

# S-adenosylmethionine (SAME) therapy in liver disease: A review of current evidence and clinical utility

Quentin M. Anstee\*, Christopher P. Day

Liver Research Group, Institute of Cellular Medicine, The Medical School, Newcastle University, Framlington Place, Newcastle-Upon-Tyne NE2 4HH, UK

## Summary

S-adenosyl-L-methionine (SAME; AdoMet) is an important, metabolically pleiotropic molecule that participates in multiple cellular reactions as the precursor for the synthesis of glutathione and principle methyl donor required for methylation of nucleic acids, phospholipids, histones, biogenic amines, and proteins. SAME synthesis is depressed in chronic liver disease and so there has been considerable interest in the utility of SAME to ameliorate disease severity. Despite encouraging pre-clinical data confirming that SAME depletion can exacerbate liver injury and supporting a hepatoprotective role for SAME therapy, to date no large, high-quality randomised clinical trials have been performed that establish clinical utility in specific disease states. Here, we offer an in-depth review of the published scientific literature relating to the physiological and pathophysiological roles of SAME and its therapeutic use in liver disease, critically assessing implications for clinical practice and offering recommendations for further research.

© 2012 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

## Introduction

### *The need for therapies to ameliorate liver injury*

Liver disease is considered acute or chronic according to the duration of the injurious process. The process of hepatic fibrogenesis is triggered by tissue damage and continues until the lesion is healed. If the damage persists or is recurrent, the repair process will persist and fibrogenesis will progress towards cirrhosis, liver failure or hepatocellular carcinoma [1,2]. Hepatocyte death, through a varying combination of oncotic necrosis and apoptosis, is a characteristic feature of most liver diseases including alco-

holic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), cholestasis, viral hepatitis, ischemia/reperfusion, liver preservation at transplantation and drug/toxin-induced injury [3]. Correction of the underlying aetiology before the development of cirrhosis and liver failure is the primary goal in managing liver disease. However, where this is not possible, treatment to ameliorate hepatocellular injury or control fibrogenesis offers an attractive therapeutic strategy that may prevent disease progression and, given that fibrosis has a reversible component, allow regression [4,5]. At present, there are no accepted anti-fibrotic agents available outside clinical trials and, beyond the use of *N*-acetylcysteine (NAC) in the treatment of acute acetaminophen (paracetamol) toxicity, there are no widely adopted agents that limit hepatocellular injury in routine clinical use.

Irrespective of aetiology, progression of liver disease is influenced by the interaction of host genetic factors, the pathogen, and other coincidental environmental influences [6]. Nutritional status is one such factor [7]. However, it has also become apparent that beyond *dietary availability* of specific nutrients and essential amino acids, an individual's *metabolic capacity* for processing them into active metabolites and the factors that influence this can profoundly affect physiology in health and disease [8]. The essential amino acid methionine and its biologically active metabolite *S*-adenosyl-L-methionine (SAME; AdoMet) are a case in point: there is evidence that SAME depletion occurs during chronic liver disease [9,10] and SAME has been proposed as treatment for certain disease states [8,11]. Due to encouraging data from early studies and the lack of other effective agents, SAME has been widely adopted in Eastern Europe, Russia, China, Southern Asia, and South America as a therapy for chronic liver disease and intra-hepatic cholestasis. It is therefore timely to discuss the role of SAME in the pathogenesis of liver disease and critically review the current evidence of clinical utility for SAME supplementation.

## S-adenosyl-L-methionine (SAME)

### *Hepatic SAME metabolism*

SAME is synthesised from dietary L-methionine and ATP by the enzyme methionine adenosyltransferase (MAT; EC 2.5.1.6) in a complex two-step reaction [12,13]. The complete triphosphosphate (PPP<sub>i</sub>) moiety is cleaved from ATP at the C-5' atom and the adenosyl moiety is transferred to methionine to form SAME;

Keywords: S-adenosyl-L-methionine; SAME; Hepatitis; Steatohepatitis; Oxidative stress.

Received 19 March 2012; received in revised form 12 April 2012; accepted 15 April 2012

\* Corresponding author. Address: Institute of Cellular Medicine, The Medical School, Newcastle University, 3rd Floor, William Leech Building, Framlington Place, Newcastle-upon-Tyne NE2 4HH, UK. Tel.: +44 (0) 191 222 7012; fax: +44 (0) 191 222 0723.

E-mail address: [quentin.anstee@newcastle.ac.uk](mailto:quentin.anstee@newcastle.ac.uk) (Q.M. Anstee).

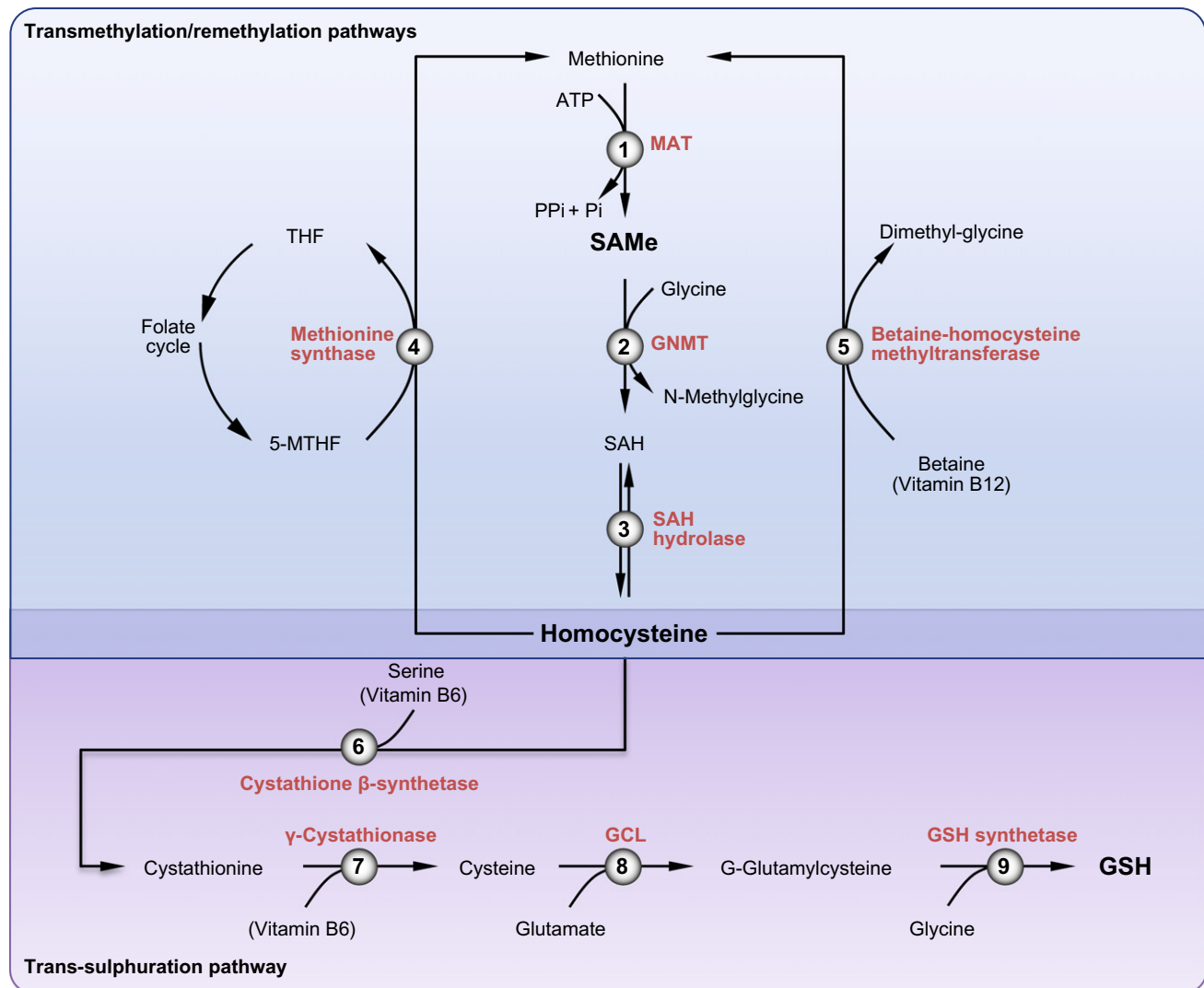


ELSEVIER

## Review

PPi is then hydrolysed to orthophosphate and pyrophosphate (PPi + Pi) at a distinct sub-site within the MAT catalytic domain; and finally S-adenosylmethionine (SAMe), orthophosphate and pyrophosphate are released (Fig. 1) [9]. In mammals, there are three separate forms of the MAT enzyme [14]. The gene *MAT1A* is predominantly expressed in the adult liver and encodes a 395 amino acid  $\alpha$ 1 catalytic subunit that is combined into either a homotetramer (MATI) or a homodimer (MATIII) [15]. In contrast, *MAT2A* is ubiquitously expressed in all mammalian tissues studied including foetal liver (and to a lesser extent in adult liver), erythrocytes, lymphocytes, brain and kidney [15–17]. It encodes a 396 amino acid  $\alpha$ 2 catalytic subunit that combines with a non-catalytic 334 amino acid regulatory  $\beta$  subunit encoded by *MAT2B* to form

the MATII isoform of the enzyme [12,15,16]. MAT is a highly conserved enzyme throughout evolution with a 59% sequence homology between *Escherichia coli* and humans [9]. Both the  $\alpha$ 1 and  $\alpha$ 2 subunits share approximately 84% amino acid sequence homology [15] however, the MATII  $\alpha$ 2/ $\beta$  dimer has lower substrate affinity (km) than MATI/III and its activity is negatively regulated by SAMe as intracellular concentration increases whilst MATI/III is not. These differences in regulatory and kinetic properties limit MATII activity, which is thought to contribute little to hepatic methionine metabolism in healthy adults under normal physiological conditions whilst the *MAT1A* coded isoforms (MATI/III) maintain high levels of SAMe synthesis (approximately 6–8 g/day) [12].



**Fig. 1. Metabolic pathways in methionine/SAMe metabolism.** SAMe is synthesised from dietary L-methionine and ATP by the enzyme methionine adenosyltransferase (MAT; ●1). In standard conditions, the majority of SAMe generated is used in transmethylation reactions. Glycine-N-methyl transferase (GNMT; ●2) is the most abundant methyltransferase in the liver. Irrespective of the specific enzyme mediating the reaction, a common product is S-adenosylhomocysteine (SAH). SAH is cleared by conversion into homocysteine and adenosine in a reversible reaction catalysed by SAH hydrolase (●3). Homocysteine is in turn metabolised through either the remethylation pathways or the transsulfuration pathways. In the former, homocysteine is remethylated by methionine synthase in a process coupled to the folate cycle (MS; ●4) or betaine methyltransferase (BHMT; ●5) to re-form methionine. Alternatively, the conversion of homocysteine to cystathionine by cystathionine  $\beta$ -synthase (CBS; ●6) begins the transsulfuration pathway leading to cysteine and ultimately glutathione (GSH) (●7, ●8, ●9). Folic acid and the co-factors vitamin B6 and B12 are required for functioning of MS (●4), CBS (●6) and BHMT (●5), respectively. Modified from [10,11].

