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REVIEW Open Access

100 years of sickle cell disease research: etiology, pathophysiology and rational drug design (part 1)

Mona A. Mahran*, Mohamed Teleb Ismail and Elwy H. Abdelkader

Abstract

Background: Sickle cell disease (SCD) is a chronic hemolytic disease caused by an altered hemoglobin molecule (HbS) and was first termed as a molecular disease. Glutamic acid in the normal hemoglobin molecule (HbA), was replaced by valine in HbS at the sixth position of both β-chains. This alteration was proved to be due to a single point mutation GTG instead of GAG in the genetic code. Since the discovery of sickle cell disease in 1910, great efforts have been done to study this disease on a molecular level. These efforts aimed to identify the disease etiology, pathophysiology, and finally to discover efficient treatment. Despite the tremendous work of many research groups all over the world, the only approved drug up to this moment, for the treatment of SCD is the hydroxyurea.

Main text: In this review, the antisickling pharmaco-therapeutics will be classified into two major groups: hemoglobin site directed modifiers and ex-hemoglobin effectors. The first class will be discussed in details, here in, focusing on the most important figures in the way of the rational drug design for SCD treatment aiming to help scientists solve the mystery of this problem and to get clear vision toward possible required therapy for SCD.

Conclusion: Despite the large number of the antisickling candidates that have been reached clinical studies yet, none of them has been introduced to the market. This may be due to the fact that hemoglobin is a large molecule with different target sites, which requires highly potent therapeutic agent. With this potency, these drugs should be safe, with acceptable oral pharmacokinetic and pharmacodynamic properties. Such ideal drug candidate needs more efforts to be developed.

Keywords: Sickle cell disease, Antisickling pharmaco-therapeutics, Alloesteric effectors, Vanillin

1 Background

Sickle cell anemia was first discovered by J. B. Herrick in 1910 [30], who noted morphological difference between normal RBC's (disc shape) and abnormal (elongated shape) of the sickled RBC's. In 1923, the reversibility of RBC's sickling was reported [65], whereas the correlation between oxygen tension and sickling was declared, many years later, by Hahn and Gillespie [27]. They stated that sickling occurs only under low oxygen tension, while erythrocytes regain its normal shape by increasing oxygen partial pressure. The breakthrough information of the sickle cell disease (SCD) was the discovery by Pauling et al. in 1949 [55] that SCD was caused by an altered hemoglobin molecule HbS and was first termed a molecular disease. The same research group also proposed a mechanism of the sickling phenomenon which happens due to the interaction of the complementary deoxy HbS molecules to form long chains that attract one another forming a crystal or liquid crystal. This postulation was supported by Harris' observation that 10% of the deoxy HbS solution consists of a polymer [29]. Using peptide mapping technique in 1956, Ingram reported that glutamic acid in the normal hemoglobin molecule (HbA) was replaced by valine in HbS at the sixth position of both β-chains [33, 34]. In 1977, Marotta research group proved that the replacement of glutamic acid by valine is due to a single point mutation GTG instead of GAG in the genetic code [42]. Indeed, electron

* Correspondence: mahranmona@yahoo.com
Department of Pharmaceutical Chemistry Faculty of Pharmacy, Alexandria University, El-Khartom square, El-Azarita, Alexandria 21521, Egypt

microscopy and X-ray diffraction analysis described the structure of HbS fiber as a 14-stranded fiber, each strand is formed of seven proto-filaments [17, 18]. Under low oxygen conditions, the mutant hemoglobin (HbS) polymerizes through intermolecular contact between the mutated βVal6 from one Hb tetramer and a hydrophobic pocket formed by β1Ala70, β1Phe85, and β1Leu88 residues on a different tetramer. The formation of these polymers results in losing the normal disc shape of RBCs with the formation of sickle-like shaped RBCs, where they become more fragile. These sickle cells are unable to pass through narrow capillaries resulting in painful vaso-occlusive crises [57]. As a result, the sickle cells undergo hemolysis leading to anemia and a shortened lifespan. The vaso-occlusive crises is associated with fever, severe pain in the extremities, chest, back, and/or abdomen. Moreover, aplastic hematologic crisis due to viral or bacterial infection, or hemolytic crisis due to infection or other etiologic reasons were reported [32]. Aplastic hematologic crisis causes injury of the bone marrow cells which in turn decreases the erythrocytes production. On the other hand, hemolytic crisis leads to destruction of the circulating erythrocytes which causes a decrease in the hematocrit values. Transfusions are lifesaving for patients in this stage of the disease. Hydroxyurea [14], a myelosuppressive agent, is the only effective drug proven to reduce the frequency of painful episodes.

