DNA methylation as a mediating mechanism in the development of neurotoxicity after methotrexate treatment

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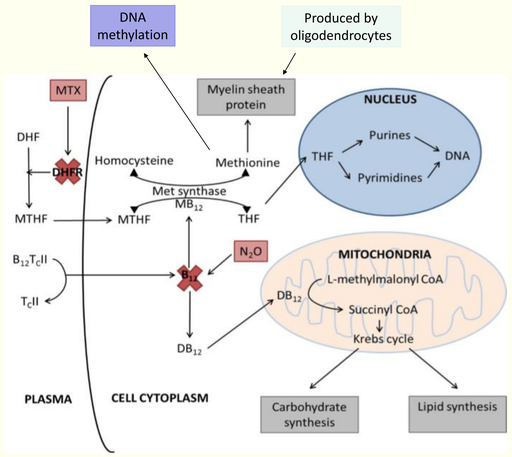
Background

- Methotrexate (MTX), an anti-folate drug, is frequently administered to treat childhood acute lymphoblastic leukaemia (ALL)
- Nitrous oxide (N_2O), which depletes vitamin B_{12}, is occasionally used in sedation during MTX treatment
- Combining these treatments has been hypothesised to ↑ neurotoxicity risk
- Since MTX and N_2O both influence one-carbon metabolism (Fig. 1), they may alter DNA methylation
- DNA methylation, the addition of a methyl group to cytosine's at CpG sites (phosphate linking cytosine to guanine), is important for gene regulation, and therefore may be one mechanism involved in treatment toxicity

Figure 1: *A summary of the biochemical reactions involving folate and B_{12} metabolism and the effects of MTX* and *N_2O administration.*



Abbreviations:
MTHF (5-methyltetrahydrofolate, levomefolic acid), MB_{12} (methyl B_{12}), THF (tetrahydrofolate, tetrahydrofolic acid), DHF (dihydrofolate, dihydrofolic acid), DHFR (dihydrofolate reductase), T_cII (transcobalamin II), dB_{12} (deoxyadenosylB_{12}), N_2O (nitrous oxide), MTX (methotrexate), Met synthase (methionine synthease).

Aims

- Investigate global and gene-specific DNA methylation in response to MTX and vitamin B_{12} depletion (as a proxy for N_2O treatment) in neuronal cells
- Investigate vitamin B_{12} supplementation on DNA methylation patterns as a potential approach to reduce toxicity risk

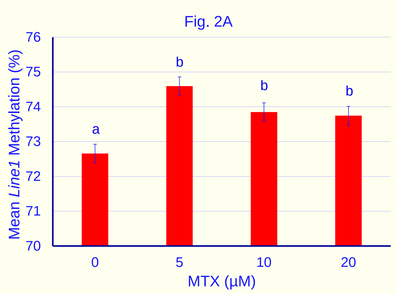
Methods

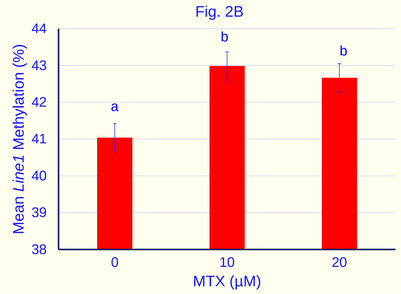
- Neuronal-like cells (SH-SY5Y, DAOY) were grown in media containing dialysed (to mimic B_{12} depletion) or normal foetal bovine serum (FBS) with varying concentrations of MTX (0, 10, 20μM), and with B_{12} reintroduced at different concentrations (0, 1, 10ng/mL) to dialysed FBS treated cells
- DNA was extracted and bisulfite modified
- Bisulfite modification deaminates cytosine to uracil, but is prevented by methylation, providing a way to discriminate methylated CpGs
- DNA methylation was assessed globally using the *Line1* assay and at specific gene loci, *ASCL2* and *SH3GL3* (previously found to have altered methylation in response to folate and/or B_{12}) using pyrosequencing
- Differences in methylation between treatments were analysed using Univariate analysis of variance with Bonferroni Post-Hoc test in SPSS.