Regulation of MAT expression and SAME synthesis

Synthesis of SAME needs significant metabolic energy given the requirement to hydrolyse the three high-energy phosphodiester bonds of ATP for each SAME molecule synthesised. To control these demands, MATI/III activity is regulated both at transcription level and through post-translational regulation. Sequencing the *MAT1A* promoter region has identified consensus binding sites for several transcription factors including C/EBP (CAAT enhancer binding protein), NF-1 (nuclear factor 1), and HNF (hepatocyte-enriched nuclear factor) [12]. However, studies have demonstrated that, although *MAT1A* expression is limited to the liver, promoter activity is present in cells from other tissues and so this is unlikely to be the mechanism through which tissue specific expression is mediated. Beyond promoter activity, gene transcription may be controlled by DNA methylation that can directly impede binding of transcription proteins and can additionally influence histone deacetylation and therefore chromatin structure. Hypermethylation of two *MAT1A* promoter CpG sites has been demonstrated in foetal liver, hepatocellular carcinoma (HCC) and extra-hepatic tissues, whilst these sites were found to be demethylated in normal adult liver where the gene is expressed [18]. Further, the degree of histone acetylation, necessary to maintain chromatin in a decondensed and active state for transcription, is modified so that the degree of H4 histone acetylation associated with the *MAT1A* promoter is approximately 15-fold greater in the liver than in tissues where *MAT1A* is not expressed such as the kidney [18]. In human hepatoma cell lines (e.g. HepG2), *MAT1A* is hypermethylated and so not expressed, while exposure of these cells to the demethylating agent 5'-aza-2-deoxycytidine or to the histone deacetylase inhibitor trichostatin A promotes gene expression [9,18]. Together, these findings indicate that tissue-specific MATI/III expression is primarily regulated through DNA methylation and histone deacetylation and that this may be affected by disease states (Fig. 2).

Beyond limiting MATI/III expression to the adult liver, where there is a high capacity to generate ATP to fuel SAME synthesis,

there is an additional need to rapidly 'fine tune' SAME synthesis according to variations in metabolic demand to prevent hepatocytes becoming ATP depleted at times of metabolic stress [12]. Reactive oxygen species (ROS) and nitric oxide (NO) can inactivate MATI/III by oxidation or S-nitrosylation of the cysteine residue at position 121 in the  $\alpha 1$  subunit, respectively, inducing a conformational change in the protein that blocks the catalytic site. This change can be equally rapidly reversed by exposure to physiological concentrations of the anti-oxidant glutathione, reactivating MATI/III [19–21]. Importantly, MATII does not possess a cysteine residue at the equivalent position and so its activity (which is regulated by SAME concentration) is not influenced by oxidative stress. Indeed, inhibition of MATI/III under conditions of oxidative stress causes SAME levels to fall, disinhibiting MATII.

Physiological role of SAME

SAME is an important, metabolically pleiotropic molecule that participates in multiple cellular reactions and influences numerous cellular functions. Biochemically, it participates in three types of reaction: transmethylation, transsulfuration and aminopropylation [22,23]. A comprehensive discussion of these metabolic pathways falls outside the scope of this review however, the key reactions will be briefly summarised.

SAME is the principle methyl donor required for methylation of nucleic acids, phospholipids, histones, biogenic amines, and proteins [23]. In standard conditions, the majority of SAME generated is used in transmethylation reactions (Fig. 1). Glycine-N-methyl transferase (GNMT; EC 2.1.1.20) is the most abundant methyltransferase in the liver and is also present in the exocrine pancreas and prostate. Irrespective of the specific enzyme mediating the reaction, a common product is S-adenosylhomocysteine (SAH). Clearance of SAH by conversion into homocysteine and adenosine in a reversible reaction catalysed by SAH hydrolase (EC 3.3.1.1) is essential as many SAME-dependent methylation reactions are strongly inhibited by SAH accumulation.

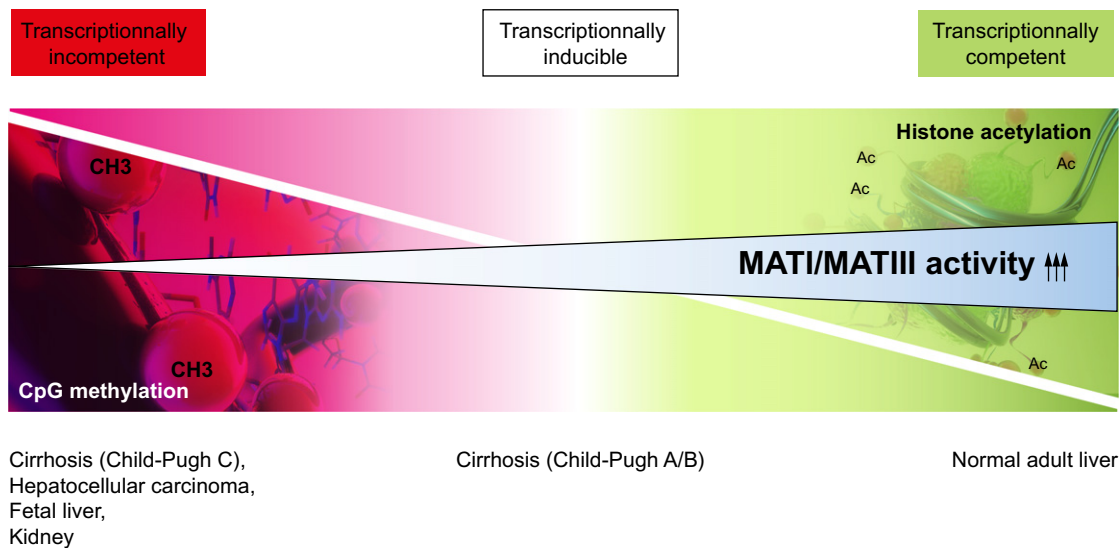


Fig. 2. Regulation of *MAT1A* expression and MATI/MATIII activity in health and disease. Tissue-specific MATI/III expression is primarily regulated through DNA methylation and histone deacetylation and this may be affected by disease states. Greater CpG methylation is observed in extra-hepatic tissues and in cirrhosis and HCC where *MAT1A* expression is suppressed and less MATI/III activity observed. Modified from [9].

## Review

Homocysteine lies at the intersection of the remethylation pathway and the transsulfuration pathway through which homocysteine may be processed to form the primary endogenous cellular antioxidant, glutathione (Fig. 1) [10]. In the former, homocysteine is remethylated by methionine synthase (MS; EC 2.1.1.13) in a process coupled to the folate cycle or betaine methyltransferase (BHMT; EC 2.1.1.5) to re-form methionine; although it should be noted that the role of BHMT is of much less significance in primates than in rodents [23]. Alternatively, the conversion of homocysteine to cystathionine by cystathionine  $\beta$ -synthase (CBS; EC 4.2.1.22) begins the transsulfuration pathway through which the methionine derived sulphur atom in SAME is processed stepwise into cysteine and ultimately glutathione (Fig. 1). These pathways are autoregulated by hepatic SAME concentration which acts as a potent inhibitor of MS and BHMT activity and an activator of CBS so that excess SAME is preferentially partitioned into glutathione [24]. Folic acid, and the co-factors vitamin B6 and B12 are required for functioning of MS, CBS, and BHMT, respectively, and so their availability will limit enzymatic activity and thus homocysteine levels and hepatic methionine handling (Fig. 1) [10]. SAME is also the precursor for the synthesis of polyamines that are needed to preserve cell viability and proliferation. Here, SAME is decarboxylated by SAME decarboxylase (EC 4.1.1.50) and the aminopropyl group used to form polyamines including the biologically active metabolite 5'-methylthioadenosine (MTA).

### *SAME metabolism in liver disease*

Recognition that methionine metabolism is impaired in patients with chronic liver disease extends back more than 60 years to studies demonstrating reduced methionine clearance after liver injury [25]. Independent of aetiology, patients with hepatic cirrhosis have been shown to have reduced *MAT1A* expression, lower MATI/III activity, with no compensatory increase in *MAT2A* expression and therefore hepatocellular accumulation of methionine and significantly reduced levels of SAME [26,27]. These changes are due to hypermethylation of the *MAT1A* promoter limiting gene expression in cirrhotic patients, however, the mechanism through which this occurs is not known (Fig. 2) [27]. Furthermore, conditions that promote oxidative stress such as alcohol consumption, viral hepatitis, septic shock, and toxin exposure; or increase nitric oxide (NO) synthesis such as hypoxia and inflammatory cytokines (e.g. TNF $\alpha$ , IL-6) will inactivate residual MATI/III [12]. Given that SAME is a biochemical intermediate in glutathione synthesis, this in turn will further downgrade hepatocellular defences against oxidative stress and worsen liver injury.

The effects of chronic SAME depletion may be studied in the *Mat1a* null (MATO) mice [28]. In these mice, hepatic hyperplasia is evident by 3 months of age; young animals are more sensitive to choline-deficient diet induced steatosis; and, consistent with the effects of methionine/choline-deficient dietary models [29], the mice develop spontaneous steatohepatitis by age 8 months [28]. Gene expression profiling reveals that, even when the liver appears histologically normal, numerous growth, dedifferentiation and acute phase response genes are upregulated including proliferating cell nuclear antigen,  $\alpha$ -fetoprotein, and *Mat2a*. MATO mice are also significantly more sensitive to the effects of carbon tetrachloride exposure than wild type animals, exhibiting higher levels of ALT, AST, and greater histological liver injury

[12]. Few human examples of spontaneous *MAT1A* deficiency have been reported, however, it is notable that neurological rather than hepatic dysfunction is the primary phenotype described (OMIM #610550).

