BBB Disruption and Permeability of Brain Tissues in MBP- Induced Experimental Autoimmune Encephalomyelitis (EAE) Model in Rats

MD Biosciences Innovalora Ltd. Israel

Isaac Levi; Lena Finkelstein; Ayelet Weksler; Avital Schauder; Talia Waxenbaum; Eva Leder; Dimitry Kovalchuk; Ayelet Dital; David Castel and Sigal Meilin

Abstract

Experimental Autoimmune Encephalomyelitis (EAE) is commonly used as a model for multiple sclerosis (MS) and as such has been a powerful tool for studying disease pathogenesis as well as potential therapeutic interventions.

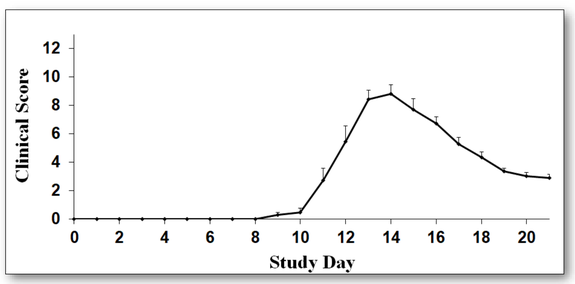
The Myelin Basic Protein (MBP) induced EAE in rat consists of inflammatory cells infiltration into the spinal cord, cerebellum and brainstem. The paralytic episodes that in this model are thought to be the result of blood–brain barrier breakdown, inflammation, and edema, but not from demyelination. This paralysis initiates approximately 10 days post induction, followed by spontaneously recovery in 5–7 days. Therefore, the therapeutic window is very short.

Our data show that while the first clinical signs of the disease were seen on study day 9 following induction with MBP, an increase in the Evans blue dye in the brain was observed 2 days following induction (17.80±3.29 µg/g). The dye content in the brain tissue remained high until study day 9 (18.40±2.16 µg/g). Its level markedly decreased on study day 11 and was similar to the level in the naïve brain.

Granulocytes, T lymphocytes and activated microglia cells were found mainly in the striatum, when clinical symptoms were started to developed.

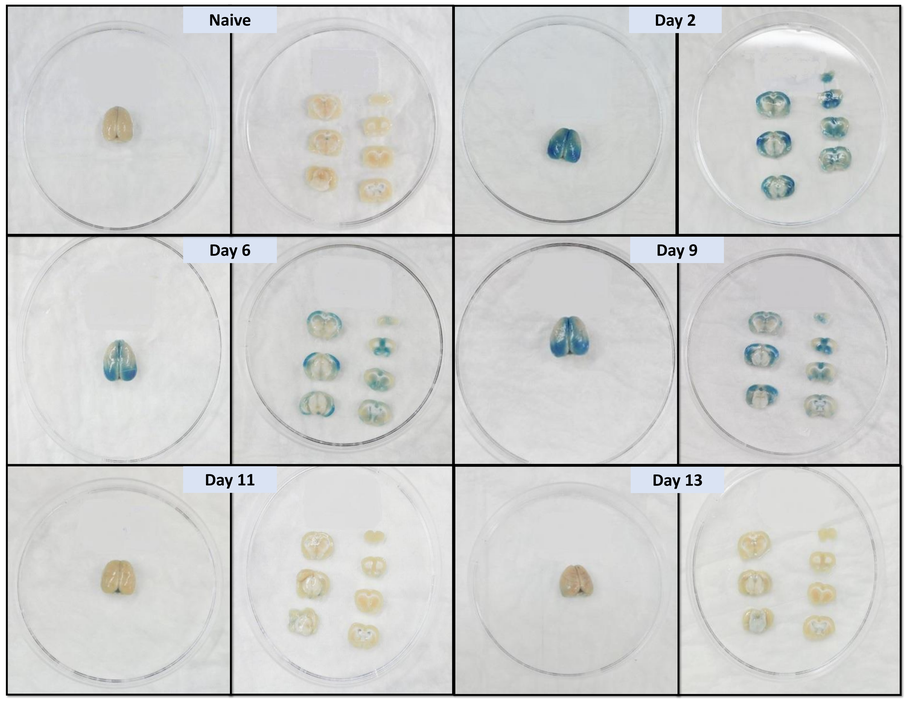
These data show dissociation between BBB permeability and the peak of the disease questioning the therapeutic vs. prophylactic traditional approach.

Results



Signs / Symptoms		Grade
Tail	No Signs	0
	Half paralyzed tail	1
	Fully paralyzed tail	2
For each of the hind or forelimbs	No Signs	0
	Weak or altered gait	1
	Paresis	2
	Fully paralyzed limb	3
Mortality	---	15

Figure 1: Clinical score in MBP-induced EAE rat. Female Lewis rats were subjected to a single inoculum injection on Study Day 0. The inoculum injection consisted of a homogenate emulsive mixture of MBP and CFA. Clinical symptoms initiate approximately 9-10 days post induction, followed by spontaneously recovery in 5–7 days.



