THE EFFECT OF THE CHEMOTHERAPY AGENT METHOTREXATE ON THE DEVELOPING BRAIN

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Introduction. Acute lymphocytic leukemia (ALL) is the most common childhood cancer and is commonly treated with a cocktail of chemotherapy agents. This treatment has improved survival to 90%. However, up to 50 % of those that recover are left with side effects, including "late effects" which can impair cognitive ability. These "late effects" are accompanied by changes in brain structure volume.[1] There has been some indication that genetics play a role in determining which patients suffer from late effects.[2] In this study, we aimed to determine if treatment with MTX at an infant stage of development in mice has consequences for brain development, measured by volume of MRI images later in life.

Methods. C57Bl/6 mice were treated with 20 mg/kg of MTX (n=13) or saline (CTL, n=9) intraperitoneally on postnatal day (P) 17. In vivo imaging was performed at P14, P24 and P42 with Mn-enhanced MRI after treatment with 50 mg/kg $MnCl_2$ 24 hours prior to imaging. Images were collected with an excitation flip angle of 45 °, a TR of 0.05 s, four averages and k-space dimensions 168x168x280 with a resolution of 125 µm for a total experiment time of 1 hour and 43 minutes. At the final time point, mice were perfusion fixed with gadolinium contrast agent (Prohance) included in the perfusate. Ex vivo images were collected with a T_2-weighted fast spin-echo sequence with k-space dimensions 360x360x450, an echo train length of 6, echo spacing of 11.8 ms, with three averages, a resolution of 56 µm and total time of 14 hours and 24 minutes.

Images were registered together nonlinearly through a series of iterative steps to produce an unbiased average[3]. In the ex vivo data, volumetric changes were computed by registering a structural atlas with 159 structures to the unbiased average image. The volumes of each of the structures in the atlas were computed by summing over the structure volume, using the Jacobian determinant at each voxel to compute volumes from individual mice. The volume of each structure in the ex vivo data was fit with a linear model, including an intercept and a categorical treatment group (MTX or CTL). The model also included a normalizing volume term accounting for the pretreatment brain volume, which was obtained from the in vivo data.

Results. Of the 159 structures we tested, 25 (or 16%) showed significant volumetric differences ($p<0.05$, uncorrected). In Figure 1, the structures that achieved significance are colored based on their relative volume change. The majority (21/25) of the differences we observed represented volume decreases in the MTX-treated group relative to the control group, with magnitude on the order of 7%. Interestingly, several cortical regions are among those changed, even though emphasis in the clinical literature has been on changes in the white matter.

Conclusion. MRI of the mouse brain detects changes in development induced by early treatment with even low dose MTX given intraperitoneally. Most of these changes represent impaired growth, and gray matter regions may be particularly affected. Further adaptation of the treatment regimen to match that of the clinic, experiments in genetically-engineered mice and testing of additional chemotherapy agents will allow a complete mouse model of chemotherapy effects on the brain to be developed. In the long-term, this will allow adaptation of treatments to minimize cognitive late effects that affect survivors' quality of life.

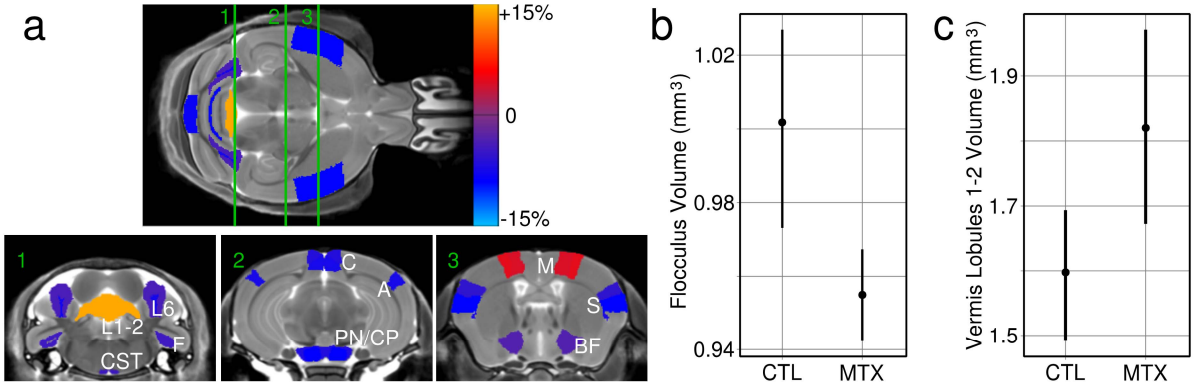

Figure 1: Structural volume differences in MTX-treated vs CTL mice. In (a), a map of significant structure differences ($p<0.05$, uncorrected) is shown with the color scale indicating percent volume change. Labeled structured include the basal forebrain (BF), somatosensory cortex (S), motor cortex (M), auditory cortex (A), cingulate cortex (C), pontine nucleus and cerebral peduncle (PN/CP), cerebellar lobule 6 (L6), lobules 1-2 (L1-2), corticospinal tract (CST) and flocculus (F). The flocculus and lobules 1-2 of the cerebellar vermis are plotted separately in (b) and (c) respectively with error bars showing 95% confidence intervals.

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