Acta Haematologica Polonica 49(1) • March 2018 • 1-8 • DOI: 10.2478/ahp-2018-0001

REVIEW ARTICLE/PRACA POGLĄDOWA journal homepage: https://content.sciendo.com/ahp

Retrospection of the effect of hydroxyurea treatment in patients with sickle cell disease

Article history:
Received: 05.07.2017
Accepted: 13.02.2018

Henu Kumar Verma[1], Saikrishna Lakkakula[2], Bhaskar V.K.S. Lakkakula[1]*

[1] *Sickle Cell Institute Chhattisgarh, Raipur, Chhattisgarh, India*
[2] *Department of Zoology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India*

Abstract

Sickle cell anemia (SCA) is one of the inherited hemoglobin disorders with substantial morbidity and early mortality. Hydroxyurea is the US Food and Drug Administration (FDA)-approved medication that has emerged as the primary disease-modifying therapy for SCA. Our purpose is to summarize the available evidence regarding the pharmacology, clinical efficacy, and safety of hydroxyurea therapy for the treatment of SCA. The electronic databases PubMed and Embase were searched from their starting dates to May 31, 2016. Databases were searched using the following terms: sickle cell, hydroxyurea, nitric oxide, dosing, therapeutic, and safety monitoring. Hydroxyurea therapy may cause severe myelosuppression when used in patients with SCA. SCA patients are initially treated with hydroxyurea at 10 or 20 mg/kg, and then the dose is escalated to mild myelosuppression using a standardized regimen. Routine blood monitoring should be performed while the patient receives hydroxyurea treatment. Hydroxyurea can increase fetal hemoglobin (HbF) level and ameliorate some of the vascular symptoms in patients with SCA. Hydroxyurea therapy may help to avoid frequent hospitalizations, especially in patients with vaso-occlusive crisis. Taken together, available evidence suggests that hydroxyurea represents an inexpensive and effective treatment option that should be offered to patients with SCA.

Keywords:
sickle cell disease, hydroxyurea; HbF, NO, side effects

Introduction

Hemoglobinopathies are a group of inherited disorders of hemoglobin, which result in either structurally abnormal or abridged synthesis of beta globin subunits (Fig. 1). A single-nucleotide transversion (A>T) in the *HBB* gene causes the change of glutamic acid (Glu) to valine (Val) at the sixth position of its protein, which leads to the production of structurally abnormal hemoglobin (HbS). HbS facilitates the polymerization of hemoglobin and distorts the red blood cells (RBCs) to assume a sickle shape, especially when under low oxygen tension and this condition is known as sickle cell anemia (SCA) [1]. Abridged or absent synthesis of the beta globin chains shows variable outcomes ranging from severe anemia to clinically asymptomatic individuals, the disorders being called beta-thalassemias (β-thalassemias) [2]. Similarly, impaired production of alpha globin chains from one, two, three, or all four of the alpha globin genes is called as alpha-thalassemia (α-thalassemia). In addition to SCA, and the beta and alpha thalassemias, there are several documented regional hemoglobinopathies, such as HbC, HbD, HbE, and HbO.

The distribution of hemoglobinopathies varies from place to place, and much of the global burden of hemoglobinopathies is mainly correlated with malaria endemicity [3]. Further, hemoglobin SS disease (SCA) is the most common cause of sickle cell disease (SCD) and is most prevalent in Africa, Asia, and Mediterranean regions [4]. Beta-thalassemia is prevalent in populations of African descent and in regions of the Mediterranean, the Middle East, Transcaucasus, Central Asia, Indian subcontinent, and the Far East. Highest incidences of beta-thalassemia are found in populations of Cyprus (14%), Sardinia (12%), and Southeast Asia [5]. Alpha-thalassemia is more common in sub-Saharan Africa, the Mediterranean Basin, the Middle East, South Asia, and Southeast Asia [6, 7, 8, 9].

In SCA, the deformed RBCs tend to get stuck in narrow blood capillaries and block the blood flow. Patients experience vaso-occlusive crisis (VOC) in their joints and bones, along with severe pain, which causes multiple organ damage (Fig. 2) in SCD patients [10]. Further, these patients – in younger age – have increased susceptibility to infections, acute chest syndrome, and stroke, while – in older age – they are susceptible to retinopathy, as well as damage to the lungs, kidney, and heart [11, 12]. In addition to VOC, sickle cell patients experience sequestration crisis (pooling of blood in an organ), aplastic crisis (reduced function of bone marrow), and hemolytic crisis (rapid breakdown of blood cells). Presence of high levels of fetal hemoglobin (HbF) inhibits polymerization in SCA patients, highlighting the role of HbF ($\alpha 2\gamma 2$) in SCD. Although the pathophysiology of SCA is well understood, its management mainly depends on supportive care. Several lines of evidence show that pharmacological induction of HbF helps in the prevention of intracellular sickling, which in turn reduces hemolysis and vaso-occlusion. Hydroxyurea (HU) is an effective and strong inducer of HbF.

