Hydrogen Sulfide in Disease Pathogenesis and Therapy: Biphasic Mechanisms, Multitarget Strategies, and Clinical Challenges

Abstract

Hydrogen sulfide (H_2S), endogenously produced by cystathionine-γ-lyase (CSE), cystathionine β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3-MST), modulates physiological homeostasis, with dysregulation linked to neurodegenerative and cardiovascular pathologies. In Huntington's disease, CSE suppression by mutant huntingtin reduces H_2S, impairing vasorelaxation and elevating oxidative stress. Alzheimer's disease involves CBS dysfunction, exacerbating amyloid-β toxicity and disrupting synaptic plasticity via NMDA receptor pathways. H_2S facilitates post-translational persulfide modifications, enhancing protein stability and mitigating oxidative damage, while oxidative stress itself inhibits 3-MST, amplifying cellular vulnerability. Mitochondrially, H_2S preserves ATP, curbs reactive oxygen species (ROS), and prevents fragmentation, countering neuroinflammatory cascades in Parkinson's disease and metabolic disorders. Therapeutic H_2S donors like GYY4137 demonstrate efficacy in ischemia-reperfusion injury by sustaining mitochondrial integrity and promoting angiogenesis. Multitarget agents, such as compounds inhibiting BACE-1 and GSK-3β, reduce Tau hyperphosphorylation by sulfhydrating GSK-3β, while hybrid molecules (e.g., ACS84) integrate H_2S release with anti-inflammatory and antioxidant actions. H_2S's biphasic effects necessitate dose precision: physiological levels enhance neuroprotection and synaptic plasticity, whereas excess H_2S induces neurotoxicity. Challenges include optimizing pharmacokinetics, blood-brain barrier penetration, and balancing dual roles in cancer—inducing apoptosis via ROS in malignancies while fostering chemoresistance in others. Innovations like liposomal AP39 and hybrid drug designs address delivery hurdles, though regulatory complexities persist. Clinical translation emphasizes biomarker-driven strategies and interdisciplinary approaches to harness H_2S's pleiotropic benefits. This review underscores the nuanced interplay of H_2S in disease pathogenesis and therapy, advocating for multitargeted, context-specific interventions to leverage its cytoprotective potential while mitigating toxicity.

I. Introduction

Hydrogen sulfide (H_2S) is endogenously synthesized through enzymatic pathways involving cystathionine-γ-lyase (CSE), cystathionine β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3-MST), which play critical roles in maintaining physiological homeostasis. Dysregulation of these enzymes is implicated in neurodegenerative and cardiovascular pathologies. In Huntington's disease (HD), mutant huntingtin (mHtt) disrupts Sp1-mediated transcription of CSE, leading to reduced H_2S production, hypertension, and impaired vasorelaxation in transgenic mice, with CSE knockout models showing a 50% decline in serum H_2S levels. Similarly, CBS dysfunction in Alzheimer's disease (AD) correlates with amyloid-β toxicity, as CBS is a primary H_2S-producing enzyme in astrocytes, modulating hippocampal long-term potentiation (LTP) via NMDA receptor activity. Enzymatic synthesis of H_2S also facilitates post-translational modifications, such as persulfide bond formation via CBS and CSE, which regulate protein conformation, suppress leukocyte adhesion, and mitigate oxidative stress through S-sulfhydration. However, oxidative stress itself inhibits 3-MST activity, exacerbating cellular vulnerability. The interplay between H_2S and mitochondrial function is evident in its role in preserving ATP levels, reducing reactive oxygen species (ROS), and preventing mitochondrial fragmentation. Disrupted sulfur metabolism, as seen in Parkinson's disease, elevates homocysteine and methionine while depleting cysteine, aggravating neuroinflammation and protein misfolding. H_2S counteracts these effects by enhancing glutathione synthesis, inhibiting monoamine oxidase, and modulating NMDA receptors, thereby promoting neuronal survival.

