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Dysregulation of methionine metabolism in multiple sclerosis

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ABSTRACT

We report a significant reduction in plasma methionine concentrations in relapse remitting multiple sclerosis (MS) patients compared to controls. *In vivo* studies demonstrate that changes in peripheral methionine levels in mice can regulate histone H3 methylation and expression of DNA methyltransferase 3A (DNMT3A) centrally, in the cerebral cortex. Therefore, we propose that decreases in circulating methionine represent one of the earliest manifestations of dysregulated methionine metabolism in MS with potential impacts on both histone H3 and DNA methylation in the central nervous system.

1. Introduction

Multiple sclerosis (MS) is an inflammatory neurodegenerative disease that results in progressive neurological disability (Noseworthy et al., 2000; Bjartmar et al., 2000). In early relapse remitting stages of disease (RRMS), inflammatory demyelination results in less efficient conduction of nerve impulses and neurological impairment. These impairments generally resolve over a period of weeks due to redistribution of sodium channels and remyelination (England et al., 1990). Over time most patients progress to secondary progressive stages of disease (SPMS) where accumulation of damage and loss of axons and neurons leads to permanent disability (Vukusic and Confavreux, 2003). It has been established that cortical pathology including extensive gray matter lesions and cortical atrophy contribute to disease and disability (Bö et al., 2006; Fisher et al., 2008). Mounting evidence points to involvement of mitochondrial deficits and metabolic disturbances in MS cortical pathology (Dutta et al., 2006; Pandit et al., 2009; Clements et al., 2008; Broadwater et al., 2011; Witte et al., 2014) and changes in B_{12}- dependent methionine metabolism have been found to be linked to mitochondrial abnormalities in MS (Singhal et al., 2015). Methionine is an essential amino acid that not only participates in protein synthesis but is also an important intermediate metabolite involved in methyl group transfer to histones and DNA. Methylation of these targets is known to regulate the expression of thousands of genes by epigenetic mechanisms. A schematic showing the reactions involved in methionine metabolism is shown in Fig. 1A. In a previous report, levels of methionine metabolites were measured by liquid chromatography tandem-mass spectrometry (LC-MS/MS) and reduced concentrations of cystathionine and the methyl donors S-adenosylmethionine (SAM) and betaine were identified in postmortem MS cortical tissue when compared to controls (Singhal et al., 2015). As a result, we suggest that methionine metabolism is dysregulated in MS cortical tissue, however, it is not presently clear whether levels of circulating methionine metabolites are also altered in MS. In the current study we have measured methionine metabolite levels in plasma from early RRMS and compared them to those obtained from control individuals. Further, we treated mice with methionine to determine whether alterations in circulating methionine levels could impact the levels of the methyl donor SAM in the brain and subsequently modify downstream methylation reactions in the brain including histone methyltransferase H3 (H3K4me3) and DNA methyltransferase 3A (DNMT3A).

