Minireview

L-glutamine for sickle cell disease: Knight or pawn?

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Impact statement

L-glutamine has been recently approved by the FDA for the prevention of acute complications in sickle cell disease (SCD). However, there are many gaps in our understanding of the biologic role of glutamine and its therapeutic implications in SCD. This review summarizes the pre-clinical and clinical evidence that can inform clinical decision-making and future research on glutamine therapy in SCD patients.

Abstract

Oxidative stress is an important contributor to the pathophysiology of sickle cell disease. The pathways involved are complex and interlinked. L-glutamine is an amino acid with myriad roles in the body, including the synthesis of antioxidants, such as reduced glutathione and the cofactors NAD(H) and NADP(H), as well as nitric oxide—so it has therapeutic potential as an antioxidant. However, the relative impact of L-glutamine on the redox environment in red blood cells in sickle cell disease is not fully understood, and there are few therapeutic trials in sickle cell disease. Following the FDA approval of L-glutamine for sickle cell disease, more research is still needed to understand its clinical effects and role in therapy.

Keywords: Sickle cell disease, sickle cell anemia, L-glutamine, glutamine, reactive oxygen species, clinical trials

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Introduction

There is increasing evidence for the role of oxidative stress in the pathophysiology of sickle cell disease (SCD).[1] Reactive oxygen species (ROS), such as superoxide, can be derived non-enzymatically (Fenton chemistry) from denatured sickle hemoglobin (Hb S) moieties and lipid peroxidation or derived enzymatically, such as by the action of NADPH oxidase. ROS damage red blood cell (RBC) membranes and decrease cell deformability,[2] which may contribute to the pathophysiology of SCD. Plasma-free hemoglobin (Hb) and iron chelates are by-products of hemolysis that can also act as oxidants.[3] So, sickle RBCs (RBCs from individuals with SCD) have higher ROS than RBCs from healthy controls and are in a vicious cycle of oxidative stress.[4,5] To counteract ROS, mammalian cells have antioxidant pathways involving reduced glutathione (GSH), NAD(H), NADP(H) and nitric oxide (NO). Conceptually, therapeutic targeting of ROS in SCD could entail the reduction of ROS production (e.g. by induction of Hb F with hydroxyurea) or augmentation of antioxidant pathways (e.g. increasing the availability of substrates for GSH, NADH, NADPH and NO).

Glutamine, an L-α-amino acid, is the most abundant amino acid in the body.[6] Although it is considered a non-essential amino acid, high RBC turnover due to hemolysis increases the demand for glutamine, which can make it a conditionally essential amino acid in SCD.[7] The main therapeutic mechanism of glutamine supplementation in SCD is thought to be its antioxidant effects. However, the relative contribution of glutamine compared to other amino acids and antioxidants to the redox environment in SCD is unknown.[8] In addition, glutamine has other metabolic roles that have received little study in SCD. The FDA recently approved L-glutamine (Endari®) for the reduction of acute complications in SCD patients (HbSS and $S\beta^0$-thalassemia) based on two clinical trials.[9,10] In this review, we summarize the relevant pre-clinical and clinical studies of glutamine. We also pose the unanswered question about glutamine: whether it is a knight or pawn in the fight against SCD?

Glutamine as a substrate for the synthesis of glutathione

Glutathione exists in a reduced (GSH) and oxidized (GSSG) form. The thiol reductant, GSH, scavenges ROS such as hydrogen peroxide and lipid peroxides.[3,11] GSH can also interact with Hb to form glutathiol hemoglobin (G-Hb) which reduces the propensity for sickling.[12] There are two pathways by which reduced glutathione (GSH) is derived: de novo synthesis which requires the amino acids glycine, glutamate and cysteine, and regeneration from oxidized glutathione (GSSG) which requires NADPH (Figure 1).

Under normal oxidative conditions, with ample NADPH availability, the total glutathione pool is maintained in a predominantly reduced state by glutathione reductase-mediated regeneration of GSH from GSSG.[13] However, in the setting of increased oxidative stress, the de novo synthesis pathway becomes more important due to the depletion of NADPH and rapid efflux of oxidized GSSG from RBCs.[14] Indeed, sickle RBCs have been shown to have reduced intracellular GSH concentration[15–17] despite higher rates of glutathione synthesis.[18] In experiments with normal RBCs exposed to chronic oxidative stress, the rate of GSSG efflux was higher than the rate of GSH synthesis. This caused an initial decline in total free glutathione (TFG) levels until a new sustained steady-state was achieved at an approximately 10% lower TFG concentration than under normal redox conditions.[19] It would be of interest to know if glutamine supplementation can augment the de novo synthesis of glutathione to return GSH levels to normal.