Properties of HU

HU is a ribonucleotide reductase inhibitor that inhibits DNA replication in a wide variety of cells. HU is an antimetabolite cytotoxic drug. HU has excellent oral bioavailability [13], with a biological half-life of about 2-4 hours in both children and adults [14, 15]. The elimination of HU from the blood is relatively rapid and appears to have an acceptable

* Corresponding author at: Senior Scientist, Sickle Cell Institute Chhattisgarh, Genetic Laboratory, Department of Biochemistry, Pt. JNM Medical College, Raipur, Chhattisgarh, India
Mobile: 8224979600, e-mail: lvksbhaskar@gmail.com

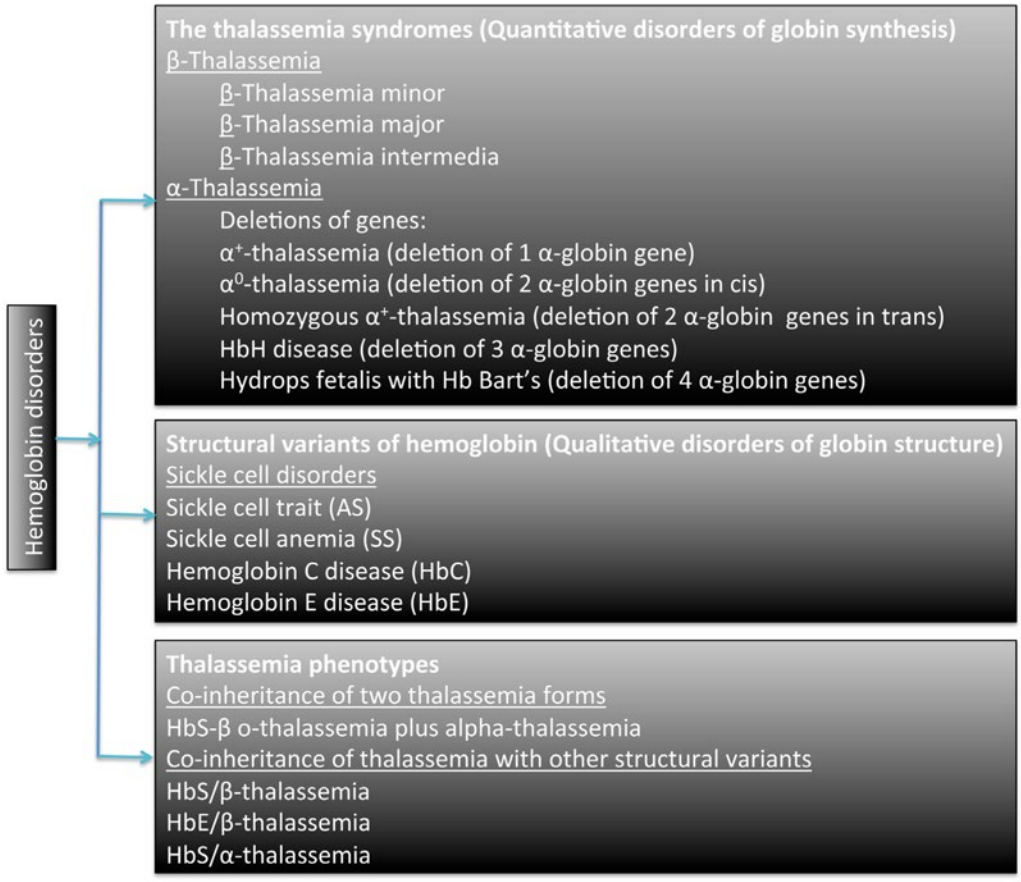

Fig. 1. Classification of inherited hemoglobin disorders

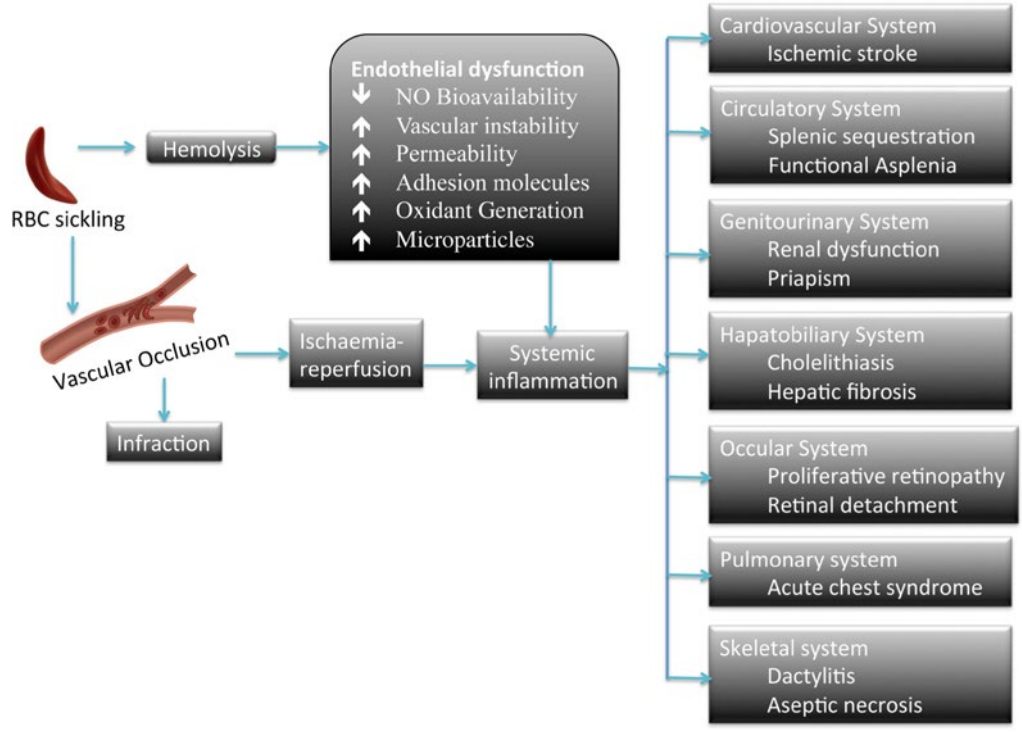

Fig. 2. Complications of sickle cell anemia

safety profile, as indicated by lower hepatic and renal toxicity [16]. Recent studies have demonstrated the involvement of drug transport proteins in the in vivo absorption, cellular distribution, and elimination of HU [17]. HU has long been utilized in both human and veterinary medicine. Although it was first synthesized in 1869, trials for testing the safety of this drug in humans started only after a century [18]. The United States Food and Drug Administration (FDA) in 1967 approved HU for the treatment of certain solid, myeloid tumors. Further, both the US FDA and, in the European Union, the European Medicines Agency (EMA) have approved HU for the treatment of SCD in 1998 and 2007, respectively. The present review focuses on the clinical benefits of HU in SCD and enhances the current understanding of the possible mechanisms of benefit for these hemoglobinopathies.