The multifactorial nature of complex disorders is underscored by overlapping mechanisms such as oxidative stress, inflammation, and metabolic dysfunction. In AD, hyperphosphorylated Tau driven by sulfhydrated GSK-3β coexists with amyloid-β toxicity, necessitating multitarget therapeutic strategies. For instance, virtual screening has identified compounds inhibiting both BACE-1 and GSK-3β, with GSK-3β sulfhydration at Cys218 reducing Tau pathology in 3xTg-AD mice. Similarly, cancer therapies target angiogenesis and chemoresistance through agents like lenvatinib, which inhibits tyrosine kinases, and molecular hybrids that overcome drug resistance. H_2S further demonstrates pleiotropic effects, suppressing pro-inflammatory cytokines like TNF-α and IL-6 in AD models while enhancing synaptic plasticity via NMDA receptor activation. Its modulation of astrocyte polarization toward an anti-inflammatory A2 phenotype reinstates glutathione levels and mitigates oxidative damage. However, H_2S exhibits context-dependent toxicity: acute exposure (>320 ppm) induces neurotoxicity and respiratory failure, whereas chronic low-level exposure alters CNS architecture. Therapeutic interventions leveraging H_2S donors, such as GYY4137, reduce ischemia-reperfusion injury by preserving mitochondrial function and promoting angiogenesis, though without synergistic effects on NO-cGMP pathways. These findings highlight the dual role of H_2S in physiological regulation and disease pathogenesis, emphasizing the need for targeted strategies that address the intricate networks underlying complex disorders.

II. Mechanisms of Multi-Target H■S-Donating Molecules

Hydrogen sulfide (H_2S) exerts its therapeutic effects through diverse molecular mechanisms, particularly via post-translational modifications that influence cellular signaling and mitochondrial function. Enzymatically produced by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), H_2S activates the Nrf2 pathway, enhancing glutathione synthesis and upregulating phase II detoxifying enzymes such as heme oxygenase-1 (HO-1) and NADPH quinone oxidoreductase 1 (NQO1), which collectively mitigate oxidative stress in diabetic and cardiovascular pathologies. A critical post-translational modification involves the sulfhydration of Cys218 on GSK3β, which inhibits its kinase activity, reducing Tau hyperphosphorylation implicated in Alzheimer's disease (AD). This mechanism aligns with findings from Bottegoni et al. [57], who identified multi-target inhibitors of β-secretase1 (BACE-1) and GSK3β, underscoring the therapeutic promise of H_2S in AD. Additionally, H_2S preserves mitochondrial integrity by maintaining membrane potential ($\Delta\Psi m$) in H9c2 cardiomyoblasts treated with NSHD donors (160 μM), reducing apoptosis and oxidative stress, as evidenced by diminished lactate dehydrogenase (LDH) release and improved viability against H_2O_2-induced damage. S-sulfhydration of mitochondrial Complex I/III further enhances respiratory efficiency by minimizing electron leakage and reactive oxygen species (ROS) generation, facilitated by sulfide:quinone oxidoreductase (SQR), which integrates H_2S catabolism into the electron transport chain, thereby bolstering antioxidant defenses through glutathione restoration.

In redox and anti-inflammatory regulation, H_2S modulates cellular antioxidant systems and inflammatory pathways. ACS84, an H_2S-releasing compound, activates the Nrf2/Keap1 axis in Parkinson's disease models, promoting cystine uptake via the Xct− transporter to elevate cysteine levels, a precursor for glutathione synthesis. This process counteracts glutamate-induced oxidative toxicity and ischemia–reperfusion injury by enhancing γ-glutamyl-cysteine synthetase activity. Concurrently, H_2S donors like ATB-346 suppress NF-κB signaling, reducing gastric COX-2 activity and pro-inflammatory cytokines such as TNF-α and IL-6, thereby alleviating NSAID-induced gastrointestinal and renal toxicity. These dual actions highlight H_2S's capacity to synergize antioxidant and anti-inflammatory responses, offering cytoprotection in chronic inflammatory conditions.