Beyond its role as a metabolic intermediate, there is evidence that the balance between MATI/III and MATII activation and therefore intracellular SAME concentration can modulate cell proliferation in the liver. SAME levels are high in quiescent hepatocytes but are much lower in proliferating cells [30]. Studies in HepG2 and HuH7 cells demonstrate that when *MAT2A* is expressed, low SAME levels promote more rapid cell growth whilst increased SAME synthesis due to *MAT1A* expression or SAME treatment is associated with slower growth [30]. These findings are also supported by reports that the potent mitogen, hepatocyte growth factor (HGF), induces acetylation of histones associated with the *MAT2A* promoter, an effect that can be overcome by administration of exogenous SAME [31,32]. *In vivo*, following partial hepatectomy in the rat, SAME levels are markedly reduced, coinciding with the start of DNA synthesis and regeneration. Once again, hepatocellular regeneration can be inhibited by SAME administration [33]. Underlining the clinical significance of the effect of SAME on hepatocellular proliferation, SAME depletion in *Mat1a* knockout mice is associated with spontaneous development of HCC [34,35] whilst development of HCC in carcinogen-exposed rats can be blocked by SAME treatment [36,37]. Thus, SAME depletion promotes increased cellular proliferation and growth. As a short lived response to an acute insult these changes may be beneficial: SAME synthesis is reset to a new lower steady state preserving ATP, hepatocyte proliferation and growth are prioritised and the original liver mass restored. However, if injury becomes chronic, SAME depletion may be deleterious as it favours malignant transformation and so SAME supplementation, at least to physiological levels, may be beneficial.

### *Role of SAME in modifying response to injury*

Given our current understanding of methionine metabolism and the physiological role of SAME, it has been proposed that supplementation could both ameliorate liver injury and reduce the development of HCC in chronic liver disease. These effects cannot be achieved by giving methionine, even a 7-fold increase in methionine consumption was unable to significantly increase hepatic SAME and could exacerbate the potentially harmful accumulation of methionine that is observed in cirrhotic patients [38,39]. Administration of SAME would, however, bypass MAT and theoretically avoid these problems. Before considering the evidence for specific disease states, we will examine the role of SAME in hepatic pathophysiology and how this may be manipulated through therapeutic supplementation.

### *Oxidative stress and cellular damage*

A common feature in the pathophysiology of most inflammatory processes is the involvement of reactive oxygen species (ROS) such as superoxide, peroxynitrite, hydrogen peroxide, and the hydroxyl radical [40]. Due to their high metabolic activity, ROS are generated continuously in hepatocytes and also, following liver injury, by cells of the innate immune system (e.g. Kupffer cells, infiltrating monocytes/macrophages, and neutrophils). These highly reactive chemical species react with cell membranes causing lipid peroxidation, induce DNA damage, activate c-jun-N-

terminal kinase (JNK) and caspase signalling to promote apoptosis and cause oncotic necrosis [40]. To counter this, intracellular ROS generation is largely confined to specific organelles including mitochondria and peroxisomes; several anti-oxidant enzyme systems have developed including superoxide dismutase (SOD), glutathione peroxidase, and catalase that detoxify ROS; and cells contain endogenous anti-oxidants which scavenge free radicals to mitigate against cellular damage, the most abundant of which is glutathione [41]. As discussed above, SAME is a precursor for the synthesis of cysteine and thus glutathione (Fig. 1) [12]. SAME has been shown to effectively increase intracellular glutathione concentration in murine models [42–45] and in patients with liver disease [46]. Importantly, SAME can replenish hepatic mitochondrial glutathione and normalise fluidity of the inner mitochondrial membrane, which is critical for maintenance of function [43,45]. It is tempting to speculate that these effects could underlie a reported amelioration of biochemical markers of chemotherapy-related liver injury described in three retrospective, observational studies from Italy, however, hepatic glutathione levels were not measured and so it would require further, prospective assessment to confirm these findings and determine mechanism of action [47–49].

There is evidence that, beyond its effect on hepatocellular ROS, SAME may have additional beneficial effects modulating the balance between pro- and anti-inflammatory cytokines in liver injury [11]. Experimental evidence of the ability of SAME to attenuate pro-inflammatory TNF $\alpha$ -mediated liver injury, likely through downregulation of transcription factor NF $\kappa$ B, comes from a number of studies [11,50]. Elevated glutathione levels may also help reduce TNF $\alpha$ -induced necrosis [44]. In rats fed a methionine–choline deficient diet to induce steatohepatitis, SAME was shown to reduce the induction of TNF $\alpha$  expression caused by exposure to bacterial lipopolysaccharide (LPS) [51]. Both SAME and its metabolite from the polyamine pathway, MTA, have also been shown to reduce TNF $\alpha$  production *in vivo* and *in vitro* following LPS stimulation of the murine monocyte cell line RAW264.7 [52,53], and to induce anti-inflammatory IL-10 production in the same models [52–54].

#### Apoptosis

The interactions between SAME and apoptotic cell death pathways are complex. SAME has been shown to inhibit bile acid-induced apoptosis *in vitro* [55,56] and both SAME and MTA can protect normal cultured rat hepatocytes against okadaic acid-induced apoptosis in a dose dependent manner, an effect thought to be mediated through reduced mitochondrial cytochrome-c release, caspase-3 activation, and poly(ADP-ribose) polymerase cleavage [11,57]. However, SAME has the opposite effect on cancer cells [58]. For example, SAME induces apoptosis in HepG2 and HuH7 cell lines via the mitochondrial death pathway [58]. The reasons for the differential effect remain incompletely understood but two mechanisms have been proposed [10]. Firstly, SAME and MTA alter cellular phosphorylation state and alternative splicing of genes in cancer cells resulting in Bcl-x<sub>s</sub> induction and apoptosis; and secondly, SAME and MTA have been shown to directly inhibit BHMT activity in cancer cells but not normal hepatocytes, perturbing homocysteine metabolism, and promoting endoplasmic reticulum stress and therefore apoptosis [10]. These effects are consistent with the observed *in vivo* chemopreventive effects of SAME in murine models of HCC where more apoptotic bodies were seen

in tumours of SAME-treated animals than in untreated controls [36,37].

#### Transmethylation reactions and intra-hepatic cholestasis

Cholestasis occurs in a number of disease states and results in an accumulation of potentially toxic bile salts in the liver and blood which in turn leads to oxidative stress, hepatocellular injury, bile duct proliferation, and ultimately hepatic fibrosis [41,59]. Methylation status is recognised to be of fundamental importance to cell membrane function with phospholipid methylation influencing membrane fluidity and transport of metabolites and transmission of signals across membranes [60–62]. As a major methyl donor, availability of SAME potentially has profound effects on these processes. Therefore, it has been suggested that SAME depletion contributes to the development of intra-hepatic cholestasis by interfering with bile salt export pump (BSEP) activity [8]. Furthermore, hepatocellular injury in cholestasis is frequently associated with glutathione depletion and so SAME may help correct this [41].

#### SAME therapy for chronic liver disease

##### *Intra-hepatic cholestasis*

The efficacy of SAME therapy has been examined in a range of chronic liver conditions, although historically the greatest interest has been in the area of intra-hepatic cholestasis (IHC). IHC is a syndrome that develops from impaired bile flow at the sub-lobular level. This may occur due to: (1) hepatocellular damage, including viral hepatitis, alcoholic hepatitis or prolonged TPN use; (2) canalicular membrane changes, often seen in drug-induced liver injury (e.g. oral contraceptives, antibiotics, etc.); (3) genetic defects in bile transporters; (4) obstruction of the canaliculi or ductules; and (5) ductopenia [63]. Clinically, IHC is characterised by the presence of pruritus or jaundice with elevated serum total bilirubin, alkaline phosphatase, and gamma-glutamyltransferase levels [63].

##### *Pre-clinical and clinical therapeutic evidence in non-pregnancy related cholestasis*

The effects of SAME treatment *in vivo* in rat models of surgical cholestasis (bile duct ligation) have shown some benefit. Rats treated with SAME and then subjected to bile duct ligation for 7 days were found to exhibit less oxidative stress as measured by thiobarbituric acid reactive substances (TBARS) and to have a reduced ratio of oxidised to total glutathione [64]. Perhaps unsurprisingly, given the nature of the model, both this and a similar study failed to detect any change in biochemical cholestasis (ALT, ALP, and bilirubin), area of hepatocellular necrosis or fibrosis [41,64,65].

The two largest studies that have examined the utility of SAME therapy in this setting have both been conducted in patients with features of IHC due to a mixture of different aetiologies [66,67]. One of the earliest was a multi-centre, double-blind placebo-controlled trial conducted in 220 IHC patients, many of indeterminate aetiology and of differing disease stage (68% cirrhosis, 26% chronic viral hepatitis, 6% PBC) [67]. This demonstrated a significant reduction in clinical biochemical indices of cholestasis and improvement in symptoms of fatigue and pruritus after oral SAME treatment (1600 mg/day). This study also

## Review

**Table 1. Selected clinical trials of SAME in non-pregnancy related IHC.**

Study, [Ref.]	Design	Subjects (n)	SAME dose (g/day)	Duration	Outcome
*Frezza <i>et al.</i> , 1990 [67]	RCT	220	1600 mg/d po vs. placebo	2 wk	RR Pruritus 0.43 (95% CI 0.27-0.69)
*Frezza <i>et al.</i> , 1987	RCT	80	800 mg/d iv vs. placebo	2 wk	RR Pruritus 0.00 (95% CI 0.25-0.53)
*Manzillo <i>et al.</i> , 1992 [68]	RCT	256 (pruritus studied in 105 patient subgroup)	800 mg/d iv vs. placebo	2 wk	Improved serum bilirubin, ALT, AST but not ALP. RR Pruritus 0.44 (95% CI 0.30-0.66)
*Qin <i>et al.</i> , 2000 [69]	RCT	110	1000 mg/d iv vs. placebo	4 wk	RR Pruritus 0.57 (95% CI 0.39-0.83)

\*Included in systematic review [70].  
RCT, randomised controlled trial.

showed that significantly more SAME-treated patients reported a >50% increase in general well-being (SAME 84% vs. placebo 29%,  $p < 0.01$ ) [67]. These findings were supported by a subsequent Italian study. Here, 640 IHC patients were allocated to one of two different parenteral dosing schedules (500 mg/day im or 800 mg/day iv) for 15 days in a non-randomised, non-placebo controlled, observational study [66]. The majority of patients recruited had chronic viral hepatitis with or without concomitant excess alcohol consumption and approximately 60% were cirrhotic at enrolment. Little additional information as to whether patients were inpatients or outpatients at the time of the study was provided. Over two-thirds of participants reported substantial improvements in subjective symptoms of pruritus and fatigue using a visual analogue scale and reductions in serum markers of cholestasis were also observed [66]. Neither parenteral regimen was found to be superior to the other. Although these studies provide supportive evidence for the use of SAME in chronic liver disease, the study cohorts were poorly defined and methodology was not to current standards for clinical trials. This is particularly so for the latter study, which was not randomised and had no control arm, making it difficult to confidently draw conclusions that influence practice. Importantly, neither study addressed duration of effect or whether this translated into any tangible prognostic benefit.

