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Elevated urinary leukotriene E₄ levels are associated with hospitalization for pain in children with sickle cell disease

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Abstract

Cysteinyl leukotrienes (CysLTs) are inflammatory mediators produced by white blood cells. Leukotriene LTE₄ is the stable metabolite of CysLTs, which can be measured in urine. We tested two hypotheses among children with sickle cell disease (SCD): (1) baseline urinary LTE₄ levels are elevated in children with SCD when compared with controls; and (2) baseline LTE₄ levels are associated with an increased incidence rate of hospitalization for SCD-related pain. Baseline LTE₄ levels were measured in children with SCD (cases) and children without SCD matched for age and ethnicity (controls). Medical records of cases were reviewed to assess the frequency of hospitalization for pain within 3 years of study entry. LTE₄ levels were obtained in 71 cases and 22 controls. LTE₄ levels were higher in cases compared with controls (median LTE₄: 100 vs. 57 pg/mg creatinine, $P < 0.001$). After adjustment for age and asthma diagnosis, a greater incidence rate of hospitalization for pain was observed among children with SCD in the highest LTE₄ tertile when compared with the lowest (114 vs. 52 episodes per 100 patient-years, $P = 0.038$). LTE₄ levels are elevated in children with SCD when compared with controls. LTE₄ levels are associated with an increased rate of hospitalizations for pain.

Introduction

Asthma increases the incidence rate of pain episodes in children with sickle cell disease (SCD) by at least twofold [1]. Similar to asthma, individuals with SCD have elevations of proinflammatory molecules. Examples of these molecules are interleukins [2], soluble vascular adhesion molecules [3,4], tumor necrosis factor [5], and C-reactive protein [6,7]. Although inflammatory proteins participate in the pathogenesis of both SCD [2,3,5–10] and asthma [11–19] the factors that mediate the relationship between asthma and increased SCD-related pain have not been elucidated.

There is evidence for a role for leukotrienes in the pathogenesis of SCD-related morbidity [2]. In a prospective cohort study, Setty et al. [2] showed that leukotriene B₄ is elevated among individuals with sickle cell anemia (HbSS) when compared with individuals without SCD. CysLTs are inflammatory mediators derived from arachidonic acid via the 5-lipoxygenase (5-LO) pathway (Fig. 1). CysLTs are produced by basophils, eosinophils, and mast cells in response to inflammatory stimuli. LTE₄ is the stable metabolite of CysLTs, which can be measured in the urine. These mediators exert their effects on vascular smooth

muscle, leading to both pulmonary and systemic vasoconstriction, as well as increased vascular permeability, mucus hypersecretion, edema, and inflammatory cell activation. CysLTs are thought to be important to the pathogenesis of asthma. In healthy individuals, inhaled CysLTs produce symptoms similar to those of asthma [20]. Further, the airways of individuals with asthma are at least 100 times more sensitive to inhaled CysLTs than airways of individuals without asthma [20], and inhaled CysLTs lowers the threshold to bronchial hyperresponsiveness to methacholine [21].

We elected to focus on the CysLT pathway because CysLTs are important to the pathogenesis of asthma and a diagnosis of asthma in children with SCD is associated with an increased rate of pain [1]. Given the potential contribution of CysLTs to SCD-related morbidity, we tested two hypotheses: first, that LTE_4 will be elevated in individuals with SCD when compared with ethnic and age-matched controls, and second, that LTE_4 levels are associated with pain episodes that require hospitalization.

Results

Demographics

Among the 97 families approached to participate in the study, 93 agreed to participate. Urine samples were collected from 71 children with SCD (44 HbSS, 19 HbSC, 1 HbS β^{thal}^0 , 6 HbS β^{thal}^+ , 1 HbS with Persistent Fetal Hb) and 22 children without SCD (10 asthmatics and 12 non-asthmatic controls). There were no statistically significant differences in age, gender, or asthma diagnosis between cases and controls (Table I). Participants with SCD were divided into approximately three equal groups based on their base-line LTE_4 levels (lowest tertile, 0–70 pg/mg creatinine; middle tertile, 71–140 pg/mg creatinine; highest tertile, 141 pg/mg creatinine). Additionally, within the three LTE_4 tertile groups, no difference was observed between groups in baseline percent hemoglobin F or white blood cell count (Table II). Five participants with SCD received regular blood transfusion therapy and six participants were on hydroxyurea therapy.

