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Impact of Arginine Therapy on Kyotorphin in Children with Sickle Cell Disease and Vasoocclusive Pain

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Abstract:

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Vasoocclusive pain episodes (VOE) are the clinical hallmark of sickle cell disease (SCD) and a leading cause of morbidity and mortality.[1] Therapies targeting underlying mechanisms of pain are lacking, which renders opioid analgesics the current standard-of-care. Multiple controlled trials in both the United States and sub-Saharan Africa support the safety and efficacy of arginine therapy in children with SCD-VOE.[2-6] Arginine is the obligate substrate for the production of nitric oxide (NO), a potent vasodilator that is low in SCD-VOE and contributes to vasoocclusive complications.[3,7] Mechanistically, arginine supplementation increases NO metabolites (NO_x),[8,9] improves mitochondrial function, and decreases oxidative stress.[10] Clinically it improves cardiopulmonary function,[4] decreases pain, and has opioid-sparing effects in children with SCD.[2,3]

Arginine is also the precursor for kyotorphin, an endogenous opioid-like analgesic first described 1979 in Kyoto, Japan,[11] produced from its amino acids precursors L-arginine and L-tyrosine by the action of the enzyme kyotorphin synthetase.[12,13] Kyotorphin exerts its analgesic effects indirectly by inducing met-enkephalin and β-endorphin that bind μ and/or δ- opioid receptors.[7,9] Oral administration of arginine (1g/kg) in wild-type mice increased kyotorphin levels in the midbrain and medulla where sites of morphine analgesia are located.[12] Subcutaneous administration of arginine inhibited carrageenin-induced hyperalgesia in a rat and mouse model, an effect that was reversed by naloxone (a δ -opioid inhibitor).[14] In addition, intracerebroventricular administration of arginine produced anti-nociception in intact mice after mechano- and thermo-nociceptive tests.[14,15] Furthermore, clinical studies have shown

persistent analgesia reversible by naloxone in chronic pain patients treated with intravenous arginine.[16,17] While kyotorphin has not been previously evaluated in SCD, these studies suggest a functional link between kyotorphin, arginine and met-enkephalin/β-endorphin in suppressing pain. Prior kyotorphin studies focused on chronic pain, however no studies to date have explored the arginine-kyotorphin relationship in acute pain.

SCD-VOE represents an acute pain model characterized by arginine deficiency.[7] Hemolysis plays a key role in arginine dysregulation;[7,18] release of erythrocyte-arginase, an arginine-metabolizing enzyme that competes with NO synthase for its obligate substrate L-arginine, hydrolyzes arginine to form ornithine and urea, while diverting away from NO production.[18,19] Low levels of the kyotorphin-precursor tyrosine have also been reported in SCD during VOE.[20] However, the relationship between arginine bioavailability and kyotorphin levels in SCD and pain is unknown. Our objective was to evaluate the impact of intravenous arginine therapy on plasma arginine, NO_x and kyotorphin concentrations in children hospitalized with SCD-VOE.

We conducted a single center, IRB-approved, prospective, randomized, open-label pharmacokinetics(pK)/pharmacodynamics(PD) study of intravenous arginine at a children's hospital in Atlanta, GA (clincialtrials.gov #NCT02447874; IND#66,943) to assess the impact of arginine therapy on plasma arginine and NO_x concentrations over time. Kyotorphin assessment was a post-hoc analysis. Patients with SCD (Hb-SS or $S\beta^0$-thalassemia) aged 7-21 years hospitalized for VOE requiring parenteral opioids were eligible. Written informed consent, and assent when appropriate, was obtained from all participants. Exclusion criteria included hemoglobin<5 gm/dL, hepatic/renal dysfunction, acute stroke, allergy to arginine, pregnancy, emergency department discharge, hospital discharge within the past 7 days, or previous enrollment into the study.

The research pharmacist performed blocked randomization using lists prepared by the biostatistician to randomize patients into one of three intravenous arginine dosing arms: 1) 100mg/kg every 8 hours (standard dose, n=4); 2) loading dose (200mg/kg) followed by standard dose (n=5); or 3) loading dose (200mg/kg) followed by continuous infusion (300mg/kg/day) (n=4). Arginine was administered over 30 minutes per manufacturer's recommendation (R-Gene10, Pfizer). Blood was obtained at 6 time points: Pre-infusion (time 0), and at 1, 1.5, 2, 4 and 8 hours after the initiation of the first arginine infusion, then at approximately 8AM daily until discharge or for 7 days, whichever came first. Plasma arginine, kyotorphin, and NO_x levels were measured through previously described methods.[7,18] pK/PD analyses were performed, including determination of arginine Cmax, Tmax, area under the curve (AUC, calculated using the trapezoid rule), rate of clearance and half-life. Numeric pain scores were extracted from the electronic medical records. Daily highest/worst, lowest and mean pain scores were assessed for correlations with peak kyotorphin concentration, arginine Cmax, change in arginine concentration from baseline to discharge (μM) and peak NO_x. Mean±SD, paired t-tests and Pearson correlation analyses between groups were performed where appropriate, using Prism-v9.5.1.