There have been several smaller studies that have also examined symptom severity (fatigue and/or pruritus) as an end point [67–69]. The value of SAME as a treatment for pruritus was addressed by the Agency for Healthcare Research and Quality in 2002 (<http://archive.ahrq.gov/clinic/tp/sametp.htm>) [70]. In a meta-analysis of four smaller, heterogeneous studies addressing IHC of mixed aetiology unrelated to pregnancy (Table 1), the authors concluded that SAME therapy was superior to placebo, significantly reducing pruritus (relative risk 0.45, 95% CI 0.37–0.55) and serum bilirubin levels (pooled estimate,  $p = 0.02$ ) however, there was insufficient evidence to compare efficacy with other agents such as ursodeoxycholic acid [70].

### Pre-clinical and clinical therapeutic evidence in cholestasis of pregnancy

Cholestasis of pregnancy is associated with a high risk of adverse perinatal outcomes, including preterm birth (19–60%), meconium passage (27%), foetal distress (22–41%), and foetal death (0.4–4.1%) [71]. Risk increases with fasting serum bile acid levels >40  $\mu\text{mol/L}$  [71]. The use of SAME to relieve symptoms and lower bile salt levels in this condition has been studied by several groups. The largest study randomised 78 patients with cholestasis of pregnancy at <36 weeks gestation to SAME, ursodeoxycholic acid (UDCA) or combination therapy until delivery. Both

therapies were equally effective at improving pruritus, however, women receiving UDCA had a significantly greater improvement in the concentration of serum bile acids ( $p = 0.001$ ) and clinical biochemistry than those receiving SAME [72]. A systematic review by members of the Cochrane Collaboration (performed in 2001 and updated 2010) identified five randomised control trials that met their inclusion criteria (these and others are listed in Table 2), however, analysis was hampered by their heterogeneity and several methodological problems were identified in the studies [73]. The drug was generally well tolerated in all studies with no serious adverse events reported, however, the authors concluded that there was insufficient evidence to support the use of SAME or UDCA in cholestasis of pregnancy [73].

### Alcoholic liver disease

Internationally, alcoholic liver disease (ALD) poses a major burden on healthcare resources. Excess alcohol consumption (typically a threshold of >20 g/day for women and >30 g/day for men is adopted) is associated with progressive liver injury ranging from steatosis, through alcoholic steatohepatitis to cirrhosis. Pathogenesis is driven by a combination of direct hepatotoxic effects of alcohol; ROS generation by cytochrome P450 (CYP2E1); bacterial translocation from the gut promoting LPS-induced Kupffer cell  $\text{TNF}\alpha$  and pro-inflammatory cytokine release; and innate immune system activation (recently reviewed elsewhere [74]). In addition, patients with ALD frequently exhibit multiple nutritional deficiencies including protein energy malnutrition and thiamine (vitamin B1), vitamin B6, vitamin B12, and folate deficiency [75]. The reasons for these deficiencies are multifactorial and include poor diet, intestinal malabsorption, and reduced hepatic uptake [75]. Additionally, specific chemical interactions also interfere with normal physiological processes, for example, the ethanol metabolite acetaldehyde displaces the active form of vitamin B6 (pyridoxal phosphatase) from its hepatic binding site [76]. As discussed above, these nutrients are essential for normal methionine metabolism and so deficiency will have important deleterious effects, impairing remethylation of homocysteine by MS and BHMT and its metabolism to form glutathione, thereby degrading defenses against oxidative stress. The consequent increase in hepatocellular homocysteine will alter the catalytic equilibrium of the reversible enzyme SAH hydrolase, altering the SAH:SAME ratio and inhibiting many SAME dependent methylation reactions (Fig. 1). Confirming this, liver biopsies from patients with alcoholic hepatitis demonstrate significant SAME depletion and 50% reduced expression of *MAT1A* as well as the genes encoding MS and CBS [77]. Similar results are found in patients with both alcoholic and non-alcoholic cirrhosis

**Table 2. Selected clinical trials of SAME in cholestasis of pregnancy.**

Study, [Ref.]	Design	Subjects (n)	SAMe dose (g/day)	Duration	Outcomes
*Nicastri <i>et al.</i> , 1998 [113]	RCT	32 women at 30-37 wk gestation	800 mg/day po vs. UDCA vs. SAMe + UDCA vs. placebo	20 d	Combination SAMe and UDCA better than either alone or placebo for bile salts, bilirubin, ALT, ALP, pruritus, neonatal and maternal outcome
*Ribalta <i>et al.</i> , 1991 [114]	RCT	18 women before 32 wk gestation	800 mg/day iv vs. placebo	20 d	No benefit of SAMe for bile salts, bilirubin, ALT, ALP, pruritus, neonatal and maternal outcome
*Frezza <i>et al.</i> , 1990 [115]	RCT	30 women	800 mg/day iv vs. placebo	Until delivery (mean 18 d)	SAMe improved bile salts, bilirubin, ALT, ALP, pruritus, neonatal and maternal outcome
*Frezza <i>et al.</i> , 1984 [116]	RCT	18 women at 28-32 wk gestation	200 mg/day po vs. 800 mg/day po vs. placebo	Until delivery	SAMe 800 mg/day improved clinical biochemistry, pruritus, neonatal and maternal outcome
*Floreani <i>et al.</i> , 1996 [117]	RCT	20 women before week 34 gestation	1 g/day im vs. UDCA	>15 d	SAMe had no effect on bile salts, bilirubin, ALT, ALP, pruritus, neonatal and maternal outcome
Lafuenti <i>et al.</i> , 1988 [118]	Open label	17 patients	1800 mg/day po and 600 mg/day po	n.a.	Manuscript out of print
Catalino <i>et al.</i> , 1992 [119]	Open label	55 patients, no comparator	800 mg/day iv	10-30 d	Improved bile salts, bilirubin, ALT, ALP, pruritus vs. baseline
Roncaglia <i>et al.</i> , 2004 [72]	RCT	46 patients, before 36 wk gestation	SAMe 1 g/day po vs. UDCA 600 mg/day	until delivery	UDCA was more effective than SAMe in lowering bile acid levels. Both improved pruritus

\*Included in Cochrane systematic review [73].  
 RCT, randomised controlled trial; n.a., not available.

[26,78] where MAT activity was suppressed, and in a range of animal models of alcoholic liver injury including mice [42], rats [44,79], baboons [80], and micropigs [81,82].

*Pre-clinical and clinical therapeutic evidence*

The effects of SAMe supplementation in ALD have been explored in a number of animal models and in several clinical trials. In general, the results of pre-clinical studies have been positive although this may be influenced by publication bias. In isolated perfused rat liver, SAMe attenuated ethanol toxicity by restoring mitochondrial and total liver glutathione [83]. SAMe supplementation has been shown to reverse SAMe and glutathione depletion in ethanol fed baboons [80] and rats [43]; reduce hepatic fibrogenesis following carbon-tetrachloride exposure in rats [84] (although as noted earlier, not following bile duct ligation [41,64,65]); restore hepatocyte mitochondrial inner-membrane fluidity in ethanol fed rats [45]; normalise mitochondrial glutathione handling in ethanol fed rats [45]; and prevent TNF $\alpha$ -mediated glutathione depletion, ameliorate steatosis, hepatocyte necrosis, and ALT elevations in ethanol fed mice [42].

Encouraged by these data, several groups have conducted clinical trials examining the efficacy of SAMe therapy across a range of different end points in patients with ALD. Many were small, observational studies with significant design flaws and have only been published in abstract form. Selected trials are summarized in Table 3. The largest of these was a 2-year Spanish multi-center study examining the effect of oral SAMe in 123 patients with cirrhosis due to alcoholic liver disease [85]. 62 patients (53 male, 9 female) were randomised to SAMe 1.2 g/day and 61 patients (53 male, 8 female) placebo. Of note, approximately a quarter of the patients also had chronic viral hepatitis [86]. Outcomes studied were all-cause mortality, liver transplantation, complications of liver disease, and clinical biochemistry.

This study found that a combined all-cause mortality/transplantation end point fell from 30% in the placebo arm to 16% in those treated with SAMe, however, the effect did not reach statistical significance ( $p = 0.077$ ) unless those with more advanced disease (Child score C) were excluded from the analysis, 29% vs. 12% ( $p = 0.025$ ) [85]. No significant difference between arms in the number of patients that experienced severe complications of cirrhosis was observed during the study.

An update of the 2001 systematic review by members of the Cochrane Collaboration identified nine randomised control trials that met their inclusion criteria (Table 3) [86]. These studies, including the trial discussed above, which was the only study considered to have used adequate methodology, recruited a total of 434 patients. No significant effect of SAMe on all cause mortality (relative risk (RR) 0.62, 95% confidence interval (CI) 0.30–1.26); liver-related mortality (RR 0.68, 95% CI 0.31–1.48); combined all-cause mortality or transplantation RR 0.55, 95% CI 0.27–1.09); or liver-related complications (RR 1.35, 95% CI 0.84–2.16) was identified [86]. The authors concluded that the available evidence was insufficient to either support or refute the benefit of SAMe therapy in ALD and that, to address this, there was a need for a large, well conducted, placebo-controlled, randomised clinical trial that stratified patients not only by alcohol consumption but also by presence of co-existent diseases such as chronic viral hepatitis.