HbF induction

HU has been in use for the treatment of SCD over many years. The main rationale behind the usage of HU for the treatment of SCD is its ability to induce HbF [19]. The possible cellular and vascular effects of HU are depicted in figure 3. Several lines of evidence suggest that HU elicits HbF induction and offers clinical benefits to SCD patients through a wide range of possible mechanisms [20]. The precise mechanism of HbF induction by HU is not fully known; however, it is mediated mainly by the redox inactivation of a tyrosyl radical on the enzyme ribonucleotide reductase [21]. The absorption, distribution, and excretion of HU vary greatly among individuals. HU causes intermittent cytotoxic suppression of erythroid progenitors and cell stress signaling, which leads to recruitment of erythroid progenitors with increased HbF levels [22, 23, 24]. HU is also involved in free radical formation, iron chelation, activation of soluble guanylyl cyclase, and direct nitric oxide (NO) production [25]. HU shows cytotoxic effects and reduces the absolute numbers of neutrophils, reticulocytes, and platelets in the bone marrow. Reduction of platelets reduces inflammation, while reduction of neutrophils and reticulocytes reduces the surface expression of adhesion receptors and alters the adhesion of RBCs to the endothelium [26].

NO production

NO plays a critical role as a molecular mediator of a variety of physiological processes, including vascular tone regulation and neurotransmission. NO synthesis results from the action of NO synthetase (NOS) on nonessential amino acids, such as arginine, and molecular oxygen [27]. Further, this free-radical gas molecule is produced in vitro by the oxidation HU by heme groups [28]. Significant

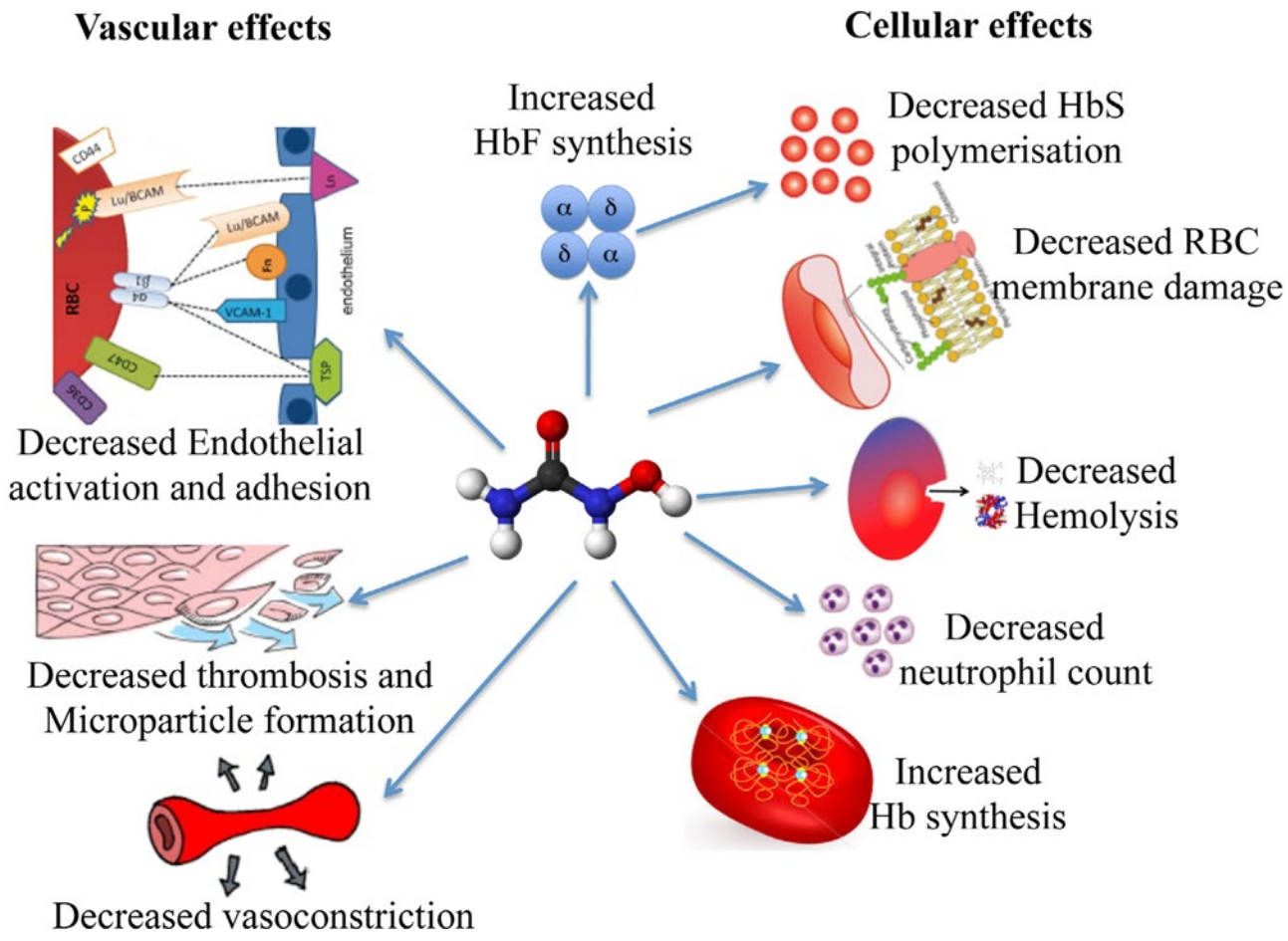

Fig. 3. Multiple effects of hydroxyurea administration in sickle cell disease patients

increase in NO-derived species after an oral dose of HU indicates NO release from HU in vivo [29]. These observations provide a strong argument for the participation of NO in the mechanism of HbF induction by HU [30]. Further, NO can promote the modification of cysteine 93 in the hemoglobin b-chain, by nitrosation [31] or transnitrosation reactions, to form glutathionyl hemoglobin [32], which inhibits HbS polymerization [33]. Downregulation of endothelial expression of vascular cell adhesion molecule (VCAM)-1 has been reported with both NO and HU therapy [34].