Sixteen patients were consented, and 13 patients were randomized. Three patients were excluded, one for elevated creatinine and two for emergency department discharge. Participant demographics, clinical characteristics, and laboratory values at initial presentation (pre-dose) are summarized in Table 1. While no statistically significant differences between randomized study arms were identified, subjects randomized to the standard dose arm (100mg/kg intravenously every 8 hours) trended younger in age, had clinically relevant lower hemoglobin levels and blood biomarkers suggesting an increased hemolytic rate that could impact arginine bioavailability. Plasma arginine and kyotorphin levels (Fig1) were significantly higher after arginine infusion, peaking at 1 hour, with no significant differences in peak concentration across study arms. Pharmacokinetics parameters are

summarized in Supplement-Table1. Mean plasma arginine peak for all subjects was 331.6±95.4 μM, 30 minutes after infusion completion (Tmax). All but one subject achieved peak plasma arginine levels above the K_m (100-150 μM) of the cationic amino acid transporter (CAT-1) after arginine infusion. The AUC was highest in the loading dose+continuous infusion arm. Kyotorphin levels strongly correlated to plasma arginine concentration (r=0.72, p<0.0001; Fig2). Arginine and kyotorphin levels over time broken down by study arm are illustrated in Supplement-Fig1. Plasma NO_x also significantly increased from pre-dose to Tmax (within 1-2 hours; mean absolute change 12.1±16.2 μM, p=0.02; Supplement-Fig2), returning to baseline by 8 hours. While NO_x increased primarily in those receiving an arginine-loading dose, no correlation was found between arginine and NO_x concentration nor with arginine Cmax and peak NO_x levels or mean change/% change in NO_x. No significant changes in the kyotorphin-precursor tyrosine were observed (Supplement-Fig3). Significant inverse correlations were identified between daily pain scores and change in plasma arginine concentration (μM) from baseline to discharge and peak Day1-kyotorphin levels when the arginine-loading dose arms were combined (Supplement-Table2). Onalo and colleagues also reported significant difference in worst pain scores after oral arginine versus placebo.[2] Non-significant inverse correlations between daily pain scores and Day1 peak kyotorphin, arginine Cmax and peak NO_x levels for all subjects were also noted (data not shown).

This is the first report of an acute increase in plasma concentration of the opioid-like analgesic kyotorphin in patients with SCD-VOE following an intravenous arginine infusion. Kyotorphin concentrations remained elevated for 2 hours before returning toward the pre-dose baseline level by 4 hours, strongly correlating to arginine concentration. Low arginine bioavailability is associated with SCD mortality and morbidity,[18] including acute pain severity.[2,3,7] Multiple phase-2 trials support the safety and efficacy of arginine therapy in children with SCD-VOE,[2-6] while marked analgesia has been reported in non-SCD patients with various forms of pain 30-40 minutes after intravenous arginine

compared to placebo, with a dose-dependent effect that lasted 6-24 hours.[16,17] As the obligate substrate for NO production, arginine's mechanism-of-action is unknown but thought to be related in-part to NO production. However, arginine is likely the rate-limiting amino acid for kyotorphin production,[11,13] potentially contributing to the efficacy of arginine therapy on pain reduction.[2,3,16] In particular, the opioid-sparing effect of arginine supplementation is not fully understood in SCD; induction of an endogenous opioid-like dipeptide like kyotorphin represents a potential mechanism of analgesia that would decrease opioid utilization during VOE. Although our study is limited by its small sample size, lack of control arm and single-center enrollment, it is a pK study meant to identify dose-dependent effects of arginine therapy and potential mechanisms-of-action, leading to a larger controlled trial in SCD-VOE.[22]

This study demonstrated that intravenous arginine rapidly increased plasma arginine concentration 2-5 times above baseline at presentation for VOE, reaching a maximum concentration within 1 hour of infusion initiation regardless of study-dose administered. While there was inter-subject variability in peak arginine levels achieved, the loading dose, which was double the standard dose, interestingly did not result in a significantly higher Cmax. However, prior studies have demonstrated a dose-dependent impact of arginine on NO_x production,[9] mitochondrial function and oxidative stress.[10] In addition, we previously demonstrated that a significantly lower peak arginine concentration was achieved in children with SCD at the onset of their acute VOE compared to levels achieved with the same arginine dose given at steady state.[9] While greater renal excretion or elevated metabolism of arginine are potential explanations not evaluated in this study, this observation may potentially reflect higher arginine intracellular transport in the loading dose arms, ultimately leading to changes in pharmacodynamic outcomes like mitochondrial function and oxidative stress that favor utilization of higher doses.[10] Kyotorphin levels strongly correlated to arginine concentration, and rapidly peaked

