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A phase 1 dose-finding study of intravenous L-citrulline in sickle cell disease: a potential novel therapy for sickle cell pain crisis

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Acute pain from vaso-occlusion (VOC) in sickle cell disease (SCD) is the most frequent cause of emergency room visits and hospital admissions, contributing to the high burden of health care costs (Lanzkron *et al*, 2010). While major advances in the care of patients with SCD have occurred over the last 30 years, very little progress has been made in the actual treatment of VOC.

Nitric oxide (NO) is a powerful vasodilator that plays a fundamental role in VOC (Morris, 2008). NO is produced from L-citrulline and L-arginine, amino acids generated through the urea cycle from the NO synthase (NOS) family of enzymes (Moncada & Higgs, 1993). There are three NOS isoforms: neuronal (nNOS) found in neuronal tissue, inducible NOS (iNOS) found in cells and tissues, and endothelial NOS (eNOS) found in vascular endothelial cells. Shen *et al* (2005) showed that citrulline was the major supply for intracellular L-arginine and endothelial NO production in murine endothelial cells. Furthermore, Wijnands *et al* (2012) showed that L-citrulline supplementation restored intracellular NO production, which was related to the degree of eNOS phosphorylation. Moreover, enhanced arginase-induced arginine consumption is believed to play an integral role in the pathogenesis of sickle cell complications. In a more recent study, L-citrulline supplementation increased NO production and improved microcirculatory flow during conditions with arginase-induced arginine deficiency (Wijnands *et al*, 2015). However, intravenous citrulline has never been evaluated in human SCD. Hence, this study aimed to characterize the pharmacokinetic (PK) and safety profile of intravenous (IV) citrulline in this unique patient population.

A single centre open label phase 1 trial of IV citrulline was performed in participants with SCD following approval from the University of Mississippi Medical Center (UMMC) institutional review board. The study was registered at ClinicalTrials.gov (NCT02314689; NCT02697240), where the inclusion and exclusion criteria are described. Th phase 1 study was performed in two steps. Step 1 included a dose escalation bolus infusion of IV citrulline in steady-state SCD to determine the PK and safety profile with a peak goal plasma citrulline concentration of 80–100 μmol/l (Barr *et al*, 2007). Step 2 of the study was performed to evaluate safety and PK during a vaso-occlusive crisis.

The study drug, L-citrulline, was administered as open label vials of 50 mg/ml (5%) isotonic solution. Plasma sampling for PK studies were collected at specific time points. Briefly, for amino acid analysis, deproteinated plasma samples were subjected to cation exchange chromatography using a 4-component pH and ionic strength graded lithium citrate buffer system on a Beckmann 7300 amino acid analyser (Beckmann, Palo Alto, CA). Data obtained for each patient was fitted to a single-compartment PK model. The appearance of citrulline in plasma was described by a zero-order process (rate of citrulline appearance, R_{app}) to account for endogenous production, whereas the removal of citrulline was determined by a first-order process (constant of citrulline removal, k_{rem}). It was assumed that the values of all parameters remained constant for each patient during the course of plasma sampling. Scientist v2.0 (Micromath Scientific Software, St. Louis, MO) was used to fit the plasma citrulline concentration to the PK model by a weighted, least squares procedure to obtain values for R_{app}, k_{rem}, and the volume of distribution (V_d). Clearance was calculated as the product of k_{rem} and V_d.

For safety assessments, the Investigator determined the intensity of any adverse event (AE) according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.03 (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf) and their causal relationship. A Data Safety Monitoring Board, comprising 3 physicians who were not related to the study, reviewed every third subject.

A total of 8 subjects with SCD were enrolled in this phase 1 study of IV citrulline: four participants were enrolled in Step 1 and another four participants in Step 2. Patient demographics, genotype and baseline blood counts are shown in Table SI. In the first cohort of four participants, the IV bolus infusion of 20 mg/kg of L-citrulline yielded a mean peak level of 259 μmol/l and trough level in the range of 20–40 μmol/l at 4 h after infusion (Fig 1). Citrulline PK parameters for Step 1 with bolus infusion are shown in Table SII. Pharmacokinetic model simulations indicated a 20 mg/kg bolus dose of IV citrulline followed by 7 mg/kg per hour continuous infusion was needed to maintain the target citrulline plasma concentration of 100 μmol/l. Subsequently, four subjects with VOC were enrolled to receive IV citrulline bolus and continuous infusion. Individuals with VOC showed significantly lower baseline citrulline levels compared to steady-state (mean ± SD: 9·37 ± 1·43 vs.