A recent North American study, published since the Cochrane analysis was completed, randomised 37 abstinent ALD patients to 1.2 g/day SAMe or placebo for 6 months. Eleven patients were excluded from the final analysis due to on-going alcohol consumption but 14 of the remaining patients had undergone paired liver biopsies at the start and end of the study. Fasting serum SAMe levels were found to have increased during the course of the study in the SAMe treatment group. The entire cohort showed

## Review

**Table 3. Selected clinical trials of SAmE in ALD.**

Study, [Ref.]	Design	Subjects (n)	SAmE dose (g/d)	Duration	Outcomes
*Altomare <i>et al.</i> , 1988 [120]	RCT	18 inpatients with severe decompensated ALD; 17 outpatients with ALD	1.2 g/d po vs. placebo	4 wk or 6 mo	Significantly increased hepatic glutathione, cysteine, taurine and reduced methionine
*Chawla <i>et al.</i> , 1999 [121]	RCT	7 patients with stable ALD cirrhosis	800 mg/d iv vs. placebo	17 d	Improved clinical biochemistry and hepatic methionine handling
*Cibin <i>et al.</i> , 1988 [122]	RCT	64 patients with alcohol dependence but not cirrhosis	200 mg/d im vs. placebo	30 d	Reduced anxiety, depression, gGT and alcohol consumption
*Corrales <i>et al.</i> , 1991 [123]	RCT	16 patients with ALD	1.2 g/d po vs. placebo	4 wk	Improved hepatic methionine handling
*Diaz Belmont <i>et al.</i> , 1996 [124]	RCT	45 alcoholic hepatitis patients	200 mg/d iv vs. placebo	15 d	Increased plasma glutathione
*Loguercio <i>et al.</i> , 1994 [125]	RCT	40 (20 non-cirrhotic, 20 cirrhotic)	2 g/d iv vs. placebo	15 d	Increased RBC glutathione and cysteine concentrations
*Mato <i>et al.</i> , 1999 [85]	RCT	123 patients with ALD cirrhosis	1.2 g/d po vs. placebo	24 mo	SAmE improved survival and reduced liver transplantation in Child-Pugh A/B patients
*Trespi <i>et al.</i> , 1997 [126]	RCT	210 patients with ALD	SAmE 1.5 g/d plus ursodeoxycholic/tauroursodeoxycholic acid vs. ursodeoxycholic/tauroursodeoxycholic alone	24 wk	Greatest clinical biochemistry improvement with SAmE plus tauroursodeoxycholic acid
*Vendemiale <i>et al.</i> , 1989 [46]	RCT	39 patients with ALD	No treatment vs. SAmE 1.2 g/day vs. placebo	24 wk	SAmE significantly increases total glutathione
Medici <i>et al.</i> , 2011 [87]	RCT	37 (14 with biopsies pre and post-treatment)	1.2 g/d vs. placebo	24 wk	No improvement in clinical, biochemical or histological disease
*Fiorelli <i>et al.</i> , 1999 [66]	NR, open label	640 IHC patients (18% ALD)	800 mg/d iv or 500 mg/d im	15 d	Clinical biochemistry and symptomatic improvement

\*Included in Cochrane systematic review [86].

\*Mixed chronic liver disease including ALD.

†Published only as abstract.

RCT, randomised controlled trial; NR, non-randomised.

an overall improvement of AST, ALT, and bilirubin levels after 24 weeks, most probably due to abstinence from alcohol, but no differences between the treatment groups in any clinical, biochemical or histological parameters (steatosis, inflammation, fibrosis, and Mallory–Denk hyaline bodies) were identified [87]. Although this study is relatively small and arguably 6-month follow-up is too little time for substantial histological changes in fibrosis to occur, the methodology used was sound. The results led the authors to conclude that ‘SAmE was no more effective than placebo’ in the treatment of ALD [75,87]. It is interesting to note that, one previous study has also performed biopsies pre- and post-6 months of SAmE therapy [46]. However, the authors did not present any histological data (despite stating in the methods section that tissue underwent histopathological assessment), only showing data that demonstrated SAmE increased intra-hepatic glutathione and improved clinical biochemistry [46].

### Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD), represents a spectrum encompassing steatosis, non-alcoholic steatohepatitis (NASH) and cirrhosis in the absence of alcohol abuse and has been

recognised as the most common cause of liver dysfunction in the majority of developed countries [6]. NAFLD is strongly associated with obesity, insulin resistance or type 2 diabetes mellitus and dyslipidaemia and so may be considered the hepatic manifestation of the metabolic syndrome [88–90]. Perhaps unsurprisingly, given the gross histological similarities between ALD and NAFLD, there is now increasing evidence to suggest that both conditions share common pathogenic processes [91]. The initiating events in NAFLD are founded on the development of obesity and insulin resistance and increased hepatic free fatty acid (FFA) flux. This imbalance between the rate of import/synthesis and the rate of export/catabolism of fatty acids leads to development of steatosis, which represents an adaptive response through which potentially lipotoxic FFAs are partitioned into relatively stable triglyceride stores [92]. When these are overwhelmed, steatohepatitis develops due to the increased oxidative stress produced during  $\beta$ - and  $\omega$ -fatty acid oxidation; direct lipotoxicity; endotoxin/TLR4 induced activation of the innate immune system causing cytokine release; and endoplasmic reticulum (ER) stress. Consequent cellular damage triggers a mixture of immune mediated hepatocellular injury and both necrotic and apoptotic cell death pathways culminating in hepatic fibrosis [3,93–95].

#### Pre-clinical and clinical therapeutic evidence

SAME may influence the pathogenesis of NAFLD both through its role as a precursor for glutathione synthesis and also as a methyl donor in the synthesis of phosphatidylcholine, which is required for VLDL assembly and hepatic triglyceride export. Evidence of a role for methionine metabolism and SAME in the pathogenesis of NAFLD has largely been based on the study of pre-clinical models. Prolonged consumption of a methionine-choline deficient (MCD) diet is associated with hepatic SAME depletion and development of a histological fibrosing steatohepatitis in rodents [29,96]. Similar changes are observed in MATO mice that, as discussed earlier, lack *Mat1a* and so are unable to synthesize SAME [28,29]. Investigation of the mechanisms underlying these observations demonstrates that, even before NAFLD is histologically apparent, there is a potent effect of *Mat1a* deletion on lipid handling [97]. Three-month old MATO mice exhibited decreased mobilization of TG stores, TG secretion in VLDL, and phosphatidylcholine synthesis via phosphatidylethanolamine *N*-methyltransferase. In addition, the authors report that administration of SAME for 7 days was sufficient to rectify the deficits in VLDL assembly and features of the secreted lipoproteins [97]. They conclude that *Mat1a* is required for normal VLDL assembly and plasma lipid homeostasis in mice and that impaired VLDL synthesis, mainly due to SAME deficiency, contributes to NAFLD development in MATO mice [97]. A recent human study has attempted to determine the extent to which methionine metabolism and SAME contribute to the pathogenesis of NASH in man [98]. Studying a cohort of 15 patients with biopsy proven NASH and 19 healthy controls, the authors determined that the rates of remethylation of homocysteine and transmethylation of methionine (Fig. 1) were significantly reduced in NASH and hypothesised that this may in part be due to inactivation of MAT1/III by increased oxidative stress [98].

No pharmacological agents are currently licenced specifically for NASH therapy and as yet there have been no clinical studies that address the utility of SAME in this condition [99]. Several studies have, however, reported encouraging results with the use of the anti-oxidant vitamin E [100,101] and so it may be that the anti-oxidant effects of SAME via augmenting hepatic glutathione synthesis could provide clinical benefit. The methyl donor betaine (trimethylglycine) is required for BMHT-mediated remethylation of homocysteine to methionine and has been shown to reduce hepatic SAH and increase SAME in animal models (Fig. 1) [11]. Betaine has been tested as a therapy for NASH in two clinical trials, an initial pilot study that yielded positive results and a subsequent large randomised placebo-control trial, which did not [102,103]. This second study randomised 55 patients with biopsy proven NASH to betaine (20 g/day) or placebo for 1 year. The analysis demonstrated that betaine therapy effectively increased serum methionine and SAME levels but did not significantly reduce SAH or ameliorate histological steatohepatitis [103]. It would seem timely to formally assess the efficacy of direct SAME supplementation in NAFLD.

#### Viral hepatitis

Several of the studies so far discussed have included in the cohorts a significant number of patients with viral hepatitis and have indicated that SAME may be effective in IHC of viral aetiology. An effect supported in a viral hepatitis cohort by an open-label study comparing SAME and traditional Chinese remedies for the treatment of jaundice [104]. Arguably of greater interest,

however, are recent studies demonstrating that SAME may be an effective adjunctive therapy in the treatment of chronic hepatitis C (HCV).

#### Pre-clinical and clinical therapeutic evidence

Despite recent advances in our understanding of genetic modifiers of host response to Pegylated IFN $\alpha$ /Ribavirin based anti-viral regimens [105–107], the mechanisms that determine response remain incompletely understood. One mechanism appears to be HCV-induced viral interference with IFN $\alpha$  signal transduction and Jak-STAT signalling due to STAT1 hypomethylation, facilitating binding of its inhibitor, protein inhibitor of activated STAT1 (PIAS1). [108]. This effect is reversed *in vitro* by SAME and betaine which restore STAT1 methylation, improve IFN $\alpha$  signalling and enhance the antiviral effect of IFN $\alpha$  in cell culture [109]. These effects have also been described *in vivo*. In an open-label pilot study of combined SAME (1200 mg/day) and betaine (6 g/day) treatment in HCV previous non-responders, there was no evidence of any direct anti-viral effect of SAME, however, SAME treatment was associated with an increase in early virological response but this did not translate into greater sustained virological response [110]. A further clinical study in a group of 24 HCV genotype 1 non-responders found that addition of SAME was associated with improved viral kinetics in the second-phase slope of viral decline (Control  $0.11 \pm 0.04 \log_{10}$  IU/ml/wk vs. SAME  $0.27 \pm 0.06$ ;  $p = 0.009$ ) and higher rates of early and sustained viral clearance [111]. This effect was associated with significantly greater interferon-stimulated gene (ISG) expression in peripheral blood mononuclear cells (PBMCs) and, once again, SAME was shown to increase induction of ISGs and the antiviral effects of interferon by increasing STAT1 methylation *in vitro* [111]. Whilst these effects will need to be replicated in large randomised-controlled trials, the authors conclude that SAME represents the first interferon sensitising agent with *in vivo* efficacy and that it may be a useful adjunct to interferon-based therapy [111].

A study examining the carcinogenic effects of hepatitis B (HBV) showed that the hepatocellular cytoplasmic level of HBV X protein (HBx) was strongly correlated with *MAT2A* expression in HCC samples [112]. *In vitro*, HBx was shown to reduce *MAT1A* gene expression and SAME production whilst directly activating *MAT2A* expression in a dose-dependent manner through binding of NF- $\kappa$ B and CREB to the *MAT2A* gene promoter. In addition, HBx and/or overexpression of *MAT2A* were able to inhibit apoptosis in HCC cells [112]. Although this has not been formally examined, these data offer a biological rationale for the use of SAME to prevent HCC in HBV. Clinical trials to validate this are, however, required.

#### Conclusions and future directions

In conclusion, there is strong pre-clinical evidence that SAME has important physiological roles in health and that liver disease of various aetiologies may perturb these. Furthermore, it is apparent that hepatocellular SAME concentration can influence diverse pathophysiological processes including tissue oxidative stress, mitochondrial function, hepatocellular apoptosis, and malignant transformation, not to mention the intriguing data suggesting that chronic viral hepatitis may modulate interferon sensitivity through SAME. These data suggest that SAME could offer substantial clinical benefits, however, very

## Review

few large, high-quality clinical trials have been performed to prove or refute this. The challenge is now to address this evidence deficit. We suggest that, rather than examining abstract features of IHC, future clinical studies should be conducted in defined, well-characterised patient groups and should be focussed on the effects of SAME on clinically relevant 'hard end points', possibly in three key areas where there is a good pre-clinical rationale for efficacy:

- The role of SAME, alone or in combination with vitamin B6/B12 and folate supplementation [75], in the treatment of inflammation in NASH;
- The utility of SAME as an adjunct to pegylated IFN $\alpha$ /Ribavirin based anti-viral therapy in chronic HCV infection. This would be of particular interest in HCV genotype 4 patients which have low sustained viral response rates with standard therapy and are currently under-represented in trials of novel protease inhibitors;
- The value of SAME as prophylaxis to reduce the incidence of HCC in chronic liver disease (e.g. HBV).