within one hour of arginine infusion initiation. While there were no significant changes in plasma tyrosine concentration after arginine infusion, we also found no significant difference in peak kyotorphin levels with our loading (200mg/kg) compared to standard dose (100mg/kg). However, since the AUC was greatest in the loading/continuous infusion group, a larger sample size might reveal a dose-dependent response. It is also possible that administration of higher doses of intravenous arginine may have a greater impact on kyotorphin production and ultimately pain relief. Given the excellent safety profile of arginine,[6] and practices utilizing up to 500mg/kg for urea cycle disorders and hyperammonemia,[23] mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS),[24] and growth hormone stimulation testing,[6] studies evaluating higher doses for SCD-VOE are indicated to potentially maximize pain management, particularly in the acute care setting.

Similar to previous reports,[7,8,25] arginine supplementation in this cohort significantly increased plasma NO_x levels, supporting a role for vasodilation as an additional potential mechanism-of-action during SCD-VOE. Finally, hemolysis depletes tetrahydrobiopterin (BH_4),[21] an essential cofactor for both tyrosine synthesis and NO production from arginine. BH_4 converts phenylalanine into tyrosine and is also a cofactor for NO synthase in the production of NO from arginine. Since BH_4 is unstable, it becomes non-enzymatically oxidized to dihydrobiopterin (BH_2) under oxidative stress,[21] disrupting both metabolic pathways and compromising tyrosine synthesis and NO production. In malaria, BH_4 is oxidized to BH_2, which contributes to endothelial dysfunction.[21] Particularly relevant to SCD, BH_4 activity warrants further study as it could disrupt both metabolic pathways, compromising tyrosine synthesis and NO production, adversely impacting kyotorphin production and potentially contributing to pain.

Our findings highlight a novel mechanism-of-action for arginine therapy in SCD-VOE that requires further research. While a phase-3 randomized controlled trial of intravenous arginine for

children and young adults with SCD-VOE is currently underway,[22] our kyotorphin-related observation has significant implications for the potential use of arginine as an opioid-sparing therapy in pain syndromes beyond SCD.

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Authorship

Contributions: C.R.M. designed research question, wrote the study protocol, obtained funding, obtained informed consent/assent, analyzed and interpreted the data, and wrote the manuscript; R.K and D.H analyzed and interpreted the data, and wrote the manuscript; L.A.S.B assisted with study protocol development, supervised sample processing, assisted with interpretation of data, and critically reviewed the manuscript; F.H. processed and analyzed biological samples, assisted with interpretation of the data, and critically reviewed the manuscript; C.A.R, D.R.A, and N.B assisted with interpretation of the data and critically reviewed the manuscript; C.D.D. assisted with study protocol design, patient enrollment and consent, interpretation of the data, and critically reviewed the manuscript.

Conflicts of Interest Statements: All authors report no conflicts of interest relevant to this manuscript. Claudia R. Morris, MD, is the inventor or co-inventor of several UCSF-Benioff Children's Hospital Oakland patents that include nutritional supplements, and is an inventor of Emory University School of Medicine patent applications for nutritional supplements for autism/apraxia, coronaviruses, kidney dysfunction, and pain, is a consultant for Roche and CSL Behring, is on the Scientific Advisory Board of TRILITY, is an editor for the sickle cell disease-fever and sickle cell disease-pain web-based reference for UpToDate, and is the Founder and Executive Director for Food as Medicine Therapeutics, LLC. Carlton Dampier, MD has received research support from Pfizer. David R. Archer, PhD has received research funding from Pfizer/Global Blood Therapeutics and DISC Medicine.

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Figure Legends

Figure 1. Impact of intravenous arginine therapy on plasma A. Arginine and B. Kyotorphin concentrations (μM) over 8 hours and daily. Both plasma arginine and kyotorphin levels peaked within 30 minutes of completion of the intravenous arginine infusion that was delivered over 30 minutes. Pooled data from the three dosing arms is represented, as there was no significant difference in peak concentration across study arms. For the 8-hour pharmacokinetics study, plasma arginine concentration troughs by 4 hours but remains significantly above baseline through 8 hours ($p=0.01$) and day 2 ($p=0.01$). Plasma kyotorphin levels were significantly elevated between 1-2 hours ($p=0.004$), before dropping towards baseline. Morning blood draws occurred at approximately 8AM daily, greater than 6 hours from the last arginine infusion, representing a trough for plasma arginine and kyotorphin levels. Subjects available for daily blood analysis varied based on clinical resolution of their vasoocclusive pain and discharge day, with 11 subjects analyzed on day 2, 7 subjects on day 3, 6 subjects on day 4, 4 subjects on day 5, and 3 subjects on day 6.