Metabolic and mitochondrial effects of H_2S are evident in its regulation of cysteine homeostasis and neuronal function. In Huntington's disease (HD), H_2S restores transsulfuration flux via CBS and mitochondrial 3-mercaptopyruvate sulfurtransferase (3-MST), balancing cysteine and H_2S production to mitigate oxidative neuronalological studies revealological studies reveal concentration-dependent biphasic effects: high-dose NaHS (≥320 μM) suppresses hippocampal synaptic transmission via

presynaptic inhibition and potassium channel modulation, causing reversible hyperpolarization, while physiological concentrations (130 μM) enhance NMDA receptor-mediated currents and long-term potentiation (LTP), underscoring its role in acute ischemic models like MCAO. Slow-releasing donors such as GYY4137 exhibit sustained therapeutic benefits, reducing hypertension in spontaneously hypertensive rats (SHR) without impairing cardiac function, indicative of its suitability for chronic applications. Donor classification further delineates H_2S's therapeutic scope: fast-releasing NaHS induces acute H_2S bursts with potential neurotoxicity at high doses, whereas GYY4137's pH-dependent release mitigates cardiac ischemia and oxidative injury during reperfusion. Hybrid molecules like ATB-346 (combining naproxen with H_2S) and Tacrine-H_2S derivatives merge acetylcholinesterase inhibition with hepatoprotective Nrf2 activation, addressing multifactorial pathologies in neurodegeneration. S-memantine integrates NMDA receptor antagonism with H_2S-mediated neuroprotection, while SG1002 and liposomal AP39 exemplify advanced delivery strategies for sustained vasodilation and mitochondrial-targeted H_2S release, respectively. These innovations highlight the evolving landscape of H_2S donors, optimizing pharmacokinetics and tissue specificity to harness H_2S's pleiotropic benefits across cardiovascular, neurodegenerative, and inflammatory diseases.

III. Therapeutic Applications in Complex Disorders

Hydrogen sulfide (H_2S) has emerged as a critical modulator in the pathophysiology and treatment of neurodegenerative diseases, particularly Alzheimer's disease (AD). The sulfhydration of GSK3β, facilitated by H_2S derived from cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS), plays a pivotal role in regulating Tau phosphorylation, a hallmark of AD. NaGYY, a slow-releasing H_2S donor, rescues CSE deficiency in 3xTg AD model mice, restoring Tau phosphorylation balance and improving cognitive performance through enhanced hippocampal long-term potentiation (LTP) and NMDA receptor modulation. This effect is further supported by the design of propargyl-modified compounds (3a and 4a), which exhibit selective inhibition of CSE over CBS, offering a targeted approach to H_2S pathway modulation. Synergistic therapeutic strategies, such as ACS84-L-Dopa, combine dopaminergic support with H_2S-mediated anti-inflammatory effects, leveraging H_2S's ability to restore glutathione levels, inhibit monoamine oxidase, and suppress pro-inflammatory cytokines like TNF-α and IL-6. This dual action enhances L-DOPA efficacy in Parkinson's disease while mitigating gastrointestinal toxicity associated with conventional anti-inflammatory agents. Similarly, rivastigmine-H_2S hybrids address cholinergic deficits by integrating cholinesterase inhibition with H_2S-driven neuroprotection, reducing oxidative stress and ischemia-reperfusion injury through glutathione upregulation and NMDA receptor potentiation. H_2S donors also protect dopaminergic neurons from 6-OHDA-induced mitochondrial dysfunction by suppressing reactive oxygen species (ROS) and preserving mitochondrial integrity, while S-memantine enhances NMDA receptor activity via CBS-derived promoting promoting synaptic plasticity and cytoprotection in hippocampal neurons. These findings underscore H_2S's dual role as a neuromodulator and neuroprotectant, distinct from other gasotransmitters like nitric oxide.

In ischemia-reperfusion injury, H_2S donors such as GYY4137 demonstrate protective effects independent of mitochondrial ATP-sensitive potassium (KATP) channels, as evidenced by their insensitivity to 4-AP and Cs+ blockade. Instead, GYY4137 acts through endothelial and vascular smooth muscle pathways, reducing infarct size without disrupting oxidative phosphorylation. ACS84, another H_2S-releasing compound, prevents dopaminergic neuron loss in 6-OHDA models by activating Nrf2-mediated antioxidant responses, elevating intracellular cysteine and glutathione levels to counteract oxidative damage. The inhibition of α-synuclein aggregation via sulfhydration of aggregation-prone residues highlights H_2S's potential in Parkinson's disease, with CSE-targeting agents modulating neurotransmitter levels and inflammation. In metabolic contexts, H_2S enhances insulin sensitivity in adipocytes through AMPK activation, suppresses leptin-driven inflammation, and regulates adipokine signaling, illustrating its systemic cytoprotective effects.