Modulation of RBC – endothelial cell interactions

VOCs are the acute complications of SCD and are initiated by the abnormal adhesion of circulating blood cells to the vascular endothelium of the microcirculation. Recent studies have shown that various signalling pathways activate erythroid cell adhesion molecules (CAMs) and their ligands. The intricate network of interactions involving adhesion molecules between sickle RBCs and the endothelial vascular wall has been documented [35]. Modulation of several cellular biophysical properties upon HU treatment has been demonstrated in previous studies [36, 37].

Myelosuppressive effect

Myelosuppression is the dose-limiting effect of HU. Although, HU therapy results in limited myelotoxicity in SCD patients [38], it decreases the level of reticulocytes, neutrophil count, and the rate of crisis [39]. Neutrophils release powerful proinflammatory mediators that play an important role in endothelial damage and release of cytokines, both of which trigger sickling activity [40]. Hence, both neutropenia and neutrophilia have long been reported as markers of severity in SCD [41]. Comparison of polymorphonuclear leukocytes (PMNs) or neutrophils from normal individuals and sickle cell patients has revealed that these cells are less deformable and more rigid in sickle cell patients [42]. HU treatment corrected the dysregulated neutrophil L-selectin expression in SCD patients [43].

Proof of efficacy

In adults, HU increases the amount of total hemoglobin as well as HbF and thereby reduces acute complications, in terms of both number and severity [44]. After studying the safety and efficacy of HU therapy in patients with SCA, HU has been approved for the treatment of adult sickle cell patients [45]. Furthermore, prolonged HU therapy in infants with SCA showed sustained hematologic benefits, reduced acute coronary syndrome (ACS) events, improved growth, and preserved organ function. The Hydroxyurea Safety and Organ Toxicity (HUSOFT) extension study revealed that patients who continued the HU therapy showed better spleen function than expected and improved growth rates [46]. Regeneration of splenic function was also demonstrated in adult patients with severe hemoglobin SC disease [47]. Many studies used level of HbF induction as a predictor of HU therapy. A substantial increase in serum erythropoietin levels has been noted, 2–3 weeks after initiation of HU treatment in SCA and HbS/beta-thalassemia patients [48].

Attenuation of organ dysfunction

Although there was a great improvement in survival for children with SCD, the failure of two or more organ systems is associated with morbidity in SCD. Sickle cell patients develop splenic dysfunction early (4-6 months of age) in the course of their disease [49]. This raises the possibility that HU therapy might be able to exert a significant disease-modifying effect in young children with SCD. The efficacy of HU in preventing acute complications and organ damage in children with SCA was assessed in a Phase III multicenter randomized controlled trial of HU (BABY HUG trial). During this trial, 20 mg HU/kg/day was given to 9- to 18-month-old children with HbSS or sickle b0-thalassemia for a period of 2 years [50]. The Stroke With Transfusions Changing to Hydroxyurea (SWiTCH) trial compared 30 months of alternative therapy (hydroxyurea and phlebotomy) with standard therapy (transfusions and chelation) in the prevention of secondary stroke and reduction of transfusional iron overload [51]. Subsequent reports suggest that HU treatment showed clinical efficacy in children with variable sickle-related organ damage, including proteinuria [26], spleen dysfunction [42], hypoxemia [52], pulmonary hypertension [53], glomerular hyperfiltration [54], neurocognitive delay [53], silent brain infarcts [41], elevated transcranial Doppler (TCD) velocities, and primary stroke [55, 56]. Furthermore, a Belgian multicenter study showed a mean hospital stay of 5.3 days in the HU-treated group and 15.2 days in the placebo group [57].

SCD management with HU

Although there is no cure for SCD, the oral chemotherapeutic drug HU is used for ameliorating the disease and improving life expectancy for most patients. The randomized BABY HUG trial has demonstrated that HU significantly reduces the incidence of VOC and dactylitis in young children [50]. There are no universally agreed indications for the initiation HU therapy in SCD patients. However, team members must review the medical history and discuss the recommendation openly with patients and families before initiating HU therapy. The initial dosage of HU for adults is 15 mg/kg/day; the dose may be reduced further to 10 mg/kg/day in patients with impaired renal function. The HUSOFT and BABY HUG trials demonstrated that 20 mg/kg/day improved hematologic parameters, provided substantial clinical benefits, and had an excellent safety profile [58, 59]. Several clinical trials have reported good clinical outcomes by using a “clinically effective dose” of 15-20 mg/kg/day [60, 61]. The positive effects of HU can be seen within weeks of commencing therapy [62]. The primary toxicity observed was neutropenia. When adjusting dosage, continuous monitoring of complete blood count (CBC) and absolute reticulocyte count (ARC) should be adopted at least every 4 weeks [63].

