



Hydrogen sulfide: Recent progress and perspectives for the treatment of dermatological diseases



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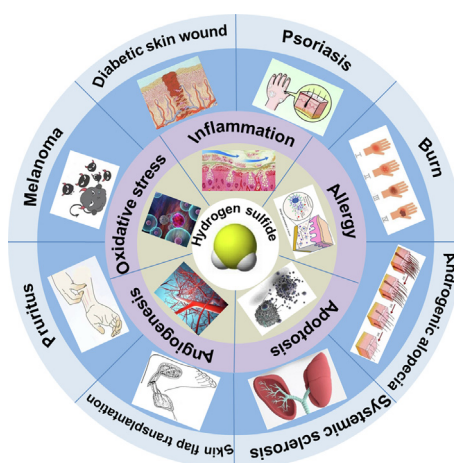
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HIGHLIGHTS

- Three hydrogen sulfide (H₂S) production enzymes including CSE, CBS and 3-MST exist in the skin.
- H₂S regulates burn, diabetic skin wound, psoriasis, systemic sclerosis, melanoma, and pruritus.
- H₂S regulates oxidative stress, inflammation, angiogenesis and apoptosis in skin diseases.
- Some ideal characteristics of H₂S-based therapeutics for topical delivery are preferred.
- Therapeutic potential of H₂S for skin disorders will be further proposed in clinical trials.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Hydrogen sulfide (H₂S) is now recognized as a vital endogenous gasotransmitter with a variety of biological functions in different systems. Recently, studies have increasingly focused on the role of H₂S in the skin.

Aim of Review: This review summarizes recent progress and provides perspectives on H₂S in the treatment of dermatological diseases.

Key Scientific Concepts of Review: Three H₂S production enzymes, cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS) and 3-mercaptopyruvate sulfur transferase (3-MST), are all present in the skin, and it is likely that different cell types in the skin express them differently. Previous studies have demonstrated that H₂S protects against several dermatological diseases, such as burns, diabetic skin wounds, psoriasis, skin flap transplantation, systemic sclerosis, melanoma, and pruritus. The mechanism might be related to the regulation of oxidative stress, inflammation, angiogenesis, apoptosis, and allergic reactions. H₂S-based therapeutics require certain characteristics for topical delivery, for example, controlled release, appropriate physicochemical properties, good storage stability, acceptable odor, and

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advanced delivery systems. H₂S-induced S-sulfhydration on proteins are potential novel targets for therapeutic intervention and drug design for the skin, which may lead to the development and application of H₂S-related drugs for dermatological diseases.

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Introduction

Hydrogen sulfide (H₂S) had long been considered as a toxic gas that pollutes the environment and induces occupational disease. However, in 1989, scientists discovered the existence of endogenous H₂S in the brains of rats and humans [1,2], which opened up research on the physiological functions of H₂S. Since then, H₂S has been regarded as a biological mediator rather than only an environmental toxin [3]. With extensive in-depth study, H₂S has been found to have a variety of biological functions in different systems, such as cardiovascular, neurological, reproductive, and endocrine systems [4–14]. H₂S is now recognized as the third endogenous gasotransmitter after nitric oxide (NO) and carbon monoxide (CO). In recent years, studies have increasingly focused on the role of H₂S in the skin. In this review, we summarize recent progress and perspectives of H₂S in the treatment of dermatological diseases.

Endogenous enzymes for hydrogen sulfide production in the skin

Endogenous H₂S is mainly produced from L-cysteine by catalysis of cystathionine- γ -lyase (CSE) and cystathionine- β -synthase (CBS). But in mitochondria, H₂S is synthesized by 3-mercaptopyruvate sulfur transferase (3-MST) in the presence of the substrate β -mercaptopyruvate pyruvate [5].

Protein quantification of forearm skin samples from healthy adults has shown that both CSE and 3-MST are expressed in the skin [15]. But another study found only CSE expression in melanoma cells and human melanoma tissues [16], which might be related to the source of melanoma cells. CSE expression increases at the later stages of healing in the granulation tissue of wounds [17], and this is significantly attenuated in ob/ob mice [18]. It is also worth noting that CSE deficiency delays wound healing [19]. However, compared with wild-type mice, the healing of burn wounds is unaffected if 3-MST is deficient [20].

