Check for updates

genetic iron-refractory iron deficiency anemia, which is associated with high hepcidin levels and iron restriction.[4]

Most of our understanding of hepcidin regulation derives from clinical studies and animal models. For example, the centrality of the bone morphogenetic protein/sons of mothers against decapentaplegic (BMP/SMAD) pathway originated from the observation that liver conditional ablation of SMAD4 fully suppressed hepcidin expression and caused severe iron overload.[5] Interestingly, the article by Mleczko-Sanecka et al[1] supports and reinforces this centrality. The crucial involvement of hemojuvelin (HJV) and of the protease that controls its functionality (TMPRSS6 or matriptase-2) was indicated by genetic studies. Similar approaches identified several other genes that participate in hepcidin control, including transferrin receptor 2 (TfR2), the inhibitory SMAD7, hypoxia inducible factor, erythropoietin, soluble factors of the transforming growth factor beta family, growth factors, and testosterone. Among them is HFE, the major cause of hereditary hemochromatosis. Although frequently studied, it has not been fully clarified how HFE affects the BMP-HJV-SMAD pathway and regulates hepcidin expression and which are the genetic and/or environmental modifiers that modify the penetrance of the C282Y mutation of HFE. This genome-wide, unbiased methodology used by the Muckenthaler-Hentze team goes some way to identify such genetic factors (see figure).

The approach used is original and is carried out in a rigorous way. Mleczko-Sanecka et al used a library of small interfering RNA pools targeting almost 20 000 human genes. It was applied in a high-throughput system to modulate the activity of the hepcidin promoter-luciferase reporter system. The response range of interest was defined in such a way that it identified 1651 putative activators and 508 putative inhibitors, which included several of the known activators and inhibitors. This long list of putative hepcidin regulators is enriched for genes involved in signal transduction, transcriptional regulation, and defense and/or inflammatory responses and is a precious source for future analyses. Bioinformatics and literature mining were used for the next difficult step to restrict the analysis to a limited number of genes. Genes involved in signal transduction and transcriptional regulation were chosen, and 15 (25%) of them were validated in stringent secondary assays.

Further analysis showed that most of the genes needed functional BMP/SMAD responsive elements to regulate the hepcidin promoter, while none were affected by the inactivation of the inflammatory interleukin-6 (IL-6) responsive element. Next, they found that the downregulation of the genes caused pronounced alteration of proteins controlling the rat sarcoma-mitogen activated protein kinase (Ras-MAPK) and rho signaling (Ras homolog signaling), which are interconnected with β-catenin and mammalian target of rapamycin (mTOR) signaling. The involvement of these pathways was verified by specific drug inhibitors, such as sorafenib, wortmannin, rapamycin, and metformin. In various hepatic cells, hepcidin messenger RNA was induced by these drugs and, conversely, it was experimentally suppressed by the stimulation of the pathways.

The study has a number of important implications. First, it confirmed that the BMP-HJV-SMAD signaling pathway is the core axis of hepcidin control and supports it to be the major target for pharmacologic control of hepcidin expression.[6] Second, it shows that the signaling can be modified by cross-talking with other pathways, including well-characterized ones such as the nutrient-sensing mTOR and proliferative Ras/RAF pathways. This may help explain the known effects of growth factors, cytokines, and liver stress conditions on hepcidin expression. Probably more important is that some of these pathways are the targets of widely used drugs, and their effects may be tested in vivo in animal models with abnormal hepcidin expression. For example, sorafenib is in clinical use for treatment of hepatocellular carcinoma, and its capacity to upregulate hepcidin may cause local iron restriction and may contribute to its antitumor activity.[7] A recent retrospective clinical study showed that the use of the mTOR inhibitor rapamycin is associated with mild microcytic anemia, likely attributable to iron restriction.[8] It remains to be demonstrated that this is linked to hepcidin excess, as predicted by Mleczko-Sanecka et al.[1]