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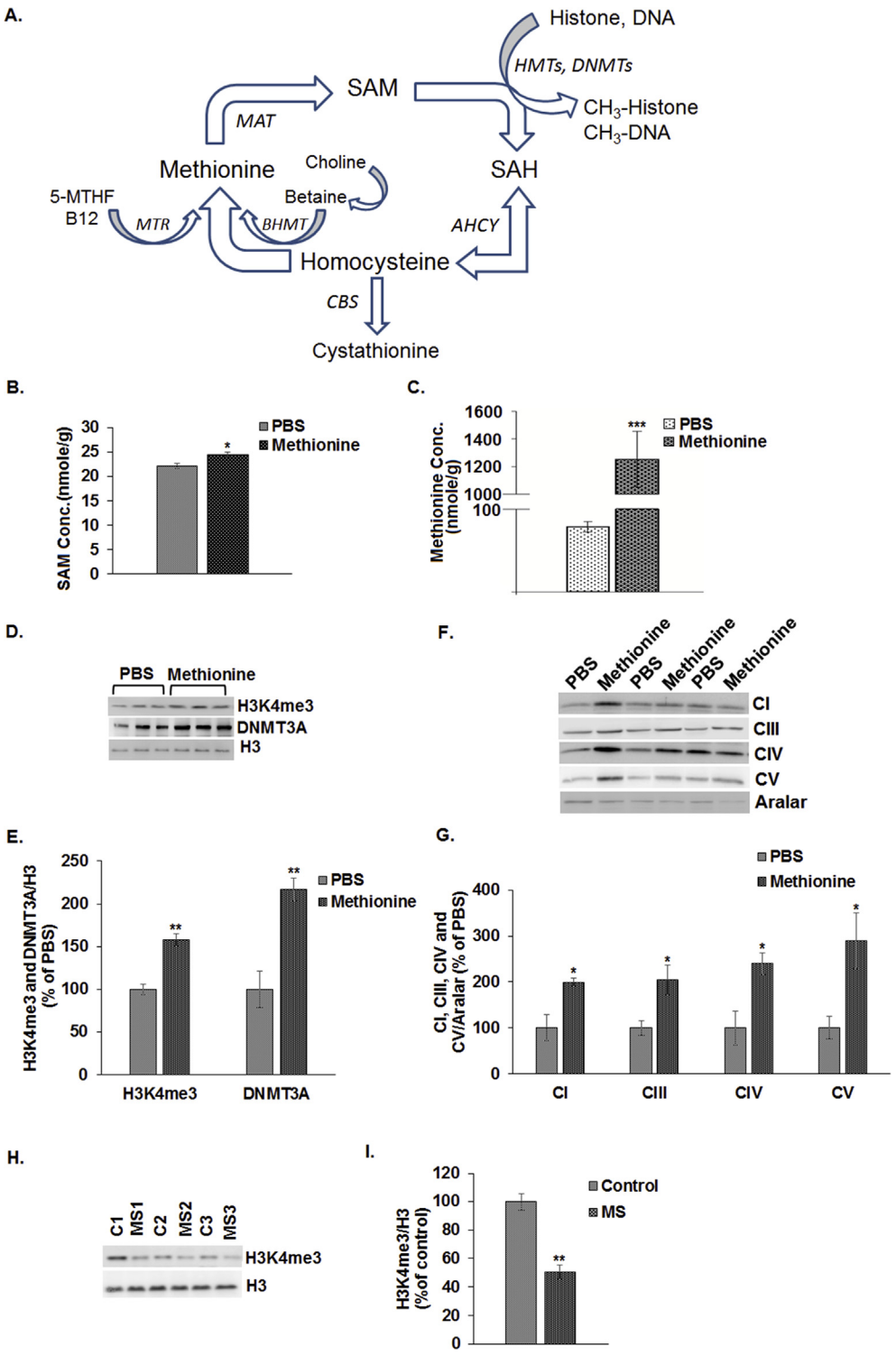

Fig. 1. Analysis of methionine metabolism metabolites and their effects on methylation in methionine treated mice and postmortem human brain tissue. **(A)** Schematic diagram depicting methionine metabolism. Homocysteine is remethylated to methionine with the transfer of methyl groups from either 5-MTHF or betaine by the enzymes MTR or BHMT respectively. Methionine is converted to SAM by MAT then SAM donates methyl groups to histone/DNA for methylation by HMT/DNMT enzymes. Once SAM donates a methyl group it is converted to SAH. Then SAH is converted to homocysteine by the enzyme AHCY. BHMT, betaine homocysteine S-methyltransferase; MTR, methionine synthase; 5-MTHF, 5-methyl tetrahydrofolate; MAT, methionine adenosyl transferase; HMTs, histone methyltransferases; DNMTs, DNA methyltransferases; AHCY, S-adenosylhomocysteine hydrolase. **(B and C)** SAM and methionine levels in brain were analyzed by LC-MS/MS in methionine treated and control mice. Both methionine and SAM were increased in methionine treated mice as compared to control **(D)** Representative Western blots for H3K4me3, DNMT3A and histone H3 from brain nuclear fractions isolated from control mice and mice treated with methionine. **(E)** Densitometry was performed for Western blots from three separate experiments. H3K4me3 and DNMT3A are increased in the brains of methionine treated mice compared with controls. **(F)** Representative Western blots for NDUFS4 (CI), UQCRC2 (CIII), COX5B (CIV), ATP5F1 (CV) and aralar from brain mitochondrial fractions isolated from control mice and mice treated with methionine. **(G)** Densitometry was performed for Western blots from three separate experiments. Protein subunits of CI, CIII, CIV, and CV are increased in the brains of methionine treated mice compared with controls. **(H)** Representative Western blots for H3K4me3 from nuclear fractions of three control and three MS cortical tissue samples. **(I)** Densitometry was performed from three separate experiments. Levels of H3K4me3 are normalized to histone H3. Data are expressed as a percentage of control with the highest control set at 100%. Error bars indicate SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

