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Scientific Comment

Haptoglobin: an emerging candidate for phenotypic modulation of sickle cell anemia?☆

Magnun Nueldo Nunes dos Santos

Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil

Sickle cell anemia (SCA) is characterized by a single homozygous mutation (A→T) in the sixth codon of the β-globin gene that results in hemoglobin S (Hb S), in which a glutamic acid residue is substituted by valine in the sixth position of the β-globin chain (HBB; glu(E)6val(A); GAG-GTG; rs334).[1] This change leads to a wide variety of symptoms, including chronic intravascular hemolysis, increased cell-free plasma hemoglobin (Hb) and heme levels and vascular alterations.[2] SCA has been characterized as a chronic inflammatory state with abnormal endothelial activation as a result of various associated factors. The mechanisms that induce the production of inflammatory mediators and the effects of these molecules on the inflammatory response are little understood in this disease.[3]

Sickled red blood cells are stiff and therefore have a predisposition to hemolysis; one third of the cells are destroyed in the intravascular space leading to increased cell-free plasma Hb and heme levels.[4] The pathophysiological effects associated with free Hb/heme are acute hemodynamic instability and acute or chronic vascular injury.[5] The toxicity and inflammatory nature of free Hb are a result of the greater nitric oxide consumption it promotes and the consequent accumulation of hydroxyl radicals and reactive oxygen species in the blood vessels. The organism's first defense mechanism against the harmful effects of free Hb involves haptoglobin (Hp), whose primary function is to bind to free Hb in the plasma, thereby preventing the excretion of iron by the kidneys and protecting blood vessels from its oxidative effects.[6,7] In addition to being an antioxidant, Hp is a positive acute-phase glycoprotein present in plasma with immunomodulatory properties.[6,7] It is mainly synthesized in hepatocytes whose production is induced by the secretion of interleukin (IL)-6, IL-1β and tumor necrosis factor-alpha (TNF-α).[8] Hp has a strong affinity for Hb, forming a highly stable complex by binding to Hb dimers (Figure 1). This Hb–Hp complex binds to CD163 receptors expressed on the surface of macrophages in the spleen, liver, bone marrow and kidneys, and is removed from the blood vessels.[9] However, when this system for eliminating free Hb is overloaded because of intravascular hemolysis, as in SCA, free Hb/heme trigger adverse clinical effects, such as nitric oxide depletion, oxidative stress, endothelial dysfunction and exposure of the kidneys to the iron in Hb/heme.[4]

In humans, the HP gene, on the long arm of chromosome 16 (16q22), is polymorphic. There are two principal codominant alleles (HP1 and HP2) resulting in three main genotypes/phenotypes (Hp1-1, Hp2-1 and Hp2-2), which have distinct biochemical and biophysical properties and have been correlated to susceptibility to and clinical evolution of various diseases.[6,7,10] The HP2 allele is a partially duplicated gene derived from a rare unequal crossover between the HP1 alleles. Because of its large size, the Hp2-2 protein has been shown to be less efficient at clearing Hb from the plasma than the Hp1-1 protein. Thus, subjects with Hp2-2 are more prone to oxidative stress.[6,11,12]

Recently, several studies have investigated the possibility of the therapeutic use of Hp in hemolytic anemias.[4,5,13] It was demonstrated in guinea pigs that Hp is efficient at preventing arterial hypertension and kidney damage induced by plasma free Hb.[4] More recently, Chintagari et al., in an *in vitro* study and in mice with sickle cell disease (SCD), showed that Hp

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Corresponding author at: Department of Clinical Pathology, School of Medical Sciences, Universidade Estadual de Campinas (UNICAMP), 13083-970 Campinas, SP, Brazil.