In oncology, H_2S donors exhibit dichotomous roles. HS-ASA induces ROS-mediated apoptosis in breast cancer by triggering mitochondrial depolarization and caspase-3 activation, while

H_2S-doxorubicin conjugates overcome chemoresistance by inhibiting P-glycoprotein efflux and enhancing drug retention. ACS48 reduces cerebral infarct size via mitoKATP activation, demonstrating cardioprotective effects in ischemia-reperfusion models. Conversely, AOAA, a CBS inhibitor, modulates endogenous H_2S to balance pro-survival and pro-apoptotic signals, offering therapeutic potential in ischemic recovery. GYY4137's vasodilatory effects, mediated through KATP channels, provide antihypertensive benefits without affecting cardiac contractility, though higher doses of AP39 reveal biphasic vascular responses, complicating dose optimization.

Pharmacokinetically, H_2S therapy faces challenges such as prolonged sulfide elevation post-poisoning and poor blood-brain barrier penetration of agents like ACS84, necessitating optimized physicochemical properties for CNS delivery. Mechanistically, the unpredictable release kinetics of H_2S donors like Met-ITC and GYY4137 complicate dosing precision, while multi-target agents such as ITH12674 encounter regulatory hurdles due to complex safety profiling and polypharmacy risks. Despite these limitations, preclinical advances in organ-on-a-chip technologies and computational modeling hold promise for translating H_2S-based therapies into clinical applications, bridging the gap between mechanistic insights and therapeutic efficacy.

V. Future Directions

The development of innovative therapeutic platforms for hydrogen sulfide (H_2S)-based interventions is rapidly advancing, with a focus on enhancing targeted delivery and therapeutic precision. Liposomal H_2S donors, such as the N-mercapto-based agent AP39, exemplify this progress by enabling controlled, moderate release of H_2S, which has shown neuroprotective and cardioprotective effects in preclinical models. Structural optimization of thirty-three AP39 derivatives has revealed that modifications to the donor scaffold can fine-tune release kinetics, stability, and bioavailability, with thiol-activated mechanisms ensuring site-specific activity. This approach mitigates oxidative damage in neuronal cells and myocardial ischemia-reperfusion injury, highlighting the potential for tailored H_2S delivery systems. Concurrently, hybrid therapeutic strategies are emerging, combining H_2S donors with immunotherapy or gene-editing technologies. For instance, PD-1 inhibitors paired with H_2S-releasing agents amplify anti-inflammatory responses, while CRISPR-Cas9-mediated restoration of cystathionine γ-lyase (CSE) addresses enzymatic deficiencies in H_2S biosynthesis. These hybrid platforms integrate synthetic biology and pharmacological design to overcome challenges such as selective enzymatic inhibition, thereby enhancing therapeutic outcomes in conditions linked to H_2S dysregulation, including cardiovascular and inflammatory diseases.

The evolution of H_2S-releasing nonsteroidal anti-inflammatory drugs (NSAIDs) further underscores the shift toward safer therapeutics. ACS14, an H_2S-aspirin hybrid, reduces gastric erosions by 75% compared to conventional aspirin, operating through a cyclooxygenase (COX)-independent mechanism that circumvents the gastrointestinal toxicity of non-selective COX inhibition. Similarly, ATB-346, an H_2S-releasing derivative of diclofenac, minimizes mucosal damage while maintaining anti-inflammatory efficacy. Unlike the slow-release donor GYY4137, ATB-346 leverages endogenous thiols to ensure controlled H_2S release, preserving gastrointestinal integrity and improving drug safety. These advancements highlight the potential of dithiolethione-based scaffolds to balance COX-2 selectivity with reduced cardiovascular and gastrointestinal risks, addressing limitations of traditional NSAIDs and coxibs. Additionally, plasma H_2S and sulfane sulfur levels are being validated as biomarkers for patient stratification, offering insights into donor efficacy and enzymatic regulation by cystathionine-β-synthase (CBS) and CSE. This biomarker-driven approach could optimize clinical trial design by correlating H_2S dynamics with therapeutic responses in vasodilation, inflammation, and cardioprotection.