Under healthy conditions, intracellular glutamate concentration in erythrocytes is well below the K_m (concentration of substrate required to reach half the maximum rate of reaction) for glutamate cysteine ligase (GCL),[19] one of the rate-limiting enzymes in glutathione synthesis[13] (Figure 1) so that increasing substrate (glutamate, L-cysteine) availability for GCL could theoretically increase the de novo synthesis of GSH. The RBC membrane is impermeable to glutamate[20] so that intracellular glutamate has to be derived from either glutamine or by the transamination of α-ketoglutarate from alanine and aspartate.

There is evidence that sickle erythrocytes have increased V_{max} (maximum rate of reaction) and decreased K_m for Na-dependent secondary active transport of glutamine into the cells.[21] Further, it has been shown that an increased rate of glutamine entry leads to increased intracellular accumulation of glutamate.[21] Mathematical modeling of experimental data showed that in normal RBCs exposed to chronic

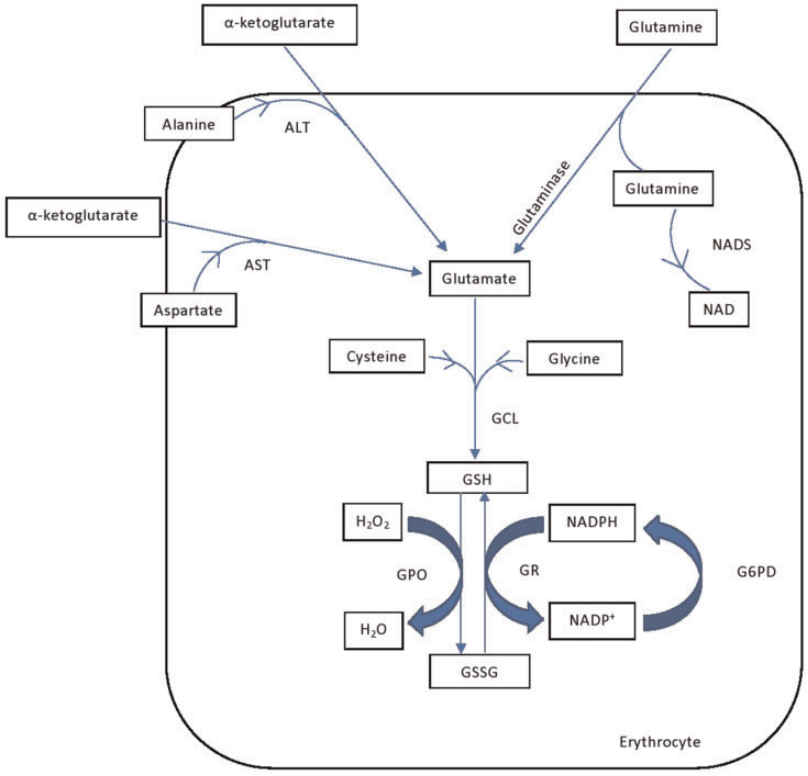

Figure 1. Glutathione synthesis in the red blood cell. De novo synthesis of reduced glutathione (GSH) is represented in top half of figure and regeneration of reduced glutathione from oxidized glutathione (GSSG) in bottom half of figure. Glutamine, α-ketoglutarate, and alanine, with smaller contribution from aspartate, are the main precursors for the intracellular synthesis of glutamate. Glutamine is also used in the synthesis of NAD^+. Cysteine, glycine, and glutamate are required for the de novo synthesis of reduced glutathione and this reaction is catalyzed by glutamate cysteine ligase (GCL), which is the rate-limiting enzyme in this pathway. GSH reduces hydrogen peroxide by the action of glutathione peroxidases, and in turn is oxidized to GSSG. Glutathione reductase catalyzes the reverse reaction with the use of NADPH. Reduced NADPH is regenerated from $NADP^+$ by the enzyme glucose-6-phosphate dehydrogenase (G6PD). Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; NADS: nicotine adenine diamide synthetase; GCL: glutamate cysteine ligase; GSH: reduced glutathione; GSSG: oxidized glutathione; GPO: glutathione peroxidase; GR: glutathione reductase; G6PD: glucose-6-phosphate dehydrogenase. (A color version of this figure is available in the online journal.)

