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The Interleukin-6-Hepcidin-Hemoglobin Circuit in Systemic Lupus Erythematosus Flares

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Abstract

Objective—Hepcidin regulates iron availability and may be responsible for the anemia of chronic disease because it is induced by interleukin-6. This study investigated the IL-6-hepcidin-hemoglobin axis in patients with lupus.

Methods—IL-6 and hepcidin were measured in serial serum samples collected before, during and after lupus flares by specific ELISAs.

Results—During renal and non-renal SLE flare cycles IL-6 did not predict hepcidin and hepcidin did not predict hemoglobin. When lupus nephritis patients were in remission, IL-6 and hepcidin were correlated, but hepcidin and hemoglobin were not.

Conclusion—Hepcidin does not contribute significantly to anemia during active lupus.

Keywords

Hepcidin; Anemia; Inflammation; Lupus Nephritis

Introduction

Hepcidin regulates iron homeostasis by preventing mobilization of iron stored in macrophages and absorption of iron from the gut ¹. Hepcidin may contribute to anemia by limiting iron availability for erythropoiesis. The main source of hepcidin is the liver, but it is made elsewhere, including kidney and macrophages ^{2, 3}. Iron is the major regulator of hepatic hepcidin production, but inflammation, through interleukin-6 (IL-6), also up-regulates hepcidin expression, and this may explain the anemia of chronic diseases ³⁻⁵.

We found that hepcidin was differentially-expressed in serial urine samples from lupus nephritis (LN) flares ⁶. In LN kidney biopsies hepcidin was expressed by infiltrating interstitial leukocytes⁶, and urine hepcidin levels correlated with the severity of renal interstitial inflammation ⁷. Other investigations also showed urine hepcidin was increased in active LN ⁸, but there is little information on serum hepcidin in lupus.

We postulated that in LN increased expression of IL-6 increases hepcidin expression and leads to anemia. To test this hypothesis the IL-6-hepcidin-hemoglobin axis was examined during LN flare cycles using prospectively collected serial serum samples from the Ohio SLE Study (OSS)⁹.

Methods

Study population

Patients had participated in the OSS, a prospective observational cohort of SLE patients followed bimonthly⁹. Patients were classified as LN if they had a previous or current kidney biopsy showing immune complex glomerulonephritis, or non-renal if they never had kidney involvement. The OSS was approved by the institutional review board and was in accordance with the Declaration of Helsinki.

SLE flare cycles were defined by the dates of renal and non-renal flares adjudicated using published criteria⁹. Serum collected 6 months from a flare was designated baseline and reflected disease stability or quiescence. Pre- and post-flare sera were from visits 4 and 2 months before and 2 and 4 months after the flare visit, respectively. A flare cycle specimen set consisted of baseline, pre-flare, flare and post-flare sera.

Hepcidin, Interleukin-6 and Hemoglobin Measurement

Specific ELISAs were used to measure the active 25 amino acid form of serum hepcidin (Bachem Kit S-1337) and IL-6 (R&D Systems). The inter-assay coefficient of variation was <20% for both assays. Hemoglobin was measured in the hospital clinical laboratory using the Coulter principle on a Beckman DXH800/LH780 instrument (Beckman-Coulter, Brea, CA, United States).

Statistical Analysis

Comparisons between two groups were done with a Student's T test. Mixed-model regression was used to determine if IL-6, hepcidin, or hemoglobin levels changed during lupus flare cycles after natural log transformation of hepcidin and IL-6. The Tukey HSD method was used to correct for multiple comparisons. These models used flare cycle time point as the predictor, level of IL-6, hepcidin, or hemoglobin as the response, and the subject as a random effect. Linear regression was used to test for associations between IL-6 and hepcidin and hepcidin and hemoglobin. Covariates considered for all regression analyses were serum creatinine concentration (SCr), age, race, and sex. P values <0.05 were considered significant.