In conclusion, this work leave us with an elegant and rigorous approach to finding genes involved in hepcidin regulatory pathways. It is expected to stimulate future work to characterize the role of the newly identified signaling pathways in the pathogenicity of iron-related diseases.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Mleczko-Sanecka K, Roche F, da Silva AR, et al. Unbiased RNAi screen for hepcidin regulators links hepcidin suppression to the proliferative Ras/RAF and the nutrient-dependent mTOR signaling pathways. *Blood*. 2014;123(10):1574-1585.
2. Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*. 2004;306(5704):2090-2093.
3. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of Mammalian iron metabolism. *Cell*. 2010;142(1):24-38.
4. Finberg KE, Heeney MM, Campagna DR, et al. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). *Nat Genet*. 2008;40(5):569-571.
5. Wang RH, Li C, Xu X, et al. A role of SMAD4 in iron metabolism through the positive regulation of hepcidin expression. *Cell Metab*. 2005;2(6):399-409.
6. Nemeth E. Targeting the hepcidin-ferroportin axis in the diagnosis and treatment of anemias. *Adv Hematol*. 2010;2010:750643.
7. Llovet JM, Ricci S, Mazzaferro V, et al; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*. 2008;359(4):378-390.
8. Sofroniadou S, Kassimatis T, Goldsmith D. Anaemia, microcytosis and sirolimus—is iron the missing link? *Nephrol Dial Transplant*. 2010;25(5):1667-1675.

© 2014 by The American Society of Hematology

Downloaded from http://ashpublications.net/blood/article-pdf/123/10/1434/1374447/1434.pdf by guest on 09 June 2024

● ● ● CLINICAL TRIALS & OBSERVATIONS

Comment on Pennell et al, page 1447

Bad liver and a broken heart

Thomas D. Coates[1,2] [1]CHILDREN'S HOSPITAL LOS ANGELES; [2]UNIVERSITY OF SOUTHERN CALIFORNIA KECK SCHOOL OF MEDICINE

In this issue of *Blood*, the CORDELIA study presented by Pennell and colleagues shows that deferasirox (DFX; Exjade, Novartis) is not inferior to deferoxamine (DFO; Desferal, Novartis) for the removal of cardiac iron in β-thalassemia.[1] CORDELIA also supports previous findings[2] that efficacy of cardiac iron removal is better if liver iron concentration (LIC) is low.

As discussed by the authors,[1] patients with transfusion-dependent anemia of any type rapidly become iron overloaded. Unless the excess iron is removed, patients develop pan-endocrine failure, liver failure, and ultimately death from cardiac failure or arrhythmia, usually in the second decade of life.

The development of noninvasive techniques to measure tissue iron (reviewed in Wood[3]) in the past decade resulted in tremendous advances in our understanding of clinical iron overload. The ability to sequentially monitor individual organ iron uptake and removal in humans and the current understanding of iron homeostasis (reviewed in Ganz[4]) allows us to begin to understand the mechanisms of transfusional iron overload in patients.

The distribution of iron loading in various organs depends in part on differences in iron-regulatory proteins. Iron levels in humans normally are controlled through modulation of iron absorption and regulation of iron recycled by macrophage phagocytosis of senescent erythrocytes. Iron enters the plasma from enterocytes and macrophages via ferroportin (FPN) and binds to plasma transferrin. Once approximately 30% of transferrin is saturated, non–transferrin-bound iron (NTBI) and labile plasma iron (LPI) begin to appear in plasma and significantly rise at Tf saturation >60%. Trf1 is plentiful in erythroid precursors and liver but is not a major regulator of uptake in endocrine organs or heart. NTBI/LPI is thought to enter the heart through voltage-regulated calcium channels and can easily enter the liver and pancreas through other non–transferrin-mediated mechanisms. Once iron enters the heart, the rate of iron transport into the heart increases significantly. FPN is the only known cellular iron exporter and is present in high levels on duodenal enterocytes, macrophages, liver, and placenta but in low levels in heart and pancreas. It is regulated at the transcriptional level and by the iron-regulatory peptide hepcidin. When hepcidin binds to FPN, it causes FPN to be internalized, blocking cellular iron export and lowering plasma iron levels. Hepcidin is made in the liver, and its expression is increased by transferrin saturation and inflammation and decreased by iron deficiency and erythroid activity. Thus, increased erythroid activity, as seen in β-thalassemia, where erythropoiesis is ineffective, decreases hepcidin, even in the face of iron overload, causing increased iron absorption (reviewed in Ganz[4]).