One study showed that H₂S promotes neovascularization after hind limb ischemia in CBS mutant mice via the peroxisome proliferator-activated receptor- γ (PPAR- γ)/vascular endothelial growth factor (VEGF) axis [21]. Many articles have demonstrated that CBS is also expressed in the skin. In 1976, CBS was found in cultured skin fibroblasts obtained from a patient with homocystinuria [22,23]. One group found lower CBS activities in cultured human skin fibroblasts of young patients with the atherosclerotic or venous disease compared to healthy controls [24]. CBS-deficient mice have wrinkled skin with hyperkeratosis of the epidermis and thinning of the dermis [25]. Another study verified that dermal fibroblasts from Down syndrome individuals have higher CBS expression than control cells. CBS localization is both cytosolic and mitochondrial [26].

Taken together, the expression of these enzymes in the skin has been poorly studied and is incompletely understood. Most likely, CSE, CBS, and 3-MST are all present and are likely expressed by different skin cell types.

Implications of hydrogen sulfide in dermatology

Burns

One study verified that H₂S levels in the plasma increase after scald burn injuries in wild-type mice, but the levels are unchanged

in 3-MST-deficient mice. Although 3-MST deletion has no obvious effect on skin wound healing after a burn, 3-MST deficiency aggravates liver, renal, and cardiac dysfunctions [20]. In any case, increasing 3-MST expression or activity might be beneficial to attenuate severe complications after a burn. However, 3-MST is only one of the endogenous enzymes for H₂S generation in the skin.

Exogenous H₂S supplementation may also have the potential to affect burns. In one study, immediately after 5% of the total body surface area (TBSA) was given a deep partial-thickness scald in rats, the H₂S donor sodium hydrosulfide (NaHS) was intraperitoneally injected [27]. The tissues in the base of the wound were collected after the burn to isolate macrophages, and it was found that NaHS increased the secretion of basic fibroblast growth factor (bFGF) and transforming growth factor β_1 (TGF- β_1), but decreased the release of tumor necrosis factor α (TNF- α) and interleukin 1 β (IL-1 β) in cultured macrophages. These data suggest that H₂S has the potential to promote growth factor secretion and wound healing.

However, the role of H₂S in inflammation during burns remains controversial. One study found that H₂S levels in the plasma significantly increased after 30% TBSA burn induction in male BALB/c mice using 95 °C water for 8 s. More importantly, intraperitoneal injection of NaHS (10 mg/kg) aggravated burn-related systemic inflammation [28]. However, whether elevated H₂S levels after a burn resulting from damaged cells or antagonize acute damage remains unknown. To complicate matters, another study has provided completely different results. After 40% TBSA of adult male C57BL/6 mice was exposed to 95 °C water for 10 s, the H₂S content in the plasma decreased. NaHS (2 mg/kg) administration 1 h after the burn significantly decreased the levels of TNF- α , IL-6, and IL-8, but increased the levels of IL-10 in the plasma [29]. This indicates that H₂S supplementation was able to inhibit inflammation post-burn, potentially providing a new strategy to improve the immune response and promote burn healing.

The above contradictory effects of H₂S on inflammation after burns might be ascribed to the burn degree of injury, the course of the burn, or the dosage or treatment time of H₂S donors. Some researchers have even proposed that H₂S may promote inflammation in the early stage of a burn but inhibit inflammation and promote healing in the late stage of a burn [30]. Another study has also shown an important role for the pro-inflammatory action of H₂S during a burn [31]. Therefore, the real role of H₂S in burns might be closely associated with the detailed properties of the burn injury. Due to the biphasic effects of H₂S on burns, H₂S supplementation in the late, not early, stage of a burn may be helpful to accelerate healing.

