Introduction

Lyme Neuroborreliosis (LNB) • LNB is a chronic infectious disease of the central nervous system (CNS) caused by a tick-borne spirochete, Borrelia burgdorferi, the same spirochete which causes the more commonly known condition Lyme disease. • LNB is a complex disease including a number of different clinical and pathological manifestations, such as meningitis, polyarthritis, cranial neuritis, and encephalomyelitis, which are all severe complica tions of Lyme disease (Kuenzle 2007).

Hypothesis

Astrocytes pretreated with anti-inflammatory agents will have a decreased expression of TLR4 when induced with LPS-PG.

Methods

Cell Culture • The mouse (Mus musculus) astrocytes strain C6STILE (ATCC) from the cerebellum type I phenotype were grown in 75 cm² flask at 37°C in 5% CO₂. • The cells were grown with Dulbecco’s Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-streptomycin, and the media was changed every two days.

Treatments of Cells • The cells were pretreated for 24 hours with the anti-inflammatory agents of garlic at 100 mg/L (Keiss et al. 2003), bromelain at 1 mg/ml (Onken et al. 2008), grape seed extract (GSE) at 0.5 mg/mL (Li et al. 2001), ginger at 1.5 mg/mL (Bratton et al. 2008), dissolved in phosphate buffer solution.

Protein Expression

Protein extracted with RIPA lysis buffer containing Sigma protease inhibitors (Pierce). • The supernatant was collected from each treatment and stored at -20°C.

Western Blot Analysis

The total protein from each treatment was quantitated using the Bradford protein assay (BioRad). • The gel was stained with Coomassie blue, and the bands were visualized using a BioRad Gel Doc and uploaded onto the computer.

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Statistical Analysis

A 1-way ANOVA test was used to analyze the average protein concentrations of the 26 treatments.

Results

Garlic + cef (+) 40.0
GSE + cef (+) 50.0
Control 2 (+) 30.0
Garlic (+) 62.5
Garlic (Bands for bromelain did not show even though protein was loaded.)

Discussion

Summary of Results • The LSP-PG had no significant effect on the concentration of protein total cell lysate of the astrocytes. • The pretreatments had no significant effect on total protein levels.

Significance • Since the western blots for the last two trials did not show TL4 expression, significance of the pretreatment cannot be determined until the Western blot procedure is optimized. • Bradford assay showed no significant difference in the protein concentration of the astrocytes from the different treatments, indicating no change in total cell mass. Cell survival assay would be needed to confirm this result.

References

Iwrovich, WestemBioTm – 625 Western Blot Kits, W10132 and W10142. Invitrogen Corporation.