PHARMACOTHERAPY

SPECIAL ARTICLE

Using L-Carnitine as a Pharmacologic Probe of the Interpatient and Metabolic Variability of Sepsis

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Objective The objective of this review is to discuss the therapeutic use and differential treatment response to Levo-carnitine (L-carnitine) treatment in septic shock, and to demonstrate common lessons learned that are important to the advancement of precision medicine approaches to sepsis. We propose that significant interpatient variability in the metabolic response to L-carnitine and clinical outcomes can be used to elucidate the mechanistic underpinnings that contribute to sepsis heterogeneity.

Methods A narrative review was conducted that focused on explaining interpatient variability in L-carnitine treatment response. Relevant biological and patient-level characteristics considered include genetic, metabolic, and morphomic phenotypes; potential drug interactions; and pharmacokinetics (PKs).

Main Results Despite promising results in a phase I study, a recent phase II clinical trial of L-carnitine treatment in septic shock showed a nonsignificant reduction in mortality. However, L-carnitine treatment induces significant interpatient variability in L-carnitine and acylcarnitine concentrations over time. In particular, administration of L-carnitine induces a broad, dynamic range of serum concentrations and measured peak concentrations are associated with mortality. Applied systems pharmacology may explain variability in drug responsiveness by using patient characteristics to identify pretreatment phenotypes most likely to derive benefit from L-carnitine. Moreover, provocation of

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sepsis metabolism with L-carnitine offers a unique opportunity to identify metabolic response signatures associated with patient outcomes. These approaches can unmask latent metabolic pathways deranged in the sepsis syndrome and offer insight into the pathophysiology, progression, and heterogeneity of the disease.

Conclusions The compiled evidence suggests there are several potential explanations for the variability in carnitine concentrations and clinical response to L-carnitine in septic shock. These serve as important confounders that should be considered in interpretation of L-carnitine clinical studies and broadly holds lessons for future clinical trial design in sepsis. Consideration of these factors is needed if precision medicine in sepsis is to be achieved.

Key Words critical care, septic shock, pharmacometabolomics, systems pharmacology.
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Epidemiology and Heterogeneity of Sepsis

Sepsis is a life threatening, dysregulated host response to infection, which is characterized by systemic organ dysfunction.[1] One in three Americans who die in the hospital have sepsis, and, in 2017, there were an estimated 48.9 million cases worldwide.[2]

The sepsis syndrome is highly heterogeneous, with patients presenting along a continuum of clinical signs, symptoms, and severity of illness.[3] The mechanism and pathophysiology underlying highly variable clinical trajectories in sepsis are complex, and the precise reason(s) some patients exhibit severe dysregulated responses while others recover from their initial infection in an uncomplicated fashion remains poorly understood. Such host-response heterogeneity muddies the interpretation of treatment response and is a major reason why novel pharmacotherapy often fails. Absence of adequate stratification of patients based on their underlying pathophysiology may contribute to this.[4] The need to advance mechanistic understanding of sepsis heterogeneity has led to calls from the National Institute of General Medical Sciences for studies that seek to determine the effect of patient characteristics on differential treatment response (NOT-GM-19-054). Teasing out this variability is necessary to bring about a precision medicine approach to sepsis.

Ample evidence suggests a hypermetabolic component and derangement of host metabolism that is central to sepsis pathophysiology.[5] Recently revised consensus guidelines define the most severe manifestation, septic shock, as infection with sustained hypotension despite recommended evidence-based treatment interventions (e.g., fluid resuscitation), and pertinent to this discussion, metabolic dysfunction and/or tissue hypoperfusion as evidenced by an elevated blood lactate concentration.[1] Hyperglycemia, protein catabolism, and lipolysis are similarly known to occur in sepsis and contribute to poor patient outcomes.[6] Although several studies have targeted lactate as a resuscitation goal,[7–9] these trials have typically utilized fluids, vasopressors, or other agents designed to improve organ perfusion under the assumption that lactate elevations are predominantly explained by ongoing tissue ischemia, which may not necessarily be true.[10] Current pharmacotherapy neither targets nor corrects these metabolic perturbations, although restoration of host bioenergetics offers a promising therapeutic target. Moreover, given the prevalence, persistent mortality, and lack of specific treatment paradigms, there is a critical need to advance understanding of the range and extent of the metabolic consequences of sepsis beyond observational studies.

Herein, we discuss clinical trials of L-carnitine, an important regulator of mitochondrial and metabolic homeostasis, for the treatment of septic shock. We consider how patient-level biological variables impact response to treatment and propose that provocation with L-carnitine offers a novel and unique opportunity to improve mechanistic understanding of the heterogeneity and metabolic consequences of sepsis.