Diabetic skin wounds

Diabetes mellitus (DM) is a common disease throughout the world, and delayed wound healing is a major complication that causes great harm to patients. The exact mechanism of delayed wound healing in DM is not fully understood, but it may be related to sustained inflammation, excessive oxidative stress, and impaired angiogenesis. Changes in the balance of H₂S play an important role in the pathogenesis of β -cell dysfunction that occurs in response to type 1 and type 2 diabetes [32]. We know that H₂S regulates inflammation, oxidative stress, and angiogenesis [33–35], suggesting a potential role for H₂S in promoting diabetic wound healing. It has been found that CSE expression and H₂S

levels in granulation tissue of wounds in ob/ob mice are significantly lower than those in controls, suggesting that H₂S production is impaired in DM wounds. Moreover, intraperitoneal injection of NaHS attenuates neutrophil and macrophage infiltration, inhibits TNF- α and IL-6 secretion, and accelerates wound healing in the ob/ob mice [18]. 3-MP (the substrate of 3-MST) supplementation increases H₂S production, facilitates wound healing, induces dermal microvessel relaxation, and increases mitochondrial bioenergetic function in rats [36]. The above research demonstrates that the enhancement of endogenous H₂S production or exogenous H₂S supplementation promotes diabetic trauma healing. During DM, reactive oxygen species (ROS) increase to activate mitogen-activated protein kinase (MAPK), including extracellular regulated protein kinases 1/2 (ERK1/2) and p38, to induce neutrophil NETosis, and finally to delay wound healing via neutrophil extracellular traps (NETs). In db/db diabetic mice, wound healing is significantly delayed, while the levels of NETs and NETosis are increased. Na₂S promotes wound healing by reducing NETs and NETosis levels, which might be due to ROS suppression followed by ERK1/2 and p38 phosphorylation inhibition in a NETosis model of neutrophils induced by phorbol 12-myristate 13-acetate (PMA) (Fig. 1) [37]. Another study also confirmed that both NaHS and 4-hydroxythiobenzamide promote wound healing in db/db diabetic mice [38]. Moreover, the endothelial progenitor cell (EPC) function of db/db diabetic mice is significantly decreased after CSE inhibitor administration or CSE silencing. NaHS significantly restores the EPC function to improve refractory wound lesions in diabetic mice (Fig. 1). Furthermore, the angiogenesis of EPC is also enhanced by NaHS via angiopoietin-1 activation [38]. This shows a direct pathway in the promotion of diabetic wound healing by H₂S. After topical application of 2% sodium bisulfide ointment, wound healing is accelerated, and superoxide dismutase (SOD), heme oxygenase-1 (HO-1), VEGF, and intercellular adhesion molecule-1 in neo-

granulation tissues are significantly increased in streptozotocin-induced diabetic rats [39]. This suggests that an H₂S donor might be a novel agent for diabetic skin ulcerations. Considering the few adverse reactions, developing H₂S-related preparations to be applied locally on the skin is desirable.

Delayed wound healing in DM is also associated with keratinocyte damage and dysfunction. In one study, HaCaT cells, a cell line of human skin keratinocytes, were exposed to methylglyoxal (MGO) to establish a diabetic wound healing model *in vitro* [40]. An N-mercapto-based H₂S donor, a novel controllable H₂S-releasing molecule, enhanced cell viability, inhibited cell apoptosis, alleviated intracellular ROS content, increased mitochondrial membrane potential, and ultimately promoted cell adhesion and migration in MGO-stimulated HaCaT cells [40]. Other researchers have designed microparticles (NaHS@MPs) that provide an *in situ* depot for the sustained release of exogenous H₂S using an emulsion method. NaHS@MPs continuously releases H₂S to inhibit oxidative stress, suppress ERK1/2 and p38, promote the proliferation and migration of epidermal/endothelial cells, improve angiogenesis, and accelerate the healing of full-thickness wounds in diabetic mice [41]. These novel H₂S-related biomaterials have great potential for the healing of diabetic wounds.