This fascinating cellular physiology has been worked out primarily in transgenic mice and cell culture. However, albeit via circumstantial evidence, these data seem to fit nicely with what is observed by magnetic resonance imaging monitoring of iron loading and unloading in humans. The liver, which can load easily via transferrin-mediated or non–transferrin-mediated processes, takes up iron very quickly. The heart and pancreas, which load primarily through non–transferrin-mediated processes, load with iron later, only after transferrin has become completely saturated, the liver has loaded, and NTBI/LPI levels have been high for a while. Conversely, iron unloads fastest (T1/2 about 4.5 months) from the liver, which expresses the iron exporter FPN, and slowest from the heart (T1/2 17 months), which contains very little FPN.[3]

What does all of this have to do with CORDELIA? First, this is a large, well-designed, and well-executed study in β-thalassemia patients with cardiac iron that clearly confirms previous single-arm studies[2,5-7] showing that DFX can reduce cardiac iron burden. Second, it demonstrates for the first time that DFX is at least as good, if not better, than DFO for the treatment of cardiac iron overload with normal cardiac function. What is most interesting about this study is the suggestion that the ability to clear cardiac iron is better in patients whose liver iron is lower to start (Figure 2D-F in Pennell et al[1]). In particular, DFX seems to be better than DFO at clearing cardiac iron when the baseline LIC is <7 mg/g. The difference between DFO and DFX, as well as the effectiveness at lowering cardiac iron, seems to disappear when the baseline LIC is higher. This is consistent with our findings that LIC and, more importantly, the ability to clear the liver of iron predicted which patients would clear their heart with DFX.[2] Considering that LPI rises quickly when LIC rises and that a decrease in LPI is associated with clearance of cardiac iron by DFX,[3] it is logical to hypothesize that a lower LIC would improve clearance of cardiac iron by reducing the pool of free iron that can re-enter the heart. Table 1 is based on rough estimates from the cited articles but shows the reduction in cardiac iron in several studies where the baseline LIC could be deduced and classified in a similar fashion to CORDELIA. All studies showed a decrease in cardiac iron after 1 year, except for the "nonresponder" group in our study.[2] With the exception of one study,[8] DFO was the least effective. Whether as a single agent[9] or in combination,[8,10] deferiprone (DFP) was the most effective at reducing cardiac iron. There is a suggestion that less cardiac clearance occurs in the high-LIC group, especially in the 2 studies that specifically addressed this question.[1,2] We cannot really tell if LIC has an effect on DFP clearance of heart iron. However, it is very clear from Table 1 that DFP is the only agent associated with significant improvement in left ventricular ejection fraction (LVEF) within 1 year.[8-10] We and

Table 1. Change in cardiac iron concentration in response to chelation as a function of baseline LIC