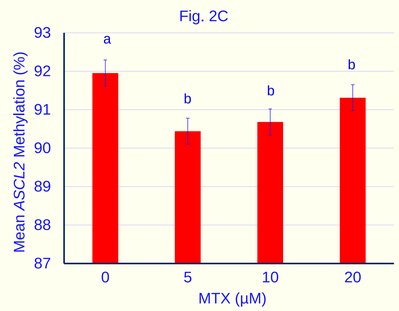
Results

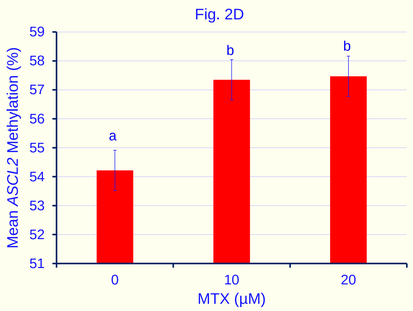
- For both cell lines, there was no significant effect of media type on *Line1* or gene specific DNA methylation
- MTX treatment significantly increased *Line1* (SH-SY5Y p< 0.001 , DAOY p=0.003) and *ASCL2* (SH-SY5Y p=0.042, DAOY p<0.001) methylation (Fig.1A-D)

Figure 2: Effect of MTX treatment on A) *Line1* methylation in SH-SY5Y cells, B) *Line1* methylation in DAOY cells, C) *ASCL2* methylation in SH-SY5Y cells and D) *ASCL2* methylation in DAOY cells. Groups not showing the same letter differ P>0.05.



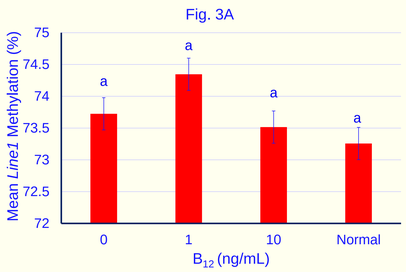


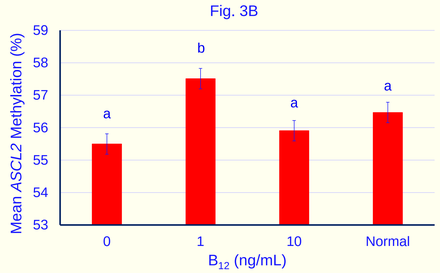




- Re-introducing B_{12} altered *Line1* DNA methylation in *SH-SY5Y cells* (*p*= 0.035), and *ASCL2* DNA methylation in *DAOY* cells (*p*<0.001), whereby 1μM B_{12} treated cells were more methylated (Fig. 3). Post-hoc tests revealed no significant differences between individual groups for *Line1* methylation in *SH-SY5Y cells*

Figure 3: A) *Line1* methylation in response to B_{12} concentrations in SH-SY5Y cells, B) *ASCL2* methylation in response to B_{12} concentrations in DAOY cells. Groups not showing the same letter differ P>0.05.





- Significant interactions were observed between MTX and B_{12} for *Line1* (*SH-SY5Y p*= 0.003), (Fig. 4A) and *ASCL2* methylation (*SH-SY5Y p*= 0.012, *DAOY p*<0.001), (Fig.4B and C), whereby 10μM levels of B_{12} appear to be protective against methylation changes in response to MTX treatment

Figure 4: Effects of an interaction between MTX treatment and B_{12} concentrations on A) *Line1* methylation in SH-SY5Y cells, B) *ASCL2* methylation in SH-SY5Y cells and C) *ASCL2* methylation in DAOY cells

Fig. 4A

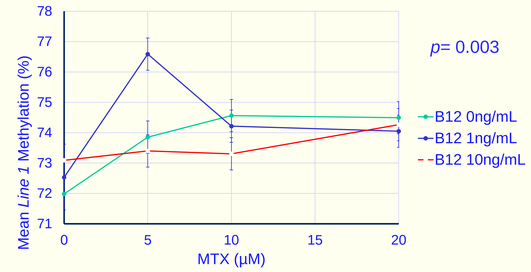


Fig. 4B

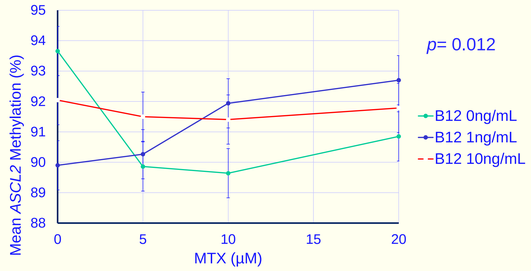
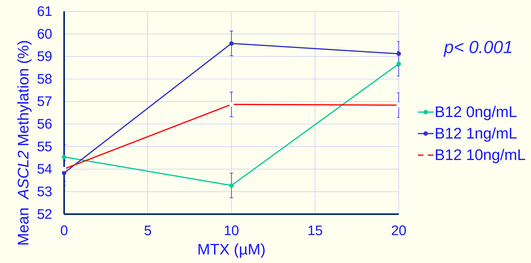


Fig. 4C



Summary

- Our data suggests altered methylation may be one mechanism mediating treatment-related neurotoxicity, and may also mediate other toxicities
- The potential protective effect of B_{12} against these changes may provide a plausible biomarker for toxicity prediction and/or targeted interventions against MTX related toxicity.
- Future replication studies and the investigation of whole genome-wide methylation will offer further insights.

Acknowledgements

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