Perturbations in one carbon metabolism may increase the risk of methotrexate-related neurotoxicity via reduced oligodendrocyte proliferation without changes to DNA methylation

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Background

- Methotrexate (MTX) is an anti-folate chemotherapy drug that can induce neurotoxicity, but the mechanisms are currently unknown.
- DNA methylation is a mechanism of gene regulation.
- Myelin is essential for proper functioning of neuronal cells.
- Metabolites in one carbon metabolism, including methionine, are essential for maintaining methylation patterns and myelin production.
- MTX can reduce available methionine for DNA methylation and myelin production, and therefore may be involved in neurotoxicity.

Hypothesis

MTX treatment may influence the risk of neurotoxicity via changes in DNA methylation in cells responsible for myelin production i.e. oligodendrocytes through reduced methionine availability.

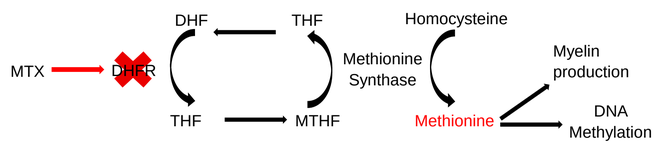


Figure 1. MTX inhibits dihydrofolate reductase (DHFR), leading to reduced conversion of dihydrofolate (DHF) to tetrahydrofolate (THF), which ultimately can reduce methionine via reduced methyl-THF (MTHF). Reduced methionine may influence DNA methylation and myelination production

Methods

- Oligodendrocyte-like cells (M03.13) were grown in dialysed (to mimic depletion of one-carbon related metabolites) and normal foetal bovine serum (FBS) supplemented media with different concentrations of MTX (0, 0.01, 0.05, 0.5, 1 and 2μM).
- To explore if the effects of MTX treatment were due to methionine availability, M03.13 cells were grown in dialysed FBS media with different methionine concentrations (0, 0.5nM, 1nM, 5nM, 10nM and 50nM).
- DNA was extracted and bisulfite modified (Fig 2).
- DNA methylation was assessed at several loci ([1]*Line-1*, [2]*ASCL2*, [3]*MBP* and [4]*MOG)* using pyrosequencing.
- Differences in methylation between treatments were analysed using Univariate analysis of variance with Bonferroni Post-Hoc test in SPSS.

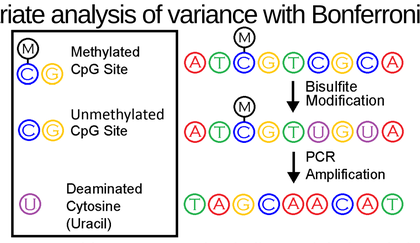


Figure 2. Bisulfite modification of DNA allows for Methylated DNA discrimination

Results

MTX treatment reduces cell proliferation in a dose dependent manner

- Higher concentrations of MTX reduced cell proliferation measured by haemocytometer counting 72 hrs post-treatment (Fig. 3).

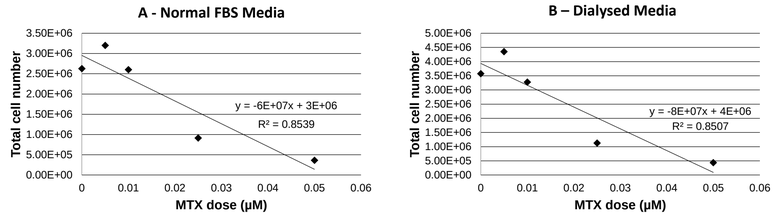


Figure 3. Total cell number 72 hrs-post treatment with varying concentrations of MTX in A) normal FBS media and B) dialysed FBS media

- There was no significant effect of either media (Fig 4) or MTX treatment (Fig 5) on DNA methylation at any of the loci investigated.

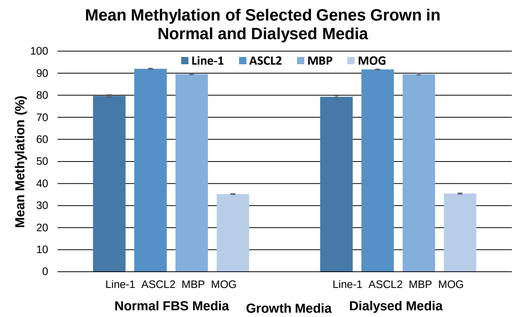


Figure 4. Mean percentage methylation of selected loci in normal FBS and dialysed media. Error bars represent standard error of the mean

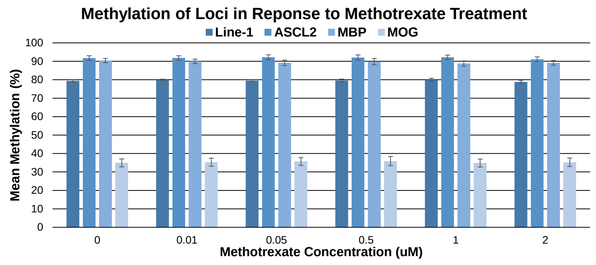


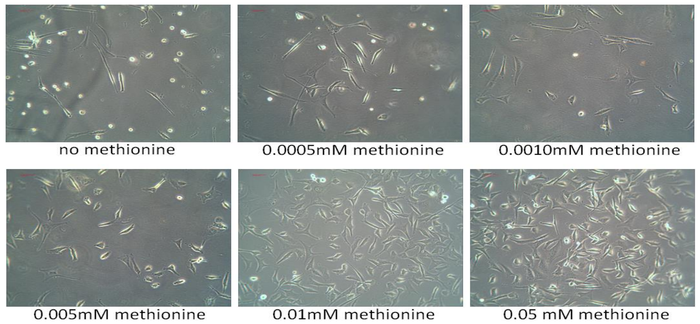
Figure 5. Mean percentage methylation of selected loci with varying MTX concentrations in normal FBS and dialysed media. Error bars represent standard error of the mean

Methionine availability influences cell proliferation

- Depleting cells of methionine resulted in reduced proliferation compared with cells treated with higher methionine concentrations (Fig 6).

Results

Figure 6. Oligodendrocyte cells after 72hrs culture in media with or without methionine at various concentrations



- There was no significant effect of methionine treatment on DNA methylation at any of the loci investigated (Fig 7).

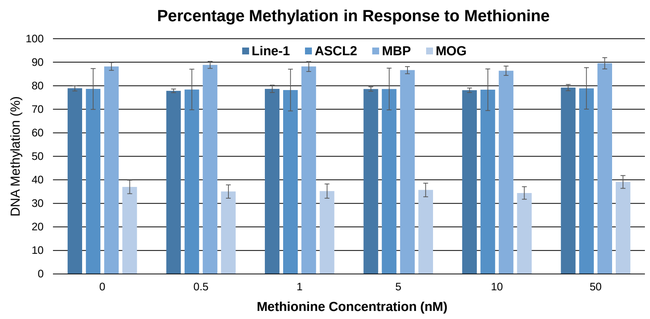


Figure 7. Mean percentage methylation in response to methionine reintroduction. Error bars represent standard error of the mean

Conclusion

- Both MTX and methionine appear to influence proliferation, but not DNA methylation, of myelin producing oligodendrocytes.
- We hypothesise that MTX-associated neurotoxicity may be due to reduced neuronal cell myelination as a result the cytotoxic effect of MTX on oligodendrocytes.

Acknowledgements

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[1]Line-1 is a genome wide repeat used to indicate global methylation levels. [2]*ASCL2 is* involved in determination of neuronal precursors in the CNS and PNS. [3]*MBP* and [4]*MOG* are myelin protein genes.