Physiological Role of Carnitine and Treatment in Sepsis Patients

Carnitine is an endogenous, polar small-molecule derived from lysine and methionine, which plays a well-established, crucial role in transport of long-chain fatty acids into the mitochondria for β-oxidation. Other key roles during times of metabolic stress include maintenance of coenzyme A homeostasis, metabolic flexibility, and promotion of normal tricarboxylic acid cycle (TCA cycle) function, and further oxidation of

fatty acids by peroxisomes.[11] A full, in-depth review of carnitine and acylcarnitine homeostasis and biochemistry is outside the scope of this paper, and it has been extensively reviewed elsewhere.[11, 12] Briefly, the carnitine shuttle allows for fatty acid entrance to the mitochondria for oxidation and subsequent energy production through transfer of acyl groups and conversion into acylcarnitines (Figure 1).

In sepsis, mitochondrial dysfunction has been increasingly reported as a critical factor in persistent organ failure and altered peripheral cell mitochondrial function is known to be associated with sepsis mortality.[14,15] Further evidence of mitochondrial dysfunction includes elevations of systemic acylcarnitines, indicating incomplete β-oxidation of fatty acids, and the presence of mitochondrial deoxyribonucleic acid (DNA) in plasma.[16,17] Sepsis alterations in mitochondrial function and lipid metabolism are associated with kidney and liver function that are driven in part through inhibition of the pyruvate dehydrogenase complex and decreased activity of carnitine palmitoyltransferase I.[18,19] Prior clinical studies of i.v. L-carnitine and acetylcarnitine given to patients in cardiogenic and circulatory shock found an overall positive effect on hemodynamic parameters and patient survival.[20–22]

These principles served as the basis for two recent clinical trials of L-carnitine in septic shock. The first was a phase I, randomized, double-blind clinical trial of L-carnitine (12 g i.v.)

