Effects of plasma transfusion on hepcidin production in human congenital hypotransferrinemia

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ABSTRACT

Hepcidin is the key regulator of systemic iron homeostasis. We describe the modulation of hepcidin production induced by plasma transfusions in a patient with congenital hypotransferrinemia that offers a unique model in which to study the mechanism of hepcidin regulation by iron and erythropoiesis. Urinary hepcidin increased from zero at baseline, when hemoglobin and serum transferrin was low, to a maximum of 98 ng/mg creatinine on day 60, and subsequently decreased. Time-course of urinary hepcidin and serum transferrin concentration suggests that hepcidin production is regulated by the combination of marrow iron requirements and iron supply by transferrin.

Key words: hypotransferrinemia, hepcidin, transferrin saturation, transfusion, eryhtropoiesis.

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Congenital hypotransferrinemia is a rare human genetic disorder characterized by a severe deficiency in serum transferrin.[1,2] The defect causes iron deficient erythropoiesis and marked iron deficiency anemia and severe iron overload in all non-hematopoietic tissues.[1-3] A similar phenotype has been observed in the hypotransferrinemic (hpx/hpx) mice that provide a model to help understand the human disease and iron homeostasis.[3,4] The presence of severe anemia in the hpx/hpx mice and patients with congenital hypotransferrinemia indicates that very little iron enters erythroid precusors through non-transferrin cycle pathways. By contrast, the non-erythroid tissues develop massive iron overload through non-transferrin mediated iron uptake, exacerbated by increased intestinal iron absorption.

Hepcidin is the key regulator of systemic iron homeostasis.[5] It inhibits iron flow from duodenal enterocytes, macrophages and hepatocytes into plasma. Hepcidin production by the liver is increased by iron and inflammation and decreased by active erythropoiesis and hypoxia. However, the molecular mechanisms of hepcidin regulation by iron, oxygen and erythropoiesis are still unclear. Hepcidin mRNA expression in hpx/hpx mice is very low[6] supporting the notion of a dominant erythroid signal in hepcidin regulation and the importance of low hepcidin levels in the development of iron overload. There are no data on hepcidin regulation in the human form of hypotransferrinemia. We describe here the modulation of hepcidin production induced by plasma transfusions in a patient with congenital hypotransferrinemia.

Design and Methods

We previously reported the case of a child affected by congenital hypotransferrinemia who has been successfully treated by monthly plasma transfusions since 1998.[2] To minimize the risk of virus infection from blood products, we selected a small group of healthy blood donors with normal serum iron indices referring to the Transfusional