Base LIC	Agent	Base cardiac T2* (ms)	Change cardiac Fe	Base LVEF	12-mo LVEF	Reference
Low (<7)	DFO	13.5	−12.06%	68.4%	68.9%	9
	DFO	13.1	−10.14%	66.4%	66.4%	1
	DFP	13	−25.79%	***69.7%***	***72.8%***	9
	DFX	12.7	−27.87%	66.9%	66.3%	1
	DFP+DFO	11	−41.20%	***65.8%***	***68.4%***‖	8
Medium (7-15)	DFO	12.4	−25.01%	64.7%	65.3%	8
	DFO	13.2	−13.03%	66.4%	66.4%	1
	DFX	10.5	−28.35%	62.1%	62.7%	2
	DFX	12	−18.57%	66.9%	66.3%	1
	DFP+DFO	5.7	−32.85%	***51.2%***	***65.6%***	10
High (>15)	DFO	11.1	−5.23%	66.4%	66.4%	1
	DFX	10.8	−9.30%	66.9%	66.3%	1
	DFX	8.25	12.33%	62.4%	62.1%	2
	DFX	11.2	−16.63	67.5%	67.7%	6

Base LIC in mg/g dry weight liver. Reference 2 classed based on "responder" ~ medium and "nonresponder" ~ high. Cardiac Fe change calculated in Pennell et al[1] from change in T2* using $[Fe] = 45 \times (T2^*)^{-1.22}$. Pre to 12-mo LVEF cells in bold italics are significantly different. $P < .01$; ‖$P < .05$.

Downloaded from http://ashpublications.net/blood/article-pdf/123/10/1434/1374447/1434.pdf by guest on 09 June 2024

others have observed LVEF improvement in selected patients after a longer treatment with chelators other than DFP.[2]

Table 1 makes it clear that several chelators are available that are effective at clearing liver and cardiac iron, and future studies will help elucidate mechanisms of action. None of these studies solve the overwhelmingly largest problem in the management of transfusional iron overload, which is the fact that patients do not take their medication and that deaths continue to occur because of poor adherence.

Conflict-of interest disclosure: T.D.C. is a consultant for Shire Pharma, Novartis, and Apo Pharma and is on the Speakers Bureau of Novartis and has received honoraria for services rendered from all 3 companies but has no stock or ownership in any of these firms.

REFERENCES

1. Pennell DJ, Porter JB, Piga A, et al; CORDELIA study investigators. A 1-year randomized controlled trial of deferasirox vs deferoxamine for myocardial iron removal in β-thalassemia major (CORDELIA). *Blood*. 2014;123(10):1447-1454.

2. Wood JC, Kang BP, Thompson A, et al. The effect of deferasirox on cardiac iron in thalassemia major: impact of total body iron stores. *Blood*. 2010;116(4):537-543.

3. Wood JC. Impact of iron assessment by MRI. *Hematology Am Soc Hematol Educ Program*. 2011;2011: 443-450.

4. Ganz T. Systemic iron homeostasis. *Physiol Rev*. 2013;93(4):1721-1741.

5. Pennell DJ, Porter JB, Cappellini MD, et al. Deferasirox for up to 3 years leads to continued improvement of myocardial T2* in patients with β-thalassemia major. *Haematologica*. 2012;97(6): 842-848.

6. Pennell DJ, Porter JB, Cappellini MD, et al. Continued improvement in myocardial T2* over two years of deferasirox therapy in β-thalassemia major patients with cardiac iron overload. *Haematologica*. 2011;96(1): 48-54.

7. Pennell DJ, Porter JB, Cappellini MD, et al. Efficacy of deferasirox in reducing and preventing cardiac iron overload in beta-thalassemia. *Blood*. 2010;115(12): 2364-2371.

8. Tanner MA, Galanello R, Dessi C, et al. A randomized, placebo-controlled, double-blind trial of the effect of combined therapy with deferoxamine and deferiprone on myocardial iron in thalassemia major using cardiovascular magnetic resonance. *Circulation*. 2007; 115(14):1876-1884.

9. Pennell DJ, Berdoukas V, Karagiorga M, et al. Randomized controlled trial of deferiprone or deferoxamine in beta-thalassemia major patients with asymptomatic myocardial siderosis. *Blood*. 2006;107(9): 3738-3744.

10. Tanner MA, Galanello R, Dessi C, et al. Combined chelation therapy in thalassemia major for the treatment of severe myocardial siderosis with left ventricular dysfunction. *J Cardiovasc Magn Reson*. 2008;10(1):12.