2 Main text

2.1 Introduction

Apparently, there is a leap in the recent research for potential treatment of SCD. This was evident by the large number of published research articles in many international journals and conferences along with several drug candidates in phase I, II, and III clinical trials [6, 13, 15, 41, 66]. This progress, in our opinion, is provoked by the discovery of many ex-hemoglobin sites which have been reported as targets to control sickling or gelling of HbS. A prime example of such sites is RBC's cell membrane-calcium-activated potassium channel (Gardos channel); one of the main routes for K^+ loss and dehydration in RBCs [24, 26, 35], which could be blocked by clotrimazole and other imidazole inhibitors [10]. Additionally, DNA methyl transferases [63] and histone deacetylase [31] are viewed as validated antisickling targets that could be inhibited for inducing fetal hemoglobin (HbF) that does not participate in the polymerization of hemoglobin subsequently decreases HbS polymerization [21, 45]. Similarly, inhibition of the rho kinase protein [40] by hydroxyfasudil shows promise in SCD treatment as it increases endothelial NO synthase levels and induces HbF [21]. Another approach is targeting adenosine signaling that is responsible for multiple pathophysiological roles in SCD through subtype 2B adenosine receptors antagonism [22]. Such discoveries opened the gate for scientists to explore new approaches and to rationally design new small molecules as potential antisickling agents [16, 21]. Thirty years ago, site-directed modification of hemoglobin was the major strategy used to design antisickling candidates targeting different hemoglobin pockets that were identified using X-ray diffraction analysis. Those hemoglobin effectors were classified according to their mode of interaction with hemoglobin into covalent and non-covalent-binding hemoglobin alloesteric effectors. Covalent modifiers are molecules that bind covalently to one or more reactive sites of hemoglobin. Examples of the covalent binding effectors are cyanates [11, 43, 49], aldehydes [4, 48], acetyl salicylates [67, 68], α,β-unsaturated carbonyls [38, 54], and nitrogen-mustard derivatives [23, 58]. On the other hand, non-covalent modifiers (e.g., aromatic amino acids [25, 59], ureas [12], alkonic acids [20, 53], and esters [9]) bind to Hb through ionic, Van der Waal, or hydrogen bond force of interaction that leads to polymer destabilization (anti-gelling) or breaking the salt bridges which results in shifting the allosteric equilibrium and the subsequent increases in HbS affinity for oxygen (antisickling).

Antisickling pharmaco-therapeutics could be generally classified into two major groups: hemoglobin site-directed modifiers and ex-hemoglobin effectors. In this part, only the first class will be discussed in more details, in order to gather all ideas, strategies, and outcomes of the research and efforts made for discover new treatment of SCD, whereas the second class is stated herein in brief and will be discussed in details in part II review.

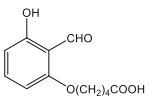
2.2 Hemoglobin allosteric modifiers

Hemoglobin allosteric modifiers (HAM) include any natural or synthetic therapeutic agent that targets hemoglobin to change its properties with the aim to inhibit its sickling and/or its polymerization to be useful for the treatment of SCD. The first attempt to synthesize stereospecific HbS modifiers was published in 1977 [39]. This work was based on the hypothesis that HbS aggregation could be abolished by an oligopeptide that mimics the amino acid sequence of the mutation site at the donor area of the HbS tetramer. This oligopeptide would competitively inhibit binding with the acceptor site of the other HbS tetramer and presumably prevent polymerization. Different series of oligopeptide amides were synthesized e.g., β 1–6, β 3–6, β 5–6, and even longer sequences of the N-terminal region of the HbS β-chains (β 1–8) [39, 69]. The obtained results indicated that oligopiptide sequence β 1–6 possesses the highest activity as inhibitor of HbS aggregation. However, when changing the sequence order of such peptide as β 125634 HbS, it maintained the same activity, which indicated lack of specify.

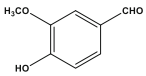
In 1984, an allosteric non-competitive DPG (2,3-diphosphoglycerate) antagonist, BW12C, was designed targeting

the α-amino terminal of oxy HbS [7]. DPG along with H^+ are the natural allosteric effectors that lower Hb affinity for oxygen. BW12C's structure was designed to have an *o*-hydroxy group to the aldehyde moiety to stabilize the formed Schiff-base between one of the α N-terminal of the protein and the carbonyl of the aldehyde group. Indeed, the carboxylic group was included in the BW12C's structure to form salt bridge with the other α N-terminal of the oxy HbS. Despite of its antisickling activity in vitro and in vivo, BW12C has very short half-life (4 h).