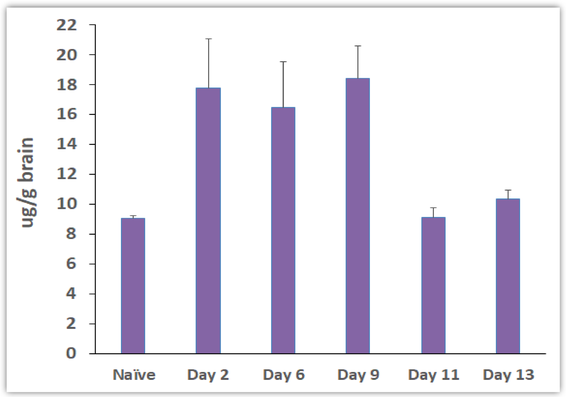


Figure 2: Evans Blue Brain penetration. Animals were taken down at different study days for the evaluation of Evans blue brain penetration. 4% Evans blue dye was injected to the jugular vein, 2 hours prior to termination, and the brain was collected and weighed. Coronal sections were performed using brain matrix for the detection of Evans blue dye in the brain. Then, the brain was immersed in Formamide at 55℃ overnight to extract the dye. Extracted dye was quantified by optical absorbance at 610 nm, and the amount of dye was calculated relative to the weight of the tissue.

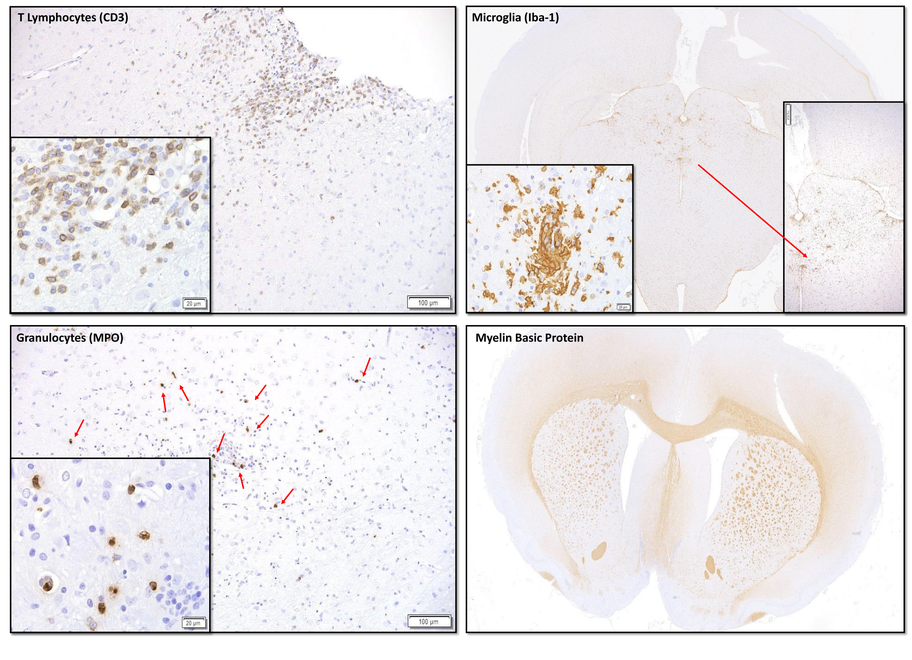


Figure 3: IHC staining of Brain tissue on day of clinical symptoms manifestation. Brains were harvested and fixed in formalin 10%. Following fixation , each brain was grossly cut to 3mm thick coronal slices using a dedicated matrix. The tissues were processed, embedded in paraffin and sectioned for IHC.

Immunohistochemical (IHC) staining was performed using Abs for CD3 (T-lymphocytes), Iba-1 (microglia), Myeloperoxidase (MPO, granulocytes) and Myelin basic protein (MBP), followed by HRP-conjugated secondary Ab.

When clinical symptoms were observed, inflammatory cells (granulocytes, T lymphocytes) and activated microglia cells were found in the tissue, mainly in the striatum.

Staining for myelin basic protein (MBP) showed that there was no demyelination of the tissue - as expected in this model.

References:

1. Andrew P. Robinson, Christopher T. Harp, Avertano Noronha, and Stephen D. Miller (2014) The experimental autoimmune encephalomyelitis (EAE) model of MS: utility for understanding disease pathophysiology and treatment. Handb Clin Neurol 122: 173–189.
2. Alan M. Palmer (2013) Multiple Sclerosis and the Blood-Central Nervous System Barrier. Cardiovascular Psychiatry and Neurology Volume 2013.
3. Mariella Errede et al (2012) Blood-Brain Barrier Alterations in the Cerebral Cortex in Experimental Autoimmune Encephalomyelitis. J Neuropathol Exp Neurol 10: 840- 854.
4. Marzena J. Fabis, Gwen S. Scott, Rhonda B. Kean, Hilary Koprowski, and D. Craig Hooper (2007) Loss of blood–brain barrier integrity in the spinal cord is common to experimental allergic encephalomyelitis in knockout mouse models. PNAS 104 (13) 5656-5661.
5. Tonra JR1, Reiseter BS, Kolbeck R, Nagashima K, Robertson R, Keyt B, Lindsay RM (2001) Comparison of the timing of acute blood-brain barrier breakdown to rabbit immunoglobulin G in the cerebellum and spinal cord of mice with experimental autoimmune encephalomyelitis. The journal of comparative neurology 430:131–14.

Conclusions

Experimental autoimmune encephalomyelitis (EAE) in Lewis rats is an acute monophasic paralytic central nervous system disease, in which most rats spontaneously recover from paralysis. Blood-Brain Barrier (BBB) disruption plays an essential role in the pathogenesis of this disease, leading to infiltration of inflammatory cells to the central nervous system.

This study shows that the disruption of the BBB is limited to approximately 7 days and precedes the initiation of clinical symptoms, caused by the infiltration of inflammatory cells. **The limited phase in which the BBB is permeable questioning the relevance of the traditional approach.**

mdbioscences.