oxidative stress, increasing the V_{max} of glutamine influx by 10% increased intracellular glutamate concentration and total free glutathione concentration to almost normal values within 10 days.[19] In contrast, other studies have shown that 89% of erythrocyte glutamate was derived from alanine aminotransferase (ALT),[22] and that α-ketoglutarate availability was more important than glutamine when RBCs were depleted of GSH.[23]

Cysteine and glycine can also enter human RBCs via specific amino acid transport systems.[20] Some studies have shown that intracellular concentrations of L-cysteine are low in SCD.[18] However, other investigators have shown that the concentration of glycine and cysteine is increased in sickle RBCs when compared to normal RBCs.[24,25] Some studies have shown that supplementation with L-cysteine can also increase de novo synthesis of glutathione.[19,26,27] Therefore, glutamine is perhaps not the only conditionally essential amino acid that could modulate the de novo synthesis of glutathione in SCD.

Glutamine as a substrate for the synthesis of NAD(H) and NADP(H)

Hb S has a higher rate of auto-oxidation than Hb A.[28] The oxidation of Hb S produces methemoglobin, superoxide, and denatured globin.[3] Superoxide, in turn, can generate hydrogen peroxide, oxygen, and hydroxyl radicals.[3,29] Methemoglobin reductases reverse this process using the cofactor NADH, and to a lesser extent, NADPH.[30] Other antioxidant pathways such as glutathione reductase, glucose-6-phosphate dehydrogenase (G6PD), 6-phosphoglucose dehydrogenase, and catalase use the cofactor NADPH.[31,32]

The conversion of glutamine to glutamate also results in the production of NAD^+ via the action of NAD synthetase (Figure 1). Sickle RBCs have a decreased NAD redox potential, demonstrated by a reduced ratio of NADH to total NAD (NAD^+ plus NADH) when compared to normal RBCs.[33] The K_m of NAD synthetase is higher than the mean concentration of intracellular glutamine, so that increasing glutamine delivery to the erythrocyte could theoretically increase NAD(H) and NADP(H) production.[34] In a small study, Niihara *et al*.[35] administered oral L-glutamine to six adult SCD patients and measured an increase in the total NADH content and NAD redox potential (NADH/total NAD) after four weeks.

NAD^+ is converted to $NADP^+$ by NAD kinase and $NADP^+$ is reduced to NADPH by the action of glucose-6-phosphate dehydrogenase (G6PD) (Figure 1). NADPH is a cofactor for the regeneration of GSH from GSSG as described earlier. NADPH is also utilized by NADPH oxidase in the generation of superoxide radicals, and this activity is upregulated in SCD.[36,37] Nevertheless, sickle RBCs have a normal ratio of NADPH to total NADP despite an increase in the total NADP content.[33] Therefore, glutamine supplementation may not affect NADP redox potential in sickle RBCs.

Glutamine as a substrate for the synthesis of nitric oxide

Enterally absorbed glutamine is the main source of intestinal citrulline production and supports renal arginine production, contributing to 15% of plasma arginine levels (Figure 2).[38–41] Arginine is transported into cells where it is a substrate for the enzyme nitric oxide synthase (NOS) that produces nitric oxide (NO). Alternatively, arginine be converted to ornithine by the enzyme arginase. Nitric oxide regulates regional blood flow through vasodilation as well as by suppression of platelet aggregation, secretion of procoagulant proteins, and expression of endothelial cell adhesion molecules.[42]

NO has both pro-oxidant and antioxidant effects.[43] However, given the evidence that SCD patients have reduced NO bioavailability,[44] its beneficial (especially antioxidant) effects in SCD have received the most attention.[42] Hemolysis releases arginase, which depletes plasma arginine,[45] while cell-free plasma Hb and ROS also scavenge NO.[42] Patients with low arginine-to-ornithine ratios have increased risk of pulmonary hypertension and early death.[46] In one study, oral glutamine administration in SCD patients was found to increase plasma and erythrocyte arginine levels, with the highest increase in arginine bioavailability (lowest arginine-to-ornithine ratio) occurring in a patient with severe pulmonary hypertension.[47]