Psoriasis

Psoriasis is a heritable inflammatory skin disease [42]. Although a large number of studies have confirmed that H₂S is a “double-edged sword” that can promote or inhibit inflammation [5,14,43], it is not clear whether H₂S plays an important role in the occurrence and development of psoriasis. One group found that H₂S levels in serum from patients with chronic progressive psoriasis are only about half of those from healthy controls, while TNF- α , IL-6, and IL-8 levels are about two-fold. More importantly, H₂S levels in the serum are negatively correlated with the severity of psoriasis [44]. However, the causal relationship between H₂S levels and psoriasis remains unknown.

Keratinocyte over-proliferation is considered to be a vital pathophysiological characteristic of psoriasis [45]. The levels of IL-6 and IL-8 increase after TNF- α stimulation in human HaCaT keratinocytes, and this is alleviated by exogenous H₂S supplementation in a concentration-dependent manner. The mechanism might be related to the inhibition of ERK, p38, and nuclear factor- κ B (NF- κ B) activation [44]. Another study also found that both NaHS and GYY4137, as two common H₂S donors, increase inducible nitric oxide synthase (iNOS) expression and promote NO secretion depending on Akt activation, thereby inhibiting ERK activation and decreasing VEGF production to attenuate the proliferation of human keratinocytes (Fig. 2) [46]. NaHS also decreases cell numbers in S phase, increases those in G2/M phase, inhibits colony formation, suppresses cell adhesion to plastic dishes, and impairs the viability of adherent cells. The mechanism may be related to the blockage of the Raf/MAPK/ERK signaling pathway and the reduction of β 4, α 2, and α 6 integrins expression [47]. Taken together, H₂S can inhibit keratinocyte growth, proliferation, and adhesion. H₂S-releasing compounds might therefore be promising therapeutic agents for psoriasis.

Previous research has verified that T cell infiltration in lesions is the initiating factor of keratinocyte proliferation. IL-17 and IL-22, which are produced from Th1/Th17 lymphocytes, promote keratinocytes to secrete IL-8, and finally enhance inflammation, which is a critical event in the pathogenesis of psoriasis [48]. H₂S inhibits IL-17 and IL-22 production and decreases IL-8 secretion to attenuate inflammation via ERK phosphorylation inhibition in human keratinocytes (Fig. 2) [49]. Although many studies have confirmed the ability of H₂S to regulate T cell activity in the immune system

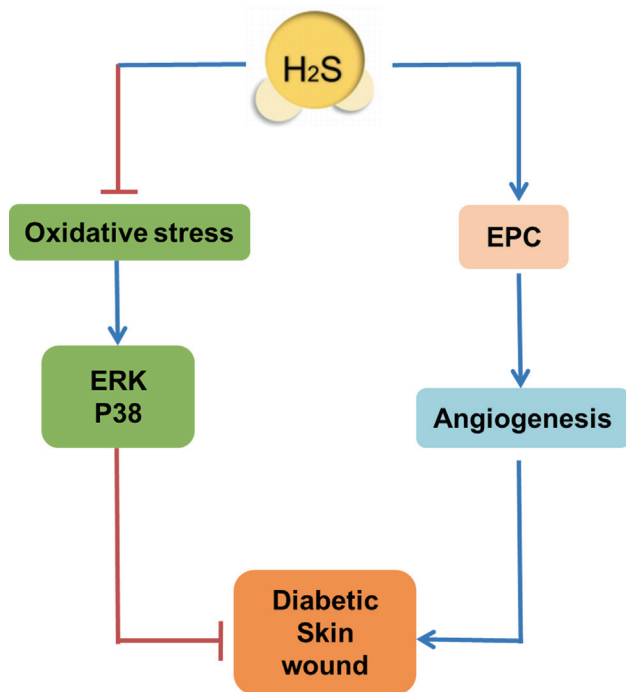


Fig. 1. Possible mechanisms of hydrogen sulfide (H₂S) in the healing of diabetic skin wounds. Increasing endogenous H₂S production or exogenous H₂S supplementation attenuates oxidative stress and inhibits extracellular regulated protein kinases (ERK) 1/2 and p38 phosphorylation to accelerate diabetic skin wound healing. H₂S also improves angiogenesis by restoring endothelial progenitor cell (EPC) function to promote skin wound healing.

