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Methionine metabolism in an animal model of sepsis

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Abstract

Background: Sepsis is a disease with high incidence and lethality and is accompanied by profound metabolic disturbances. In mammalian methionine metabolism, S-adenosylmethionine (SAM) is produced, which is important in the synthesis of neurotransmitters and glutathione and as an anti-inflammatory agent. The degradation product and antagonist of SAM is S-adenosylhomocysteine (SAH). In this study, we investigated changes in methionine metabolism in a rodent model of sepsis.
Methods: Sepsis was induced in male Wistar rats (n=21) by intraperitoneal injection of bacterial lipopolysaccharide (10 mg/kg). Controls (n=18) received vehicle only. Blood was collected by cardiac puncture 24 h later. Puncture of the suboccipital fossa was performed to collect cerebrospinal fluid (CSF). Methionine metabolites were measured using stable isotope dilution tandem mass spectrometry. Plasma total homocysteine and cysteine were measured by HPLC using fluorescence detection. Glutathione was assayed using a modified enzymatic microtiter plate assay.
Results: We observed significantly higher plasma levels of SAM ($p<0.001$) and SAM/SAH ratio ($p=0.004$) in septic animals. In CSF, there was also a trend for higher levels of SAM in septic animals ($p=0.067$). Oxidative stress was reflected by an increase in the ratio of oxidized/reduced glutathione in septic animals ($p=0.001$).
Conclusions: Sepsis is associated with an increase in SAM/SAH ratio in plasma and CSF in rodents. This indicates an altered methylation potential during sepsis, which may be relevant for sepsis-associated impairment of transmethylation reactions, circulation and defense against oxidative stress. If verified in humans, such findings could lead to novel strategies for supportive treatment of sepsis, as methionine metabolism can easily be manipulated by dietary strategies.
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Keywords: S-adenosylmethionine; DNA methylation; homocysteine metabolism; glutathione; oxidative stress; sepsis; transmethylation.

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Introduction

Despite considerable efforts, sepsis is still one of the leading causes of death in intensive care units (1, 2). Sepsis markedly alters metabolism, in particular amino acid and protein metabolism (3, 4). During transmethylation of methionine to homocysteine, methionine is transferred to S-adenosylmethionine (SAM; Figure 1). SAM occupies a central position in the metabolism of all cells as an essential methyl donor to maintain normal methylation of DNA, RNA, proteins, phospholipids, histones and neurotransmitters, as well as a multitude of small molecules necessary for normal cell function and viability. SAM can control the expression of multiple genes in a dose-dependent manner by binding to riboswitches that control transcription and translation (5). In sepsis, SAM inhibits the decrease in circulating immunoactive cells and the increase in the pro-inflammatory cytokine interleukin-1 (IL-1) (6). The degradation product of SAM is S-adenosylhomocysteine (SAH), which is reversibly hydrolyzed to homocysteine (Hcy). Hcy can be transsulfurated into cystathionine via cystathionine β-synthase (CBS). Furthermore, via cystathionine γ-lyase, cystathionine is catalyzed to cysteine as a component of glutathione (GSX), which is important for the cellular redox system. As an alternative to transsulfuration, Hcy can be recycled by remethylation to methionine via methionine synthase. This requires vitamin B12 and 5-methyltetrahydrofolate, which is synthesized by 5,10-methylenetetrahydrofolate reductase (MTHFR) from 5,10-methylenetetrahydrofolate. In addition, the latter folate derivative is necessary for nucleic acid synthesis. Supraphysiological doses of vitamin B12 and vitamin B2, a cofactor of MTHFR, are beneficial in sepsis (7, 8). Lipopolysaccharide (LPS) is a major cell-wall constituent of Gram-negative bacteria. The LPS model of sepsis induction involves binding to the LPS-binding protein, endothelial activation through a receptor complex of Toll-like receptor 4, the LPS receptor molecule (CD14), and MD2, and early activation of nuclear factor-κB, finally resulting in the production of various pro-inflammatory mediators and possibly cell injury (9).

