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Ethanol inhibits methionine adenosyltransferase II activity and *S*-adenosylmethionine biosynthesis and enhances caspase-3-dependent cell death in T lymphocytes: relevance to alcohol-induced immunosuppression☆

Prachi T. Hote[a], Rashmita Sahoo[b], Tanvi S. Jani[a], Smita S. Ghare[c], Theresa Chen[a], Swati Joshi-Barve[b], Craig J. McClain[a,b,d], Shirish S. Barve[a,b,*]

[a]*Department of Pharmacology and Toxicology, University of Louisville Medical Center, Louisville, KY 40292, USA*
[b]*Department of Internal Medicine, University of Louisville Medical Center, Louisville, KY 40292, USA*
[c]*Department of Microbiology and Immunology, University of Louisville Medical Center, Louisville, KY 40292, USA*
[d]*Louisville VA Medical Center, Louisville, KY 40292, USA*

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Abstract

An important aspect in alcohol abuse-associated immune suppression is the loss of T helper $CD4^+$ lymphocytes, leading to impairment of multiple immune functions. Our work has shown that ethanol can sensitize $CD4^+$ T lymphocytes to caspase-3-dependent activation-induced cell death (AICD). It has been demonstrated that the formation of *S*-adenosylmethionine (SAMe) catalyzed by methionine adenosyltransferase (MAT) II is essential for $CD4^+$ T-cell activation and proliferation. Since ethanol is known to affect SAMe metabolism in hepatocytes, we investigated the effect of ethanol on MAT II activity/expression, SAMe biosynthesis and cell survival in $CD4^+$ T lymphocytes. We demonstrate for the first time that ethanol at a physiologically relevant concentration (25 mM) substantially decreased the enzymatic activity of MAT II in T lymphocytes. Ethanol was observed to decrease the transcription of *MAT2A*, which encodes the catalytic subunit of MAT II and is vital for MAT II activity and SAMe biosynthesis. Furthermore, correspondent to its effect on MAT II, ethanol decreased intracellular SAMe levels and enhanced caspase-3-dependent AICD. Importantly, restoration of intracellular SAMe levels by exogenous SAMe supplementation considerably decreased both caspase-3 activity and apoptotic death in T lymphocytes. In conclusion, our data show that MAT II and SAMe are critical molecular components essential for $CD4^+$ T-cell survival that are affected by ethanol, leading to enhanced AICD. Furthermore, these studies provide a clinical paradigm for the development of much needed therapy using SAMe supplementation in the treatment of immune dysfunction induced by alcohol abuse.

Keywords: Ethanol; Methionine adenosyltransferase; *S*-Adenosylmethionine; $CD4^+$ T cells; Caspase-3; Activation-induced cell death

1. Introduction

Excessive alcohol consumption is known to have deleterious effects on the immune system [1–5]. Chronic alcohol administration in experimental animal systems leads to a decrease in the absolute number of $CD4^+$ T lymphocytes from the periphery and the spleen, as well as to a reduction in their immune function [6–12].

In human studies, alcohol-dependent patients have significantly reduced numbers of $CD4^+$ T lymphocytes, and the recovery of $CD4^+$ T-lymphocyte count after alcohol withdrawal has been noted in several studies, suggesting that ethanol can directly affect $CD4^+$ T-lymphocyte survival [13–18]. $CD4^+$ T lymphocytes are the central regulators of the immune system, controlling both cell-mediated and humoral immunity. While experimental and clinical studies

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* Corresponding author. Department of Medicine, Pharmacology and Toxicology, University of Louisville Medical Center, Louisville, KY 40202, USA. Tel.: +1 502 852 5245; fax: +1 502 562 4271.
E-mail address: shirish.barve@louisville.edu (S.S. Barve).

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have documented that alcohol intake can cause depletion of $CD4^+$ T lymphocytes, the mechanisms underlying this alcohol effect are only beginning to be understood. Recently, our work has shown that the in vitro exposure of human $CD4^+$ T cells to physiologically relevant concentrations of ethanol enhances their susceptibility to Fas-induced and activation-induced cell death (AICD) by augmenting FasR- and TCR-CD3-mediated caspase-3 activation [19,20]. The present work was carried out to further clarify the mechanisms, and to identify the molecular components, involved in the ethanol-mediated enhancement of the AICD of $CD4^+$ T cells, which are the central regulators of the immune system, by controlling both cell-mediated and humoral immunity.