Further study is needed to evaluate the long-term treatment effects on growth and development, as well as on kidney, lung, and central nervous system function. A randomized, placebo-controlled trial in adults did not demonstrate a significant improvement in the time to resolution of VOC [64]. Adults with SCD should be evaluated for known stroke risk factors and managed according to the 2011 American Heart Association/American Stroke Association (AHA/ASA) primary stroke prevention guidelines. HU or bone marrow

transplantation is the only option for children at high risk for stroke in whom RBC transfusion is contraindicated [65]. HU therapy decreases TCD flow velocities [66], and this decrease may be associated with decreased turbulent flow and the consequent endothelial damage around the stenosis. An open-label pilot study revealed that long-term HU therapy improved cerebral oxygen saturation [67]. However, this improved oxygen saturation may raise the threshold for infarction by augmenting the oxygen reservoir [68]. The BABY HUG trial reported that cerebrovascular events occur only in about 10% of SCD children taking HU therapy [50]. Hence, HU seems to be a highly useful alternative and is relatively free of serious side effects.

Adverse effects of HU

Results of the BABY HUG Trial revealed that HU has an excellent safety profile, and side effects of HU therapy in young patients with SCD are usually low. As HU causes severe myelosuppression, patients should be monitored during treatment for cytopenias very carefully, particularly while seeking the maximum tolerated dose [69]. In children receiving HU therapy, kidney and liver toxicity was not statistically significant compared to the placebo group [70]. Further, these groups showed similar rates of cytopenia, including severe neutropenia, thrombocytopenia, and anemia with reticulocytopenia. Furthermore, a MSH study reported hair loss, skin rash, gastrointestinal disturbance, and fever in the HU-treated group, but it was not statistically significant compared to the placebo group [71]. Cutaneous side effects include nail hyperpigmentation, as well as increased skin pigmentation on the palms and soles [72]. Further, leg ulceration has been reported as a rare cutaneous manifestation of HU therapy in a few studies [73, 74, 75]. Assessment of renal function and the pharmacokinetics of HU indicate that the renal impairment results in increased systemic exposure and decreased urinary recovery of the drug [16]. Some patients receiving HU therapy showed mild albuminuria, with an increase in white cells and granular casts, as well as occasional red cells, in the urine [76]. However, the BABY HUG trial demonstrated that HU is associated with better urine-concentrating ability and less renal enlargement, in addition to improvement in overall renal function [58]. Studies in animal models revealed that HU therapy inhibits spermatogenesis and results in hypogonadism [77]. Semen analysis of SCD patients demonstrated impaired sperm count, motility, and morphology while taking HU therapy [78].

There is increasing concern about the occurrence of malignancy or myelodysplasia in patients with SCD on HU therapy [79, 80]. Several scattered reports document the malignancy that occurs in both children and adults with SCD but do not provide complete information on the incidence of various cancer types [81, 82, 83, 84, 85]. Furthermore, a multicenter study that assessed the risks and benefits for up to 9 years of HU treatment did not show development of secondary leukemia in adults [86]. This indicates that the carcinogenic potential of HU in clinical settings is much less influential. HU is a potent teratogen in all animal species yet tested and thus qualifies as a universal teratogen [87]. The teratogenicity of HU was demonstrated by documenting various anomalies in the central nervous system, palate, as well as the genitourinary, cardiac, ocular, and multiple skeletal systems [88, 89, 90]. As very large doses (> 250 mg/kg per 24 hours) have been reported as teratogenic, the safety of HU therapy in pregnancy remains unclear. Outcome of pregnancy with HU treatment in 31 cases revealed that the there was no major malformation in the case series with exposure to HU [91]. However, this study documented significant rates of intrauterine growth retardation (IUGR), fetal death, and prematurity; hence, careful follow-ups with physical, biological, and sonographic examination are warranted. A follow-up study of the original MSH trial revealed that exposure of the fetus to HU does not cause teratogenic changes [92].

Conclusions

HU is available by prescription in oral tablet, capsule, or oral syrup form. Dose concentrations of HU vary greatly in sickle cell patients, so it is critical to follow the prescription as directed by the doctor in order to see assured treatment results. Hence, SCD patients are initially treated with HU at 10 or 20 mg/kg and then dose-escalated to mild myelosuppression using a standardized regimen. Routine blood monitoring should be performed while the patient receives HU treatment. Treatment with HU should not be initiated if bone marrow function is markedly depressed. Despite the continued and growing clinical experience with HU therapy, several important areas call for further research to overcome the barriers to HU utilization among SCD patients.

Conflict of interest

There are no conflicts of interests.

Authors' contributions

All authors have contributed equally and approve the manuscript.

References

[1] Pauling L, Itano HA, et al. Sickle cell anemia, a molecular disease. Science 1949;110(2865):543–8.

[2] Ruangrai W, Jindadamrongwech S. Genetic factors influencing hemoglobin F level in beta-thalassemia/HB E disease. The Southeast Asian journal of tropical medicine and public health 2016;47(1):84–91.

[3] Kwiatkowski DP. How malaria has affected the human genome and what human genetics can teach us about malaria. Am J Hum Genet 2005;77(2):171–92.

[4] Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. Lancet 2010;376(9757):2018–31.

[5] Cao A, Moi P. Genetic modifying factors in beta-thalassemia. Clin Chem Lab Med 2000;38(2):123–32.

[6] Dehbozorgian J, Moghadam M, Daryanoush S, et al. Distribution of alpha-thalassemia mutations in Iranian population. Hematology 2015;20(6):359–62.