SCD patients treated with oral L-glutamine for at least four weeks had reduced adhesion of sickle erythrocytes to human umbilical vein endothelial cells compared to untreated patients.[48] The explanation for this finding is unclear, and it could be related to improvement in the NAD redox state that reduces inflammation or increased NO synthesis (both processes could lead to reduced expression of endothelial cell adhesion molecules). However, further studies are needed to corroborate these findings and understand the mechanisms involved.

Other metabolic roles of glutamine

Glutamine is a substrate for nucleotide synthesis including purine, pyrimidines, and amino sugars and, as such, it is a conditionally essential amino acid in catabolic conditions such as malnutrition and surgery.[6] Several studies have explored the role of glutamine supplementation in critical illness, surgical recovery, and immune deficiency states (described in the sections below). As there is high cell turnover related to hemolysis and inflammation in SCD, glutamine may have a metabolic (and therapeutic) role that is separate from its antioxidant effects.

Glutamine also plays a role in the immune system where immune cells consume glutamine at rates similar to glucose. It is required for a myriad of functions, such as lymphocyte proliferation, cytokine production, phagocytic activity, and bactericidal reactions.[49] For example, glutamine enhances superoxide production in neutrophils via the generation of ATP and the regulation of NADPH oxidase expression.[50] The immunomodulatory effects of glutamine have been explored in infection and trauma[6,51] but not in SCD.

