INTRODUCTION

Neuroinflammation is a severe and debilitating aspect of several diseases, including sepsis, Alzheimer’s disease, encephalopathy (HE). 70% of human sepsis survivors report cognitive impairment (CI) at their hospital discharge, and 45% report CI at one year post-discharge. There are few statistics on sepsis in veterinary species, but it is a significant clinical consideration and often results in death. AD is a severe neurodegenerative disease with no cure. HE is a confounding syndrome associated with portosystemic shunts (PSS), a congenital condition of dogs that is often lethal. Previous studies in a rat model have shown that long-term neuroinflammation results in altered blood-brain barrier (BBB) permeability, long-term decreased perfusion, and increased free radical production. The goal of this study was to evaluate the effectiveness of OKN-007, a neuroprotective, anti-inflammatory compound that is capable of crossing the BBB. Injection of lipopolysaccharide (LPS) into rats was used as a model for evaluating long-term neuroinflammation. Stimulus-evoked functional magnetic resonance imaging (fMRI) was used to assess oxygen consumption in cortical brain regions of saline treated, LPS treated, and LPS and OKN-007 treated rats during a normal oxygenated state and a hypercapnic state. fMRI distinguishes between the paramagnetic oxyhemoglobin and deoxyhemoglobin. Deoxyhemoglobin is converted from oxyhemoglobin when there are cells actively taking up oxygen in that area, and is thus a marker for cellular activity. Oxyhemoglobin has a brighter signal intensity than deoxyhemoglobin, and changes in concentration of both can be detected in real time with fMRI. In addition to fMRI, perfusion was also used to analyze the blood flow rate throughout the cortex region. Increased perfusion rate indicates vasodilation while decreased perfusion indicates vasoconstriction. Prior research has indicated that there is acute increased perfusion followed by long-term decreased cerebral perfusion associated with neuroinflammation.

MATERIALS AND METHODS

Male Sprague-Dawley rats (8-10 weeks old) were treated with either LPS (10 mg/kg; IP; n=4), saline (0.9%; IP; n=5), or LPS (10 mg/kg; IP) and OKN-007 (18 mg/kg/day; drinking water, n=5), and were assessed using fMRI at 3 weeks following LPS administration. MRI experiments were done on a 7.0 Tesla, 30 cm horizontal-bore Bruker Biospin Biospec MRI. Rats were anesthetized at the 3 week time point using isoflurane in oxygen and then restrained in the MRI cradle. First, a T1 and perfusion scan were done. Then a T2 scan was done for morphology, followed by an EPI and a T1* scan done at 20 repetitions each lasting 16 minutes for the normal baseline state. Oxygen was then switched to 10% CO2, and an additional EPI and T1* scan at 20 repetitions each lasting 16 minutes were done to evaluate the presence of oxyhemoglobin during hypercapnia. Images generated by the T1* scans were then added together to generate one pre- and one post-hypercapnia image for each subject. Selective ROIs were placed in the rostral, mid, and caudal cortex regions bilaterally, then a percent difference and standard deviation for each region was calculated. Difference images between the pre- and post-hypercapnia scans were also generated to further evaluate morphological changes relating to the hypercapnia.

RESULTS

Long-term oxygen use changes in the brain were observed as a significantly more negative percent change in the mid (p=0.0015) and caudal (p=0.0150) cortex regions of the brain for LPS rats compared to LPS & OKN-007 rats. This indicates that the more positive (close to zero) the difference, the more normal the brain tissue in that region. Additionally, there was no significant change between saline and LPS & OKN-007 rats in the mid (p=0.7779) or caudal (p=0.4088) cortex regions, indicating that rats treated with OKN-007 had comparable oxygen usage to the control animals in those brain regions. No significant changes found between any of the three cohorts for the rostral cortex region. For perfusion, there was a significant decrease in perfusion for the LPS cohort compared to the saline (p=0.002) and the LPS & OKN-007 (p=0.0024) groups.

REFERENCES