[7] Zhang Q, Fan X, He S, et al. [Gene distribution characteristics of deletional alpha-thalassemia in Guangxi region]. Zhonghua Xue Ye Xue Za Zhi 2014;35(10):941–3.
[8] Kendzhaev AT, Tsibul'skaia MM, Turaev AT, Tadzhieva ZA. [Distribution of alpha-thalassemia in children in Uzbekistan]. Gematol Transfuziol 1993;38(7):37–9.
[9] Zeng YT, Huang SZ, Chen MJ. The types and distribution of alpha--thalassemia-2 in China. Hemoglobin 1988;12(5-6):455–8.
[10] Fuggle P, Shand PAX, Gill LJ, Davies SC. Pain. Quality of Life and coping in Sickle Cell Disease. Archieves of Disease in Childhood 1996;75:199–203.
[11] Kaul DK FM, Nagel RL. Microvascular sites and characteristics of sickle cell adhesion to vascular endothelium in shear flow conditions: pathophysiological implications. Proc Natl Acad Sci USA 1989;86:3356–60.
[12] Steven I, Reger AS, Adams TC, Endredi J, Ranganathan V, Yue GH, Sahgal V, Finneran MT. Classification of large array surface myoelectric potentials from subjects with and without low back pain. Journal of Electromyography and Kinesiology 2006;16(4):392–401.
[13] Rodriguez GI, Kuhn JG, Weiss GR, et al. A bioavailability and pharmacokinetic study of oral and intravenous hydroxyurea. Blood 1998;91(5):1533–41.
[14] De Montalembert M, Bachir D, Hulin A, et al. Pharmacokinetics of hydroxyurea 1,000 mg coated breakable tablets and 500 mg capsules in pediatric and adult patients with sickle cell disease. Haematologica 2006;91(12):1685–8.
[15] Charache S, Dover GJ, Moore RD, et al. Hydroxyurea: effects on hemoglobin F production in patients with sickle cell anemia. Blood 1992;79(10):2555–65.
[16] Yan JH, Ataga K, Kaul S, et al. The influence of renal function on hydroxyurea pharmacokinetics in adults with sickle cell disease. J Clin Pharmacol 2005;45(4):434–45.
[17] Walker AL, Franke RM, Sparreboom A, Ware RE. Transcellular movement of hydroxyurea is mediated by specific solute carrier transporters. Experimental Hematology 2011;39(4):446–56.
[18] Fishbein WN, Carbone PP, Freireich EJ, Misra D, Frei E, 3rd. Clinical Trials of hydroxyurea in patients with cancer and leukemia. Clin Pharmacol Ther 1964;5:574–80.
[19] Afrin LB. Utility of hydroxyurea in mast cell activation syndrome. Exp Hematol Oncol 2013;2(1):28.
[20] Ware RE. How I use hydroxyurea to treat young patients with sickle cell anemia. Blood 2010;115(26):5300–11.
[21] Funamizu N, Kamata Y, Misawa T, et al. Hydroxyurea decreases gemcitabine resistance in pancreatic carcinoma cells with highly expressed ribonucleotide reductase. Pancreas 2012;41(1):107–13.
[22] Ware RE. How I use hydroxyurea to treat young patients with sickle cell anemia. Blood 2010;115(26):5300–11.
[23] Cokic VP, Smith SR, Beleslin-Cokic BB, et al. Hydroxyurea induces fetal hemoglobin by the nitric oxide-dependent activation of soluble guanylyl cyclase. J Clin Invest 2003;111(2):231–9.
[24] Dover GJ, Charache S. Hydroxyurea induction of fetal hemoglobin synthesis in sickle-cell disease. Semin Onco 1992;19(3):61–6.
[25] Gladwin MT, Kato GJ. Cardiopulmonary complications of sickle cell disease: role of nitric oxide and hemolytic anemia. Hematology Am Soc Hematol Educ Program 2005:51–7.
[26] Agrawal RK, Patel RK, Shah V, Nainiwal L, Trivedi B. Hydroxyurea in sickle cell disease: drug review. Indian J Hematol Blood Transfus 2014;30(2):91–6.
[27] Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 2006;113(13):1708–14.
[28] Kim-Shapiro DB, King SB, Shields H, Kolibash CP, Gravatt WL, Ballas SK. The reaction of deoxy-sickle cell hemoglobin with hydroxyurea. Biochim Biophys Acta 1999;1428(2-3):381–7.
[29] Gladwin MT, Shelhamer JH, Ognibene FP, et al. Nitric oxide donor properties of hydroxyurea in patients with sickle cell disease. Br J Haematol 2002;116(2):436–44.
[30] Lepoivre M, Flaman JM, Bobé P, Lemaire G, Henry Y. Quenching of the tyrosyl free radical of ribonucleotide reductase by nitric oxide. Relationship to cytostasis induced in tumor cells by cytotoxic macrophages. J Biol Chem 1994;269(34):21891–7.
[31] Gladwin MT, Schechter AN, Shelhamer JH, et al. Inhaled nitric oxide augments transport of sickle cell hemoglobin without affecting oxygen affinity [see comments]. J Clin Invest 1999;104:937–45.
[32] Kim-Shapiro DB, King SB, Shields H, Kolibash CP, Gravatt WL, Ballas SK. The reaction of deoxy-sickle cell hemoglobin with hydroxyurea. Biochim Biophys Acta 1999;1428(2-3):381–7.
[33] Garel MC, Domenget C, Caburi-Martin J, Prehu C, Galacteros F, Beuzard Y. Covalent binding of glutathione to hemoglobin. I. Inhibition of hemoglobin S polymerization. J Biol Chem 1986;261(31):14704–9.
[34] Strouse JJ, Heeney MM. Hydroxyurea for the treatment of sickle cell disease: efficacy, barriers, toxicity, and management in children. Pediatric Blood & Cancer 2012;59(2):365–71.
[35] Chaar V, Laurance S, Lapoumeroulie C, et al. Hydroxycarbamide decreases sickle reticulocyte adhesion to resting endothelium by inhibiting endothelial lutheran/basal cell adhesion molecule (Lu/BCAM) through phosphodiesterase 4A activation. J Biol Chem 2014;289(16):11512–21.
[36] Ballas SK, Dover GJ, Charache S. Effect of hydroxyurea on the rheological properties of sickle erythrocytes in vivo. Am J Hematol 1989;32(2):104–11.
[37] Hosseini P, Abidi SZ, Du E, et al. Cellular normoxic biophysical markers of hydroxyurea treatment in sickle cell disease. Proc Natl Acad Sci USA 2016;113(34):9527–32.
[38] Charache S, Barton FB, Moore RD, et al. Hydroxyurea and sickle cell anemia: Clinical utility of a myelosuppressive 'switching' agent. Hematology 1996;27:300–26.
[39] Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med 1994;330:1639–44.
[40] Rodgers GP, Dover GJ, Uyesaka N, Noguchi CT, Schechter AN, Nienhuis AW. Augmentation by erythropoietin of the fetal hemoglobin response to hydroxyurea in sickle cell disease. N Engl J Med 1993;328:73–80.
[41] Platt OS. Easing the suffering caused by sickle cell disease. N Engl J Med 1994;17(11):783–4.
[42] Hofstra TC, Kalra VK, Meiselman HJ, Coates TD. Sickle erythrocytes adhere to polymorphonuclear neutrophils and activate the neutrophil respiratory burst. Blood 1996;15(10):4440–7.
[43] Halsey C, Roberts IA. The role of hydroxyurea in sickle cell disease. Br J Haematol 2003;120(2):177–86.