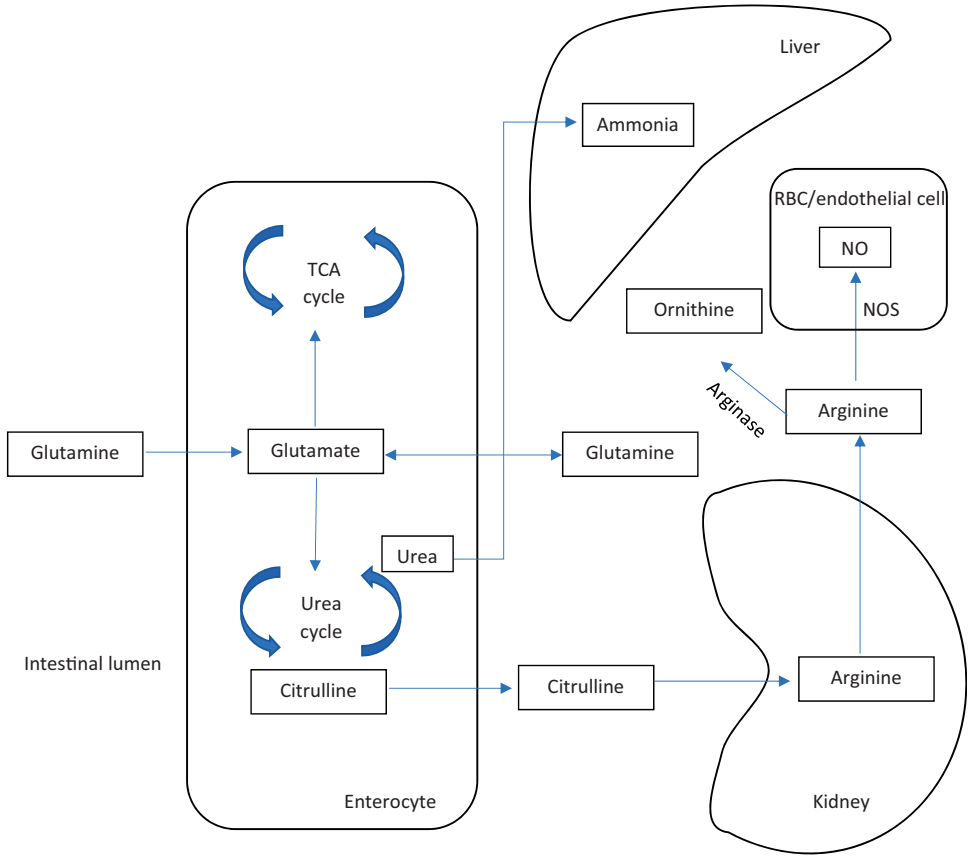

Figure 2. Intestinal and renal metabolism of glutamine. Enteral glutamine is taken up by intestinal cells where some is metabolized as an energy source. Glutamate generated from glutamine can also be directed into the urea cycle producing citrulline that is released into the bloodstream. Urea generated through this process is transported to the liver via the portal vein where it is converted into ammonia. Citrulline is taken up by renal tubular cells where it is converted into arginine. Plasma arginine is used in the synthesis of nitric oxide by the action of nitric oxide synthase (NOS) in red blood cells and endothelial cells. Plasma arginine levels are depleted by the action of arginase producing ornithine. NO: nitric oxide; NOS: nitric oxide synthase; RBC: red blood cell. (A color version of this figure is available in the online journal.)

There has also been long-standing interest in the effects of glutamine on cardiovascular diseases. Glutamine has been shown to augment endothelial cell production of ammonia, which triggers heme oxygenase-1 (HO-1) transcription. HO-1 catalyzes the conversion of heme to carbon monoxide (CO), iron, and biliverdin. CO and biliverdin (and its derivative, bilirubin) have vasodilatory effects and improve vascular function by suppressing inflammation, oxidative stress, apoptosis, and vascular smooth muscle cell proliferation and migration.[52] However, glutamine can also mediate harmful angiogenic responses by fueling the Krebs cycle and stimulating the proliferation and migration of vascular cells and deposition of extracellular matrix. This could lead to vascular remodeling that is associated with conditions such as pulmonary arterial hypertension.[53] Whether this occurs in SCD is unknown.

Intestinal metabolism and pharmacokinetics of glutamine

Although glutamine is enterally absorbed, intestinal cells are the major consumers of glutamine in the body. Based on studies of glutamine absorption under healthy conditions, a large amount (50–70%) of enterally administered glutamine is metabolized by mitochondrial-associated glutaminase (GLS) in intestinal cells (Figure 2).[54] However, enterally administered glutamine is still effective in raising blood concentrations of glutamine in a dose-related manner.[54–56] Fasting, malnutrition, and catabolic stress can alter the kinetics of absorption in the gut.[57] However, glutamine absorption in SCD has not been thoroughly studied.

Ziegler *et al.*[56] conducted a series of dose-response studies of L-glutamine in healthy volunteers. They evaluated single enteral doses of 0.1 and 0.3 g/kg, intravenous doses of 0.0125 and 0.025 g/kg/h given over 4 h, and glutamine-enriched total parenteral nutrition (TPN) with 0.285 and 0.570 g/kg/day administered over five days. Whole blood glutamine and ammonia levels tended to rise with the glutamine dose. After an enteral dose, blood glutamine levels peaked at 30 to 45 min and then declined steadily to the normal range in 90 to 120 min (low dose, 0.1 g/kg) or in 180 to 240 min (high dose, 0.3 g/kg).

Morris *et al.*[47] studied the pharmacokinetics of a single oral dose of 10 g of L-glutamine in SCD patients with elevated tricuspid regurgitant jet velocities, specifically evaluating plasma and erythrocyte glutamine and arginine levels. In five SCD patients, three of whom were tested with and without glutamine supplementation, plasma glutamine levels peaked 30 min after ingestion, decreased to

a plateau by 2 h and remained higher than baseline by 8 h. Plasma arginine concentration peaked by 4 h and remained elevated through 8 h. Erythrocyte glutamine levels began to increase by 8 h, while erythrocyte arginine concentration peaked at 8 h. One patient with severe pulmonary hypertension had the greatest improvement in intracellular arginine bioavailability. There are no published reports on multiple-dose pharmacokinetics of glutamine in SCD.