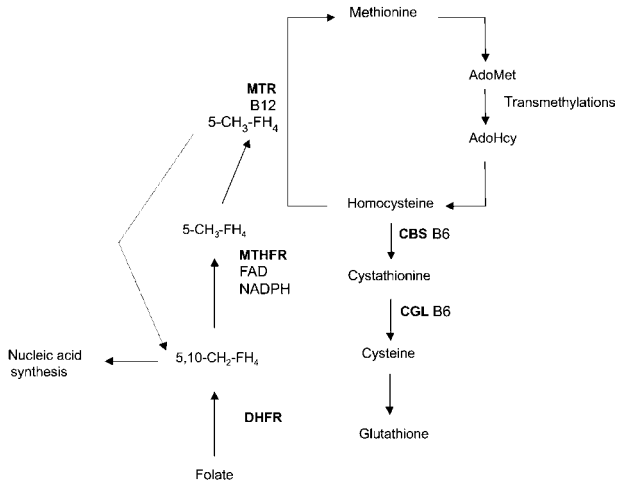

Figure 1 Methionine metabolism.
The sulfur-containing amino acid methionine is activated to S-adenosylmethionine (AdoMet), which is an ubiquitous methyl-group donor. The degradation product of AdoMet is S-adenosylhomocysteine (AdoHcys), which is hydrolyzed to homocysteine. Homocysteine can be remethylated to methionine and AdoMet via methionine synthase (MTR), which depends on derivatives of folate and vitamin B12 as cofactors. Dihydrofolate reductase (DHFR) converts folate into 5,10-methylenetetrahydrofolate, which is further converted into 5-methyltetrahydrofolate by 5,10-methylenetetrahydrofolate reductase (MTHFR). Alternatively, homocysteine can be transsulfurated by vitamin B6-dependent cystathionine-beta-synthase (CBS) and cystathionine γ-lyase (CGL) to cysteine as a component of glutathione.

In view of the central importance of methionine metabolism for overall cellular viability and oxidative defense and the beneficial role of cofactors of important enzymes of methionine metabolism during sepsis, we investigated the effects of sepsis on essential components of methionine metabolism. We compared SAM/SAH and oxidized/reduced glutathione (GSSG/GSH) ratios and Hcy and cysteine levels in LPS-treated and control rats. The SAM/SAH ratio is important because it is an indicator of the cellular methylation potential, as SAM and SAH are the substrate and product, respectively, of essential methyltransferase reactions, and SAH effectively antagonizes SAM-dependent transmethylation reactions. The GSSG/GSH ratio is a common measure of cellular redox potential, and Hcy and cysteine levels are indicative of transsulfuration activity in methionine metabolism (10). As central nervous system (CNS) involvement is a common complication of sepsis and is associated with higher mortality in sepsis patients (11, 12), we also measured SAM and SAH in cerebrospinal fluid (CSF).

Materials and methods

Male Wistar rats (Charles River, Sulzfeld, Germany) weighing 250–300 g were housed in groups of four under standard conditions at 22±1°C and a 12-h light/dark cycle with free access to standard food pellets (Altromin, Soest, Germany) and tap water.

To initiate experimental sepsis, rats received LPS (0127:B8, *E. coli*, 10 mg/kg) dissolved in 1 mL of 0.9% sodium chloride intraperitoneally (i.p.; n=22). Control animals received the vehicle (sodium chloride) alone (n=18). At 24 h after initiation of experimental sepsis, animals were anesthetized i.p. with ketamine (75 mg/kg) and xylazine hydrochloride (10 mg/kg). Puncture of the suboccipital fossa was performed to collect approximately 100 μL of CSF. Blood (1 mL) was collected by cardiac puncture and stored in EDTA-containing vials. All animals were sacrificed by decapitation. Septic and control animals were treated in parallel in groups of 10 each. The animal experiments were approved by the local government.