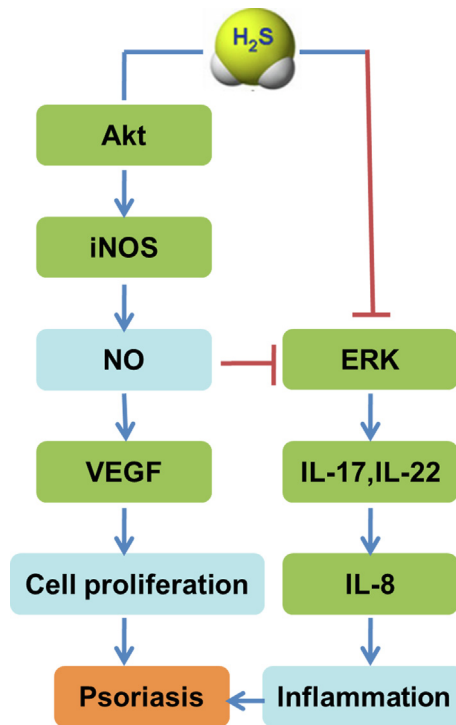


Fig. 2. Possible mechanisms of hydrogen sulfide (H_2S) action against psoriasis. Inflammation and over-proliferation of keratinocytes are vital pathophysiological mechanisms of psoriasis. H_2S increases inducible nitric oxide synthase (iNOS) expression and promotes nitric oxide (NO) secretion depending on Akt activation, thereby inhibiting extracellular regulated protein kinases (ERK) activation and decreasing vascular endothelial growth factor (VEGF) production to attenuate the proliferation of human keratinocytes. H_2S also inhibits IL-17 and IL-22 production and decreases IL-8 secretion to attenuate inflammation via ERK phosphorylation inhibition in human keratinocytes.

[50–52], the real role of H_2S in T cells of the skin needs further study.

A complex interaction between keratinocytes and T cells is involved in psoriasis. However, whether H_2S can regulate the interaction between keratinocytes and T cells to inhibit and even block the pathways for keratinocytes proliferation or differentiation requires further investigation.

Skin flap transplantation

Skin flap transplantation is a common manner of reconstructive surgery. However, the obstruction of local microcirculation and blood flow reperfusion injury after successful transplantation limit the application of skin flaps. Some researchers have found that H_2S pretreatment alleviates apoptosis in mouse-derived fibroblasts after hypoxia-reoxygenation in an *in vitro* model of cutaneous tissue transplantation [53]. This suggests that H_2S administration before ischemia significantly protects against reperfusion injury, indicating the potential applicability of H_2S in skin transplantation.

Recently, one team intuitively investigated the effects of H_2S on tissue necrosis and skin microcirculation by stereofluorescence microscopy and intravital fluorescence microscopy in an axial pattern ear flap model of hairless mice. They found that GYY4137 significantly dilates capillaries, increases functional capillary density, reduces auricular tissue necrosis, and improves microcirculation [54]. In summary, H_2S improves microcirculation and maintains the vitality of transplanted skin flaps. H_2S administration is therefore a potential means to improve the success rate and therapeutic effect of skin flaps.

Systemic sclerosis

Systemic sclerosis (SSC) is a serious connective tissue disease with unknown etiology. It is characterized by multiple organ fibrosis owing to inflammation, vascular injury, and excessive collagen deposition. Skin sclerosis is one of the main manifestations of SSC. One study found that H_2S concentrations in plasma significantly decrease in a bleomycin-induced mouse model of SSC [55]. After NaHS treatment, the pathological structure of the skin is improved, the dermis becomes thinner and the lipid layer thicker, and inflammatory cell infiltration and collagen deposition are alleviated. The mechanism may be related to the reduction of extracellular matrix accumulation via TGF- β_1 inhibition [55]. This provides a new modality for the clinical treatment of SSC-related organ fibrosis.

Melanoma

H_2S shows a bimodal pharmacological character in cancer, in that it both inhibits metabolite biosynthesis and increases their concentrations to a certain threshold to exert anticancer effects [56]. Previous studies have demonstrated an emerging role for H_2S in various cancers including colon cancer, ovarian cancer, urothelial carcinoma, renal cell carcinoma, and prostate cancer [57,58]. In dermatology, H_2S is a potential chemopreventive agent against melanoma development.