Pharmaceutical sources of L-glutamine

Pharmaceutical-grade, free glutamine (Endari®, Nutrestore®) is available as a powder for oral suspension or by mixing with food and taken twice daily. Endari® has an FDA indication for SCD. Glutamine is also available in more stable, dipeptide forms such L-glycyl-L-glutamine, L-arginyl-L-glutamine, and L-alanyl-L-glutamine.[52] These dipeptide forms use the high-capacity human oligopeptide transporter 1 on enterocytes, which may facilitate a higher bioavailability of the dipeptide form than free glutamine.[58] Perhaps the use of L-arginyl-L-glutamine is suitable for use in SCD where both arginine and glutamine deficiencies have been noted.[59]

Clinical studies of glutamine in non-SCD populations

Glutamine supplementation has been used to enhance athletic performance for many years. A recent meta-analysis, however, concluded that there was no effect on aerobic performance, body composition, or immune function, but glutamine supplementation was associated with increased weight loss and, at high doses (>200 mg/kg body weight), with reduced neutrophil numbers.[60]

L-glutamine (NutreStore®) has an FDA indication for short bowel syndrome (SBS) in conjunction with human growth hormone. In this setting, glutamine supplementation improved weight gain and energy absorption in SBS, but its effects were temporary, and the evidence is inconclusive for long-term therapy.[61]

Glutamine becomes conditionally essential in catabolic states and its supplementation has been studied in trauma, post-operative recovery, and other critically ill patients. When studied in surgical ICU patients, glutamine supplementation was found to be safe but did not affect clinical outcomes such as mortality.[62] Before the year 2013, meta-analyses of randomized trials supported improved clinical outcomes and a survival benefit associated with glutamine supplementation in critically ill patients.[63,64] However, in a large multicenter trial published in 2013 ($n = 1223$), glutamine supplementation did not improve clinical outcomes and was associated with increased mortality in critically ill patients with multiorgan failure.[65] The mechanism for increased mortality is unclear. Ammonia levels were not measured in this study, but patients with severe liver dysfunction were excluded. Patients with renal failure were included in the analysis, which were not part of earlier studies of glutamine in critically ill patients.[66] There is ongoing debate about the safety and efficacy of glutamine in critical illness.[67]

The role of glutamine in cardiometabolic disease has also been explored.[52] Metabolic profiling of large study cohorts has evaluated the glutamine-to-glutamate ratio (Gln:Glu) and its association with cardiovascular risk factors. A high Gln:Glu ratio has been positively correlated with high density lipoproteins and reduced incidence of type 2 diabetes mellitus (DM), whereas a low Gln:Glu ratio is correlated with increased body mass index (BMI), blood pressure, circulating triglycerides and insulin.[68,69] Oral administration of glutamine in patients with type 2 DM improved their glucose tolerance and body composition.[70,71] Further, Ma *et al*.[72] reported the results of two large prospective studies in the US demonstrating that increased dietary intake of glutamine and an increased plasma Gln:Glu ratio were associated with reduced risk of cardiovascular mortality, independent of other dietary or lifestyle factors.[72]

Glutamine supplementation was found to be safe in pediatric oncology patients, but it had no effect on the incidence of oral mucositis or the incidence of infections.[73,74] Similarly, in adult oncology and bone marrow transplant (BMT) patients treated with glutamine, there were no differences in clinical outcomes such as incidence of oral mucositis, hematologic recovery, and length of hospital stay.[75–77] However, one study showed reduced need for parenteral nutrition and a suggestion of improved long-term survival with glutamine supplementation.[78] In another study in pediatric BMT patients, there was a reduced incidence of infections and bacterial colonization when glutamine was added to parenteral nutrition.[79]

Clinical studies of glutamine in SCD

Four clinical studies have evaluated L-glutamine therapy in SCD (Table 1). Niihara *et al*.[35] reported the first clinical study of glutamine supplementation that assessed the incidence of acute SCD complications. Oral L-glutamine was administered at a dose of 30 g/day for four weeks to seven adults with SCD. The primary outcome was assessment of the NAD redox potential (ratio of NADH to $NAD^+ + NADH$), in which there was a statistically significant improvement. Six of the seven patients also reported subjective improvements in chronic pain, reduced daily opioid use, and improved energy levels. The small sample size and lack of controls make it difficult to interpret these results. The study team went on to conduct lager trials that lead to the FDA approval of Endari®.

Williams *et al*.[80] studied the effects of 24 weeks of oral glutamine supplementation at 600 mg/kg/day on the metabolic status of children and adolescents with SCD. They reported a 6% reduction in resting energy expenditure (REE) in treated patients when compared to their baseline, but there was no difference in the total Hb concentration and SCD-related clinical outcomes were not studied.

