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Sickle Cell Vaso-occlusive Crisis Induces the Release of Circulating Serum Heat Shock Protein-70

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Abstract

Inflammation may play an important role in the pathophysiology of sickle cell disease (SCD), and recent studies have identified the 70-kDa heat shock protein (Hsp70) as an important mediator of inflammatory responses. Here we demonstrate a significant increase in circulating serum Hsp70 level in SCD during vaso-occlusive crisis (VOC) as compared with baseline steady-state levels ($P < 0.05$) and a significant increase in Hsp70 levels in SCD at baseline compared with normal controls ($P < 0.05$). Taken together, these results indicate that circulating serum Hsp70 might be a marker for VOC in SCD.

Keywords

chaperokine; heat shock proteins; inflammation; sickle cell disease; vasoocclusive crisis

INTRODUCTION

The pathophysiology of sickle cell disease is initiated by sickle hemoglobin (HbS) polymerization, sickling of the red blood cell under hypoxic conditions, and vaso-occlusive disease (VOC). Pro-inflammatory mediators including superoxide, hydrogen peroxide, and peroxynitrite, and the hydroxyl radical may contribute to sickle acute chest syndrome [1,2]. Whereas the induction in expression of intracellular Hsp70 has clearly been shown to confer protection against diverse forms of cellular and tissue injury, no study to date has addressed a role of extracellular Hsp70 in SCD. This is important because a paradigm shift in the understanding of the function of intracellular versus extracellular Hsp70 has been initiated; it is now clear that Hsp70 can exit mammalian cells and tissues, interact with cells of the immune system, and exert pro-inflammatory and immunoregulatory effects, known as the chaperokine activity of Hsp70 (for review see [3]). Because sickle vaso-occlusion is a form of reperfusion injury, we examined the possibility that extracellular Hsp70 might play a role in SCD in general

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and specifically in VOC. In this study, we demonstrate that circulating serum Hsp70 levels are elevated in sickle cell disease and increases further during VOC, suggesting that circulating serum Hsp70 might be a marker for VOC in SCD.

STUDY DESIGN

On study entry, patients had signed an institutional review board-approved informed consent. We recruited five subjects with SCD: three were homozygous for HbS and two had HbSC disease. Three African-American subjects with normal hemoglobin AA served as controls (hemoglobin phenotype was determined by HPLC). Samples were procured either during VOC or at “steady state” 2–4 weeks removed from VOC. Circulating serum Hsp70 levels were assayed using a sandwich ELISA technique (StressGen Biotechnologies, Victoria, British Columbia, Canada) with minor modifications and quantified using Softmaxpro (Sunnyvale, CA). The total protein content within the serum was determined by Bradford analysis using bovine serum albumin as a standard. Statistical analysis and calculation of means, Student's *t*-test, standard deviation, confidence intervals, and *P* values of the samples were determined using standard statistical software (ANOVA).

RESULTS AND DISCUSSION

SCD is characterized by a shortened erythrocyte life span and red cell damage leading to vaso-occlusion. The most common complication of SCD is the painful episode [4]. By the age of 4.9 and 7.1 years, approximately 50% of patients with sickle cell anemia and HbSC disease have experienced a painful episode, respectively. This diagnosis is largely still based on history from the patient coupled with a physical examination that occasionally demonstrates evidence of local inflammation. In uncomplicated painful episodes, there is no evidence of active infection. Five subjects with SCD were recruited into the study: three homozygous for HbS and two with HbSC disease. Controls were three African-America subjects with normal hemoglobin. Samples were procured either during VOC or at “steady state,” 2–4 weeks after the VOC episode (Table I). Three of the five patients with SCD at “steady state” had significantly higher levels of Hsp70 compared with normal controls ($P < 0.05$). Compared with “steady-state” levels, during VOC 5/5 patients had greater than 9-fold mean increase in circulating serum Hsp70 ($P < 0.05$) (Table I). Interestingly, one patient had a mean Hsp70 level of 104.2 ± 4 ng/mL during “steady state” (Table I). One week after the sample was taken, this subject developed severe VOC requiring hospitalization, and the mean Hsp70 level increased to 185.0 ± 6 ng/mL. If this trend continues in a larger patient population, it would be interesting to speculate that perhaps circulating serum Hsp70 might be a predictive marker for VOC in patients with SCD; however, further studies with a larger patient population are required before this can be affirmed.

Because exogenously administered Hsp70 stimulates the release of pro-inflammatory mediators from macrophages [5,6], it is possible that repeated episodes of vaso-occlusion and reperfusion may produce tissue injury [7,8], results in the release of Hsp70 into the circulation, which due to its chaperokine activity will perpetuate the inflammatory process. In line with this, we show that even at baseline SCD subjects have a significantly higher circulating serum Hsp70 levels as compared with normal controls ($P < 0.05$) and patients with VOC have significantly higher circulating serum Hsp70 levels as compared with steady-state SCD ($P < 0.05$) (Table I).

To date, this is the first description of a putative role of circulating serum Hsp70 in the VOC in SCD. We are currently expanding this study in a larger number of patients to fully understand their diagnostic and prognostic value.

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TABLE I
Hemoglobin Phenotype and Circulating Serum Hsp70 in SCD and Normal Control Subjects

Patient ID number	Hemoglobin Type	Mean serum Hsp70 concentration (ng/ml) $\pm$ SD from respective subjects ^a		
		VOC	Steady state	Control
BU-01	SC	185.0 $\pm$ 6 *	104.2 $\pm$ 4 *	—
BU-02	SS	180.0 $\pm$ 8 *	12.4 $\pm$ 1	—
BU-03	SS	160.0 $\pm$ 16 *	31.3 $\pm$ 3 *	—
BU-04	SC	225.0 $\pm$ 23 *	21.9 $\pm$ 2 *	—
BU-05	SS	185.0 $\pm$ 12 *	16.0 $\pm$ 2	—
BU-06	AA	—	—	4.0 $\pm$ 2
BU-07	AA	—	—	14.0 $\pm$ 4
BU-08	AA	—	—	8.0 $\pm$ 2

^aResults are serum concentration of Hsp70 $\pm$ SD of quadruplicate samples.

* $P < 0.05$ vs Control (Student's *t*-test).