2. Materials and methods

2.1. Analysis of methionine metabolites by LC-MS/MS

Blood was collected in collaboration with the Oak Clinic for MS from 13 RRMS patients and 13 controls under an approved IRB protocol. The average age was 37.62 years (SEM ± 2.11) for MS patients and 42.73 years (SEM ± 3.0) for the controls. Plasma was separated from blood cells by centrifugation and stored at −80 °C within 1 h of the blood draw. Stable-isotope dilution liquid chromatography-electrospray ionization (ESI)-tandem mass spectrometry (LC-ESI-MS/MS) was performed to determine SAM, SAH (S-adenosyl-homocysteine), methionine, cystathionine, choline and betaine in human plasma as previously described (Inoue-Choi et al., 2013). Total plasma homocysteine (tHcy) was measured by LC-MS/MS as previously described (Ducros et al., 2006). Analysis of samples were done blind as to the disease diagnosis. Statistical significance of changes in average methionine metabolite concentrations between MS and control samples or between control and methionine treated mice was determined with a two-tailed T-test with $p \leq 0.05$ considered significant.

2.2. Methionine treatment

Adult male C57Bl/6 mice (30 days old) were treated with either L-methionine (5.2 mmol/kg) (Sigma) or phosphate buffer saline (PBS) by subcutaneous injections once a day for 7 days. All procedures involving animals were approved by the animal care and use committee at Kent State University. 24 h after the last injection of methionine, mice were perfused with PBS and the cerebral cortex was removed. Methionine and SAM concentrations were measured, from one half of the cortex by LC-MS/MS as previously described (Singhal et al., 2015). H3K4me3 and DNMT3A proteins were isolated from the other half of brain cortex and analyzed by Western blotting. Three controls (PBS injected) and four methionine treated mice were analyzed.

2.3. Western blotting

Nuclear and mitochondrial fractions were isolated from half brains (sagittally cut) from control and treated mice. Nuclear fractions were also isolated from control and MS postmortem cortex (obtained from Rocky Mountain MS Center and Brain and Spinal Cord Resource Center at UCLA). Proteins were separated by gel electrophoresis, and Western blotted with antibodies to either DNMT3A and H3K4me3 (AbCam, Cambridge, MA) for nuclear fractions or different electron transport chain complexes including NADH dehydrogenase [ubiquinone] iron-sulfur protein 4 (NDUFS4 or complex I), ubiquinol-cytochrome c reductase complex (UQCRC2 or complex III), cytochrome C oxidase 5B (COX5B or complex IV) and, ATP synthase subunit b (ATP5F1 or complex V) (ABclonal, Woburn, MA) for mitochondrial fractions. Densitometry was performed from three separate experiments with Image J. Relative protein levels were normalized to histone H3 for nuclear fractions and aralar for mitochondrial fractions. Statistical significance was determined with a two-tailed T-test with $p \leq 0.05$ considered significant.

3. Results

Plasma was collected from 13 RRMS and 13 control subjects. We measured plasma methionine metabolites including methionine, SAM, SAH, tHcy, choline, cystathionine and betaine and found a significant decrease in methionine levels in plasma from RRMS patients (20.1 μmol/L) compared to controls (29.2 μmol/l), $p < 0.05$ (Table 1). The methionine metabolism pathway is shown in Fig. 1A. To determine whether changes in circulating methionine levels can impact downstream methylation reactions in the CNS, we treated mice with subcutaneous injections of L-methionine every day for 7 days. The concentration of methionine and SAM was measured in the brains of these mice after perfusion with PBS to eliminate blood contamination. Injections of methionine resulted in a significant increase in concentrations of methionine in the brain (Fig. 1B and C). Levels of brain methionine were increased 15 fold in methionine treated animals from 78.7 nmol/g (±11.3) in controls to 1252.5 nmol/g (+/− 406.0) in methionine treated. Since SAM is the sole methyl-group donor for methylation reactions in cells, we also measured SAM concentration in mouse brain tissue to determine whether increasing methionine could have an effect on SAM levels. We found that SAM concentration was increased modestly but significantly from 22.1 nmol/g (+/− 0.97) to 24.5 nmol/g (+/− 0.93) in controls and methionine treated mice respectively. We then examined levels of histone H3 methylation and expression of the DNA methyltransferase DNMT3A in the cortex of these mice by Western blotting. We found a significant increase in levels of H3K4me3 and DNMT3A in the cortex of mice treated with methionine compared to controls (Fig. 1D and E). Furthermore, methionine administration up-regulated the expression of mitochondrial electron transport complex proteins. We found that levels of NDUFS4 (complex I), UQCRC2 (complex III), COX5B (complex IV), and ATP5F1 (complex V) were increased in mitochondrial fractions isolated from brains of methionine treated mice compared to controls (Fig. 1F and G). These data suggest that levels of circulating methionine can impact downstream methylation reactions and gene expression not only in the liver (Mentch et al., 2015) but also in the cortex. Our results in mice suggest that circulating methionine can impact histone methylation in the brain. Since we found reduced plasma methionine in RRMS, we then measured the level of H3K4me3 in postmortem MS cortex compared to controls. We confirmed that H3K4me3 is reduced in MS cortex as previously reported (Singhal et al., 2015), supporting a link between decreased methionine concentration in plasma and levels of H3K4me3 in the cortex in MS (Fig. 1H and I).