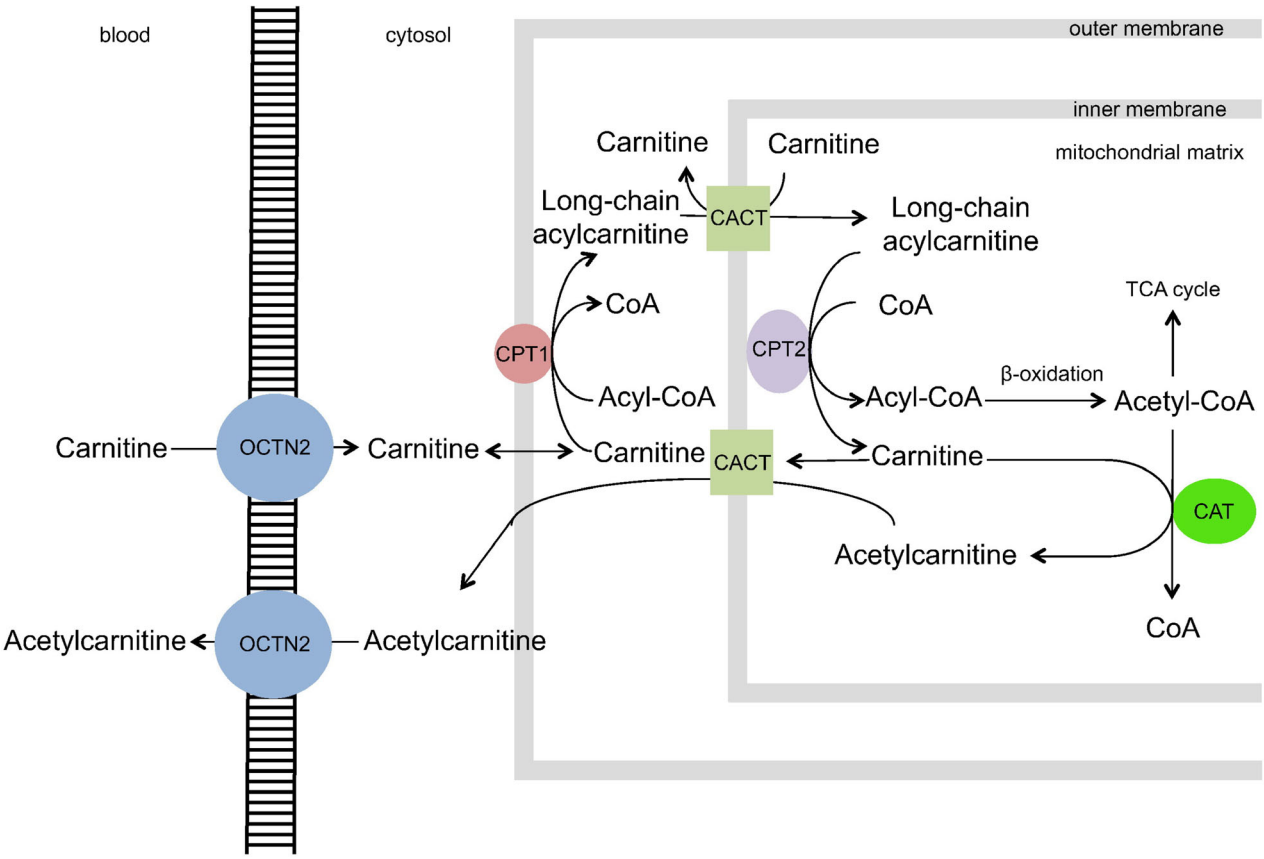

Figure 1. Overview of carnitine transport and enzymatic conversions in the cell. Carnitine enters the cell from the blood through an organic cation transporter (OCTN2), after which carnitine palmitoyl transferase I (CPT-1) facilitates the conversion of carnitine and long-chain fatty acid-coenzyme As (CoAs) to acylcarnitines and CoA. The transporter carnitine-acylcarnitine translocase (CACT) moves the newly formed long-chain acylcarnitines into the mitochondrial matrix in exchange for free carnitine. Here, long-chain acyl groups are transferred back to CoA by carnitine palmitoyl transferase II (CPT-II). The newly regenerated acyl-CoA undergoes β-oxidation into Acetyl-CoA, which feeds into the tricarboxylic acid cycle (TCA) cycle. Alternatively, carnitine acetyl-transferase (CAT) converts free carnitine and acetyl-CoA to acetylcarnitine, which can freely diffuse through CACT and OCTN2 back into the bloodstream. This latter process may be enhanced during sepsis and times of metabolic stress, serving as a crucial sink for excess acetyl groups that may be toxic to the cell. The ladder cartoon represents the plasma membrane separating the blood and the cytosol of the cell, whereas grey boxes represent the outer and inner membranes of the mitochondria. (Open-source through the Creative Commons Attribution, obtained with permission from https://doi.org/10.1016/j.ebiom.2017.01.026.)[13] [Color figure can be viewed at wileyonlinelibrary.com]

versus saline placebo conducted in 31 patients with septic shock enrolled within 16 hours of diagnosis.[23] Study drug was given as an i.v. bolus (33% of total dose), followed by a 12-hour infusion that delivered the remaining drug. This study found no difference in the reduction of Sequential Organ Failure Assessment (SOFA) score at 24 hours, but there was an improvement in mortality at 28 days (4/16 vs 9/15, p=0.048) and 1-year (8/16 vs 12/15, p=0.081) in L-carnitine treated patients. Adverse events sometimes attributable to L-carnitine, including gastrointestinal distress, body odor, and an decreased seizure threshold, were not observed in the study. In addition, serious adverse events were not significantly different between the L-carnitine and placebo treatment arms. A follow-up phase II multicenter, double-blind, adaptive dose-finding trial randomized 250 patients within 24 hours of identified septic shock to i.v. L-carnitine (6, 12, or 18 g) versus placebo.[24] In the primary analysis, the highest dose (18 g) of L-carnitine was not found to be superior to placebo in reducing the total SOFA score at 48 hours, and the predicted probability of success of a subsequent phase III trial in reducing mortality at 28 days did not exceed the a priori threshold of 90%. The 6 and 12 g L-carnitine doses underperformed in the trial and were adaptively dropped from the randomization scheme as the trial progressed. Three, interim, preplanned safety and futility analyses were completed by an independent data safety monitoring board.

However, the primary end points of both clinical studies do not describe a critical component of drug response to supplemental L-carnitine in patients with septic shock. The pharmacometabolomics data from the phase I trial reveal substantial interpatient variability in serum carnitine and acetylcarnitine concentrations postinfusion.[25,26] Patients receiving L-carnitine in the phase I study had 24-hour postinfusion (T24) serum carnitine levels ranging from 30 μM to over 1600 μM (median = 368 μM). The temporal changes in carnitine and acetylcarnitine for the treatment and placebo arms are shown in Figure 2. Critically, L-carnitine treated nonsurvivors (based on 1-year mortality) had elevated carnitine and acetylcarnitine (C2), short chain acylcarnitines (C3, C4, and C5), and long chain acylcarnitines (C14 and C16) compared with L-carnitine treated survivors. This suggests the observed variability in measured peak concentrations and metabolic response profiles are associated with clinical outcomes. As such, identification of the patient-level factors associated with peak carnitine/acylcarnitine concentrations may help identify patients most likely to derive a mortality benefit from L-carnitine and inform the design of future clinical studies.