Elevated LTE_4 levels in SCD

LTE_4 levels were significantly higher in children with SCD (mean LTE_4 : 171, 95% CI: 132–209 pg/mg creatinine) compared with controls (mean LTE_4 : 64, 44–86 pg/mg creatinine), $P < 0.001$ (Fig. 2). Individuals with either Hb SS, S beta thalassemia or Hb SC were combined into one group because no significant difference was noted when comparing LTE_4 levels ($P = 0.742$) HbS with Persistent Fetal Hb. Similarly, the asthmatic and non-asthmatic groups were combined into one control group because they were found to have no difference in LTE_4 levels ($P = 0.998$).

We subsequently evaluated the presence of LTE_4 in children with SCD and asthma in order to determine whether these children had an increased level of LTE_4 when compared with children without SCD, but with asthma. LTE_4 levels were significantly higher in children with SCD and asthma (mean LTE_4 : 151, 112–190 pg/mg creatinine) compared with children without SCD but with asthma (mean LTE_4 : 61, 46–76 pg/mg creatinine), $P = 0.0001$ (Fig. 3). There were no differences in LTE_4 levels when stratifying for inhaled corticosteroid or montelukast use (data not shown).

Elevated LTE_4 levels were associated with pain episodes

The incidence rate of pain was greater when comparing the highest LTE_4 tertile group to the lowest tertile LTE_4 group. Children with higher LTE_4 group had more painful episodes that required hospitalization (66 episodes per 100 patient-years; 95% CI, 31–138) compared with

children who had lower LTE₄ levels (30 episodes per 100 patient-years; 95%CI, 20–41), $P=0.038$ (Table III).

A multivariate model of the leukotriene level was associated with pain after adjustment for risk factors for pain in this cohort. The goodness of fit test showed that pain counts of events were overdispersed, $P<0.05$; therefore, results were calculated using deviance scale option to correct for the overdispersion in the Poisson regression model. The final adjusted value included the covariates that were significant in the univariate model: age ($P=0.001$) and asthma ($P=0.09$). Again, we confirmed that the leukotriene LTE₄ group when divided into tertiles was associated with an increase rate pain after adjustment for age and the presence of asthma (Table IV).

Discussion

The relationship between CysLT levels and SCD is poorly defined. In this study, we tested the hypotheses that baseline levels of LTE₄ are elevated among children with SCD when compared with controls, and that levels of LTE₄ are associated with an increased rate of pain. Our results support our hypotheses. For the first time, we have demonstrated that CysLT levels, which are elevated at baseline among children with SCD, are associated with an increased rate of previous hospitalizations for pain.

We can only postulate as to why there is an association between LTE₄ levels and pain among children with SCD. CysLTs are primarily produced by leukocytes and activate two receptors known as CysLT receptor 1 and CysLT receptor 2 (CysLTR-1 and CysLTR-2). CysLTR-1 is expressed within the lungs, leukocytes, and spleen [22]. CysLTR-2 has been found within leukocytes, spleen, heart, and placenta [23]. Activation of these receptors results in broncho- and vascular constriction, mucous secretion, vascular permeability and inflammatory cell recruitment, which may promote vaso-occlusion and therefore pain among children with SCD. Our findings demonstrate that baseline values of CysLTs predict pain episodes; however, the physiologic role of CysLTs in vaso-occlusion may be quite dynamic. There is significant evidence that LTA₄ released from leukocytes can be taken up by endothelial cells, erythrocytes, or platelets in a process termed “transcellular biosynthesis” and metabolized to CysLTs [24,25]. Thus, the cell–cell interactions critical to the pathogenesis of vaso-occlusion may also serve to increase synthesis of CysLTs, thereby augmenting an inflammatory response and leading to vascular occlusion and pain.

