in the vasoconstriction and vasodilation response to hypoxia. It is thought that the rate of metabolism of H2S serves as the oxygen sensor in vascular smooth muscle. In Olson's proposed model, the concentration of vasoactive H2S is regulated by the balance between vascular H2S production and its oxidation by available oxygen. Clearly under hypoxic conditions the lack of oxygen would decrease the oxidation of H2S and its vascular effects would persist. Perhaps competition between oxygen and H2S for binding to cytochrome oxidase in mitochondria could also be involved. If this model is correct then there could be implications for pre-eclampsia.

CBS appears to be essential for female reproductive function, as it has been shown that *CBS-/-* knockout mice are infertile (103).

Endometriosis, one of the most frequently encountered gynecological disorders affecting up to 10% of the reproductive-aged female population, is characterized by the presence and growth of endometrial tissue outside the uterine cavity. Ectopic endometrium expresses a high level of CBS and CSE. Meanwhile, we identified that exogenous and endogenous H2S could induce cell proliferation, and this pro-proliferation effect was mediated by activation of NF-κB signaling (104).

29. Hydrogen sulfide and Clinical trials

Postoperative pain relief (105) facilitates recovery from injury, but optimal perioperative or postoperative treatments remain problematic. In recent years, the biological importance of H2S in many organs and systems has become increasingly clear. Extensive studies have documented the many beneficial antiinflammatory effects of this gaseous mediator. Several groups have developed novel H2S-releasing drugs in the hope that they will be useful for treatment of a range of diseases .

There is a large body of evidence regarding the ability of H2S to reduce inflammation and pain (106). In the Gastro Intestinal (GI) tract, H2S has been shown to reduce inflammation and accelerate healing of damaged tissue (such as ulcers), while suppression of H2S production in the GI tract results in impaired healing of tissue injury and exacerbation of inflammation (107).

The plasma H2S levels were measured in 154 outpatients with type 2 diabetes and 66 outpatients without diabetes (66). The plasma H2S levels were measured using the methylene blue assay. The patients with type 2 diabetes showed a progressive reduction in the plasma H2S levels (45.1±15.5 μM versus 54.0±26.4 μM, $p<0.05$), which paralleled poor glycemic control. There was a significant correlation between a reduction in the plasma H2S levels and the HbA1c levels ($\beta=-0.505$, $p<0.01$), Furthermore, a reduction in the plasma H2S levels was found to be related to a history of cardiovascular diseases in patients with type 2 diabetes (39.9±13.8 μM versus 47.5±15.9 μM, $p<0.01$) (66).

A proof-of-concept, Phase 2 clinical trial of the gastrointestinal safety of a hydrogen sulfide-releasing anti-inflammatory drug (107): 244 healthy volunteers completed a 2-week, double-blind study, taking either ATB-346 (250 mg once daily) or naproxen (550 mg twice daily), with upper GI ulceration being examined endoscopically. ATB-346 is an H2S-releasing derivative of naproxen. Naproxen is among the most widely used NSAID for treatment of arthritis and other conditions characterized by inflammation and pain. In 2016, Antibe performed a Phase 2 clinical trial, in osteoarthritis patients, to test the hypothesis that ATB-346 was more potent and long acting than naproxen. The study involved 12 osteoarthritis patients taking ATB-346 (250 mg) once daily for 10 days, with measurements of the patients' level of pain during the 10-day period. Once daily administration of ATB-346 at a dose of 250 mg could produce substantial relief of pain in osteoarthritis patients (108).

Of the 126 subjects treated with naproxen, 53 had at least one ulcer greater than 3 mm in diameter. Of the 118 subjects treated with ATB-346, three had at least one ulcer greater than 3 mm in diameter. The incidence of ulcers between the two groups was significantly different ($P < 0.05$). There were also far more gastric ulcers (50-fold) in the naproxen group than in the ATB-346 group. There was a total of 210 gastric ulcers observed in the 53 subjects that developed ulcers, while in the ATB-346 group, there was a total of only four gastric ulcers in the three subjects that developed ulcers. Ulceration of the duodenal mucosa was also observed in seven of the subjects treated with naproxen (with an average of 3.4 duodenal ulcers per subject), while duodenal ulcers were not observed in any of the subjects treated with ATB-346.

Plasma levels of H2S levels in subjects treated with ATB-346 were significantly greater ($P < 0.05$) than those in subjects treated with naproxen. Plasma H2S levels were measured by a modified MBB method. Hence, this biomolecule-bound sulfide pool serves as a sulfide buffer system by responding to concentration changes via shifting chemical equilibria. Importantly, when sulfide is consumed in a biological process, the sulfide pool will also likely serve as a slow endogenous sulfide donor system and maintain steady-state sulfide levels via similar mechanisms. Therefore, the differences measured by these methods are highly relevant and represent the sum of differences in (a) free sulfide levels and (b) the ability of the sulfide pool to release sulfide.

Cumulative data from the three clinical trials in which ATB-346 has been administered at 250 mg once daily for 10–14 days reveal a 4.7% overall incidence of clinically significant increases in liver transaminases. This is comparable to what has been observed with the use of NSAIDs such as diclofenac, naproxen, and piroxicam (107).

30. Conclusions

Hydrogen sulfide (H2S) is a signaling molecule that is actively synthesized in the tissues and is involved in the regulation of vascular tone, neuromodulation, cytoprotection, inflammation and apoptosis. In recent years, new data on animal and human H2S metabolism and function under the effect of various endogenous and exogenous factors, including drugs were collected. H2S metabolism and regulation, peculiarities of transport, signaling, biological role and participation in pathogenesis are focus topics of research. It was demonstrated that vitamin-microelement and microelement complexes with H2S-synthesizing enzymes cofactors and activators represent a promising approach for H2S content correction in tissues. Although the field of H2S biology has dramatically expanded over the last decade, many topics and issues remain that will demand continuing attention. Mainly due the presence of H2S, STWs might be an advantageous and promising option as an add-on non-pharmacological complementary therapy for relatively long-lasting improvement of clinical parameters in patients with reviewed diseases. Furthermore, H2S-rich STWs have various effects upon inflammatory and immunological parameters that may contribute toward their clinical efficacy.

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32. Author contribution

All authors have consistently contributed to this article, CM is the lead author who suggested the idea of the article. CM and DM participated in the process of systematic review, writing and verification of the article. GO coordinated and advised the progress of the article.

33. Declaration of interests

This article does not contain any studies with human or animal subjects. This study did not require written consent from patients. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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