Niihara *et al*.[10] conducted two placebo-controlled clinical trials: a phase II study ($n = 70$)[9] and a phase III trial ($n = 230$). Patients with sickle cell anemia (89.9%) and sickle-β^0-thalassemia were randomized to receive L-glutamine at 0.3 g/kg orally twice daily for 48 weeks followed by a three-week taper (136 exposed for six months,

Table 1. Clinical studies of glutamine in sickle cell disease.

Study	Population/study design	Glutamine dose	Main findings
Niihara *et al*.[35]	7 SCD patients Age: 19–60 years Open-label, uncontrolled	30 g/day administered orally in three divided doses for 4 weeks	NAD redox potential (ratio of NADH to $NAD^+ + NADH$) increased from $47.2 \pm 3.7\%$ to $62.1 \pm 11.8\%$ ($P < 0.01$) NADH level increased from 47.5 ± 6.3 to 72.1 ± 15.1 nmol/mL RBC ($P < 0.01$) 6 of 7 patients reported improvement in chronic pain and reduced daily narcotic use
Williams *et al*.[80]	27 SCD patients Aged: 5.2–17.9 years Open-label, uncontrolled	600 mg/kg/day orally for 24 weeks	6% reduction in median resting energy expenditure compared to baseline ($P = 0.053$); greatest reduction in patients with less than 90% ideal body weight ($P < 0.03$)
Niihara *et al*.[9]	81 SCD patients Aged: 9–58 years Randomized 1:1 to glutamine or placebo	0.3 g/kg orally twice daily for 48 weeks	Mean number of painful crises was 2.5 and 5.5 for L-glutamine and placebo groups respectively ($P = 0.060$) at week 24 Mean number of hospitalizations was 0.8 and 1.3 for L-glutamine and placebo, groups, respectively ($P = 0.036$) at week 24.
Niihara *et al*.[10]	230 SCD patients Aged: 5–58 years Randomized 2:1 to glutamine or placebo	0.3 g/kg orally twice daily for 48 weeks	Median of 3 pain crises in the glutamine group and 4 in the placebo group ($P = 0.005$) Median of 2 hospitalizations in the glutamine group and 3 in the placebo group ($P = 0.005$) Reduced number of SCD complications in glutamine group compared to placebo: fewer cumulative days in the hospital (6.5 vs. 11, $P = 0.02$), prolonged time to first pain crisis (84 vs. 54 days, $P = 0.02$), and second pain crisis (212 vs. 133, $P = 0.03$), reduced episodes of acute chest syndrome ($P = 0.003$)

SCD: sickle cell disease (HbSS and sickle-β^0-thalassemia genotypes only).

109 exposed for at least a year) or placebo ($n = 111$). Both trials excluded patients with hepatic or renal insufficiency, recent blood transfusions, pregnancy, or lactation. The phase II trial failed to show a significant difference in its primary end point of frequency of sickle cell pain crises between treatment and control groups. There was a small but statistically significant decrease in the number of hospitalizations for pain crisis at week 24 (mean number of hospitalizations was 0.8 in L-glutamine group vs. 1.3 in placebo group ($P = 0.036$)), but this difference was not seen at week 48 ($P = 0.07$). There were no statistical differences in Hb, hematocrit, or reticulocyte count between treatment and placebo groups. Overall, L-glutamine was well tolerated. There was one death in the glutamine arm due to multiorgan failure that was deemed unrelated to the drug.

The phase III trial by Niihara *et al*.[10] showed a statistically significant reduction in the median number of sickle cell crises in the glutamine group versus the placebo group (3 vs. 4, $P = 0.005$) and a decreased median number of hospitalizations for painful events (2 vs. 3, $P = 0.005$). There was a statistically significant reduction in other SCD complications (see Table 1). There were again no statistically significant between-group differences in the Hb, hematocrit, or reticulocyte count. Overall, glutamine was well tolerated in this study. However, there were two sudden deaths in the glutamine group; both patients were in their mid-40s with history of chronic organ failure. Most patients on these trials were on hydroxyurea therapy. Both trials had very high withdrawal rates (over 50% in the phase II study, 32% in the phase III study) which make it difficult to interpret their results. The therapeutic effects of L-glutamine in SCD genotypes other than HbSS and sickle-β^0-thalassemia have not been studied.