E-mail addresses: magnun@fcm.unicamp.br, magnun.nunes@gmail.com

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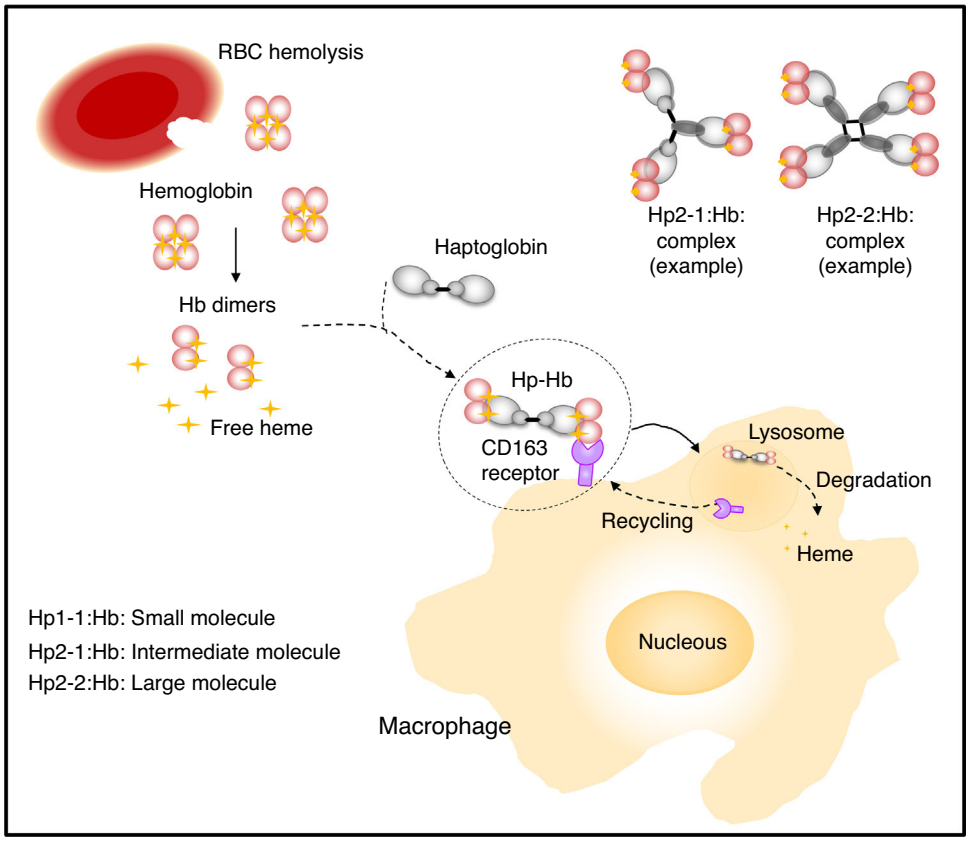

Figure 1 – The Hp–Hb complex is cleared by the CD163 receptors expressed on the surface of macrophages and digested in lysosomes to release heme. The Hp2-2 protein removes iron from circulation more slowly because it is a larger molecule. Consequently, in individuals with this phenotype, free Hb remains in the circulation longer and causes greater oxidative stress. On the other hand, the Hp1-1 phenotype may be advantageous because the Hp1-1:Hb complex is removed to the extravascular space more rapidly than the other Hp complexes.

attenuates the expression of heme oxygenase-1 (HO-1), an important antioxidant and anti-inflammatory protein that, stimulated by plasma free Hb, mediates the degradation of heme to biliverdin, iron, and carbon monoxide.[14] These results suggest that treatment with Hp can reduce the toxicity of free Hb after hemolysis.[9] Preclinical studies are being carried out with a view to evaluate how Hp could be used as a specific modulator in SCD and investigate possible adverse effects of its use. A better understanding of this mechanism could provide more knowledge to exploit the therapeutic potential of Hp to neutralize free Hb/heme in hemolytic anemias.[4,5]

In this volume of the *Revista Brasileira de Hematologia e Hemoterapia*, Pierrot-Gallo et al.[15] analyze the possible influence of Hp genotypes on the secretion of IL-6 and IL-8 in 60 patients with stable SCA (aged 15–50 years old, 53% males; recruited in the Hereditary Anemia Outpatient Clinic of the Hematology and Blood Transfusion Unit, Universidade Federal de São Paulo – UNIFESP, Brazil). A group of 74 apparently healthy individuals (aged 17–60 years old, 53% males; from the same Brazilian region) was used as a control. In the study, SCA patients were found to have significantly higher levels of IL-6 and IL-8 than controls, corroborating other studies. However, no associations between Hp genotypes and these cytokines were identified. Furthermore, an investigation of the prevalence of Hp genotypes did not reveal any significant differences.

This difficulty in evaluating a candidate phenotype modulator (the Hp polymorphism) in a multifactorial disease such as SCA can be seen from the authors' comments on the lack of agreement between the results for the distribution of the Hp genotypes in studies of this kind. The Hp2-2 genotype was found to be less represented in SCD patients in Nigeria ($n = 54$)[16] and in SCA patients in the northeast of Brazil ($n = 599$).[17] On the other hand, the Hp1-1 genotype was less represented in SCD patients from Kuwait ($n = 82$)[16] and in SCA patients from the southeast of Brazil ($n = 60$).[15] This disagreement may reflect the genetic background of the patients as well as the influence of environmental factors and, especially, the sample size, characteristics and quality in each study. The representativeness of the number of patients analyzed, the homogeneity of the criteria used to select patients, the presence of pediatric or adult patients in the sample and the extent to which the control groups are formed and numerically equal to or greater than the patient groups are some of the factors that can cause heterogeneity when making this type of comparison. Furthermore, in multifactorial diseases the effects of multiple genes and numerous environmental factors are expected to be additive and to be associated with each other,

leading to a multiplicity of effects that quite often makes it virtually impossible to analyze each factor in isolation.