Clinical and regulatory advancements are accelerating the translation of H_2S therapeutics into practice. Phase I/II trials for AP39, targeting mitochondrial dysfunction, and ATB-346, evaluated in inflammatory bowel disease, exemplify the transition from ligand discovery to clinical validation. Computational tools are being leveraged to refine drug design, enabling multi-target strategies akin to the success of ladostigil and rasagiline in Alzheimer's disease. Mechanistically, H_2S stabilizes SIRT2 via the ALDH2-AMPK axis, restoring glutathione levels and mitochondrial function to counteract oxidative

stress and apoptosis, as demonstrated in neuronal and cardiovascular models. Furthermore, H_2S enhances GLUT4 translocation in skeletal muscle through Nrf2/Trx pathway activation, boosting insulin sensitivity by upregulating cystine transporters and γ-glutamylcysteine synthetase. These effects mirror the neuroprotective actions of L-DOPA in Parkinson's disease, underscoring the broad therapeutic potential of H_2S in metabolic and neurodegenerative disorders. Collectively, these developments signal a paradigm shift toward precision medicine, integrating biomarker-guided strategies and multi-target therapeutics to address complex disease mechanisms.

VI. Conclusion

The development of multi-target hydrogen sulfide (H_2S) donors represents a significant advancement in therapeutic strategies for complex diseases. Compounds such as NSHD-1, NSHD-2, and NSHD-6, which feature N-mercapto (N-SH) moieties, demonstrate the ability to release H_2S in a controlled manner through targeted structural modifications. This tunable release profile enables precise modulation of H_2S bioavailability, which is critical for mitigating oxidative stress and inflammation—key contributors to neurodegenerative and cardiovascular pathologies. For instance, in models of Huntington's disease, these donors enhance cellular defense mechanisms by upregulating antioxidant pathways, such as Nrf2 signaling, while suppressing pro-inflammatory mediators like NF-κB. By simultaneously addressing the oxidative-inflammatory axis, these compounds exemplify the potential of multi-target agents to disrupt interconnected pathological cascades, offering a more holistic therapeutic approach compared to single-target interventions.

Strategic hybrid drug design further amplifies this potential by integrating pharmacophoric elements from distinct bioactive molecules into single entities. Compounds like THS and ITH12674 exemplify how molecular hybridization leverages complementary pharmacological properties to achieve synergistic effects. Advances in quantitative structure-activity relationship (QSAR) modeling and high-throughput screening (HTS) have streamlined the identification of optimal hybrid configurations, balancing efficacy with safety. For example, THS combines structural motifs from anti-inflammatory and antioxidant agents, resulting in enhanced pharmacokinetic profiles and reduced off-target interactions. This approach not only improves therapeutic outcomes in conditions such as cancer but also circumvents resistance mechanisms by engaging multiple targets, thereby reducing the likelihood of adaptive cellular responses.

The translation of these innovations into clinically viable therapies necessitates interdisciplinary collaboration. Chemists, neurologists, and pharmacologists must synergize expertise to align molecular design with disease pathophysiology. Computational tools, including cheminformatics and virtual screening, accelerate the identification of lead compounds, while biological assays validate target engagement and safety. Such integration is particularly vital in neurodegeneration and oncology, where disease complexity demands multi-target strategies. For instance, collaborative efforts have enabled the optimization of H_2S donors to cross the blood-brain barrier, ensuring sufficient CNS bioavailability for neurodegenerative applications.

A critical consideration in this paradigm is the narrow therapeutic index of conventional H_2S donors like sodium hydrosulfide (NaHS), which exhibits efficacy at ≤100 μM but significant toxicity at concentrations ≥200 μM. This underscores the importance of dose optimization and mechanistic studies to elucidate drug-target interactions. Multi-target agents may mitigate these risks by enabling lower effective doses through additive or synergistic actions on complementary pathways. For example, coupling H_2S release with anti-inflammatory activity could reduce the required concentration of each component, minimizing off-target effects. Such strategies not only enhance safety but also address the limitations of single-target therapies, which often fail in complex diseases due to compensatory mechanisms. By prioritizing multi-target engagement, drug development can achieve a balance between potency and tolerability, paving the way for safer, more effective treatments.