Genetic or environmental factors likely explain differences in baseline values of LTE₄ among children with SCD in our study. Baseline LTE₄ levels in this group varied widely from 15 to 751 pg/mg. In the general population, polymorphisms in the leukotriene pathway have been associated with asthma predisposition [26]. Given the disease-modifying effects of asthma in SCD, future studies of genetic variations in leukotriene genes may provide insight into the pathogenesis of pain in children with SCD.

Several limitations exist in this cross-sectional observational study. A single urine sample may not reflect an accurate baseline level of LTE₄, and thus our results will need to be confirmed in another population. In addition, the true association between LTE₄ levels and SCD-related morbidity may be underestimated, given that 11 children with SCD received either chronic transfusion therapy ($n=6$) or hydroxyurea ($n=5$); however, these interventions are more likely to bias the results toward the null hypothesis because they are associated with a decrease in pain episodes. Despite the association between LTE₄ levels and pain incidence, we cannot establish a temporal relationship of this association. Such a limitation is inherent within a cross-sectional study design and subsequent prospective studies are underway to address this limitation. We did not account for tobacco smoke

exposure in this study, although active or passive tobacco smoke may increase LTE₄ levels [27].

LTE₄ levels are associated with a greater number of pain episodes in children with SCD. CysLTs offer a potential therapeutic target to lessen SCD-related morbidity. Montelukast, a leukotriene D₄ receptor antagonist, blocks the action of cysteinyl leukotrienes. This class of agents may be of some clinical utility among children with SCD because the therapy may attenuate the inflammatory response. Additional clinical studies to replicate our findings and seek a better understanding of the basis of the relationship between LTE₄ levels and pain are required for potential targeted therapies in SCD to be implemented.

Materials and Methods

Study design

This study was approved by the Human Research Protection Office at Washington University School of Medicine and informed consent was obtained prior to enrollment. The target population was children (4–21 years of age) with SCD seen for routine care in the hematology clinic at St. Louis Children's Hospital. The study was conducted between December 2006 and April 2007. Individuals with SCD were eligible to participate in the study if clinically well and able to provide a urine sample at time of enrollment. Specifically, all participants were without pain and reported not taking any pain medication at the time of enrollment. Individuals taking hydroxyurea and those who were receiving blood transfusion therapy were included in the study because we had no reason to believe that leukotrienes levels would be decreased in these participants.

Interval health questionnaire

A questionnaire regarding current state of health, hemoglobin phenotype, past medical history, and current medication use was administered to all participants prior to urine sample collection. Since Montelukast [28] is known to reduce LTE₄, specific questions about asthma medication use were included in the questionnaire to account for this effect. Questions about asthma status, pain episodes, and acute chest syndrome (ACS) were also included [1].

Measurement of urinary LTE₄

Approximately 10 ml of urine was obtained from each participant. Collected samples were centrifuged for 15 min at 4°C, aliquoted, and frozen at –80°C until assay. Enzyme-linked immunosorbent assay was used to detect LTE₄ levels (Cayman Chemical, Ann Arbor, MI). All samples were assayed in duplicate.

Determination of pain and ACS episodes rates

A 3-year retrospective medical record review was conducted for all children with SCD using the date of urine collection as the index date to determine the association between hospitalizations for pain and LTE₄ levels. A painful episode was defined as an event that required hospitalization and treatment with an opioid. Outpatient visits for pain were not included. To ensure independence of painful events, any pain event requiring hospitalization within 14 days of a previous pain event was counted as one event. For individuals in whom a diagnosis of both pain and ACS were documented in the medical record, the event was not counted as a painful event.

Hematologic variables

Baseline measurements when the patient was well and greater than 2 years of age for white blood cell count, platelet count, absolute eosinophil count, absolute monocyte count, and percent hemoglobin F were obtained.

Statistical analysis

Student's *t* test and analysis of variance (ANOVA) were used to compare baseline characteristics. Student's *t* test was also used to compare LTE₄ levels between children with SCD and controls. Log transformed LTE₄ levels were calculated to adjust for the non-normal distribution of LTE₄ levels.