The results reported by Pierrot-Gallo et al.[15] do not support a conclusion that the Hp genotypes can modulate the inflammatory response in the patients they studied. Nevertheless, the possibility that Hp polymorphisms may contribute to the clinical diversity observed in this condition, in combination with other genetic and environmental factors, should not be excluded. Studies of the inflammatory components using more representative sample sizes together with experimental analyses could help to elucidate important aspects of the clinical behavior and survival of SCA patients.

Conflicts of interest

The author declares no conflicts of interest.

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REFERENCES

1. Steinberg MH. Sickle cell anemia, the first molecular disease: overview of molecular etiology, pathophysiology, and therapeutic approaches. ScientificWorldJournal. 2008;8:1295–324.
2. Camus SM, De Moraes JA, Bonnin P, Abbyad P, Le Jeune S, Lionnet F, et al. Circulating cell membrane microparticles transfer heme to endothelial cells and trigger vasoocclusions in sickle cell disease. Blood. 2015;125(24):3805–14.
3. Lanaro C, Franco-Penteado CF, Albuqueque DM, Saad ST, Conran N, Costa FF. Altered levels of cytokines and inflammatory mediators in plasma and leukocytes of sickle cell anemia patients and effects of hydroxyurea therapy. J Leukoc Biol. 2009;85(2):235–42.
4. Schaer DJ, Buehler PW, Alayash AI, Belcher JD, Vercellotti GM. Hemolysis and free hemoglobin revisited: exploring hemoglobin and hemin scavengers as a novel class of therapeutic proteins. Blood. 2013;121(8):1276–84.
5. Schaer DJ, Vinchi F, Ingoglia G, Tolosano E, Buehler PW. Haptoglobin, hemopexin, and related defense pathways-basic science, clinical perspectives, and drug development. Front Physiol. 2014;5:415.
6. Melamed-Frank M, Lache O, Enav BI, Szafranek T, Levy NS, Ricklis RM, et al. Structure–function analysis of the antioxidant properties of haptoglobin. Blood. 2001;98(13):3693–8.
7. Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. Clin Chem. 1996;42(10):1589–600.
8. Van Vlierberghe H, Langlois M, Delanghe J. Haptoglobin polymorphisms and iron homeostasis in health and in disease. Clin Chim Acta. 2004;345(1-2):35–42.
9. Chintagari NR, Nguyen J, Belcher JD, Vercellotti GM, Alayash AI. Haptoglobin attenuates hemoglobin-induced heme oxygenase-1 in renal proximal tubule cells and kidneys of a mouse model of sickle cell disease. Blood Cells Mol Dis. 2015;54(3):302–6.
10. Goldenstein H, Levy NS, Levy AP. Haptoglobin genotype and its role in determining heme-iron mediated vascular disease. Pharmacol Res. 2012;66(1):1–6.
11. Asleh R, Ward J, Levy NS, Safuri S, Aronson D, Levy AP. Haptoglobin genotype-dependent differences in macrophage lysosomal oxidative injury. J Biol Chem. 2014;289(23):16313–25.
12. Veiner HL, Gorbatov R, Vardi M, Doros G, Miller-Lotan R, Zohar Y, et al. Pharmacogenomic interaction between the Haptoglobin genotype and vitamin E on atherosclerotic plaque progression and stability. Atherosclerosis. 2015;239(1):232–9.
13. Alayash AI, Andersen CB, Moestrup SK, Bulow L. Haptoglobin: the hemoglobin detoxifier in plasma. Trends Biotechnol. 2013;31(1):2–3.
14. Araujo JA. HO-1 and CO: fighters vs sickle cell disease? Blood. 2013;122(15):2535–6.
15. Pierrot-Gallo BS, Vicari P, Matsuda SS, Adegoke SA, Mecabo G, Figueiredo MS. Haptoglobin gene polymorphisms and interleukins 6 and 8 levels in patients with sickle cell anaemia. Rev Bras Hematol Hemoter. 2015;37(5):329–35.
16. Adekile AD, Haider MZ. Haptoglobin gene polymorphisms in sickle cell disease patients with different betaS-globin gene haplotypes. Med Princ Pract. 2010;19(6):447–50.
17. Santos MN, Bezerra MA, Domingues BL, Zaccariotto TR, Oliveira DM, Costa FF, et al. Haptoglobin genotypes in sickle-cell disease. Genet Test Mol Biomarkers. 2011;15(10):709–13.