Intracellular *S*-adenosylmethionine (SAMe) is known to be critical for normal cell development and function. SAMe levels vary under different biologic conditions of differentiation and proliferation, and are regulated by biosynthesis and utilization [21]. SAMe is of pivotal importance to cellular metabolism and serves as the principal biologic donor of methyl groups in transmethylation reactions, thereby supporting the synthesis and modification of several key cellular components, including proteins, lipids, RNA and DNA. Moreover, SAMe controls essential metabolic pathways by regulating several important enzymatic reactions, including those involved in polyamine biosynthesis and single carbon metabolism [21]. Methionine adenosyltransferase (MAT) is a key enzyme in cellular metabolism because it catalyzes the only reaction that generates SAMe from L-methionine (L-Met) and ATP [21].

An important consequence of chronic alcohol abuse is the abnormal metabolism of hepatic SAMe due to decreased hepatic methionine adenosyltransferase (MAT–MAT I) expression and activity, resulting in hepatic SAMe deficiency and hepatotoxicity [22]. However, the effects of ethanol on nonhepatic MAT (MAT II) and SAMe levels in T lymphocytes have not been examined. In the case of T lymphocytes, both the SAMe pool size and the rate of SAMe utilization are known to increase upon T-cell activation [23]. A key mechanism for the increase in SAMe biosynthesis in T lymphocytes is increased transcription of *MAT2A*, which encodes the catalytic subunit of MAT II and is vital for MAT II activity and SAMe biosynthesis [24,25]. *MAT2A* is constitutively expressed in actively dividing/proliferating T cells, and its expression is inducible upon T-cell activation [26]. Hence, in the present work, we examined the effect of ethanol on MAT II activity expression and SAMe biosynthesis and its consequent impact on activation-induced $CD4^+$ T-cell death. Our data show for the first time that MAT II activity and SAMe biosynthesis are essential for T-cell survival and that their down-regulation by ethanol leads to enhancement of caspase-3-dependent apoptotic cell death. Importantly, exogenous SAMe supplementation significantly attenuates the ethanol-induced enhancement of caspase-3-dependent apoptotic cell death, indicating its potential therapeutic use in the treatment of alcohol-induced immune suppression.

2. Materials and methods

2.1. Cell culture and treatment

Jurkat T cells (clone E6-1; ATCC, Rockville, MD, USA) and MOLT-4 T cells (ATCC) were cultured in RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 10 U/ml penicillin and 10 μg/ml streptomycin (Invitrogen Corporation, Carlsbad, CA, USA) at 37°C in a 5% CO_2 environment. Jurkat and MOLT-4 cells were resuspended in 1×10^6 cells/ml prior to treatment.

2.1.1. Peripheral blood lymphocyte isolation

After informed consent had been obtained from healthy nonalcoholic donors, fresh whole blood was drawn into Vacutainer tubes (Becton Dickinson Vacutainer; Becton Dickinson, Franklin Lakes, NJ) containing EDTA. Peripheral blood mononuclear cells (PBMCs) were isolated using Histopaque-1077 (Sigma-Aldrich Chemicals, St. Louis, MO, USA). A total of 5×10^6 PBMCs/well were distributed into six-well plates (Corning, Inc., Costar, NY, USA) and allowed to adhere in a 5% CO_2 incubator at 37°C for 2 h in 2 ml of RPMI 1640. Nonadherent peripheral blood lymphocytes (PBLs) were removed from adherent cells (monocytes), washed carefully twice with prewarmed RPMI 1640 and resuspended at a concentration of 2×10^6 cells/ml.

2.2. Reagents and antibodies

Cell culture reagents were obtained from Invitrogen Corporation. Ethyl alcohol and phytohemagglutinin (PHA) were obtained from Sigma (St. Louis, MO, USA). SAMe, as 1,4-butanedisulphonate salt, was provided by Dr. R. O'Brian (Knoll Pharmaceuticals, Piscataway, NJ, USA) and Dr. G. Stramentionoli (Knoll Farmaceutici, Milan, Italy).

2.3. Reverse transcriptase–polymerase chain reaction and real-time PCR

Reverse transcriptase–polymerase chain reaction (RT-PCR) assays were used to assess *MAT2A* mRNA levels in Jurkat cells. Total RNA was isolated from treated cells after 3 h using TRIZOL (Invitrogen Corporation), and real-time PCR was performed as described elsewhere [27]. Specific primers were designed for human GAPDH and *MAT2A* using Primer3 software program. The following primers were used in real-time PCR:

GAPDH-RT-FP: 5′-TGGGCTACACTGAGCACCAG-3′
GAPDH-RT-RP: 5′-GGGTGTCGCTGTTGAAGTCA-3′
MAT2A-RT-FP: 5′-ACAATCTACCACCTACAGCCA-AGT-3′
MAT2A-RT-RP: 5′-GCATAAGAGACCTGAACAAGA-ACC-3′.

The parameter C_t (threshold cycle) was defined as the fraction cycle number at which fluorescence passed the threshold. The relative gene expression of *MAT2A* was