Results

Patient Characteristics

The patients (n=48) were 94% female, 65% White, 31% Black and 4% Asian. A kidney biopsy showing LN had been done in 37 patients, while 11 never had any evidence of kidney involvement (non-renal patients). The LN group experienced 39 renal flares and 25 extra-renal flares, and the non-renal group had 11 flares. Clinical data and medication use at

baseline and flare are presented in Table 1. Significant proteinuria was only observed during LN flares. SCr was numerically but not significantly higher at LN flare. Complement was generally lower in LN patients. Most patients were receiving prednisone at the time of flare. Immunosuppressive use was higher at flare in LN patients than in non-renal patients.

Serum Hepcidin, Hemoglobin and Interleukin-6 Levels Expression in SLE Flare Cycles

No differences were seen when baseline and flare levels of hepcidin or IL-6 were compared for any flare type (Table 2). There were no differences in hemoglobin between baseline and renal flare or extra-renal flare in non-renal patients. There was a small but statistically significant decrease in hemoglobin during extra-renal flares in LN patients (Table 2). Hepcidin, hemoglobin and IL-6 did not change over time during SLE flare cycles (Supplemental Figure 1). Hemoglobin was significantly lower in LN patients who developed renal flares compared to non-renal patients at all time points (Supplemental Figure 1B), and IL-6 levels were higher at baseline in patients who had LN flares than in patients who had extra-renal flares (Supplemental Figure 1C, Table 2). SCr was a significant co-variate for hemoglobin and IL-6 in LN flare cycles ($P=0.024$; $P=0.003$, respectively). At baseline 23% of the patients had impaired renal function, as indicated by a SCr between 1.25 mg/dl and 2.5 mg/dl.

Predictors of Hemoglobin and Hepcidin

To test the integrity of the IL-6-hepcidin-hemoglobin circuit in SLE flares the influence of IL-6 on hepcidin and the influence of hepcidin on hemoglobin during lupus flare cycles was determined. There was no significant relationship between hepcidin and hemoglobin for any flare type, nor did IL-6 predict hepcidin level in lupus flares (Table 3). Interestingly, when LN patients were evaluated at baseline, there was a significant positive correlation between IL-6 and serum hepcidin ($r^2=0.42$, $P=0.017$ after log transformation). This relationship was not seen at baseline in non-renal patients ($r^2=0.28$, $p=0.28$) and was also not evident at the time of renal or extra-renal flare in LN patients ($r^2=0.10$, $p=0.29$). Hepcidin only showed a trend toward a weak inverse correlation with hemoglobin at baseline in LN patients ($r^2=0.09$, $P=0.073$).

Discussion

The IL-6-hepcidin-hemoglobin axis does not appear to be functional during lupus flare cycles. This unexpected conclusion is based on the observation that serum hepcidin levels did not increase as renal or extra-renal lupus activity increased, and that no coherent relationship between IL-6, hepcidin, and hemoglobin could be demonstrated during SLE flare cycles. Other investigations have also called the relationship between IL-6, hepcidin and anemia into question. Only one previous study examined serum hepcidin levels in lupus. No differences were seen compared to healthy individuals and there was no correlation with hemoglobin, however pro-hepcidin was measured in contrast to active hepcidin ²⁵¹⁰. Hepcidin is increased in patients with chronic and end-stage kidney disease (CKD, ESKD) ¹¹, and correlates with IL-6 ¹², because CKD and ESKD are inflammatory conditions. An association of hepcidin with anemia in CKD/ESKD is however less clear, supported by some ¹³ but not all studies ¹². In a breast cancer investigation hepcidin was increased but did not

correlate with IL-6¹⁴, and in a chronic obstructive pulmonary disease (COPD) study hepcidin was elevated but did not increase further with acute exacerbations¹⁵. There was a modest correlation between IL-6 and hepcidin at COPD exacerbation. Lastly, in rheumatoid arthritis hepcidin did not correlate with hemoglobin, but IL-6 correlated weakly, and after treatment with an IL-6 antagonist hepcidin levels fell and hemoglobin increased¹⁶.