It is hoped that, by addressing these, the therapeutic potential of SAME can be translated from bench to the bedside.

### Conflict of interest

CPD has received consultancy fees and both authors have received speakers' fees from Abbott Laboratories. The authors declare that this work was supported by an educational grant from Abbott Laboratories.

### Key Points

- S-Adenosyl-L-methionine (SAME; AdoMet) is a metabolically pleiotropic molecule that is the precursor for the synthesis of the anti-oxidant glutathione and is also an important methyl donor
- Hepatocellular SAME concentration can influence diverse pathophysiological processes including tissue oxidative stress, mitochondrial function, hepatocellular apoptosis and malignant transformation
- SAME synthesis is depressed in chronic liver disease. Pre-clinical studies indicate this could exacerbate liver injury and so supplementation may be a useful therapy
- SAME has been widely adopted in Eastern Europe, Russia, China, Southern Asia and South America as a therapy for chronic liver disease and intra-hepatic cholestasis
- To date no large, high-quality, randomised placebo-controlled clinical trials have been performed that definitively establish clinical utility in specific disease states. Further clinical studies in well-characterised, disease-specific patient groups are needed and should be focussed on measuring the effects of SAME supplementation on clinically relevant 'hard end-points'

### Acknowledgements

Q.M.A. is the recipient of a Clinical Senior Lectureship Award from the Higher Education Funding Council for England (HEFCE). This work was supported by an educational grant from Abbott Laboratories.

### References

- [1] Malhi H, Gores GJ. Cellular and molecular mechanisms of liver injury. *Gastroenterology* 2008;134 (6):1641–1654.
- [2] Anstee QM, Wright M, Goldin R, Thursz MR. Parenchymal extinction: coagulation and hepatic fibrogenesis. *Clin Liver Dis* 2009;13 (1):117–126.
- [3] Malhi H, Gores GJ, Lemasters JJ. Apoptosis and necrosis in the liver: a tale of two deaths? *Hepatology* 2006;43 (2 Suppl. 1):S31–44.
- [4] Benyon RC, Iredale JP. Is liver fibrosis reversible? *Gut* 2000;46 (4):443–446.
- [5] Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S, et al. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. *J Clin Invest* 1998;102 (3):538–549.
- [6] Anstee QM, Daly AK, Day CP. Genetics of alcoholic and nonalcoholic fatty liver disease. *Semin Liver Dis* 2011;31 (2):128–146.
- [7] Plauth M, Cabre E, Riggio O, Assis-Camilo M, Pirlich M, Kondrup J, et al. ESPEN guidelines on enteral nutrition: liver disease. *Clin Nutr* 2006;25 (2):285–294.
- [8] Lieber CS. S-adenosyl-L-methionine: its role in the treatment of liver disorders. *Am J Clin Nutr* 2002;76 (5):1183S–1187S.
- [9] Martinez-Chantar ML, Garcia-Trevijano ER, Latasa MU, Perez-Mato I, Sanchez del Pino MM, Corrales FJ, et al. Importance of a deficiency in S-adenosyl-L-methionine synthesis in the pathogenesis of liver injury. *Am J Clin Nutr* 2002;76 (5):1177S–1182S.
- [10] Mato JM, Lu SC. Role of S-adenosyl-L-methionine in liver health and injury. *Hepatology* 2007;45 (5):1306–1312.
- [11] Purohit V, Abdelmalek MF, Barve S, Benevenga NJ, Halsted CH, Kaplowitz N, et al. Role of S-adenosylmethionine, folate, and betaine in the treatment of alcoholic liver disease: summary of a symposium. *Am J Clin Nutr* 2007;86 (1):14–24.
- [12] Mato JM, Corrales FJ, Lu SC, Avila MA. S-adenosylmethionine: a control switch that regulates liver function. *FASEB J* 2002;16 (1):15–26.
- [13] Catoni GL. S-adenosylmethionine; a new intermediate formed enzymatically from L-methionine and adenosinetriphosphate. *J Biol Chem* 1953;204 (1):403–416.
- [14] Mudd SH. Activation of methionine for transmethylation. V. The mechanism of action of the methionine-activating enzyme. *J Biol Chem* 1962;237:1372–1375.
- [15] Kotb M, Mudd SH, Mato JM, Geller AM, Kredich NM, Chou JY, et al. Consensus nomenclature for the mammalian methionine adenosyltransferase genes and gene products. *Trends Genet*: TIG 1997;13 (2):51–52.
- [16] Kotb M, Geller AM. Methionine adenosyltransferase: structure and function. *Pharmacol Ther* 1993;59 (2):125–143.
- [17] Gil B, Casado M, Pajares MA, Bosca L, Mato JM, Martin-Sanz P, et al. Differential expression pattern of S-adenosylmethionine synthetase isoenzymes during rat liver development. *Hepatology* 1996;24 (4):876–881.
- [18] Torres L, Avila MA, Carretero MV, Latasa MU, Caballeria J, Lopez-Rodas G, et al. Liver-specific methionine adenosyltransferase MAT1A gene expression is associated with a specific pattern of promoter methylation and histone acetylation: implications for MAT1A silencing during transformation. *FASEB J* 2000;14 (1):95–102.
- [19] Avila MA, Mingorance J, Martinez-Chantar ML, Casado M, Martin-Sanz P, Bosca L, et al. Regulation of rat liver S-adenosylmethionine synthetase during septic shock: role of nitric oxide. *Hepatology* 1997;25 (2):391–396.
- [20] Sanchez-Gongora E, Ruiz F, Mingorance J, An W, Corrales FJ, Mato JM. Interaction of liver methionine adenosyltransferase with hydroxyl radical. *FASEB J* 1997;11 (12):1013–1019.
- [21] Ruiz F, Corrales FJ, Miquero C, Mato JM. Nitric oxide inactivates rat hepatic methionine adenosyltransferase in vivo by S-nitrosylation. *Hepatology* 1998;28 (4):1051–1057.
- [22] Lu SC. S-adenosylmethionine. *Int J Biochem Cell Biol* 2000;32 (4):391–395.
- [23] Lieber CS, Packer L. S-adenosylmethionine: molecular, biological, and clinical aspects – an introduction. *Am J Clin Nutr* 2002;76 (5):1148S–1150S.
- [24] Ou X, Yang H, Ramani K, Ara AI, Chen H, Mato JM, et al. Inhibition of human betaine-homocysteine methyltransferase expression by S-adenosylmethionine and methylthioadenosine. *Biochem J* 2007;401 (1):87–96.