Melanoma is a common skin cancer with high mortality. Compared with normal human epidermal melanocytes, CSE expression is increased in melanoma cells. Immunohistochemical analysis of human melanoma samples shows that the highest levels of CSE expression are in primary tumors. However, CSE expression is decreased in metastatic lesions and almost absent in non-lymph node metastases, indicating that the occurrence and metastasis of melanoma may be related to the H_2S pathway [16]. H_2S donors can inhibit melanoma cell proliferation by regulating the cell cycle, promoting apoptosis, inhibiting NF- κ B activation and downstream anti-apoptotic-related protein expression, and reducing AKT and ERK phosphorylation [16]. The inhibitory effect of H_2S on melanoma may offer a promising alternative to existing therapies.

Acetyl deacetylase disulfide (ADA), a novel H_2S -releasing compound, inhibits human melanoma cell proliferation and invasion by inducing apoptosis. Further studies have confirmed that ADA reduces NF- κ B nuclear translocation and activation, decreases anti-apoptotic protein expression, and inhibits Akt and ERK phosphorylation. *In vivo*, B16/F10 melanoma cells were injected into C57BL/6 mice via the tail vein, and ADA administration reduced the volume of lung metastatic foci in inoculated mice in a concentration-dependent manner [59]. These studies suggest that the H_2S donor ADA significantly inhibits the progression of melanoma and could represent an important lead compound for the design and development of novel anti-metastatic agents.

A “combination therapy” based on the H_2S pathway may be a potential treatment for melanoma. One group successfully designed several H_2S -releasing non-steroidal anti-inflammatory drugs (H_2S -NSAIDs) with the properties of H_2S release and cyclooxygenase (COX) inhibition. For example, ATB-346 [2-(6-methoxynaphthalen-2-yl)-propionic acid 4-thiocarbamoyl phenyl ester] significantly inhibits melanoma proliferation via NF- κ B and Akt suppression [60]. Naproxen-4-hydroxybenzodithioate (NAP-HBTA) exhibits an inhibitory effect on melanoma cell proliferation. Further experiments have shown that NAP-HBTA enhances apoptosis and inhibits cell motility, invasion, and cell colony formation. NAP-HBTA also significantly suppresses melanoma growth and progression in mice. The mechanism may be related to the inhibition of chemokine (C-X-C motif) ligand 1 (CXCL1), matrix metalloprotein (MMP)-2, and MMP-13 expression [61]. Considering the good pharmacokinetic properties of naproxen, a naproxen