Candidate Mechanisms of Interpatient Variability of Drug Response in Sepsis

Pharmacogenomics

Pharmacogenomics seeks to explain variability in drug exposure and response based on genetic differences between individuals. Genetic variation in drug metabolizing enzymes, transporters, and targets impact an individual's exposure and/or response to a given pharmacologic therapy, which can manifest as distinct drug-response phenotypes. Genetic variability is also known to alter patient response across disease states and medications commonly seen in the intensive care unit (ICU).[28] Treatment and dosing paradigms, which incorporate patient-specific pharmacogenomic data, hold promise in decreasing adverse drug events (ADEs) and improving efficacy.[29] Moreover, rationale clinical trial enrollment based on pharmacogenomic phenotypes can foster a more homogenous patient cohort and target patient populations most likely to benefit from therapy (Table 1).

Genetic variability in a number of enzymes and transporters could contribute to L-carnitine drug response, including those highlighted in the carnitine shuttle (Figure 1). Carnitine acts intracellularly and is highly sequestered in skeletal muscle and other tissues of the body.[11] Given the polar structure of carnitine, active sodium-dependent transport by organic cation/carnitine transporters (OCTNs) is required for entry from the blood into the cell and subsequent facilitation of fatty acid β-oxidation. The primary carnitine transporter, OCTN2, thus represents the focus of this section.

The OCTN2 transporter is encoded by the *SLC22A5* gene located on chromosome 5q31.1. Spanning 25 kb, the 10 exons of this gene encode the full length 557 amino acid protein. Numerous autosomal recessive mutations in the *SLC22A5* gene are responsible for primary carnitine deficiency and results in low serum carnitine levels due to the kidney's impaired ability to reabsorb the molecule.[30] Missense mutations are exceedingly rare, result in severe metabolic and