Side effects

The main side effects of L-glutamine reported in the SCD clinical trials were constipation, nausea, headache, abdominal pain, cough, extremity pain, back pain, and chest pain. Adverse reactions leading to treatment discontinuation included one case each of hypersplenism, abdominal pain, dyspepsia, burning sensation, and hot flashes.[9,10] The three deaths in L-glutamine-treated SCD patients are reported above.

Glutamine is involved in nitrogen exchange via ammonia transport between tissues.[6] Intestinal cells are efficient at handling large amounts of enterally administered glutamine. Ammonia released from the activity of intestinal glutaminase is extracted by the liver before it enters the bloodstream.[57] However, an excess of glutamine can exacerbate defects in ammonia metabolism in the setting of hepatic insufficiency.[81] In addition, long-term glutamine supplementation in non-SCD patients may adversely affect the homeostasis of other amino acids as well.[82] Patients with renal and hepatic impairment were excluded from the clinical trials of glutamine in SCD that lead to the FDA approval.[9,10] Therefore, clinicians should be cautious when administering L-glutamine to SCD patients with renal and hepatic impairment until these populations are studied further.

Therapeutic role in SCD

There is increasing evidence to substantiate an adverse role of oxidative stress in the pathophysiology of SCD, and it is reasonable to suspect that antioxidants could be beneficial. However, given the complexity of the redox environment and the multitude of intermediates in interlinked pathways, it will require pre-clinical and large clinical trials to

find the optimal antioxidant(s), if any, for SCD. Whether glutamine fulfills this role remains to be determined. Although there is sound biologic rationale based on experimental data that glutamine contributes to the synthesis of GSH, its relative importance compared to other amino acids such as α-ketoglutarate and L-cysteine has not been established, especially in SCD. Although the effects of glutamine on surrogate markers of oxidative stress such as NADH/NAD ratio have been studied, direct measurement of RBC ROS and RBC lifespan has not been conducted. In addition, glutamine has other metabolic roles in protein and nucleotide synthesis that are poorly studied, if at all, in SCD.

There is conflicting evidence on the role of glutamine in critical illness and concerns about its association with increased mortality in patients with multiorgan failure. In fact, many clinical trials conducted over multiple years in critical care centers have been unsuccessful in reaching a consensus. The SCD population may be less heterogenous than critical care patients, making it easier to study the effects of glutamine. However, unlike hydroxyurea, there are no validated clinical biomarkers to assess response to glutamine therapy. In addition, multi-dose pharmacokinetic studies have not been conducted to establish the optimal dosing regimen. Whether a dipeptide formulation of glutamine, such as L-arginyl-L-glutamine, has improved bioavailability and greater benefit than L-glutamine should also be investigated.

A reasonable line of inquiry would be to investigate the additive benefit of L-glutamine to optimized hydroxyurea therapy. As the proposed mechanism of action and toxicity profile of glutamine is different than hydroxyurea, the combination of these drugs may be theoretically advantageous and safe.[83] Additionally, for the minority of patients who do not tolerate hydroxyurea, L-glutamine may be a reasonable alternative. However, L-glutamine therapy comes with a significant cost: approximately $3000 per month for adults and $1000 per month for children, which is 20-times more expensive than hydroxyurea.[84]

Another factor to consider is medication adherence, which is a vital issue for patients with chronic illnesses like SCD.[85,86] The necessity of twice daily administration of powdered L-glutamine may lead to poor adherence in clinical trials as well as clinical practice. Moreover, the addition of L-glutamine to hydroxyurea therapy may adversely affect adherence to hydroxyurea. Given that multiple studies show a survival benefit for adults and children who take hydroxyurea, the addition of any intervention without established survival benefit that might, even if unintentionally, reduce adherence to hydroxyurea should be considered thoughtfully.

Conclusion

The randomized trials of glutamine by Niihara *et al.*[9,10] and the availability of a new therapy for SCD are a welcome advance. However, larger and longer-term studies are required to assess the impact of glutamine on many clinically relevant outcomes, including mortality. Additional studies are required to establish an optimal or individualized dosing regimen, identify biomarkers of response to glutamine therapy, and describe its effects in sickle-hemoglobin C disease and other SCD genotypes. Currently, there are too many unanswered questions to know if glutamine is a knight or but a pawn in the therapeutic fight against SCD.

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