Rates of pain episodes among children with SCD were determined. The SCD group was divided into three groups of approximately the same size based on LTE₄ levels. These groups were created as opposed to using continuous measurements of LTE₄ because the relationship between LTE₄ levels and pain was nonlinear. The log-linear Poisson regression model was employed to determine the incidence rate of pain per 100 patient-years with adjustment of the potential confounders: LTE₄ groups (tertiles groups based on LTE₄), age (continuous), gender, asthma status (present or absent), white blood cell count, baseline hemoglobin, and hemoglobin F level. The crude and adjusted pain rates with their 95% confidence interval were calculated. In a subgroup analysis, controls were grouped according to asthma status, asthmatic controls, and non-asthmatic controls. Controls were excluded if they were known to have an inflammatory disease other than asthma that may affect urinary LTE₄ levels.

Associations between LTE₄ and hematologic variables and LTE₄ and pain episodes were tested using Spearman's correlations. A *P*-value of 0.05 was considered statistically significant. Data were analyzed using SPSS 14.0 and SAS 9.1.

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Figure 1.
5-Lipoxygenase pathway.

Figure 2. Elevated urinary leukotriene E₄ levels in children with SCD compared with children without SCD; *P* value = 0.001; error bars = 95% CI.

Figure 3. Elevated urinary leukotriene E₄ levels in children with SCD and asthma compared with children without SCD with asthma.

TABLE I

Comparison of Baseline Characteristics in Children With Sickle cell Disease (SCD) Versus Children Without SCD

	SCD (N = 71)	Controls (N = 22)
Mean age (years), range	10.8 (4–20)	11.1 (4–19)
Male (%)	54	46
Asthma diagnosis (%)	65	46
ICS use (%)	47	46
Montelukast use (%)	14	27
Prednisone use (%)	6	5

TABLE IIBaseline Characteristics of Children With SCD Divided Into Tertiles Based on Leukotriene E₄ Levels

	Lowest tertile (N = 22), 0–70 pg/ mg creatinine	Middle tertile (N = 24), 71–140 pg/ mg creatinine	Highest tertile (N = 25), 3141 pg/ mg creatinine
Mean age (years), range	11.0 (5–19)	10.9 (5–20)	10.4 (4–20)
Male (%)	59	54	48
Asthma diagnosis (%)	64	67	64
Hemoglobin F (%)	4.4	6.8	7.3
White blood cell (k/mm ³)	10.3	11.2	10.2

TABLE IIIAssociation of Leukotriene E₄ Levels (Tertiles) and Incidence Rate of Pain that Required Hospitalization

Leukotriene E ₄ group divided into tertiles	Pain (events per 100 patient-years)			
	Crude	95% CI	Adjusted	95% CI
Lowest tertile (<i>N</i> = 22), 0–70 pg/mg creatinine	30	(20, 41)	30	(20, 41)
Middle tertile (<i>N</i> = 24), 71–140 pg/mg creatinine	42	(29, 54)	38	(17, 85)
Highest tertile (<i>N</i> = 25), >141 pg/mg creatinine	61	(46, 77)	66 [*]	(31, 138)

The adjusted rate is adjusted for age and the presence of asthma.

^{*} *P* < 0.05.

TABLE IV

Poisson Regression Model: Association of LTE₄ Levels and Incidence Rate of Pain Episodes—Final Model
Univariate and each Predictor in a Multivariate Model

	Univariate model		Multivariate model	
	Rate ratio (95% CI)	P-value	Rate ratio (95% CI)	P-value
Leukotriene E ₄ at highest level (0–70 pg/mg creatinine)	2.01 (0.9, 4.48)	0.086	2.20 (1.05, 4.62)	0.038
Leukotriene E ₄ at middle level (71–140 pg/mg creatinine)	1.38 (0.58, 3.25)	0.47	1.27 (0.57, 2.83)	0.55
Age (years)	1.11 (1.04, 1.18)	0.003	1.13 (1.04, 1.18)	0.001
Asthma (yes or no)	1.82 (0.89, 3.78)	0.10	1.79 (0.92, 3.52)	0.088