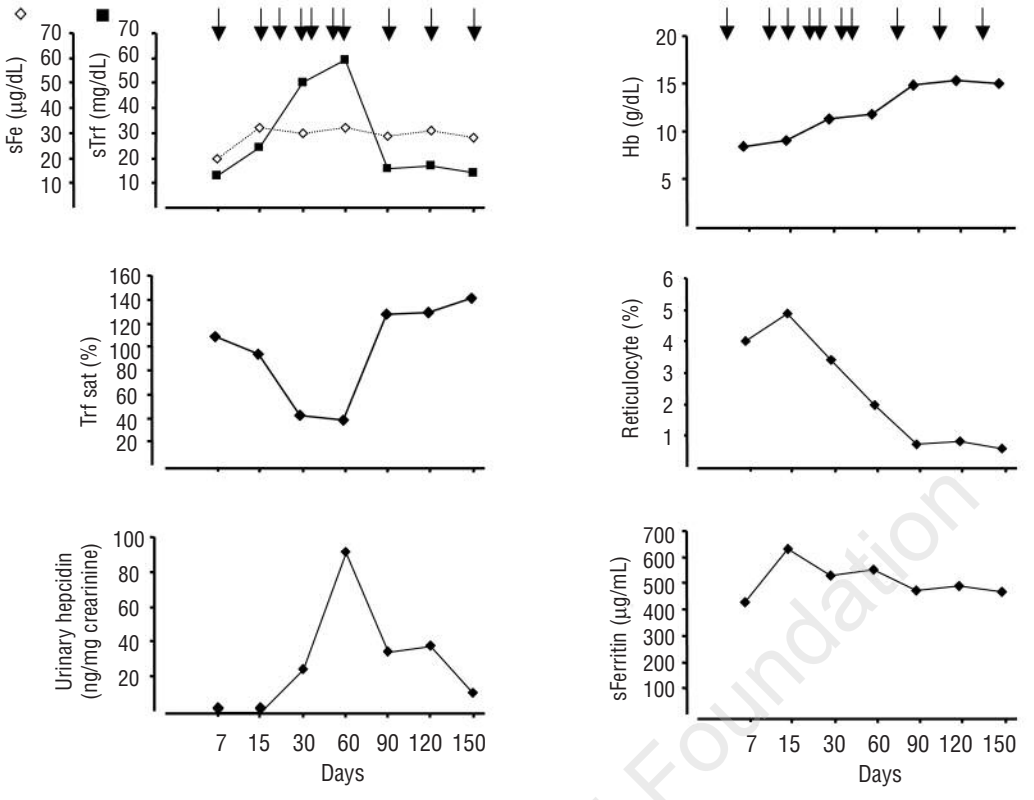

Figure 1. Time-course of serum iron (sFe) (normal value 60-140 µg/dL), transferrin (sTrf) (normal value 200-360 mg/dL), transferrin saturation (Trf sat) (normal value 16-45%), urinary hepcidin (normal value 10-200 ng/mg creatinine) (12), hemoglobin (Hb) (normal value 13.5-18 g/dL), reticulocyte count (normal value 0.5-2%) and ferritin (normal value 13-200 µg/L) during the study. Each arrow represents one plasma transfusion (400 ml).

Centre of the Hospital as previously reported.[2] The affected child maintained normal hemoglobin level and stable serum ferritin level, showing only mild hepatic iron overload by SQUID analysis until the end of 2003. At that time (age 10 years, body weight 43 kg), he began to show a progressive decrease of hemoglobin probably due to a growth-related increase of erythroid iron demand. In May 2004, his hemoglobin level and MCV were 7.3 g/dl and 64 fl respectively. Pre-transfusion transferrin was 0 mg/dl. There was no evidence of blood loss or intestinal malabsorption. We then increased the amount of plasma transfused from 300 ml to 400 ml and the frequency of transfusions to every week for two months to get a sufficient serum transferrin concentration to maintain normal erythropoiesis. As hemoglobin normalized, the frequency of plasma transfusions were rescheduled to 400 ml every month. During this period, we collected patient's urine before plasma transfusion for hepcidin measurement on days 7, 15, 30, 60, 90, 120 and 150. Twenty-five ml urine was preserved with sodium azide (0.01%) and frozen. Urinary hepcidin levels were measured at the University of California (Los Angeles, USA) as previously described.[7] As usual, fasting serum iron, transferrin and transferrin saturation, serum ferritin, cell blood and reticulocyte counts were measured before transfusion. Soluble transferrin receptor was measured by an immuno-nephelometric method (Dade Behring, Liederbach, Germany). Serum iron, transferrin and urinary hepcidin were also measured one hour after transfusion. Transferrin saturation was indirectly calculated using the formula serum iron (µg/dL)/serum transferrin (mg/dL) /1.40, based on the atomic weight of iron and the molecular weight of transferrin. To exclude the effects of inflammatory cytokines on urinary hepcidin variations, common indicators of inflammation (ESR, PCR, α1- and α2-globulin) were routinely examined in blood donors and in the affected child.

Results and Discussion

Patient pre-transfusion hemoglobin, reticulocyte count, serum iron, transferrin, transferrin saturation, serum ferritin and urinary hepcidin during the period of the study are shown in Figure 1. Serum iron increased by 50% by the second time-point (day 15) and remained stable throughout the study. Serum ferritin did not change. Hemoglobin progressively increased reaching normal values during the third month.

Urinary hepcidin rose from zero at baseline to a maximum of 98 ng/mg creatinine on day 60 and subse-

Table 1. Time-course of serum transferrin (sTrf), transferrin saturation (Trf sat) and urinary hepcidin before (Pre) and after (Post) plasma transfusion.

Day	Hepcidin (ng/mg creatinine)		s-Transferrin (mg/dl)		Transferrin saturation (%)	
	Pre	Post	Pre	Post	Pre	Post
7	0	0	13	58	>100	46
15	0	0	24	79	94	40
30	24	24	50	106	43	28
60	92	98	59	106	40	25
90	35	44	16	58	>100	58
120	38	22	17	63	>100	70
150	11	9	14	57	>100	71

quently decreased. The time-course of urinary hepcidin and serum transferrin were on the whole superimposable, while urinary hepcidin and transferrin saturation had an inverse course.

