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Modulation of pain in pediatric sickle cell disease: Understanding the balance between endothelin mediated vasoconstriction and apelin mediated vasodilation

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ABSTRACT

Children with sickle cell disease (SCD) have painful vaso-occlusive episodes (VOEs), which often reoccur across the individual's lifespan. Vaso-constrictive and vaso-dilatory molecules have been hypothesized to play a role in VOEs. Endothelin-1 (ET-1) is a potent vasoconstrictor that is released during VOEs and is correlated with pain history. Apelin is a vaso-dilatory peptide that also has a modulatory role in pain processing. We hypothesize that the ratio between vaso-dilatory and vaso-constrictive tone in children with SCD may be a marker of pain sensitization and vaso-occlusion. Plasma endothelin and apelin levels were measured in 47 children with SCD. Procedural pain and baseline pain were assessed via child- and caregiver-reports and observational distress. Pain history was assessed using retrospective chart review. Plasma apelin was related to age, with decreased levels in older children. The ratio between apelin and ET-1 was negatively correlated to observational baseline pain. The ratio between apelin and Big ET was negatively correlated to caregiver ratings of baseline pain and positively correlated to history of VOEs, which is possibly due to hydroxyurea treatment. These results suggest that an imbalance in the apelin and endothelin systems may contribute to an increasing number of VOEs and baseline pain in children with SCD.

Introduction

Sickle cell disease (SCD) is a genetic blood disorder that is characterized by recurrent vaso-occlusive events (VOEs) during which sickle-shaped red blood cells occlude small blood vessels. One of the major characteristics of VOEs is severe pain that often peaks at the height of the VOE and then slowly returns to normal over the course of days to weeks. It has been suggested that in SCD, there may be an imbalance in the production of local vasoconstrictors and vasodilators, with a shift in the balance towards the production of vasoconstrictors [1]. One of these vasoconstrictors thought to be involved is endothelin-1 (ET-1), one of the most potent endogenous vasoconstrictors. Studies have shown that ET-1 levels are increased in patients with SCD compared to healthy controls [2,3] and these increased levels are even more pronounced during an acute VOE [1]. Down-regulation of ET-1 gene expression and a reduction in the half-life of circulating ET-1 have been found in patients with SCD treated with hydroxyurea (HU), which is a drug that has been shown to decrease the rate and intensity of VOEs [4–6]. In addition to its vasoactive properties, ET-1 also causes nociception when injected into both humans and animals [7–13]. Interestingly, the time course of ET-1 plasma elevations parallels VOE-associated pain symptoms with a peak in plasma levels and pain at the height of the VOE and then a slow return to baseline that requires several weeks [3].

Previously, we have shown that endothelin variables in the plasma of pediatric patients with SCD are related to baseline pain such that higher plasma levels of ET-1 and its precursor, Big ET, were found in children with higher baseline pain [14]. In contrast, lower plasma Big ET levels were found in children with a higher frequency of recent VOEs, possibly a result of recent conversion to vasoactive ET-1. From these findings of excessive ET mediated vasoconstriction in children with SCD we were interested in the role of vasodilatory systems in SCD. We have recently begun to explore the role of apelin, a novel vasoactive peptide with vasodilatory properties, in the pain associated with SCD. Apelin is formed from the 77 amino acid preproapelin, which is cleaved by angiotensin-converting enzyme 2 to form apelin-36 and other biologically active fragments such as apelin-17 and apelin-13 [15,16]. Apelin is the endogenous ligand for the orphan G-protein coupled receptor, APJ. The apelin–APJ system has also been recently implicated in having a modulatory role in nociception since apelin and its receptor are found in several brain regions associated with pain [17]. In the vasculature,

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apelin's interaction with its receptor causes nitric-oxide (NO) dependent vasodilation when acting on endothelial cells [16,18,19]. Recently, it has been proposed that there is a shift in favor of ET-1-mediated vasoconstriction and away from NO-mediated vasodilation during an acute VOE in SCD patients [1]. To date, there have been no studies that explore the potential relationship between apelin and ET-1 in relation to pain in SCD.

The goal of this study was to examine the balance between ET-1 or its precursor, Big ET, and apelin in a cohort of pediatric patients with SCD and how this balance correlates with acute chest syndrome, procedural pain, and pain history. No directional hypotheses are offered as this is the first known study to evaluate apelin in relation to SCD outcomes and both positive and negative associations could be justified based on existing data.