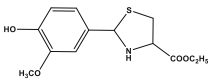
In 1991, vanillin, a nutraceutical agent, was picked up as a safe antisickling lead compound [3], following the previous results reported by Zauggand and Beddel research groups [8, 70]. Vanillin has a moderate antisickling effect and was proved to bind covalently with HbS, increasing its oxygen affinity as well as decreasing RBC's sickling. However, due to its poor oral bioavailability, vanillin possessed weak antisickling effect after oral administration [5, 19, 36, 64]. To overcome such drawback, a vanillin pro-drug was designed to have a thiazolidine protection of the aldehyde group to bypass the gastrointestinal metabolism. Such compound showed significant improved oral pharmacokinetics and pharmacodynamics, yet, it still suffered from some degradation in the digestive tract [71].



(BW12C)

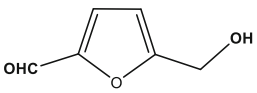


Vanillin



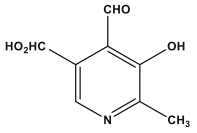
Thiazolin prodrug of vanillin

5-Hydroxymethyl-2-furfural (5-HMF, a vanillin isoster) was reported to have a remarkable antisickling activity. It is several times more potent than vanillin in inhibiting sickling and protecting sickle mice from hypoxia [2, 60]. 5-HMF is currently in clinical trials in SCD patients [28].

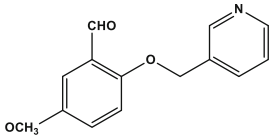


5-HMF

The structure activity relationship study of a series of 5-HMFs as antisickling agents indicated that replacing the hydroxymethyl group at the 5-position of the furan ring as in 5-HMF by hydrophobic moieties (for example alkyl or alkoxy groups) decreases its activity, while its removal destroys the activity. This observation was confirmed by the crystallographic results implied the importance of the hydroxyl moiety of 5-HMF in the stabilization of the relaxed R state of hemoglobin [61]. Based on these results and using vanillin and pyridoxal (previously studied antisickling non-toxic aldehyde) [37] as scaffold, several pyridyl derivatives (INN) were developed and evaluated for their antisickling activity [1, 50, 62]. Amazingly, some of these compounds showed as much as 90 and 2.5-foldpotency compared to vanillin and 5-HMF, respectively, although they bind at the same site of hemoglobin as 5-HMF [1].



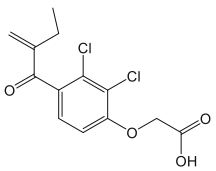
Pyridoxal



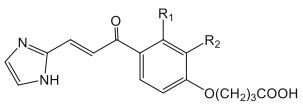
INN-312

In the same study, it was stated that the allosteric activity of these pyridyl derivatives is highly related to the position of the methoxy and pyridyl groups with respect to the aldehyde function. Generally, ortho-pyridyl benzaldehyde derivatives having a meta or para-methoxy substitution showed the highest activity, as in compound INN312, which act as a stereospecific inhibitor of the deoxy-HbS polymer while efficiently increasing the Hb affinity for oxygen [50].

In a trial to overcome the poor oral bioavailability of aldehydes, a series of imidazolylacryloyl derivatives were designed using ethacrynic acid (ECA); 2-(2,3-dichloro-4-(2-methylenebutanoyl)phenoxy)acetic acid, as a pharmacophore [54]. ECA, a diuretic, was reported to inhibit HbS polymerization [38, 56]. However, its diuretic effect opposed its chance to be used in the treatment of SCD. The imidazolylacryloyl derivatives, referred as KAUS, have an α, β-unsaturated ketone moiety, which was expected to undergo Michael addition on the thiol group of βCys93 in the same manner of ECA leading to the inhibition of sickling. Although those compounds did not show the expected activity, co-crystallization of deoxygenated or carbonmonoxy Hb with KAUS-12 or KAUS-1 showed an unexpected mode of Michael addition on the N-terminal αVal1 at the α-cleft of the T-state structures of hemoglobin.