- [25] Kinsell LW, Harper HA, Barton HC, Hutchin ME, Hess JR. Studies in methionine and sulfur metabolism. I. The fate of intravenously administered methionine, in normal individuals and in patients with liver damage. *J Clin Invest* 1948;27 (5):677-688.
- [26] Duce AM, Ortiz P, Cabrero C, Mato JM. S-adenosyl-L-methionine synthetase and phospholipid methyltransferase are inhibited in human cirrhosis. *Hepatology* 1988;8 (1):65-68.
- [27] Avila MA, Berasain C, Torres L, Martín-Duce A, Corrales FJ, Yang H, et al. Reduced mRNA abundance of the main enzymes involved in methionine metabolism in human liver cirrhosis and hepatocellular carcinoma. *J Hepatol* 2000;33 (6):907-914.
- [28] Lu SC, Alvarez L, Huang ZZ, Chen L, An W, Corrales FJ, et al. Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. *Proc Natl Acad Sci USA* 2001;98 (10):5560-5565.
- [29] Anstee QM, Goldin RD. Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. *Int J Exp Pathol* 2006;87 (1):1-16.
- [30] Cai J, Mao Z, Hwang JJ, Lu SC. Differential expression of methionine adenosyltransferase genes influences the rate of growth of human hepatocellular carcinoma cells. *Cancer Res* 1998;58 (7):1444-1450.
- [31] Latasa MU, Boukaba A, Garcia-Trevijano ER, Torres L, Rodriguez JL, Caballeria J, et al. Hepatocyte growth factor induces MAT2A expression and histone acetylation in rat hepatocytes: role in liver regeneration. *FASEB J* 2001;15 (7):1248-1250.
- [32] Garcia-Trevijano ER, Latasa MU, Carretero MV, Berasain C, Mato JM, Avila MA. S-adenosylmethionine regulates MAT1A and MAT2A gene expression in cultured rat hepatocytes: a new role for S-adenosylmethionine in the maintenance of the differentiated status of the liver. *FASEB J* 2000;14 (15):2511-2518.
- [33] Huang ZZ, Mao Z, Cai J, Lu SC. Changes in methionine adenosyltransferase during liver regeneration in the rat. *Am J Physiol* 1998;275 (1 Pt 1):G14-21.
- [34] Martínez-Chantar ML, Corrales FJ, Martínez-Cruz LA, García-Trevijano ER, Huang ZZ, Chen L, et al. Spontaneous oxidative stress and liver tumors in mice lacking methionine adenosyltransferase 1A. *FASEB J* 2002;16 (10):1292-1294.
- [35] Chen L, Zeng Y, Yang H, Lee TD, French SW, Corrales FJ, et al. Impaired liver regeneration in mice lacking methionine adenosyltransferase 1A. *FASEB J* 2004;18 (7):914-916.
- [36] Pascale RM, Marras V, Simile MM, Daino L, Pinna G, Bennati S, et al. Chemoprevention of rat liver carcinogenesis by S-adenosyl-L-methionine: a long-term study. *Cancer Res* 1992;52 (18):4979-4986.
- [37] Pascale RM, Simile MM, De Miglio MR, Nufiris A, Daino L, Seddaiu MA, et al. Chemoprevention by S-adenosyl-L-methionine of rat liver carcinogenesis initiated by 1,2-dimethylhydrazine and promoted by orotic acid. *Carcinogenesis* 1995;16 (2):427-430.
- [38] Finkelstein JD, Martin JJ. Methionine metabolism in mammals. Adaptation to methionine excess. *J Biol Chem* 1986;261 (4):1582-1587.
- [39] Hardwick DF, Applegarth DA, Cockcroft DM, Ross PM, Cder RJ. Pathogenesis of methionine-induced toxicity. *Metabolism* 1970;19 (5):381-391.
- [40] Jaeschke H. Reactive oxygen and mechanisms of inflammatory liver injury: present concepts. *J Gastroenterol Hepatol* 2011;26 (Suppl. 1):173-179.
- [41] Coppie BL, Jaeschke H, Klaassen CD. Oxidative stress and the pathogenesis of cholestasis. *Semin Liver Dis* 2010;30 (2):195-204.
- [42] Song Z, Zhou Z, Chen T, Hill D, Kang J, Barve S, et al. S-adenosylmethionine (SAME) protects against acute alcohol induced hepatotoxicity in mice. *J Nutr Biochem* 2003;14 (10):591-597.
- [43] Garcia-Ruiz C, Morales A, Colell A, Ballesta A, Rodes J, Kaplowitz N, et al. Feeding S-adenosyl-L-methionine attenuates both ethanol-induced depletion of mitochondrial glutathione and mitochondrial dysfunction in periportal and perivenous rat hepatocytes. *Hepatology* 1995;21 (1):207-214.
- [44] Colell A, Garcia-Ruiz C, Miranda M, Ardite E, Mari M, Morales A, et al. Selective glutathione depletion of mitochondria by ethanol sensitizes hepatocytes to tumor necrosis factor. *Gastroenterology* 1998;115 (6):1541-1551.
- [45] Colell A, García-Ruiz C, Morales A, Ballesta A, Ookhtens M, Rodés J, et al. Transport of reduced glutathione in hepatic mitochondria and mitoplasts from ethanol-treated rats: effect of membrane physical properties and S-adenosyl-L-methionine. *Hepatology* 1997;26 (3):699-708.
- [46] Vendemiale G, Altomare E, Trizio T, Le Grazie C, Di Padova C, Salerno MT, et al. Effects of oral S-adenosyl-L-methionine on hepatic glutathione in patients with liver disease. *Scand J Gastroenterol* 1989;24 (4):407-415.
- [47] Santini D, Vincenzi B, Massacesi C, Picardi A, Gentiluoci UV, Esposito V, et al. S-adenosylmethionine (AdoMet) supplementation for treatment of chemotherapy-induced liver injury. *Anticancer Res* 2003;23 (6D):5173-5179.
- [48] Vincenzi B, Daniele S, Frezza AM, Berti P, Vespasiani U, Picardi A, et al. The role of S-adenosylmethionine in preventing oxalipatin-induced liver toxicity: a retrospective analysis in metastatic colorectal cancer patients treated with bevacizumab plus oxalipatin-based regimen. *Support Care Cancer* 2012;20 (1):135-139.
- [49] Vincenzi B, Santini D, Frezza AM, Berti P, Vespasiani U, Picardi A, et al. The role of S-adenosyl methionine in preventing FOLFOX-induced liver toxicity: a retrospective analysis in patients affected by resected colorectal cancer treated with adjuvant FOLFOX regimen. *Expert Opin Drug Saf* 2011;10 (3):345-349.
- [50] Veal N, Hsieh CL, Xiong S, Mato JM, Lu S, Tsukamoto H. Inhibition of lipopolysaccharide-stimulated TNF-alpha promoter activity by S-adenosylmethionine and 5'-methylthioadenosine. *Am J Physiol Gastrointest Liver Physiol* 2004;287 (2):G352-362.
- [51] Chawla RK, Watson WH, Eastin CE, Lee EY, Schmidt J, McClain CJ. S-adenosylmethionine deficiency and TNF-alpha in lipopolysaccharide-induced hepatic injury. *Am J Physiol* 1998;275 (1 Pt 1):G125-129.
- [52] Song Z, Uriarte S, Sahoo R, Chen T, Barve S, Hill D, et al. S-adenosylmethionine (SAME) modulates interleukin-10 and interleukin-6, but not TNF, production via the adenosine (A2) receptor. *Biochim Biophys Acta* 2005;1743 (3):205-213.
- [53] Hevia H, Varela-Rey M, Corrales FJ, Berasain C, Martínez-Chantar ML, Latasa MU, et al. 5'-Methylthioadenosine modulates the inflammatory response to endotoxin in mice and in rat hepatocytes. *Hepatology* 2004;39 (4):1088-1098.
- [54] Song Z, Barve S, Chen T, Nelson W, Uriarte S, Hill D, et al. S-adenosylmethionine (AdoMet) modulates endotoxin stimulated interleukin-10 production in monocytes. *Am J Physiol Gastrointest Liver Physiol* 2003;284 (6):G949-955.
- [55] Benz C, Angermüller S, Kloters-Plachky P, Sauer P, Stremmel W, Stiehl A. Effect of S-adenosylmethionine versus tauroursodeoxycholic acid on bile acid-induced apoptosis and cytolysis in rat hepatocytes. *Eur J Clin Invest* 1998;28 (7):577-583.
- [56] Webster CR, Boria P, Usechak P, Anwer MS. S-adenosylmethionine and cAMP confer differential cytoprotection against bile acid-induced apoptosis in canine renal tubular cells and primary rat hepatocytes. *Vet Ther* 2002;3 (4):474-484.
- [57] Ishii H, Adachi M, Fernandez-Checa JC, Cederbaum AI, Deaciuc IV, Nanji AA. Role of apoptosis in alcoholic liver injury. *Alcohol Clin Exp Res* 2003;27 (7):1207-1212.
- [58] Ansoarena E, Garcia-Trevijano ER, Martínez-Chantar ML, Huang ZZ, Chen L, Mato JM, et al. S-adenosylmethionine and methylthioadenosine are antiapoptotic in cultured rat hepatocytes but proapoptotic in human hepatoma cells. *Hepatology* 2002;35 (2):274-280.
- [59] Golovanova EV, Petrakov AV, Noskova KK. Intrahepatic cholestasis in chronic liver diseases. *Eksp Klin Gastroenterol* 2011;2:58-67.
- [60] Hirata F, Axelrod J. Phospholipid methylation and biological signal transduction. *Science* 1980;209 (4461):1082-1090.
- [61] Hirata F, Toyoshima S, Axelrod J, Waxdal MJ. Phospholipid methylation: a biochemical signal modulating lymphocyte mitogenesis. *Proc Natl Acad Sci USA* 1980;77 (2):862-865.
- [62] Hirata F, Viveros OH, Diliberto Jr EJ, Axelrod J. Identification and properties of two methyltransferases in conversion of phosphatidylethanolamine to phosphatidylcholine. *Proc Natl Acad Sci USA* 1978;75 (4):1718-1721.
- [63] Dooley JS, Lok AS, Burroughs AK, Heathcote EJ, editors. *Sherlock's diseases of the liver & biliary system*. Wiley-Blackwell; 2011.
- [64] Gonzalez-Corraea JA, De La Cruz JP, Martín-Aurioles E, Lopez-Egea MA, Ortiz P, Sanchez de la Cuesta F. Effects of S-adenosyl-L-methionine on hepatic and renal oxidative stress in an experimental model of acute biliary obstruction in rats. *Hepatology* 1997;26 (1):121-127.
- [65] Yang H, Ramani K, Xia M, Ko KS, Li TW, Oh P, et al. Dysregulation of glutathione synthesis during cholestasis in mice. molecular mechanisms and therapeutic implications. *Hepatology* 2009;49 (6):1982-1991.
- [66] Fiorelli G. S-adenosylmethionine in the treatment of intrahepatic cholestasis of chronic liver disease: a field trial. *Curr Ther Res Clin Exp* 1999;60 (6):335-348.
- [67] Frezza M, Surrenti C, Manzillo G, Fiaccadori F, Bortolini M, Di Padova C. Oral S-adenosylmethionine in the symptomatic treatment of intrahepatic cholestasis. A double-blind, placebo-controlled study. *Gastroenterology* 1990;99 (1):211-215.
- [68] Manzillo G, Piccinino F, Surrenti C, Frezza M, Giudici GA, Le Grazie C. Multicentre double-blind placebo-controlled study of intravenous and oral s-adenosyl-L-methionine (SAME) in cholestatic patients with liver disease. *Drug Invest* 1992;90-100.