Methods

Participants — apelin plasma

This study used a subsample of 47 children with SCD ages 2 to 18 ($M = 9.98$, $SD = 4.78$; 22 males, 25 females) that were participating in a larger study of procedural pain in SCD. Due to the goals of the larger study, children between the ages of 10 and 12 (the transition from pre- to post-pubescence) were excluded. All protocols were approved by the Institutional Review Board of Palmetto Richland Hospital, which provides approval concomitantly with the University of South Carolina. All participants were recruited from a Hematology Clinic located in Columbia, South Carolina over a 9-month period. Forty-seven children had adequate plasma available for apelin analysis by ELISA. For the ratios, 46 children had both ET-1 and apelin data and 43 children had both Big ET and apelin data available. The ET-1 and Big ET ELISAs were conducted as part of the previous study and details about those results can be found in Schlenz et al. [14]. For child ratings discussed below, only children over the age of 5 completed ratings, resulting in a sample of 37 children for the apelin/ET-1 analysis and 36 children for the apelin/Big ET analysis.

Procedures

Children and their caregivers were approached at routine hematologist visits for participation. Children routinely receive venipuncture at these visits. Venipuncture was chosen to represent a standardized painful stimulus. After consent and assent procedures were completed, children and caregivers completed baseline (pre-venipuncture) ratings of pain and caregivers completed a background questionnaire. Once the venipuncture was completed, children and caregivers completed ratings of the child's pain during the procedure. Children were video recorded from the time they entered the exam room to the end of the venipuncture, in order to obtain observational ratings of pain. Medical record reviews were conducted after the child's visit using a structured coding method.

Measures

Caregivers completed a background information questionnaire to obtain demographic information. Children rated their pain using the Wong Baker Faces Scale [20]. Caregivers rated their child's pain using a visual analog scale (VAS). Observational ratings of pain were also taken at baseline and during the procedure using the modified version of the Observational Scale of Behavioral Distress [21]. Reviews of children's medical charts were used to establish history of acute chest syndrome, hydroxyurea status, and VOEs. Of the 47 children in this sample, 12 had a history of acute chest syndrome and 35 did not. For recent VOE history, we measured the number of hospitalizations, emergency department visits, and outpatient contacts for pain in the previous 24 months.

ELISA

Blood collection and plasma separation for this sample of participants have been described previously [14]. Briefly, blood was collected into EDTA vacutainer tubes and placed on ice for plasma isolation within 30 min of blood collection. After isolation, plasma was stored at −80 °C until further analysis by ELISA. ELISA kits for ET (1–21) (Cat no. Bl-20052) and Big ET (Cat no. Bl-20082) were purchased from ALPCO Immunoassays and apelin-36 (EK-057-15), which recognizes apelin-12, -13, and -36, was purchased from Phoenix Pharmaceuticals. ELISAs for ET-1 and Big ET-1 were performed in triplicate and ELISAs for apelin-36 were performed in duplicate according to the respective assay protocols. A standard curve was plotted from the standards of each kit using Prism software (GraphPad Software Inc., San Diego, CA), which was then used to extrapolate the sample concentrations from each plasma sample.

Data analysis

This study used an exploratory analysis to examine relationships between apelin and ratios of apelin to ET-1 and Big ET to the primary outcomes: baseline pain, procedural pain, acute chest syndrome, recent VOE history, and hydroxyurea status. We were particularly interested in providing effect sizes that could be used for future research in this area. For VOE history, there was one outlier (a child with 58 documented pain episodes) who was removed from the analysis. The relationships between apelin, the two ratios, baseline pain, procedural pain, and VOE history were assessed using correlations. The relationships between apelin, the two ratios, acute chest syndrome, and hydroxyurea status were assessed via t-test. Descriptive information on age and gender differences for apelin is also provided using correlation and t-test analysis, respectively. Information on age and gender for ET-1 and Big ET can be found in a prior publication [14].

The ratio variables demonstrated a positive skew that was corrected with log-transformation. Additionally, to ensure that the ratios were equally associated with both apelin and the endothelin variables, we examined correlations between the ratios, apelin, ET-1, and Big ET. The log-transformed ratio for apelin/ET-1 was highly associated with ET-1 ($r = -.84$) whereas the untransformed ratio was more equally associated with both apelin ($r = .41$) and ET-1 ($r = -.47$). We reported the untransformed results for this ratio descriptively below; however, both the log-transformed and untransformed results can be found in Table 1. For outcome variables, the three baseline and procedural pain ratings all demonstrated a positively skewed distribution and were log-transformed. In addition, because baseline ratings of pain were associated with significantly greater procedural pain, regression was used to remove variance in procedural pain that could be explained by baseline ratings. This approach allowed for the procedural pain ratings to solely reflect the pain from the procedure. Finally, due to the exploratory nature of this study, we were careful to evaluate the impact of outliers (defined as values exceeding three standard deviations from the mean of the variable).

Results

Table 1 provides all correlation results and these findings are explained descriptively below. t-Test results are reported here.

Apelin

Apelin levels ranged from 0.87 to 6.94 ng/mL and were significantly related to age, with older age associated with lower apelin levels. There was no statistically significant difference in mean apelin levels between male ($M = 2.53$, $SD = 1.42$) and female ($M = 2.61$, $SD = 1.12$) children, $t(45) = -.22$, $p = .829$.