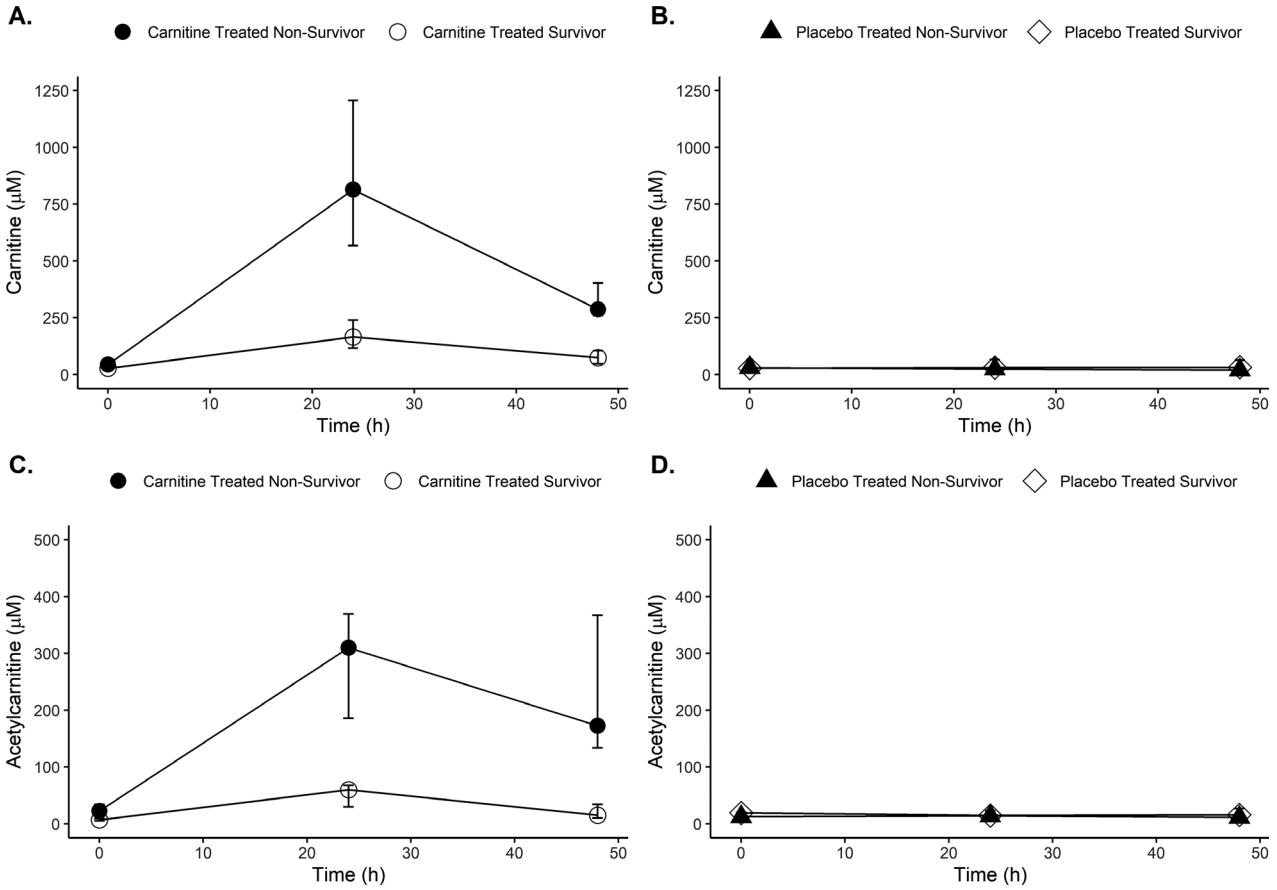

Figure 2. Carnitine treatment induces a metabolic phenotype whereby serum carnitine and acetylcarnitine concentrations are elevated in sepsis nonsurvivors. Serum concentrations of carnitine and acetylcarnitine are plotted over time for patients treated with either L-carnitine (panels A and C) or saline placebo (panels B and D). Data plotted are the median, 25th, and 75th percentile of observed serum concentrations, and the Mann–Whitney *U* test was used to determine significant differences between non-survivors and survivors at each timepoint. All p values are corrected for multiple comparison using a false discovery rate method according to Storey and colleagues[27] and are reported as q values. L-carnitine treated nonsurvivors (N=7–8) at 1-year had significantly higher concentrations of carnitine relative to survivors (N=8) at baseline (BL, q = 0.02); 24-hours (T24, q = 0.004); and 48-hours (T48, q = 0.02) posttreatment. Similar trends were observed for acetylcarnitine (BL, q = 0.01; T24, q = 0.003; and T48, q = 0.02). No significant differences in carnitine or acetylcarnitine concentrations were observed between placebo treated non-survivors (N=8–12) and survivors (n=3).

mitochondrial dysfunction, and manifest clinically as a primary carnitine deficiency at a young age. As such, loss of function mutations are unlikely to play a role in explaining variability in L-carnitine concentrations or response in clinical studies of adults with septic shock. Nonetheless, given the vital role of OCTN2 in carnitine uptake into the cell, and considering the large doses administered in these trials, more common genetic polymorphisms in OCTN2 resulting in reduced function and / or expression may improve understanding of the mechanisms that explain the broad dynamic range of carnitine concentrations after supplementation.

Common polymorphisms (i.e., minor allele frequency greater than 1%) in the *OCTN2* gene and their impact on carnitine transport outside the context of primary carnitine deficiency are rare.[31–33] Three single-nucleotide polymorphisms (SNPs; Phe17Leu, Tyr449Asp, and Val481Asp) were associated with reduced OCTN2 function compared with wild-type, and a SNP in the promoter region of the gene (−207C>G) was associated with increased carnitine transport capacity and trended toward increase mRNA expression in cell lines.[31] Out of these, only the promoter region variant (−207C>G, rs2631367) could be considered common according to the National Center for Biotechnology Information database of genetic variation (database [db]SNP).[34] Further studies have observed a tissue-specificity to the −207C>G variant's effect on mRNA expression levels.[32,33]

Table 1. Impact of Patient-level Variables That Could Influence the Outcome of Future Clinical Trials of Sepsis Therapeutics

Candidate mechanisms of interpatient variability of drug response in sepsis	Impact on L-carnitine trial design and interpretation	Influence on improving precision medicine in sepsis
Pharmacogenomics	Genetic variance in the transport receptor of L-carnitine (OCTN2) may influence drug concentration at site of action	Stratify patients by genotype at the time clinical trial enrollment
Drug interactions	Co-administration of OCTN2 inhibitors, including commonly used antibiotics, and vasopressors, may influence drug concentrations	Thorough screening for potential drug interactions by clinical pharmacists at time of trial enrollment and post hoc
Pharmacometabolomics	Baseline and dynamic metabolic signatures are associated with elevated drug concentrations and patient mortality	Target metabolic subgroups for trial enrollment and measure metabolic response signatures post drug administration
Morphomics	Patient muscle mass and body composition may influence metabolic adaptability, energetic stores, and drug distribution	Consider variation in body size and composition when testing targeted metabolic therapeutics
Renal function and PKs	Altered renal clearance and reabsorption of drug and acyl-metabolites may influence drug concentrations and patient outcomes	Embedded clinical pharmacology studies to quantify sepsis pathophysiology induced alterations in drug PK

OCTN2 = organic cation/carnitine transporter; PK = pharmacokinetic.

To supplement the limited literature regarding common polymorphisms effecting OCTN2, we conducted a systematic bioinformatics search for potentially relevant SNPs. We queried the Genotype-Tissue Expression (GTEx) Project (available at https://gtexportal.org/home/), which seeks to explain variability in mRNA expression levels from previously healthy human cadavers with whole genome sequencing.[35] The goal of this query was to determine common genetic variants (i.e., SNPs) that significantly alter gene expression of the OCTN2 transporter. Using expression quantitative trait loci (eQTL) analysis, ~1500 variants were found to be associated with altered gene expression at the tissue level. Summing across more than 6000 SNP/tissue pairs, the variant with the largest effect on net *OCTN2* gene expression was the promoter region variant (−207C>G, rs2631367).