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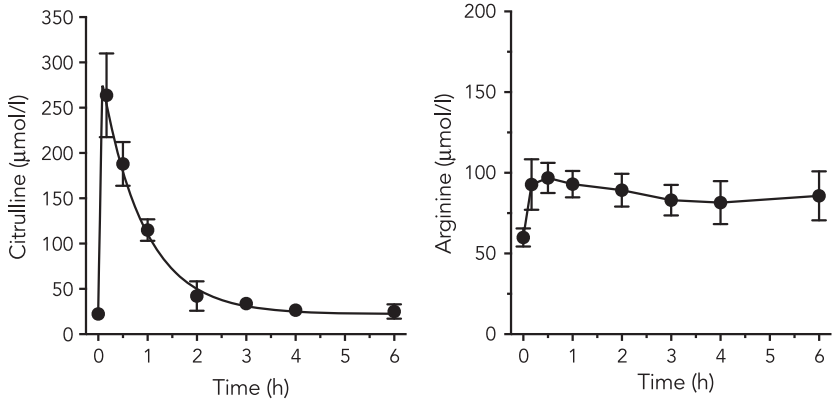

Fig 1. Graphs showing plasma citrulline and arginine levels over time after an intravenous bolus infusion of 20 mg/kg L-citrulline.

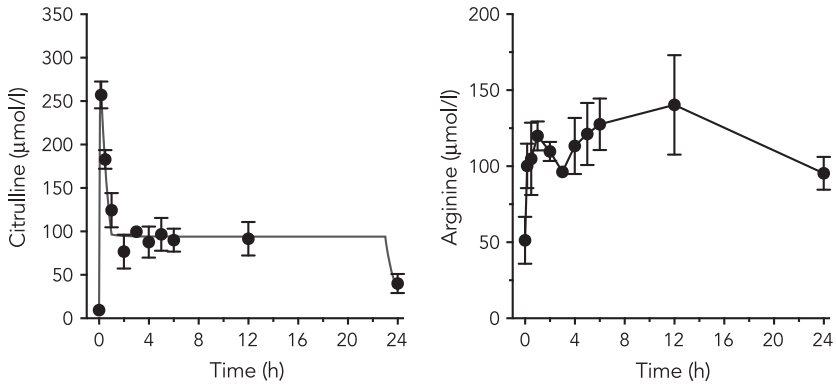

Fig 2. Graphs showing plasma citrulline and arginine levels over time after an intravenous bolus of 20 mg/kg followed by a continuous infusion of 7 mg/kg per hour L-citrulline.

22·28 ± 6·8, $P = 0·01$) (Figure S1). After the IV bolus, mean peak plasma concentrations was 257 μmol/l and citrulline plasma concentrations of approximately 100 μmol/l were achieved during a 7 mg/kg per hour continuous infusion. Furthermore, there was a robust and durable rise in both citrulline and arginine levels (Fig 2). The citrulline PK parameters for Step 2 with the bolus and IV continuous infusion are shown in Table SIII. The endogenous citrulline appearance rate (Rapp) was significantly lower in the VOC cohort compared to steady-state (5·0 μmol/h/kg vs 11·1 μmol/h/kg, $P = 0·014$).

Overall, intravenous citrulline in SCD was well tolerated and safe. There were no AEs ≥ grade 2 level of toxicity. Drowsiness was noted in 6 participants, but not severe enough to discontinue study medication. One patient reported feeling cold and one patient had nausea (no vomiting) associated with the drowsiness. Due to the theoretical risk of vasodilation from the NO boost, vital signs were followed closely (Figure S2). In one subject, the diastolic blood pressure transiently dropped >20% from baseline during the first 30 min of drug administration but normalized within 1 h without any intervention. There were no significant changes in the complete blood count and renal function tests (Table SIV). However, one subject was readmitted about 2 weeks later with right upper quadrant painelevated alanine transaminase and aspartate transaminase, peaking at 367 and 335 u/l respectively, which resolved by day 31. The subject developed fever during hospitalization; tests revealed cytomegalovirus (CMV) IgM positivity and CMV polymerase chain reaction of 6300 copies, suggesting acute CMV infection as an aetiology. This was reported to the US Food and Drug Administration as a serious AE.

This is the first report on the use of intravenous citrulline in SCD. While this intervention was well tolerated, drowsiness was a potential side effect of unclear aetiology that could be related to vasodilation of the cerebral vasculature or, perhaps, improvement in pain. Given the potential benefit of boosting endothelial NO and contributing to microcirculatory vasodilation, studies are needed to evaluate the efficacy of intravenous citrulline during a vaso-occlusive crisis.

Authorship

SM designed the study and wrote the manuscript. TRG performed the PK analysis, MH and NS conducted the study. JD analysed the data. MS designed the study. GC performed the amino acid testing. DD, RN and AC critically revised the manuscript. FB designed the study.

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Disclosures

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Box-plot showing baseline plasma citrulline levels in steady-state sickle cell subjects ($n = 4$) and during a sickle cell pain crisis ($n = 4$); $P = 0{\cdot}01$.

Figure S2. Vitals signs (mean ± standard error) in the first 4 subjects after a bolus infusion of L-citrulline.

Table SI. Showing the demographic, genotype and baseline complete blood count of participants

Table SII. Citrulline pharmacokinetic model parameter estimates for I.V. bolus cohort.

Table SIII. Citrulline pharmacokinetic model parameter estimates for intravenous bolus plus continuous infusion cohort. R_{app}, rate of citrulline appearance; k_{rem}, constant of citrulline removal.

Table SIV. Showing the laboratory profile after receiving the bolus and continuous intravenous L-citrulline at baseline and at 24 h (end of infusion).

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