These data suggest IL-6 can induce hepcidin in inflammatory conditions, but this does not necessarily result in anemia. In LN, IL-6 did correlate with hepcidin, but only when lupus was quiescent. This may be explained by the observation that some inflammatory cytokines like TNF can downregulate hepcidin expression¹. In the cytokine-rich milieu of an LN flare it is conceivable that the balance of cytokines favors hepcidin inhibition. Chronic anemia in SLE, seen mainly in LN, probably reflects chronic kidney damage as SCr was a significant covariate of hemoglobin in LN flare cycles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Clinical Data and Medication Use at Baseline and Flare

	Lupus Nephritis Patients		Lupus Nephritis Patients		Non-Renal Patients	
	Baseline	Renal Flare	Baseline	Non-Renal Flare	Baseline	Flare
C3 (mg/dl) ¹	90±34	76±28	99±27	95±34	132±50	128±57 ⁴
C4 (mg/dl) ¹	16±11	14±8	15±7.3	16±10	24.8±10.4	26±12 ⁵
UPCR (mg/mg) ²	0.82±1.52	3.6±3.4 ⁶	0.18±0.12	0.32±0.34	Not Done	Not Done
SCr (mg/dl) ³	0.92±1.23	1.25±0.52	0.92±0.27	1.02±0.45	0.98±0.22	0.92±0.29
Prednisone (%)	80	92	85	96	50	70
Azathioprine (%)	7	20	20	28	10	0
MMF (%)	87	77	55	52	30	27
Hydroxychloroquine (%)	47	44	55	64	30	36

¹ Complement components C3 and C4;

² Urine protein to creatinine ratio from a 24-hour urine collection;

³ Serum creatinine concentration;

⁴ P<0.001 vs renal flare and P<0.05 vs extra-renal flare in renal patients;

⁵ P<0.01 vs renal flare and P<0.05 vs extra-renal flare in renal patients;

⁶ P<0.0001 vs baseline and P<0.0001 vs extra-renal flare in renal patients

Table 2

Hepcidin, Interleukin-6 and Hemoglobin in SLE

	Hepcidin (ng/ml)			Hemoglobin (g/dL)			Interleukin-6 (pg/ml)		
	Baseline	Flare	P	Baseline	Flare	P	Baseline	Flare	P
RF¹	19.6±15.2	20.7±19.2	0.40	11.6±1.2	11.4±1.4	0.57	7.98±4.2	8.25±6.4	0.51
ERF-R²	19.1±17.2	23.6±26.1	0.84	12.6±1.0	12.2±1.5	0.03	2.75±3.6	3.6±4.7	0.98
ERF-NR³	16.4±16.8	32.8±44.1	0.09	13.5±2.0	13.6±2.1	0.30	2.02±1.4	4.19±5.1	0.40
P (RF vs ERF-R)	NS	NS		<0.01	<0.05		<0.05	NS	
P (RF vs ERF-NR)	NS	NS		<0.01	<0.01		<0.01	NS	
P (ERF-R vs ERF-NR)	NS	NS		NS	<0.05		NS	NS	

¹ Renal Flare;

² Extra-Renal Flare in Renal Patients;

³ Extra-Renal Flare in Non-Renal Patients

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Table 3
P Values from Mixed-Model Analysis to Determine Predictors of Hepcidin and Hemoglobin During SLE Flare Cycles

Predictor	Hepcidin			Hemoglobin		
	RF ¹	ERF-R ²	ERF-NR ³	RF	ERF-R	ERF-NR
IL-6 ⁴	0.25	0.13	0.37			
Hepcidin ⁵				0.23	0.33	0.64

¹ Renal Flare;
² Extra-Renal Flare in Renal Patients;
³ Extra-Renal Flare in Non-Renal Patients;
⁴ Interleukin-6 was tested as a predictor of hepcidin;
⁵ Hepcidin was tested as a predictor of hemoglobin