In previously unpublished data from our group, patients treated with L-carnitine in the phase I trial[23] were genotyped for the OCTN2 (−207C>G) SNP. In this preliminary study, 14 patients had both genomic and serum carnitine concentrations measured at 24 hours (T24). Among these, 4 patients were wild-type (CC), whereas 10 carried 1 or 2 copies of the G allele. Patients with the C/G or G/G genotype trended toward lower T24 plasma levels of L-carnitine ($p=0.11$), suggesting that genetic variation in the OCTN2 transporter may contribute to variability and persistent elevations in L-carnitine after supplementation during septic shock. More pharmacogenetic studies are needed and are underway in the phase II trial[24] to determine if variation in OCTN2 and other carnitine-specific enzymes and / or transporters explain interpatient variability in L-carnitine drug response.

Drug Interactions

Drug interactions occur when the activity, exposure, or effectiveness of a drug is impacted by the presence of another drug. Co-administered drugs may inhibit or induce expression of important enzymes or transporters, compete at target binding sites, or act in a synergistic or antagonistic fashion. Different combinations of drugs and their interactions introduce variability in the pharmacokinetic (PK) and pharmacodynamic (PD) response to pharmacologic therapy, which may put patients at increased risk of ADEs and either mitigate or enhance therapeutic efficacy. Critically ill patients are at increased risk of drug interactions and subsequent complications given comorbidities and disease complications that are often present (e.g., renal failure) and the requisite complex treatments regimens prescribed.[36,37] In other disease states, such as cancer, there is a high prevalence of drug interactions in patients enrolled in clinical trials.[38] Drug interactions in critically ill patients may pose a similar threat to trial validity and patient health and should be systematically screened and considered (Table 1).

For L-carnitine, several drugs are reported to inhibit the OCTN2 transporter and, therefore, could contribute to interpatient variability in

exposure. These drugs can also cause secondary carnitine deficiency through inhibition of the OCTN2 transporter in the kidneys leading to decreased efficiency of reabsorption.[39] Of particular interest, in the setting of sepsis, are two widely used classes of medications, namely antibiotics and vasopressors. Previous reports have demonstrated that cefepime and levofloxacin inhibit OCTN2 in vitro.[40,41] Although the choice of antibiotic therapy in sepsis depends on a number of patient-specific factors, cefepime and levofloxacin are two commonly used antibiotics in the United States and are both recommended options in evidence-based best practices. Vasopressors, such as norepinephrine and other catecholamines, used to maintain blood pressure support, and other commonly used medications, including omeprazole and valproic acid, inhibit OCTN2 and could similarly impact L-carnitine drug response.[39] In addition to omeprazole, other proton-pump inhibitors, including pantoprazole and lansoprazole, have been shown to inhibit similar organic ion transporters but whether they interfere with the function of OCTN2 and carnitine transport has not been reported.[42]

Propofol, a short-acting hypnotic and sedative that is widely used in the ICU, may also play a critical role in understanding variable drug response to L-carnitine. Propofol is known to inhibit carnitine palmitoyltransferase I and the mitochondrial electron transport chain, which leads to incomplete β-oxidation of fatty acids.[43] The induced metabolic disruptions have been linked to propofol infusion syndrome (PRIS), a severe adverse effect of propofol that includes bradycardia, arrhythmias, rhabdomyolysis, metabolic acidosis, hepatomegaly, hyperlipidemia, and organ failure. Moreover, animal and in vitro experiments have suggested a role for L-carnitine and acetylcarnitine in restoring propofol inhibition of fatty acid metabolism.[44,45]

Variable exposure to one or more of these drugs could influence resulting blood concentrations and subsequent metabolic response to supplemental L-carnitine. Other mechanisms are certainly possible such that other concomitant medications and variable patient feeding may further confound the clinical studies discussed above. Presently, the clinical relevance of such interactions and how they should be managed is currently unknown. Further investigation into the use of these drug inhibitors and the effect on L-carnitine concentrations in the phase II study is underway.

Pharmacometabolomics

Metabolomics seeks to identify and quantify small molecules, the full collection of which define the metabolome, in a given biofluid.[46] The metabolome constitutes a read-out of underlying cellular and biochemical events that reflect the genetic makeup of the host, transcriptomic, and proteomic influence, as well as variability in the microbiome and environmental exposure. As such, metabolomics represents the culmination of these important regulators on the host. In addition, given that metabolism is dynamic on a practical and physiological time scale, this sensitivity can inform heterogeneity in disease trajectory and treatment response. Pharmacometabolomics exploits this paradigm and is aimed at understanding and predicting response to drug treatment. In short, clinical application of metabolomics holds great promise in improving the diagnosis and risk stratification of critically ill patients, furthering drug discovery through metabolic signatures of drug response and/or ADEs, and elucidating biochemical pathways involved in the pathophysiology of critical illness (Table 1).