4. Discussion

Dysregulation of methionine metabolism has been reported in a number of neurological disorders, including Alzheimer's disease, dementia, subacute combined degeneration of the spinal cord (SACD), HIV-related neuropathies and in patients with metabolic disorders such as methylenetetrahydrofolate reductase deficiency (Bottiglieri and Hyland, 1994). We have previously reported dysregulation of methionine metabolism in MS (Singhal et al., 2015). Studies on postmortem brain tissue have also shown that changes in methylation of DNA and histone H3 contribute to MS pathology

Table 1
Methionine metabolite concentrations.

	tHcy (μmol/L)	SAM (nmol/L)	SAH (nmol/L)	SAM/SAH	Cys (nmol/L)	Betaine(μmol/L)	Choline (μmol/L)	Met (μmol/L)
Controls (n = 13)	7.3	70.0	7.8	13.4	247.1	35.0	6.5	29.2
RRMS (n = 13)	6.8	81.1	9.7	11.4	147.6	37.2	5.5	20.1*

The asterisks denote significance between MS and control concentrations ($p < 0.05$).

(Huynh et al., 2014; Singhal et al., 2015). Changes in DNA methylation in T-cells has been reported in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS (Li et al., 2017). In the present study however, we have examined the relationship between alterations in circulating methionine metabolites and markers of methylation linked to MS pathology. In this study, we found a significant reduction in plasma methionine levels in early stage RRMS patients compared to controls. Plasma methionine was reduced from 29.2 μM in controls to 20.2 μM in RRMS. This finding is consistent with a previous report which showed that levels of methionine, SAM, SAH, and vitamin B_{12} were decreased in plasma from MS patients at different disease stages (Gardner et al., 2013). Further, we have previously linked a dysregulation in methionine metabolism with alterations of histone methylation in MS cortex. We found reductions in the methyl donors SAM and betaine in MS cortical gray matter tissue and decreased levels of H3K4me3 in neurons in MS cortex (Singhal et al., 2015). Reduced H3K4me3 was subsequently linked to decreased expression of mitochondrial genes in neuronal cell culture, as well as in MS cortex (Singhal et al., 2015) suggesting that strategies that support methionine metabolism and prevent reductions in H3K4me3 may provide neuroprotection in MS. In the current study, we investigated the therapeutic potential of methionine supplementation on maintaining methionine metabolism and downstream methylation reactions. We found that peripheral administration of methionine in mice can increase levels of H3K4me3 and expression of DNMT3A and mitochondrial complex proteins in the cortex. Our findings are supported by a previous study reporting that altering dietary methionine levels can have an effect on histone methylation and gene expression in cell culture and in the liver of mice (Mentch et al., 2015). In this study, mice fed a methionine restricted diet had decreased levels of circulating methionine and decreased levels of H3K4me3 in the liver. SAM administration has also been shown to restore DNMT expression in lung cancer cells which was reduced by 5-fluorouracil (Ham et al., 2013).

The impact of methionine supplementation on methylation changes in the CNS has not been addressed previously. Our data demonstrate that methionine metabolism is dysregulated in MS both peripherally as well as centrally indicated by the reduction in plasma methionine levels in early RRMS and the reduction in H3K4me3 in the cortex. Our data suggest that strategies to enhance methionine metabolism through dietary supplementation may prevent the reduction of H3K4me3 in MS and the resulting changes in gene expression in neurons.

Conflict of interest

The authors declare that they have not any conflict of interest.

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