© 2014 by The American Society of Hematology

Comment on Mereuta et al, page 1479

LECT2 makes the amyloid list

Raymond L. Comenzo[1] [1]TUFTS UNIVERSITY

In this issue of *Blood*, Mereuta et al report that we must now add leukocyte cell–derived chemotaxin 2 (LECT2) to the list of proteins that can cause systemic amyloidosis,[1] a fibrillar protein deposition disease that leads to end-organ damage and related symptoms and requires a tissue diagnosis demonstrating apple-green birefringence in Congo red–stained sections viewed microscopically under polarized light.[2] When dissected from affected tissue, digested into protein fragments of different lengths for proteomic analysis, and assessed by mass spectrometry for their original constituents, amyloid deposits reveal a unique signature of chaperones such as apolipoprotein E and serum amyloid P-component (arrows) as well as the identity of the critical main culprit: the amyloid-forming protein (red boxes).[3]

This application of mass spectrometry is most useful because the types of amyloid cannot be routinely distinguished in the clinic and because the different types are managed or treated differently.[4] Common types of amyloid-forming proteins are immunoglobulin light chains, transthyretin (TTR) (both mutant and wild-type), serum amyloid A (SAA), and now LECT2. Despite our limited knowledge of LECT2-associated amyloidosis (ALect2) pathobiology, the findings Mereuta et al report immediately impact our clinical approach to certain patients with a tissue diagnosis of amyloidosis.[1]

The *LECT2* gene has been conserved throughout vertebrate evolution and in humans is inducible in the liver in association with fatty infiltration, hepatocellular carcinoma, and regeneration. LECT2 may also participate in innate immunity.[4,5] The basis for the 16-kDa LECT2 protein forming amyloid has not been studied in detail, but the presumption is that, as in inflammation-related AA amyloid due to SAA, an inducible acute-phase protein, increased production of LECT2 under certain circumstances is a predisposing factor. ALect2 amyloid has been identified in patients with hepatic and, less frequently, renal involvement, many of whom in both instances were Hispanic, a perplexing fact because no pathogenic mutations have been found, although all cases have been homozygous for a common LECT2 polymorphism. Importantly ALect2 amyloid can cause hepatic and renal failure.[4,6] Whether it can cause cardiac amyloid, albeit unlikely, remains on open question.

In the series of 130 consecutive cases of hepatic biopsies with amyloid sent for mass-spectrometry typing reported by Mereuta et al, 62% (n = 81) had immunoglobulin light-chain amyloidosis (AL) and 25% (n = 32) ALect2, whereas in the 285 cases of renal biopsies with amyloid reported by Larsen et al 86% (n = 246) had AL and 2.5% (n = 7) ALect2.[1,7] Of the 32 cases of ALect2 hepatic amyloid, 7 had steatosis or steatohepatitis, 5 had chronic active hepatitis (4 hepatitis C, 1 unknown), and over one-third had liver function test abnormalities; data on monoclonal gammopathies in these patients were not available from the testing center, but hepatitis C can be associated with clonal B-cell disorders, notably cryoglobulinemia.[8] This point is relevant because the requirement and challenge for hematologists dealing with patients who have clonal plasma cell neoplasms and organ damage is to determine whether and how the two are linked in order to explain the illness to patients and their families and to endorse a plan of therapy.

When dealing with potential AL patients, many of whom evolve from prior monoclonal gammopathies (MG) such as monoclonal gammopathy of undetermined significance (MGUS), smoldering and symptomatic multiple myeloma (MM), or Waldenstrom macroglobulinemia (WM), the hematologist must evaluate whether the amyloid-related organ damage is due to AL or non-AL disease;

Downloaded from http://ashpublications.net/blood/article-pdf/123/10/1434/1374447/1434.pdf by guest on 09 June 2024