A pharmacometabolomic approach was utilized to understand baseline metabolic differences in patients treated in the phase I study of L-carnitine.[26] Patients treated with L-carnitine who had low baseline levels of the ketone levels, 3-hydroxybutyrate, also had lower posttreatment carnitine levels at 24 hours. The L-carnitine treated, low-ketone patients also had better clinical outcomes as evidenced by a timelier reduction in vasopressor requirement and decreased 1-year mortality. An untargeted metabolomics approach was then conducted in male patients from the phase I study.[47] The L-carnitine treated non-survivors were found to have posttreatment elevations in metabolites related to vascular inflammation, including histamine, allysine, and fibrinopeptide A. Along with the differential metabolic response of survivors and nonsurvivors highlighted in Figure 2, these data suggest both baseline metabolic signatures and metabolic profiles over time may be predictive of L-carnitine treatment responsiveness.

Morphomics

Analytic morphomics is a new and rapidly growing scientific discipline within precision pharmacotherapy that studies how variation in body size, composition, and structure are associated with drug

and disease response.[48] In sepsis, two recent meta-analyses have observed a paradox between body composition and survival, whereby particularly overweight (body mass index [BMI] between 25 and 29.9 kg/m^2), and to a lesser extent obese (BMI between 30 and 40 kg/m^2), patients tend to have better mortality outcomes compared with normal weight individuals (BMI between 18.5 kg/m^2 and 24.9 kg/m^2).[49,50] Notably, underweight (BMI less than 18.5 kg/m^2) and morbidly obese (BMI greater than 40 kg/m^2) patients were found to have similar risk of mortality relative to normal weight individuals. Neither measured peak concentrations of L-carnitine nor mortality were significantly associated with BMI in patients who received study drug in the phase I study. However, the observed "obesity paradox" reinforces the concept of a metabolic and energy-driven component to sepsis pathophysiology and has a number of possible pathophysiological explanations, including increased energy stores, antiinflammatory mediator release from adipose tissue, and lipoprotein binding of bacterial cellular components.[51]

Another possible explanation is that increased muscle mass offers energetic and metabolic adaptability to patients within a window of the BMI spectrum. Protein catabolism and subsequent myopathy are observed in critically ill patients, and skeletal muscle, an important energetic source to the host, experiences mitochondrial injury over the course of sepsis.[52] Indeed, recent studies have found an association between low muscle mass and increased risk of mortality for patients with sepsis. In 74 patients with liver cirrhosis and sepsis, patients with low muscle mass (defined as mid-arm muscle circumference lower than the fifth percentile of the population) had increased mortality compared with patients with normal muscle mass (47% compared with 26%, p=0.06).[53] In a separate retrospective review of 627 patients with a diagnosis of sepsis and an available abdominal computed tomography scan of the psoas muscle, muscle mass depletion was associated with 28-day mortality in both univariate and multivariate logistic regression (odds ratio [OR] = 2.79, p=0.01).[54] Given the extent of protein catabolism, the sepsis-obesity paradox, and the known sequestering of carnitine into muscle tissue, morphomics and variability in body composition offers a currently untapped field that could aid in explaining the observed variability in response to supplemental L-carnitine and patient mortality in sepsis broadly (Table 1).

Pharmacokinetics and Renal Function

Pharmacokinetics (PKs) as a science seeks to understand what the body does with and to drugs. More specifically, it is the study of how drugs are absorbed, distributed, metabolized, and eliminated from the body. Previous studies have highlighted that there is profound sepsis-induced variation in drug PKs. The reasons for this are likely multifaceted but include altered protein binding, perturbed vascular and tissue permeability, decreased hepatic and renal blood flow, and lower activity of drug metabolizing enzymes.[55] High interpatient variability in drug PK in sepsis clinical trials contributes to overall heterogeneity of the patient cohort and may confound trial results unless careful analysis of drug exposure is considered (Table 1).

