

Lenaldekar Prevented Relapses in Experimental Autoimmune Encephalomyelitis Mice: A Diffusion Basis Spectrum Imaging Study

Xiaojie Wang¹, Matthew Cusick², Robert S Fujinami², and Sheng-Kwei Song^{3,4}

¹Chemistry, Washington University, St. Louis, MO, United States, ²Pathology, University of Utah, Salt Lake City, UT, United States, ³Radiology, Washington University, St. Louis, MO, United States, ⁴Hope Center for Neurological Disorders, Washington University, St. Louis, MO, United States

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease with axonal injury causing permanent neurologic disability. Infiltrating T cells and macrophages are found in active MS lesions and correlate with the extent of demyelination.¹ Lenaldekar (LDK), 1H-indole-3-carbaldehyde quinolin-8-yl-hydrazone, is a robust inhibitor of T cell expansion effectively preventing relapses in experimental autoimmune encephalomyelitis (EAE)-affected SJL/J (SJL-EAE) mice.² Diffusion basis spectrum imaging (DBSI) proved by both phantom and in vivo animal studies to be able to distinguish and quantify complex pathologies (axon and myelin injury, cell infiltration and vasogenic edema) associated with mouse EAE.³ In this work, SJL-EAE mice were treated with LDK or vehicle at the onset of first relapse mimicking the therapeutic intervention of MS patients. At the end of the study, mice were perfusion fixed and ex-vivo 99-direction DBSI was applied to evaluate LDK's efficacy in improving SJL-EAE mice spinal cord white matter integrity.

Methods

Animal preparation: EAE was induced in SJL/J mice via injection of PLP₁₃₉₋₁₅₁ peptide emulsified in complete Freund's adjuvant (CFA) with *Mycobacterium tuberculosis*. Control mice received CFA and *Mycobacterium tuberculosis* in the absence of PLP. Mice were also injected with *Bordetella pertussis* on day 0 and day 2. Daily treatment was initiated at the onset of EAE-relapse by injection of 40 mg/kg LDK, or vehicle (DMSO), throughout the study duration. Mice were evaluated daily for neurological deficits using the standard EAE clinical score (CS) scales. On the 37th day post immunization (dpi), mice were subjected to intra-cardiac perfusion fixation using 0.01M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1M PBS. Mouse vertebral columns were excised, post-fixed overnight. **MRI:** Fixed mouse cords underwent ex vivo DBSI examination on a 4.7 T scanner. A solenoid coil was used as both transmit and receive coil. Images of 4 contiguous transverse slices covering T13 through L1 vertebrae were acquired using the following parameters: TR 1.0 sec, TE 38 ms, Δ 20 ms, δ 5 ms, slice thickness 1.0 mm, data matrix 128x128 (zero filled to 256x256), field of view = 1 cm², total data acquisition time ~ 3.0 hr, diffusion gradient were applied along 99 directions on a 3D grid with maximum b value of 3000 s/mm².

Results

The LDK-treated EAE mice demonstrated a remarkably diminished relapse severity indicated by returning clinical scores to 0 at 30 dpi while the vehicle-treated mice were at the peak of the relapse (Fig. 1). The effect of LDK treatment is readily seen by examining the color maps of $\lambda_{||}$, λ_{\perp} , cell ratio and edema water ratio of the representative cords at L1 (vertebral) level, from control, LDK-treated and vehicle-treated SJL-EAE mice (Fig.2). Without LDK treatment, EAE mice exhibited significantly decreased $\lambda_{||}$ and increased λ_{\perp} (Fig. 2c) reflecting extensive axon and myelin injury. The presence of inflammation was reflected by DBSI determined increases in cell ratio and edema water ratio (Fig. 2c). LDK treatment effectively reduced inflammation (reduced cell ratio and edema water) in SJL-EAE mice, in addition to preserving axonal integrity (Fig. 2b). Shown from the group-averaged data, all the DBSI-derived metrics were improved towards those of normal control. No difference was seen between control and LDK-treated group in $\lambda_{||}$ (Fig.3a) suggesting axon was well preserved in LDK-treated mice. LDK treatment improved λ_{\perp} of the SJL-EAE mice by 27%. However, the λ_{\perp} of LDK-treated SJL-EAE was still 20% higher than λ_{\perp} of the control (Fig. 3b), probably reflecting the incomplete remyelination after the initial attack. LDK reduced the extent of edema by 28%, comparing to that of vehicle-treated SJL-EAE mice, although it was still 30% higher than that of the control (Fig. 3c). The cell ratio of the LDK-treated group is 7% lower than that of vehicle-treated SJL-EAE mice, comparable to that of control group, indicating effective anti-inflammatory effect of LDK.