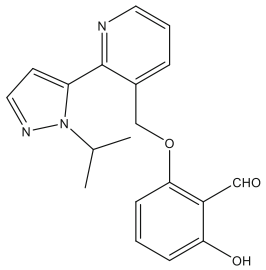
ECA



KAUS12, R_1= R_2= Cl
KAUS15, R_1= H, R_2= Cl

Recently, Metcalf's research team, using molecular modeling, has designed a new series of aldehydes having a bicylic ether link ortho to the aldehyde group. Their strategy was based on the ability of the aldehyde's carbonyl function group to form a Schiff base with the two N-terminal valines in both *α*-chains in HbS, while the bicyclic moiety would fit more into the intradomain cavity [44]. This work was concluded successfully with the discovery of a new potent allosteric modifier of HbS, GBT440, having a pyrazol-5-yl-pyridine ether link. This compound was able to highly increase the hemoglobin affinity for oxygen

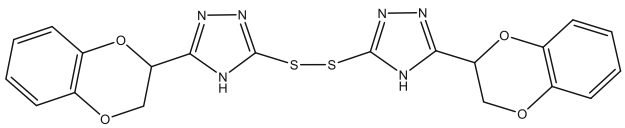
which resulted in the decline of the polymerization of deoxy-HbS.



GBT440

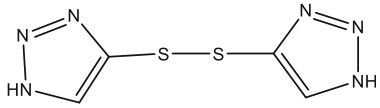
The X-ray analysis revealed that GBT440 [52] binds covalently to a single α-chain in a 1:1 stoichiometry to the HbS tetramer. It is worth mentioning that all of the previously reported aldehydes bind covalently to the HbS chain in a 2:1 stoichiometry. It was reported that compound GBT440 possesses a high oral bioavailability in rats (60%), with more than 19 h half-life. In addition, GBT440 partitions highly and preferentially into the red blood (RBC/plasma ratio is ~ 150). Therefore, the authors proposed that GBT440 would be a superior antisickling hemoglobin modifier that specifically targets RBCs and exerts its effect in a relatively low therapeutic dose. Currently, GBT440 is in phase III clinical trials in SCD patients.

Interestingly, another class of covalent binding Hb allosteric modifiers was discovered having a symmetric structure mediated by a disulfide link [47, 51]. Those modifiers were identified after a random biological screening of 38, 700 compounds using small molecule microarrays, followed by a high-throughput assay to test the selected molecules that modified Hb affinity for oxygen. TD-1(di(5-(2,3-dihydro-1,4-benzodioxin-2-yl)-4*H*-1,2,4-triazol-3-yl)disulfide) was the capstone of the evaluated compounds where it showed a greater effect even than 5-HMF, on oxygen affinity of human hemoglobin.



TD-1

The X-ray crystallographic analysis of Hb-TD-1 complex indicated that a monomer of the TD-1 structure reacts covalently to both β-Cys93 and β-Cys112. Indeed, it was found also that TD-1 reacts in a monomeric pattern, but non-covalently to the central water cavity of the Hb tetramer, stabilizing the relaxed (R) state, and disturbing the salt-bridge interaction between β-His146 and β-Asp94, destabilizing the tens (T) stat. TD-1, was also reported to prevent sickling of human sickle cells. Encouraged by these results, another triazole disulfide, TD-3, was published by the same researcher group, to bind covalently to the Hb as TD-1 does but has no superior effect than the later as a hemoglobin modifier [46].



TD-3

2.3 Conclusion

Hundreds of covalent binding hemoglobin allosteric modifiers that successfully increase HbS oxygen affinity and decrease its polymerization were published. Many of these modifiers have reached human clinical trials, but unfortunately none has been introduced to the market yet. This could be attributed to the fact that hemoglobin is a large molecule having different target sites, which requires highly potent therapeutic agent (nanomolar affinity). In addition, these drugs should be safe and possess suitable oral pharmacokinetic and pharmacodynamic properties. Such ideal drug candidate needs more efforts to be identified and developed.

Abbreviations

5-HMF: 5-Hydroxymethyl-2-furfural; DPG: 2,3-Diphosphoglycerate; ECA: Ethacrynic acid; HAM: Hemoglobin allosteric modifiers; Hb: Hemoglobin; HbA: Normal hemoglobin molecule; HbS: Sickled hemoglobin; RBC: Red blood corpuscles; SCD: Sickle cell disease

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Authors' contributions

EA and MT were responsible for collecting data, summarized it, and helped in revising the written manuscript. MM was responsible for writing the manuscript, format its design, arrange its ideas, and prepare it for publication. All authors read and approved the final manuscript.

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Consent for publication

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Competing interests

The authors declare that they have no competing interests

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