Multiple Sclerosis Research Center of New York

Intrathecal methotrexate (ITMTX) reduces astrogliosis in a non-inflammatory demyelination model

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Objective

We hypothesize that the benefits of intrathecal methotrexate on progressive MS is only partially related to its anti-inflammatory properties. Consequently, we investigated its influence on a non-inflammatory CNS demyelination model.

Background

MS patients with a progressive disease respond poorly to currently available anti-inflammatory disease modifying agents. Intrathecal administration of the antifolate drug methotrexate has a beneficial impact on the disease progression of severe primary and secondary progressive MS cases.
Astrocytic activation and subsequent astrogliosis occur in progressive MS as well as in the cuprizone-induced model of corpus callosum demyelination and are considered to be hallmarks of scar formation in the CNS.

Methods

To induce corpus callosum demyelination, male C57Bl/6 mice were fed with cuprizone mixed into ground chow. Methotrexate was administered intracerebroventricularly (icv) by osmotic pumps. Brain tissue sections were stained for astrocytic markers, myelin and microglial cells. Five random sections representing the corpus callosum were analysed per animal by a blinded investigator.

Results

Administration of ITMTX during cuprizone-induced demyelination: Mice were fed with cuprizone for four weeks. Simultaneously, the animals received ITMTX and control animals icv PBS. ITMTX significantly reduced the number of GFAP+ astrocytes (**Figure 1**) and decreased the cuprizone-induced demyelination of the corpus callosum (**Figure 2**). On the other side, the number of MAC3+ macrophages and/or microglial cells in the corpus callosum was not significantly affected by ITMTX (**Figure 3**).
Administration of ITMTX for two weeks after withdrawal of cuprizone: A four-week icv methotrexate administration after a cuprizone feeding period of 6 weeks did neither influence the number of astrocytes in the corpus callosum nor did it delay the remyelination or reduction of the presence of MAC3+ cells (**Figure 4**).

Conclusion

The pathophysiological basis of the cuprizone-induced demyelination model is not primarily related to inflammatory mechanisms. Similarly, the progressive forms of MS are less dependent on inflammatory events than its relapsing forms.
We were able to establish an inhibition of demyelination and astrogliosis by ITMTX in the corpus callosum of cuprizone fed mice. This corroborates the idea that the beneficial impact of ITMTX on disease progression is not solely mediated by its anti-inflammatory properties. Moreover, the inhibition of astroglial activation suggests that ITMTX may influence the generation of astrocytic scars in MS lesions.

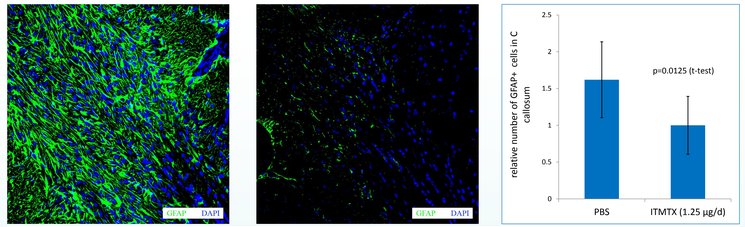

Figure 1) ITMTX reduces astrogliosis. Left: GFAP staining of a selected brain slice of a control mouse. Middle: GFAP staining of a brain slice of a ITMTX treated mouse. Right: Quantitative analysis of the number of astrocytes in the corpus callosum (n=7 per group)

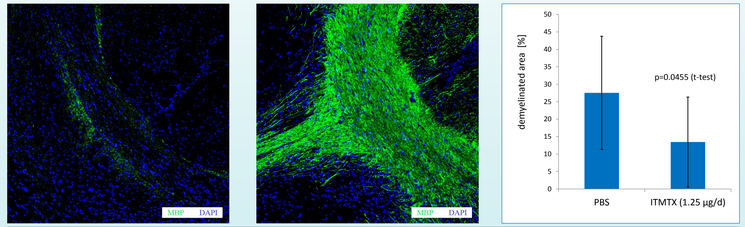

Figure 2) ITMTX reduces cuprizone-induced demyelination. Left: MBP staining of a brain slice of a selected control mouse. Middle: MBP staining of a brain slice of a ITMTX treated mouse. Right: Quantitative analysis of the demyelinated area

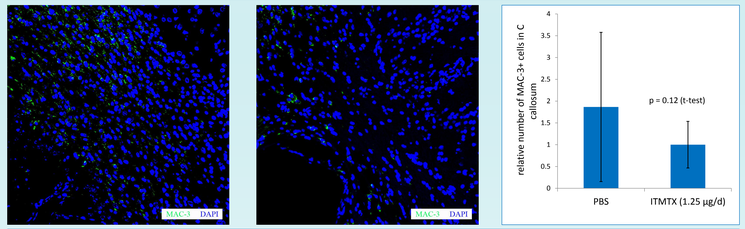

Figure 3) ITMTX does not influence the infiltration of MAC3+ cells into the corpus callosum. Left: MAC-3 staining of a brain slice of a selected control mouse. Middle: MAC-3 staining of a brain slice of a ITMTX treated mouse. Right: Quantitative analysis of the number of MAC3+ macrophages or microglial cells in the corpus callosum.

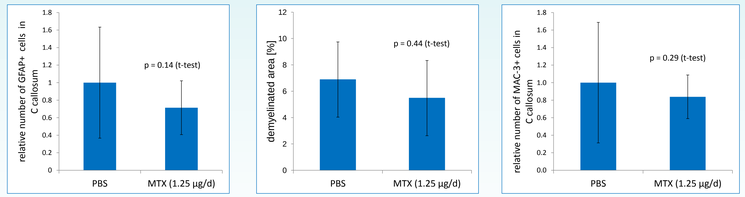

Figure 4) ITMTX administered after cuprizone withdrawal does not inhibit remyelination. Left: Quantitative analysis of GFAP+ cells in the corpus callosum. Middle: Quantitative analysis of the demyelinated area. Right: Quantitative analysis of the number of MAC3+ macrophages or microglial cells in the corpus callosum.

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