To obtain plasma, blood was centrifuged for 15 min at 4000 *g* and 4°C immediately after collection. The supernatant was deproteinized by addition of 10% perchloric acid. All aliquots were stored at –80°C for 1–2 months before shipment on dry ice. Deproteinized plasma and CSF were used for simultaneous determination of SAM and SAH using stable isotope dilution tandem mass spectrometry as previously reported. The inter-assay coefficient of variation was 6.8% for SAM and 6.9% for SAH (13). Owing to the limited amounts of CSF available, we restricted CSF analysis to the key parameters SAM and SAH. Plasma total Hcy and cysteine were measured by HPLC using fluorescence detection. The inter-assay coefficient of variation was 1.8% for Hcy and 3.6% for cysteine (14). GSX was assayed as total cellular GSX, i.e., the sum of reduced and oxidized forms, using a modified enzymatic microtiter plate assay (15, 16). Briefly, pellets of EDTA blood were treated with sulfosalicylic acid prior to centrifugation and freezing of the supernatant. Total cellular GSX content was determined after addition of dithiobisnitrobenzoic acid to aliquots of the supernatant (final concentration 10 mM) by measuring the change in absorbance at 405 nm for 10 min. To determine GSSG, GSH was derivatized with 2.5% 2-vinylpyridine for 60 min prior to kinetic measurement. Protein content was determined using the Lowry method (17). GSH was calculated as the difference between total GSX and GSSG.

Statistics

Differences between groups were calculated using a two-sided t-test, and correlation analysis was performed using Pearson's correlation coefficient. A p-value of ≤ 0.05 was considered statistically significant.

Results

Experimental induction of sepsis led to typical symptoms such as piloerection, tachypnoea and social withdrawal. One rat in the sepsis group died during the observation period. All other rats survived LPS and vehicle treatment. Thus, groups of LPS-treated and control animals included 21 and 18 rats, respectively.

The plasma and CSF results are listed in Table 1. In LPS-treated rats, the plasma SAM/SAH ratio was significantly higher ($p=0.004$) than in control animals because of higher SAM levels ($p<0.001$). Comparable, but not significantly different results were obtained for changes in SAM ($p=0.067$) and SAH levels in CSF. SAM levels in plasma and CSF were significantly correlated in both septic animals ($r=0.460$; $p=0.041$) and controls ($r=0.691$; $p=0.006$). There was

Table 1 Comparison of LPS-treated and control animals.

Parameter	Control rats (n = 18)	LPS-treated rats (n = 21)	t-Test (two-sided)
SAM, nmol/L	178.6 ± 33.8	367.2 ± 152.0	$p < 0.001$
SAH, nmol/L	39.32 ± 25.6	41.20 ± 28.0	$p = 0.832$
SAM/SAH ratio	6.55 ± 3.74	11.90 ± 6.39	$p = 0.004$
CSF: SAM, nmol/L	324.09 ± 159.67	420.64 ± 141.37	$p = 0.067$
CSF: SAH, nmol/L	25.14 ± 25.65	22.05 ± 21.62	$p = 0.702$
CSF: SAM/SAH	20.17 ± 11.20	29.87 ± 17.35	$p = 0.068$
Hcy, μmol/L	2.78 ± 0.81	4.44 ± 2.62	$p = 0.063$
Cysteine, μmol/L	178 ± 15	218 ± 52	$p = 0.093$
GSH, nmol/mg protein	216.57 ± 70.0	102.55 ± 38.46	$p < 0.001$
GSSG, nmol/mg protein	59.67 ± 58.36	117.79 ± 77.46	$p = 0.022$
GSSG/GSH	0.24 ± 0.17	0.48 ± 0.09	$p = 0.001$

Values are reported as mean ± SD.

no significant difference in Hcy or cysteine between the groups. GSX levels were also not significantly different. However, relative amounts of GSSG were significantly higher ($p = 0.022$) and of GSH were significantly lower ($p < 0.001$), so that the GSSG/GSH ratio was significantly higher ($p = 0.001$) in septic animals.