## Review

- [69] Qin B, Guo S, Zhao Y, Zou S, Zhang Q, Wang Z, et al. A trial of ademetionine in the treatment of intrahepatic biliary stasis viral hepatitis. *Zhonghua Gan Zang Bing Za Zhi* 2000;8 (3):158–160.
- [70] Hardy ML, Coulter I, Morton SC, Favreau J, Venuturupalli S, Chiappelli F, et al. S-adenosyl-L-methionine for treatment of depression, osteoarthritis, and liver disease. *Evid Rep Technol Assess (Summ)* 2003;64:1–3.
- [71] Pust T, Beuers U. Intrahepatic cholestasis of pregnancy. *Orphanet J Rare Dis* 2007;2:26.
- [72] Roncaglia N, Locatelli A, Arregghini A, Assi F, Cameroni I, Pezzullo JC, et al. A randomised controlled trial of ursodeoxycholic acid and S-adenosyl-L-methionine in the treatment of gestational cholestasis. *BJOG* 2004;2:17–21.
- [73] Burrows RF, Clavisi O, Burrows E. Interventions for treating cholestasis in pregnancy. *Cochrane Database Syst Rev* 2001;4:CD000493.
- [74] Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology* 2011;141 (5):1572–1585.
- [75] Halsted CH, Medici V. Vitamin-dependent methionine metabolism and alcoholic liver disease. *Adv Nutr* 2011;2 (5):421–427.
- [76] Lumeng L. The role of acetaldehyde in mediating the deleterious effect of ethanol on pyridoxal 5'-phosphate metabolism. *J Clin Invest* 1978;62 (2):286–293.
- [77] Lee TD, Sada MR, Mendler MH, Bottiglieri T, Kanel G, Mato JM, et al. Abnormal hepatic methionine and glutathione metabolism in patients with alcoholic hepatitis. *Alcohol Clin Exp Res* 2004;28 (1):173–181.
- [78] Cabrero C, Duce AM, Ortiz P, Alemany S, Mato JM. Specific loss of the high-molecular-weight form of S-adenosyl-L-methionine synthetase in human liver cirrhosis. *Hepatology* 1988;8 (6):1530–1534.
- [79] Barak AJ, Beckenhauer HC, Tuma DJ, Badakhsh S. Effects of prolonged ethanol feeding on methionine metabolism in rat liver. *Biochem Cell Biol* 1987;65 (3):230–233.
- [80] Lieber CS, Casini A, DeCarli LM, Kim CI, Lowe N, Sasaki R, et al. S-adenosyl-L-methionine attenuates alcohol-induced liver injury in the baboon. *Hepatology* 1990;11 (2):165–172.
- [81] Halsted CH, Villanueva JA, Devlin AM, Niemela O, Parkkila S, Garrow TA, et al. Folate deficiency disturbs hepatic methionine metabolism and promotes liver injury in the ethanol-fed micropig. *Proc Natl Acad Sci USA* 2002;99 (15):10072–10077.
- [82] Halsted CH, Villanueva J, Chandler CJ, Stabler SP, Allen RH, Muskhelishvili L, et al. Ethanol feeding of micropigs alters methionine metabolism and increases hepatocellular apoptosis and proliferation. *Hepatology* 1996;23 (3):497–505.
- [83] Gigliozzi A, Romeo R, Fraioli F, Cantafora A, Delle Monache M, Cardilli A, et al. Effect of S-adenosyl-L-methionine and dilinoleoylphosphatidylcholine on liver lipid composition and ethanol hepatotoxicity in isolated perfused rat liver. *Dig Dis Sci* 1998;43 (10):2211–2222.
- [84] Corrales F, Gimenez A, Alvarez L, Caballeria J, Pajares MA, Andreu H, et al. S-adenosylmethionine treatment prevents carbon tetrachloride-induced S-adenosylmethionine synthetase inactivation and attenuates liver injury. *Hepatology* 1992;16 (4):1022–1027.
- [85] Mato JM, Camara J, Fernandez de Paz J, Caballeria L, Coll S, Caballero A, et al. S-adenosylmethionine in alcoholic liver cirrhosis: a randomized, placebo-controlled, double-blind, multicenter clinical trial. *J Hepatol* 1999;30 (6):1081–1089.
- [86] Rambaldi A, Gluud C. S-adenosyl-L-methionine for alcoholic liver diseases. *Cochrane Database Syst Rev* 2006 (2):CD002235.
- [87] Medici V, Virata MC, Peerson JM, Stabler SP, French SW, Gregory 3rd JF, et al. S-adenosyl-L-methionine treatment for alcoholic liver disease: a double-blinded, randomized, placebo-controlled trial. *Alcohol Clin Exp Res* 2011;35 (11):1960–1965.
- [88] Sanyal AJ. American gastroenterological A. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002;123 (5):1705–1725.
- [89] Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, et al. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 1999;107 (5):450–455.
- [90] Powell EE, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology* 1990;11 (1):74–80.
- [91] Nagata K, Suzuki H, Sakaguchi S. Common pathogenic mechanism in development progression of liver injury caused by non-alcoholic or alcoholic steatohepatitis. *J Toxicol Sci* 2007;32 (5):453–468.
- [92] Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology* 2007;45 (6):1366–1374.
- [93] Anstee QM, Concas D, Kudo H, Levene A, Pollard J, Charlton P, et al. Impact of pan-caspase inhibition in animal models of established steatosis and non-alcoholic steatohepatitis. *J Hepatol* 2010;53 (3):542–550.
- [94] Farrell GC, Larter CZ, Hou JY, Zhang RH, Yeh MM, Williams J, et al. Apoptosis in experimental NASH is associated with p53 activation and TRAIL receptor expression. *J Gastroenterol Hepatol* 2009;24 (3):443–452.
- [95] Iredale JP. Models of liver fibrosis: exploring the dynamic nature of inflammation and repair in a solid organ. *J Clin Invest* 2007;117 (3):539–548.
- [96] Wortham M, He L, Gyamfi M, Copple BL, Wan YJ. The transition from fatty liver to NASH associates with SAME depletion in db/db mice fed a methionine choline-deficient diet. *Dig Dis Sci* 2008;53 (10):2761–2774.
- [97] Cano A, Buque X, Martinez-Una M, Aurrekoetxea I, Menor A, Garcia-Rodriguez JL, et al. Methionine adenosyltransferase 1A gene deletion disrupts hepatic very low-density lipoprotein assembly in mice. *Hepatology* 2011;54 (6):1975–1986.
- [98] Kalhan SC, Edmison J, Marczewski S, Dasarthy S, Gruca LL, Bennett C, et al. Methionine and protein metabolism in non-alcoholic steatohepatitis: evidence for lower rate of transmethylation of methionine. *Clin Sci* 2011;121 (4):179–189.
- [99] Anstee QM, McPherson S, Day CP. How big a problem is non-alcoholic fatty liver disease? *BMJ* 2011;343:d3897.
- [100] Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010;362 (18):1675–1685.
- [101] Lavine JE, Schwimmer JB, Van Natta ML, Molleston JP, Murray KF, Rosenthal P, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. *JAMA* 2011;305 (16):1659–1668.
- [102] Abdelmalek MF, Angulo P, Jorgensen RA, Sylvestre PB, Lindor KD. Betaine, a promising new agent for patients with nonalcoholic steatohepatitis: results of a pilot study. *Am J Gastroenterol* 2001;96 (9):2711–2717.
- [103] Abdelmalek MF, Sanderson SO, Angulo P, Soldevila-Pico C, Liu C, Peter J, et al. Betaine for nonalcoholic fatty liver disease: results of a randomized placebo-controlled trial. *Hepatology* 2009;50 (6):1818–1826.
- [104] Bao-en W. Ademetionine 1,4 butanedisulphonate vs traditional Chinese medicine for the treatment of hepatocellular jaundice complicating chronic viral hepatitis. *Clinical Drug Invest* 2001;21 (11):765–773.
- [105] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009;41 (10):1105–1109.
- [106] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009;41 (10):1100–1104.
- [107] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;461 (7262):399–401.
- [108] Duong FH, Filipowicz M, Tripodi M, La Monica N, Heim MH. Hepatitis C virus inhibits interferon signaling through up-regulation of protein phosphatase 2A. *Gastroenterology* 2004;126 (1):263–277.
- [109] Duong FH, Christen V, Filipowicz M, Heim MH. S-adenosylmethionine and betaine correct hepatitis C virus induced inhibition of interferon signaling in vitro. *Hepatology* 2006;43 (4):796–806.
- [110] Filipowicz M, Bernsmeier C, Terracciano L, Duong FH, Heim MH. S-adenosyl-methionine and betaine improve early virological response in chronic hepatitis C patients with previous nonresponse. *PLoS One* 2010:e15492.
- [111] Feld JJ, Modi AA, El-Diwany R, Rotman Y, Thomas E, Ahlenstiel G, et al. S-adenosyl methionine improves early viral responses and interferon-stimulated gene induction in hepatitis C nonresponders. *Gastroenterology* 2011;140 (3):830–839.
- [112] Liu Q, Chen J, Liu L, Zhang J, Wang D, Ma L, et al. The X protein of hepatitis B virus inhibits apoptosis in hepatoma cells through enhancing the methionine adenosyltransferase 2A gene expression and reducing S-adenosyl-methionine production. *J Biol Chem* 2011;286 (19):17168–17180.
- [113] Nicastrì PL, Diaferia A, Tartagni M, Loizzi P, Fanelli M. A randomised placebo-controlled trial of ursodeoxycholic acid and S-adenosylmethionine in the treatment of intrahepatic cholestasis of pregnancy. *Br J Obstet Gynaecol* 1998;105 (11):1205–1207.
- [114] Ribalta J, Reyes H, Gonzalez MC, Iglesias J, Arrese M, Ponichak J, et al. S-adenosyl-L-methionine in the treatment of patients with intrahepatic cholestasis of pregnancy: a randomized, double-blind, placebo-controlled study with negative results. *Hepatology (Baltimore, MD)* 1991;2: 1084–1089.

- [115] Frezza M, Centini G, Cammareri G, Le Grazie C, Di Padova C. S-adenosylmethionine for the treatment of intrahepatic cholestasis of pregnancy. Results of a controlled clinical trial. *Hepatogastroenterology* 1990;37 (Suppl. 2):122–125.
- [116] Frezza M, Pozzato G, Chiesa L, Stramentinoli G, di Padova C. Reversal of intrahepatic cholestasis of pregnancy in women after high dose S-adenosyl-L-methionine administration. *Hepatology* 1984;4 (2):274–278.
- [117] Floreani A, Paternoster D, Melis A, Grella PV. S-adenosylmethionine versus ursodeoxycholic acid in the treatment of intrahepatic cholestasis of pregnancy: preliminary results of a controlled trial. *Eur J Obstet Gynecol Reprod Biol* 1996;67 (2):109–113.
- [118] Lafuenti G, Plotti G, Nicolanti G, Gaglione R, Tibollo FG, Mancuso S. Evaluation of the obstetrical risk in pregnant women with intrahepatic cholestasis treated with S-adenosyl-L-methionine. *Recent Prog Med* 1988;79 (10):420–423.
- [119] Catalino F, Scarponi S, Cesa F, Loiacono G, Bortolini M. Efficacy and safety of intravenous S-adenosyl-L-methionine therapy in the management of intrahepatic cholestasis of pregnancy. *Drug Invest* 1992;4:78–82.
- [120] Altomare E, Vendemiale G, Marchesini G, Legrazie C, Dipadova C. Increased bioavailability of sulfurated compounds after S-adenosylmethionine (same) administration to alcoholics. *Biomed Soc Aspects Alcohol Alcohol* 1988;805:353–356.
- [121] Chawla RK, Gaetke L, McClain CJ. S-adenosylmethionine (Adomet) and plasma methionine clearance in alcoholic subjects. *Hepatology* 1999;30 (4):397A.
- [122] Cibir M, Gentile N, Ferri M, Canton G, Legrazie C, Gallimberti L. S-adenosylmethionine (same) is effective in reducing ethanol abuse in an outpatient program for alcoholics. *Biomed Soc Aspects Alcohol Alcohol* 1988;805:357–360.
- [123] Corrales F, Pajares M, Pliego M, Ortiz P, Moreno J, Puerta J, et al. Effect of S-adenosylmethionine treatment on methionine intolerance in alcoholic cirrhosis. *J Hepatol* 1991;13 (Suppl. 2):S111.
- [124] Diaz Belmont A, Dominguez Henkel R, Uribe Ancira F. Parenteral S-adenosylmethionine compared to placebos in the treatment of alcoholic liver diseases. *Ann Med Intern* 1996;13 (1):9–15.
- [125] Loguercio C, Nardi G, Argenzio F, Aurilio C, Petrone E, Grella A, et al. Effect of S-adenosyl-L-methionine administration on red blood cell cysteine and glutathione levels in alcoholic patients with and without liver disease. *Alcohol Alcohol (Oxford, Oxfordshire)* 1994;597–604.
- [126] Trespi E, Vigoni R, Matti C, Colla C. TUDCA, UDCA and ademetionine (Ade) in the treatment of alcohol-induced liver damage. *J Hepatol* 1997;26 (Suppl. 1):128.