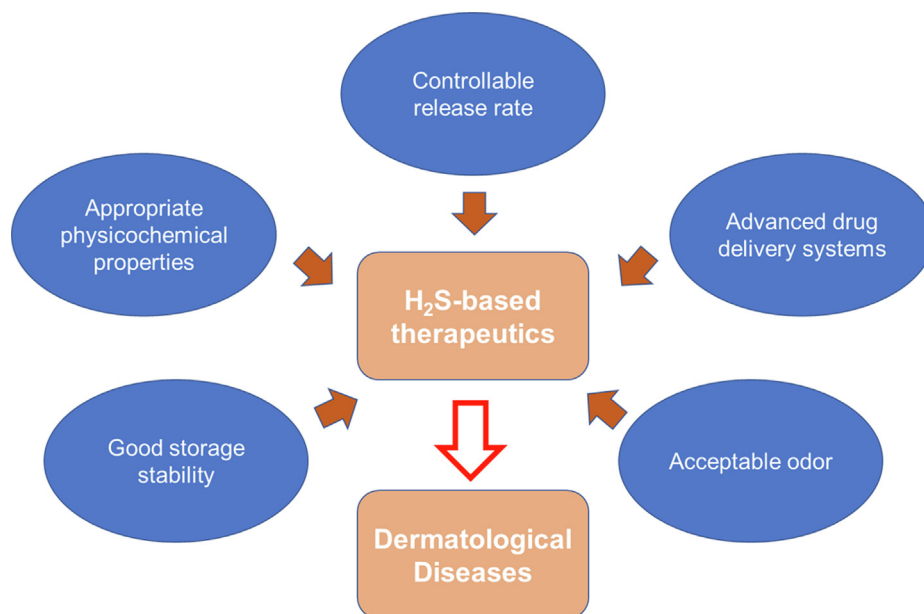


Fig. 3. Ideal characteristics of hydrogen sulfide (H_2S)-based therapeutics for dermatological diseases. Some characteristics of H_2S -based therapeutics for topical delivery would be ideal; for example, these molecules should present the controllable release of H_2S , appropriate physicochemical properties, good storage stability, and acceptable odor. These features could serve as reference points to evaluate existing donors and guide future drug design. Formulating H_2S -based therapeutics for dermatological diseases into advanced drug delivery systems that increase cutaneous bioavailability and decrease systemic exposure is preferred.

H_2S -derivative might alleviate the toxicity of H_2S to organs, which provides a new method for the prevention and treatment of melanoma and other diseases.

Other diseases

Androgenic alopecia is a chronic, progressive condition affecting millions of individuals worldwide. Sodium thiosulfate (STS), known to generate H_2S , accelerates hair growth in the “telogen model” of mice, suggesting that H_2S stimulates telogen hair follicles to reenter the anagen phase of hair growth [62]. H_2S may represent a novel and beneficial remedy for hair loss in androgenic alopecia.

Pruritus is a distressing physiological self-protective mechanism similar to pain. However, the role of H_2S in pruritus remains controversial. Some scholars have found that subcutaneous injection of NaHS or Na_2S increases scratching behavior in a μ -opioid receptor-dependent and histamine-independent manner in mice [63], while others have reported that Na_2S significantly ameliorates histamine- or compound 48/80 (C48/80)-induced pruritus in male BALB/c mice [64]. Further studies are needed to clarify the exact effect and detailed mechanism of H_2S on pruritus.

Conclusions and future perspectives

Over the last few decades, significant progress has been achieved in delineating the potential effect of H_2S on dermatological diseases such as burns, diabetic skin wounds, psoriasis, skin flap transplantation, systemic sclerosis, and melanoma. The regulation of oxidative stress, inflammation, angiogenesis, apoptosis, and allergic reactions might be responsible for the distinct roles of H_2S in the skin. However, the specific signaling molecules involved in the above processes need to be further explored. Recently, a novel post-translational modification of specific cysteine residues of target proteins induced by H_2S , named S-sulfhydration (or S-persulfidation), has been proposed [5,65,66]. As of now, specific proteins with S-sulfhydration as well as their physiological function in dermatology are not well known.

H_2S -based therapeutics have been evolving to deliver H_2S to desired locations at appropriate concentrations and with appropriate pharmacokinetics. Instead of taking H_2S -based therapeutics orally, the application of small molecules on the skin may provide targeted local therapy for skin disorders and decrease the risk of off-target side effects. Successful topical drug delivery of H_2S -based therapeutics allows the molecules to be conveyed directly to the site of action, providing relevant therapeutic concentrations and avoiding unnecessary systemic circulation, first-pass hepatic metabolism, and off-target side effects. Some characteristics of H_2S donors should be improved. First, controllable release rates of H_2S are essential, since its release directly affects the concentration of H_2S in the target site and thus its pharmacological functions. Second, topical delivery is often challenging because organs exposed to the environment possess effective barrier functions that hinder the entry of xenobiotics. Third, targeted intraepidermal delivery using advanced formulation strategies is desired, since it not only fine-tunes the release of an H_2S donor from the small formulation “depot” before being triggered by certain environments but also enables improved cutaneous delivery via specific transport pathways with minimal risk of systemic exposure. Other aspects such as appropriate physicochemical properties, good storage stability, acceptable odor (odorless or without unpleasant smell) are also important for drugability (Fig. 3). In future studies, the therapeutic potential of H_2S for skin disorders will be explored in clinical trials.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Compliance with ethics requirements

Not applicable.

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