In exploratory analysis of data for septic animals, we found correlation between plasma Hcy and SAH levels ($r = 0.601$; $p = 0.011$), between Hcy plasma and total cellular GSX ($r = 0.633$; $p = 0.011$), and a trend between SAM plasma and total cellular GSX ($r = 0.434$; $p = 0.064$).

Discussion

Protein metabolism is markedly changed during sepsis, as characterized by generalized protein catabolism in muscle and enhanced elimination of amino acids from the intravascular space, mainly through the liver (3, 18). Proteolysis might contribute to the increase in SAM observed. SAM is methyl donor for the manifold methyltransferases. Sepsis-induced inhibition of these methyltransferases may lead to impaired SAM utilization and contribute to the increase in SAM levels. Increased Hcy remethylation to methionine and finally SAM during sepsis is unlikely, as oxidative stress leads to lower methionine synthase and betaine-Hcy S-methyltransferase activity (19–21). In addition, we would expect Hcy levels to be lower in septic animals if increased remethylation occurred during sepsis. High SAM levels down-regulate MTHFR, a key enzyme in Hcy remethylation, making it unlikely that remethylation is increased (22). Finally, the increase in SAM might be due to enhanced synthesis of SAM from methionine during sepsis. The present data do not allow any conclusion on the underlying mechanism.

Because SAM and SAH are the substrate and product of essential methyltransferase reactions, and because SAH is an effective antagonist of transmethylation enzymes, the SAM/SAH ratio is frequently used as an indicator of cellular methylation potential (23, 24). The SAM/SAH ratio of 6.55 observed for controls in the current study corresponds well to SAM/SAH ratios observed in humans in studies under physiological conditions (24, 25). However, this ratio increases during sepsis, indicating an increased methylation potential that influences numerous reactions in cellular metabolism, DNA methylation, membrane stability and neurotransmitter synthesis (26–28).

In CSF of septic rats, we also observed increased SAM levels and a trend for an increased SAM/SAH ratio. SAM levels in plasma and CSF were correlated in control and LPS-treated animals, suggesting that SAM measurement in plasma is suitable for determining SAM availability in the CNS under physiological conditions and during sepsis.

GSX is one of the most important cellular redox systems, and oxidative stress plays a key role in various diseases including sepsis (29–33). During sepsis the amount of GSSG increased, the amount of GSH decreased, and thus the GSSG/GSH ratio increased, indicating relevant oxidative stress during sepsis. In the liver, approximately 50% of the cysteine in GSX is derived from Hcy via the transsulfuration pathway (34). The positive correlation between Hcy and GSX during sepsis, but not in controls, suggests that Hcy is required for GSX production during sepsis (Figure 1).

Transsulfuration is enhanced by high SAM levels, as SAM physiologically activates CBS and decreases MTHFR activity (10, 22). Accordingly, we found a trend for increases in Hcy and cysteine levels. Thus, our results indicate that higher levels of SAM lead to a higher transsulfuration rate to allow GSX synthesis for defense of oxidative stress induced by sepsis. In addition, oxidative stress leads to an increased Hcy flux through the transsulfuration pathway by up-regulation of CBS activity (35) and decreased activity of methionine synthase and betaine-Hcy S-methyltransferase (19–21). Increased transsulfuration rates during experimentally induced sepsis were also shown by injection of radiolabeled cysteine and methionine in rats (36, 37).

Methionine metabolism is markedly altered during LPS-induced sepsis in rodents. The validity of our data is limited, as the LPS model of sepsis does not accurately reflect human sepsis (38). Furthermore, blood and CSF samples were taken at only one time-point. However, the data observed encourage future

studies of methionine metabolites in human patients over time to explore the association between sepsis and methionine metabolism in more detail. As methionine metabolism can easily be manipulated by dietary strategies such as supplementation with vitamins or SAM, such studies could lead to strategies for additive treatment of sepsis.

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