Figure 2. Pearson correlation between plasma arginine and kyotorphin levels (μM) for all available timepoint values. A strong correlation exists between plasma arginine and plasma kyotorphin concentration ($r=0.72$, $p<0.0001$). When an outlier timepoint with a peak kyotorphin level of 5.0μM was excluded from the analysis, the correlation was even stronger ($r=0.77$, $p<0.0001$). Filled circles represent data at 1,1.5, and 2 hours after initiation of arginine infusion, reflective of the significant acute increase in plasma kyotorphin levels. Unfilled circles represent Time 0 (pre-dose), 4 and 8 hours after initiation of arginine infusion and daily values for patients remaining in the hospital.

Figure 1

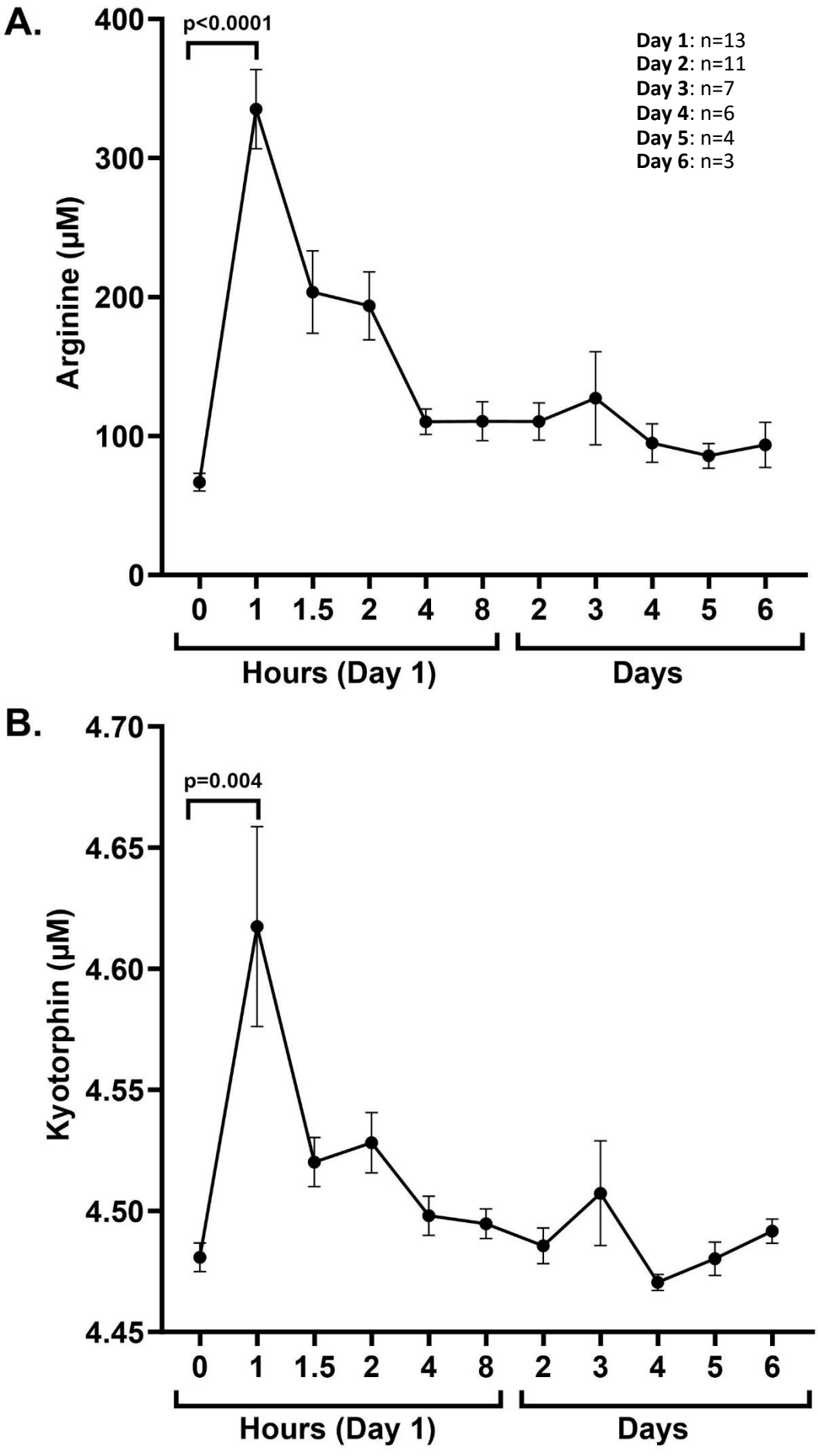

Figure 2