The absence of urinary hepcidin at the beginning of the study is consistent with the findings in hpx/hpx mice.[6] This could be explained by the iron deficiency (low transferrin, low or no iron available), the increased iron demand by the bone marrow, the increased erythropoietic activity (as indicated by the high reticulocyte count) and the presence of anemia.[5,6] Tissue iron overload had no substantial effect confirming that erythropoietic control overwhelms the effect of high iron stores on hepcidin production. The administration of plasma every week resulted in a progressively higher amount of serum transferrin, well above the 10 mg/dl considered to be the smallest amount required to support adequate erythropoiesis in these patients[1] (Table 1). Accordingly, as iron availability to the erythron improved, patient's hemoglobin increased. On day 30 and 60, the patient was still anemic with an elevated reticulocyte count indicating slightly increased erythropoiesis. This would be expected to maintain hepcidin suppression, but urinary hepcidin showed progressive and marked increase. Infection and inflammation were excluded as causes of hepcidin variation because common indicators of inflammation were always normal in patient and in blood donors. In addition, hepcidin levels, measured before and after transfusions (Table 1) did not significantly change. This suggests that they were not influenced by donors' plasma. The probable explanation for hepcidin regulation in this phase is that the erythropoietic drive was diminishing as transferrin concentration and iron delivery to the erythron was increasing, and that iron supply eventually met erythron iron requirements. Accordingly, transferrin saturation temporarily decreased to normal values. This might be due to reduced iron release into the plasma determined by the normal/high hepcidin concentration and the increased use of iron by the bone marrow. It is possible that the decrease in transferrin saturation may reflect the addition of transferrin to the system. However, this was more evident, as expected, just after transfusion when, compared with pre-transfusion values, transferrin saturation markedly decreased (Table 1).

The achievement of a normal erythropoietic activity, as shown by constant normal hemoglobin levels (14.8, 15.4 and 15 g/dL), normal reticulocyte count and soluble transferrin receptor concentration (<4 mg/L) on day 120 and 150, in the presence of tissue iron overload would be expected to upregulate hepcidin synthesis. However, urinary hepcidin fell again as transferrin level decreased due to the less frequent plasma transfusion. Since the erythropoietic drive was not increased, the decrease of hepcidin in this phase was probably related to the low transferrin concentration that causes a decreased iron supply in response to normal marrow iron requirements. This agrees with the concept that iron supply to the erythron is the most important factor influencing iron absorption and iron release from stores.[8] Low hepcidin would allow increased iron flows into plasma, leading to the saturation of transferrin, and consequently non-transferrin-bound-iron formation and non-erythropoietic tissue iron overload.[4] One limitation of our work is that the time-frame for changes in hepcidin levels was on a scale of hours[5] while the time-frame of the study was days to weeks. A more frequent measurement of the various parameters would give more information, but this was not possible. Nevertheless, altogether these findings suggest that hepcidin production is regulated by the balance between iron requirements of the erythroid marrow and iron supply by transferrin. A recent study in the hemoglobin-deficit mouse model showed that diferric transferrin is a key indicator of body iron requirement and is the possible link between bone marrow and liver to modulate hepcidin synthesis according to erythropoietic changes.[9] A level of transferrin saturation below 20% was associated with reduced hepcidin expression in the same model[9] and a deficient iron supply can be detected by a transferrin saturation of less than 16% in humans.[8] In hypotransferrinemia plasma transferrin is fully saturated, but the amount of diferric transferrin is largely below the threshold for normal erythropoiesis. Studies in animal models support the idea that TfR2 is a sensor of diferric transferrin concentrations that, in turn, regulates hepcidin production.[9-11] As predicted by animal studies,[10] we demonstrated that plasma transfusions increased hepcidin production by increasing transferrin concentration and iron supply to the bone marrow. These findings also have therapeutic implications, as they indicate that maintaining a higher serum transferrin level might relieve the suppression of hepcidin and reduce intestinal iron absorption and tissue iron overload in these patients.

Authors' Contribution

PT contributed to conception and design, interpretation of data, to drafting the article, and final approval of the manuscript; TC contributed to analysis of data, drafting the article and final approval of the version to be published; EN contributed to analysis and interpretation of data, and to revising the article and final approval of the version to be published; RM contributed to design, interpretation of data and final approval of the version to be published; AB contributed to revising the article and final approval of the manuscript; TG contributed interpretation of data, revising the article and final approval of the version to be published; AP contributed to conception and design, interpretation of data, revising the article and final approval of the version to be published.

Conflicts of Interest

The authors reported no potential conflicts of interest.

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