[44] Ferster A, Tahriri P, Vermylen C, et al. Five years of experience with hydroxyurea in children and young adults with sickle cell disease. Blood 2001;97(11):3628–32.

[45] de Montalembert M. [Hydroxyurea treatment in patients affected with sickle cell anemia: efficacy and safety]. Transfus Clin Biol 2008;15(1-2):34–8.

[46] Hankins JS, Ware RE, Rogers ZR, et al. Long-term hydroxyurea therapy for infants with sickle cell anemia: the HUSOFT extension study. Blood 2005;106(7):2269–75.

[47] Huang Y, Ananthakrishnan T, Eid JE. Hydroxyurea-induced splenic regrowth in an adult patient with severe hemoglobin SC disease. American Journal of Hematology 2003;74(2):125–6.

[48] Papassotiriou I, Voskaridou E, Stamoulakatou A, Loukopoulos D. Increased erythropoietin level induced by hydroxyurea treatment of sickle cell patients. Hematol J 2000;1(5):295–300.

[49] Adekile AD, Owunwanne A, Al-Za'abi K, Haider MZ, Tuli M, Al--Mohannadi S. Temporal sequence of splenic dysfunction in sickle cell disease. American Journal of Hematology 2002;69(1):23–7.

[50] Wang WC, Ware RE, Miller ST, et al. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). Lancet 2011;377(9778):1663–72.

[51] Ware RE, Eggleston B, Redding-Lallinger R, et al. Predictors of fetal hemoglobin response in children with sickle cell anemia receiving hydroxyurea therapy. Blood 2002;99(10-14).

[52] Hankins J, Aygun B. Pharmacotherapy in sickle cell disease – state of the art and future prospects. Br J Haematol 2009;145(3):296–308.

[53] Venkataraman A, Adams RJ. Neurologic complications of sickle cell disease. Handb Clin Neurol 2014;120:1015–25.

[54] Haymann JP, Stankovic K, Levy P, et al. Glomerular hyperfiltration in adult sickle cell anemia: a frequent hemolysis associated feature. Clin J Am Soc Nephrol 2010;5(5):756–61.

[55] Nichols FT, Jones AM, Adams RJ. Stroke prevention in sickle cell disease (STOP) study guidelines for transcranial Doppler testing. J Neuroimaging 2001;11(4):354–62.

[56] Hussain S, Nichols F, Bowman L, Xu H, Neunert C. Implementation of transcranial Doppler ultrasonography screening and primary stroke prevention in urban and rural sickle cell disease populations. Pediatric Blood & Cancer. Epub 2014. doi: 10.1002/pbc.25306.

[57] Ferster A, Tahriri P, Vermylen C, et al. Five years of experience with hydroxyurea in children and young adults with sickle cell disease. Blood 2001;97:3628–32.

[58] Alvarez O, Miller ST, Wang WC, et al. Effect of hydroxyurea treatment on renal function parameters: results from the multi-center placebo--controlled BABY HUG clinical trial for infants with sickle cell anemia. Pediatric Blood & Cancer 2012;59(4):668–74.

[59] Wang WC, Ware RE, Miller ST, et al. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). Lancet 2011;377(9778):1663–72.

[60] Ferster A, Vermylen C, Cornu G, et al. Hydroxyurea for treatment of severe sickle cell anemia: a pediatric clinical trial. Blood 1996;88(6):1960–4.

[61] de Montalembert M, Belloy M, Bernaudin F, et al. Three-year follow--up of hydroxyurea treatment in severely ill children with sickle cell disease. The French Study Group on Sickle Cell Disease. Journal of Pediatric Hematology/Oncology 1997;19(4):313–8.

[62] Ballas SK, McCarthy WF, Guo N, et al. Exposure to hydroxyurea and pregnancy outcomes in patients with sickle cell anemia. Journal of the National Medical Association 2009;101:1046–51.

[63] Yawn BP, Buchanan GR, Afenyi-Annan AN, Ballas SK, Hassell KL, James AH, Jordan L, Lanzkron SM, Lottenberg R, Savage WJ, Tanabe PJ, Ware RE, Murad MH, Goldsmith JC, Ortiz E, Fulwood R, Horton A, John-Sowah J . Management of sickle cell disease summary of the 2014 Evidence-Based Report by Expert Panel Members. JAMA 2014;312(10):1033–48.