The PKs of L-carnitine has been explored; however, no studies have determined the precise PKs of L-carnitine in sepsis or at such high intravenous doses. As discussed above, OCTN2 is a critical carnitine transporter that is responsible for carnitine uptake into cells/tissues, however, it is also responsible for reabsorption of carnitine in the kidney proximal tubule. As such, kidney function may play a vital role in the interpatient variability in serum carnitine concentrations that result after supplementation. Previous reviews report an average renal clearance of endogenous carnitine of 1 to 3 ml/min, indicating that at physiologically relevant concentrations up to 99% of carnitine is reabsorbed by the kidneys.[56] Exogenous carnitine administered to healthy volunteers, increased renal clearance of carnitine and acetylcarnitine, indicating saturation of the OCTN2 transporter and the reabsorption process, may be relevant for supraphysiologic doses of intravenous carnitine like those given in septic shock trials.[56] Unfortunately, urine samples were not collected in these studies, which prevents us from estimating renal clearance of relevant carnitine species in these patients. Both studies reported similar serum creatinine levels among survivors and nonsurvivors indicating renal function alone does not explain heterogeneity in L-carnitine and acylcarnitine concentrations among patients. However, the reliability of creatinine as a biomarker in the setting of acute kidney injury (AKI), sepsis, and other critical illnesses, and in drug development broadly have been called into question.[57,58] New investigations of biomarkers of kidney injury and function are underway, but have yet to be widely adapted or clinically validated. Further

investigations of the variability in L-carnitine drug response stratified by the presence of AKI and acute liver injury, and among other measures of organ dysfunction are warranted before precise clinical recommendation can be made in these patient groups. Moreover, modeling the impact of patient-level biological variables, such as sex, age, and race, is critical to understand the observed heterogeneity in L-carnitine drug response.

Metabolic Provocation with Supplemental L-carnitine

Although the approaches outlined above offer an opportunity to identify patients with sepsis most likely to respond to L-carnitine, understanding the metabolic response signature of L-carnitine treated patients holds value beyond a potential therapeutic benefit. Outside of sepsis, the concept of provoked metabolic testing is used to uncover latent disease phenotypes. For example, a glucose tolerance test is used to diagnosis a previously undetectable pre-diabetic phenotype in pregnant women. As seen in Figure 2, the metabolic response profiles of the placebo arm did not differentiate patient mortality at 1-year, as they did for L-carnitine treated patients. Critically, this finding suggests the possibility that treatment with L-carnitine amplifies or incites a phenotype of sepsis mortality and underlying derangement in carnitine homeostasis. Indeed, elevations in plasma acylcarnitines are understood to be a measure of mitochondrial dysfunction and altered coenzyme A homeostasis in other metabolic diseases and elevated acetylcarnitine were recently found to be predictive of plasma cytokine levels, blood culture positivity, multiorgan dysfunction, and mortality in patients with sepsis.[59] Others have shown that short chain acylcarnitines levels are related to plasma mitochondrial DNA, an indicator of cellular damage, and that acylcarnitines are predictive of mortality in critically ill patients.[16,17] Together, these data suggest derangements of the carnitine/acylcarnitine pool may be indicative of metabolic dysfunction and/or worsening sepsis that is predictive of mortality.

A metabolic test with supplemental L-carnitine can provoke biochemical pathways in sepsis and amplify signals of underlying mitochondrial dysfunction and perturbed energy pathways. A more complete investigation of other metabolite profiles that are disrupted upon treatment may also lead to new insights into underlying disease mechanism and pathophysiology. Although there are a number of sepsis metabolomics studies that confirm the substantial metabolic disturbances of the disease, they do not inform distinct sepsis phenotypes in the way that a metabolic provocation test could. The substantial variability in response to L-carnitine exposure and subsequent mortality differences indicate phenotypic differences between groups. In aggregate, this observation introduces the principle that even in the presence of a disease like sepsis, which is known to induce a substantial metabolic perturbation, provocation of metabolism is required to bring the full dynamic range into view.

Conclusion and Future Directions

L-carnitine and acylcarnitine concentrations are highly variable after L-carnitine supplementation in septic shock, and the observed interpatient variability is associated with patient mortality. The heterogeneity of sepsis and drug response complicates the interpretation of a therapeutic value of L-carnitine and other potential sepsis pharmacotherapies. Currently, a careful analysis of the phase II clinical trial to inform the design of, and the results from, a phase III trial are needed before L-carnitine treatment can be recommended for a specific sepsis patient population. However, even though more work needs to be done, a strategy using the patient-level factors and biological variables that impact L-carnitine drug response could be used in the a priori identification of patients who are most likely to derive the greatest benefit from treatment. Well defined phenotypes of drug response could serve as inclusion-exclusion criteria and aid in the design and interpretation of future phase III clinical studies of L-carnitine. Such information will need to be balanced with threats to clinical and external validity, as well as consideration to the ability to recruit a sufficient patient population.

The approach outlined here is applicable to other emerging sepsis therapeutics and could aid in developing a precision medicine approach to sepsis and the design of early-phase clinical trials in critical illness. Moreover, provoking metabolism in septic shock with L-carnitine supplementation offers a unique opportunity to define metabolic signatures of survival and elucidate biochemical pathways deranged in the sepsis syndrome. Such an approach offers a novel

mechanism to further the understanding of sepsis pathophysiology and progression, as well as elucidate drug response phenotypes.

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