Conclusion

LDK was able to suppress the disease activity when treated SJL-EAE mice at the onset of relapse through the anti-inflammatory (evidenced by reduced cell ratio and edema), axonal preservation (normalized $\lambda_{||}$), and remyelination (improved λ_{\perp}) actions. Thus, it is a potent drug for treating EAE and could potentially be applied to treat MS. Meanwhile, DBSI-derived metrics could serve as sensitive biomarkers for assessing treatment efficacy of novel drugs.

References

1. Frischer, JM et al., Brain 132: 1175-1189 (2009). 2. Cusick, MF et al., J Neuroimmunology 244: 35-44 (2012). 3. Wang, Y et al, Brain, 134: 3590-3601 (2011).

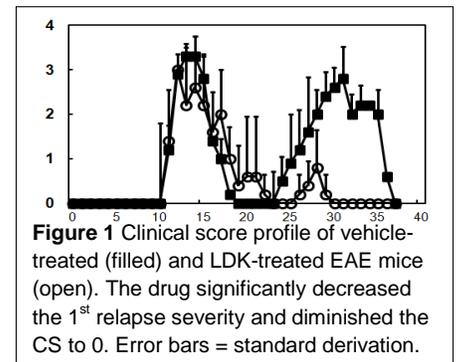


Figure 1 Clinical score profile of vehicle-treated (filled) and LDK-treated EAE mice (open). The drug significantly decreased the 1st relapse severity and diminished the CS to 0. Error bars = standard derivation.

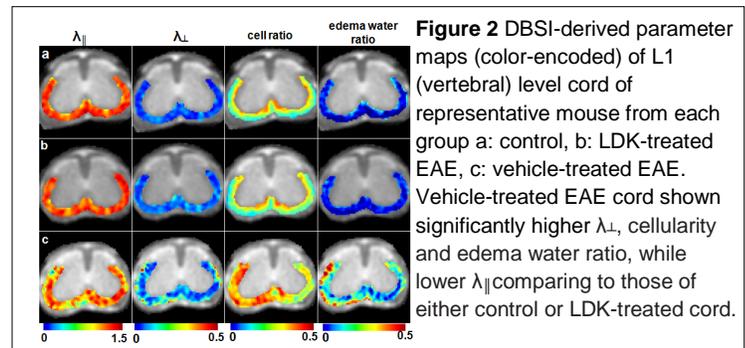


Figure 2 DBSI-derived parameter maps (color-encoded) of L1 (vertebral) level cord of representative mouse from each group a: control, b: LDK-treated EAE, c: vehicle-treated EAE. Vehicle-treated EAE cord shown significantly higher λ_{\perp} , cellularity and edema water ratio, while lower $\lambda_{||}$ comparing to those of either control or LDK-treated cord.

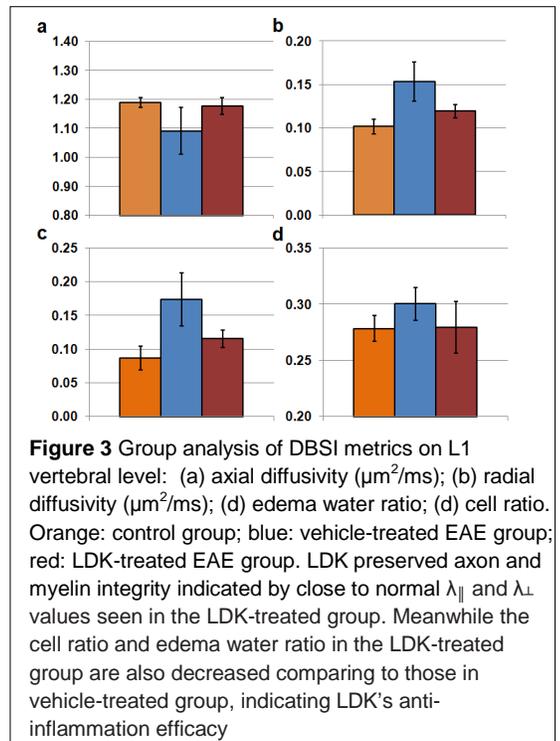


Figure 3 Group analysis of DBSI metrics on L1 vertebral level: (a) axial diffusivity ($\mu\text{m}^2/\text{ms}$); (b) radial diffusivity ($\mu\text{m}^2/\text{ms}$); (c) edema water ratio; (d) cell ratio. Orange: control group; blue: vehicle-treated EAE group; red: LDK-treated EAE group. LDK preserved axon and myelin integrity indicated by close to normal $\lambda_{||}$ and λ_{\perp} values seen in the LDK-treated group. Meanwhile the cell ratio and edema water ratio in the LDK-treated group are also decreased comparing to those in vehicle-treated group, indicating LDK's anti-inflammation efficacy