[64] Gladwin MT, Kato GJ, Weiner D, et al. Nitric oxide for inhalation in the acute treatment of sickle cell pain crisis: a randomized controlled trial. JAMA 2011;305(9):893–902.

[65] Goldstein LB, Bushnell CD, Adams RJ, et al. Guidelines for the primary prevention of stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2011;42(2):517–84.

[66] Kratovil T, Bulas D, Driscoll MC, Speller-Brown B, McCarter R, Minniti CP. Hydroxyurea therapy lowers TCD velocities in children with sickle cell disease. Pediatric Blood & Cancer 2006;47(7):894–900.

[67] Tavakkoli F, Nahavandi M, Wyche MQ, Castro O. Effects of hydroxyurea treatment on cerebral oxygenation in adult patients with sickle cell disease: an open-label pilot study. Clin Ther 2005;27(7):1083–8.

[68] Wagner DD, Frenetle PS. The vessel wall and its interactions. Blood 2008;111(11):5271–81.

[69] Vichinsky EP, Luban NL, Wright E, et al. Prospective RBC phenotype matching in a stroke-prevention trial in sickle cell anemia: a multicenter transfusion trial. Transfusion 2001;41(9):1086–92.

[70] Wang WC, Wynn LW, Rogers ZR, Scott JP, Lane PA, Ware RE. A two--year pilot trial of hydroxyurea in very young children with sickle-cell anemia. The Journal of Pediatrics 2001;139(6):790–6.

[71] Kutlar A, Woods KF, Clair B, et al. Long term use of hydroxyurea in adults with sickle cell disease: a large single centre experience. Blood 2000;96(10).

[72] O'Branski EE, Ware RE, Prose NS, Kinney TR. Skin and nail changes in children with sickle cell anemia receiving hydroxyurea therapy. J Am Acad Dermatol 2001;44:859–61.

[73] Kikuchi K, Arita K, Tateishi Y, Onozawa M, Akiyama M, Shimizu H. Recurrence of hydroxyurea-induced leg ulcer after discontinuation of treatment. Acta Derm Venereol 2011;91(3):373–4.

[74] Dissemond J, Hoeft D, Knab J, Franckson T, Kroger K, Goos M. Leg ulcer in a patient associated with hydroxyurea therapy. Int J Dermatol 2006;45(2):158–60.

[75] Poros A, Nadasdy K. Leg ulcer in hydroxyurea-treated patients. Haematologia (Budap) 2000;30(4):313–8.

[76] Samuels ML, Howe CD. Renal Abnormalities Induced by Hydroxyurea (Nsc-32065). Cancer Chemother Rep 1964;40:9–13.

[77] Jones KM, Niaz MS, Brooks CM, et al. Adverse effects of a clinically relevant dose of hydroxyurea used for the treatment of sickle cell disease on male fertility endpoints. Int J Environ Res Public Health 2009;6(3):1124–44.

[78] Grigg A. Effect of hydroxyurea on sperm count, motility and morphology in adult men with sickle cell or myeloproliferative disease. Intern Med J 2007;37(3):190–2.

[79] Schultz WH, Ware RE. Malignancy in patients with sickle cell disease. American Journal of Hematology 2003;74(4):249–53.

[80] Stricker RB, Linker CA, Crowley TJ, Embury SH. Hematologic malignancy in sickle cell disease: report of four cases and review of the literature. American Journal of Hematology 1986;21(2):223–30.

[81] Goldin AG, Kelty KC, Beard MF. Sickle cell anemia terminating in acute myeloblastic leukemia. Ann Intern Med 1953;39(4):920–8.

[82] Labi M, Haponik EF, Welsh RA, Summer WR. Alveolar cell carcinoma complicating sickle cell anemia: a chance occurrence? American Journal of Hematology 1989;32(3):222–5.

[83] Marsh A, Golden C, Hoppe C, Quirolo K, Vichinsky E. Renal medullary carcinoma in an adolescent with sickle cell anemia. Pediatric Blood & Cancer 2014;61(3):567.

[84] Avendano-Garcia M, Mercado U, Marin ME. A case of Peutz-Jeghers syndrome associated with duodenal carcinoma and sickle cell anemia. Am J Gastroenterol 2002;97(3):762–3.

[85] Baron BW, Mick R, Baron JM. Hematuria in sickle cell anemia – not always benign: evidence for excess frequency of sickle cell anemia in African Americans with renal cell carcinoma. Acta Haematol 1994;92(3):119–22.

[86] Steinberg MH, Barton F, Castro O, et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. JAMA 2003;289(13):1645–51.

[87] Lankas GR, Wise LD, Cartwright ME, Pippert T, Umbenhauer DR. Placental P-glycoprotein deficiency enhances susceptibility to chemically induced birth defects in mice. Reprod Toxicol 1998;12(4):457–63.

[88] Chaube S, Murphy ML. The effects of hydroxyurea and related compounds on the rat fetus. Cancer Res 1966;26(7):1448–57.

[89] Khera KS. A teratogenicity study on hydroxyurea and diphenylhydantoin in cats. Teratology 1979;20(3):447–52.

[90] Wilson JG, Scott WJ, Ritter EJ, Fradkin R. Comparative distribution and embryotoxicity of hydroxyurea in pregnant rats and rhesus monkeys. Teratology 1975;11(2):169–78.

[91] Thauvin-Robinet C, Maingueneau C, Robert E, et al. Exposure to hydroxyurea during pregnancy: a case series. Leukemia 2001;15(8):1309–11.

[92] Ballas SK, McCarthy WF, Guo N, et al. Exposure to hydroxyurea and pregnancy outcomes in patients with sickle cell anemia. J Natl Med Assoc 2009;101(10):1046–51.