An Acute Inflammatory Response in a Diabetic Alzheimer’s Disease Model

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INTRODUCTION

Alzheimer’s disease (AD) is the most common form of dementia, accounting for 50 to 80 percent of all dementia cases. This neurodegenerative disease leads to neuronal death and tissue loss in the brain, resulting in the slow deteroration of memory, thinking, and eventually even the ability to perform daily tasks. While it is not a normal part of aging, AD is mostly diagnosed in people over the age of 65; thus, the main risk factor for Alzheimer’s disease is increasing age, though it is likely other additional factors also contribute (Heesie & Akatsu, 2008). Neuropathological hallmarks of AD include neurofibrillary tangles (NFTs) formed by the aggregation of hyperphosphorylated tau protein, and amyloid plaques formed by the accumulation of β-amyloid (AP) proteins (Cvetkovic-Dzic et al., 2001). To date, the etiology of AD remains unknown; but several models investigating multiple pathways have been used to better understand the disease. In this experiment, two specific AD risk factors, Type 2 diabetes mellitus (DM) and inflammation of the brain, are of concern. Recent evidence has indicated a connection between Alzheimer’s disease and Type 2 DM. The use of the DM model to investigate AD represents a useful tool to examine AD pathologies. In Type 2 DM, cells fail to use insulin properly, resulting in insulin resistance. This resistance consequently affects the metabolism of tau and Ap proteins, potentially leading to formation or acceleration of NFTs and amyloid plaques characteristic seen in AD (Gasparrini et al., 2002).

Chronic brain inflammation, commonly referred to as neuroinflammation, induces a neurotoxic effect by increasing Ap proteins, nerve plaques, and neuronal damage, leading to exacerbated symptoms and a more rapid disease progression. Alternatively, acute inflammatory responses have been suggested to serve a protective role in degenerative disorders (Frank-Cannon et al., 2009). A critical question in AD research is what facets of chronic neuroinflammation exacerbate pathological features, as well as if an acute inflammatory response may play a neuroprotective role in reducing the aggregation of Ap, and thus lowering the likelihood of cell loss. The purpose of this study was to investigate the role that brain inflammation plays in a diabetic model of AD. To examine this, we injected an immune response induced by (lypopoicysarlis, (LPS)) would alter AD-related behavioral or pathological features using the streptozotocin (STZ) diabetic model of AD.

EXPERIMENTAL PROCEDURE

ANIMALS

Forty-four male Sprague-Dawley (~9 weeks old) were used throughout the studies. The animals were single-housed in a standard animal facility with a 12–12 hours light-dark cycle, with food and water available ad libitum. All procedures were performed during the light phase and in accordance with the University of Nevada, Las Vegas Animal Care and Use Committee and NHL guidelines for ethical treatment of research subjects.

DRUG ADMINISTRATION

Rats were randomly distributed into four groups. To induce DM, streptozotocin surgeries were performed under aseptic conditions and ketamine/xylazine anesthesia (Kimmy et al., 2003). Sabath et al. (2001) in two of the groups, bilateral injections of streptozotocin (STZ, 25 mg/ml; 8 ml) were slowly administered on each site using sterile syringes: 0.7 mm posterior, 1.4 mm lateral to Blbma, and 3.5 mm ventral to the surface of the skull (Paxinos & Watson, 2009). The remaining two groups were infused with artificial cerebrospinal fluid (ACSF) to serve as a control. A week after the surgery, a single intraperitoneal injection of LPS (1.0 mg/kg) was administered, resulting in the four groups: ACSF/Saline, STZ/Saline, ACSF/LPS, and STZ/LPS.

TEMPERATURE AND WEIGHT RECORD

To ensure LPS induced an immune response, temperatures and weights were recorded after LPS injections. Rectal temperatures were monitored for the first 72 hours and then one week later. Body weights were recorded throughout the course of the experiment.

MORRIS WATER MAZE

Spatial learning and memory was tested using the Morris Water Maze spatial task two weeks after LPS or saline injection. The water maze was conducted using a white, circular polythene tube (1.5 m in diameter, 76 cm in height, 4.77 mm thick) filled with tap water and slightly tinted pink, and divided into four quadrants for analysis. Four different large, colorful shapes and posters on the walls were used as distal spatial cues. Path length, swim speed, perimeter time (hippocampus), latency, and quadrant location were recorded.

In the experimental trials, rats were allowed to swim for 60 seconds to find the hidden platform (a 10 cm x 10 cm square clear plastic located 1.0 cm below the water’s surface) located in one of the four quadrants. If a rat was not able to locate the platform after 60 seconds, they were guided to the platform. Once the platform the animal was given 20 seconds to orientate and tamamize itself to the spatial cues. The trial was repeated in identical fashion three more times, with a 30 second interval between trials. Four consecutive trials were performed each day for each animal. A tracking system was used to record all trials. After five days of training, a probe trial was done where the hidden platform was taken out and animals were allowed to search the maze for 60 seconds. The tracking system recorded the amount of time subjects spent in all four quadrants, as well as the time the animals path crossed over the former platform location versus analogous locations in other quadrants. Visible platform training was then conducted wherein the platform was raised above water level and ensure motoric and visual function was equivalent between groups.

TISSUE COLLECTION

Rats were individually euthanized via CO2 asphyxiation, decapitated, and hippocampi and cortices were immediately dissected out and frozen. Hippocampi and cortices were homogenized using RIPA buffer (Bolton et al., 2012). Homogenization was performed using a handheld Polytron tissue homogenizer (Kinematica Inc.). Lysates were centrifuged at 14,000 g for 15 minutes at 4°C. The supernatant was then collected and protein concentrations were determined using the bicinchoninic acid assay (BCA; Pierce). Samples were frozen and stored at -80°C. Tissue was collected four weeks after LPS and saline injections.

SDS PAGE/WESTERN BLOT

Samples (20 µg) from the hippocampus tissue were separated on 10% SDS-PAGE gels. Proteins were electro-transferred to nitrocellulose membranes and blocked for 2 hours. Membranes were then incubated overnight in primary antibody (Aβos/Actin: 1:1,000, Cell Signaling; rabbit anti-pTau Ser404: 1:1,500; Cell Signaling; mouse anti-NMDAR2B, 1:1,000; Cell Signaling; rabbit anti-GluR, 1:1,000, Cell Signaling; rabbit anti-NMDAR2B, 1:1,000, Cell Signaling; rabbit anti-GluR, 1:1,000, Cell Signaling; at 4°C). Following incubation, membranes were washed three times with phosphate buffer saline and TWEEN (PBT) for 5 minutes each, and then placed in secondary antibody (IR dye goat anti-rabbit 680, 1:10,000, Bio-Rad, IR dye goat anti-mouse 800, 1:10,000, Bio-Rad) at 4°C for 1 hour, and then washed again in PBT. In three trials for 5 minutes each and a final 10 minute PBS wash prior to imaging. Membranes were imaged using Odyssey (LI-COR Infrared Imaging System [LI-COR]). Intensity was scanned and normalized using the ImageJ software. Western blot analysis was performed on the hippocampus using antibodies against Ap, pTau, Ap, NMDAR subunits, and NMDAR. Western blot images were analyzed using ImageJ software. The intensities were measured in gray level units for each band and normalized to actin (Figure 3E, F).

RESULTS

1. The body weights of the animals injected with LPS were lower than the control animals (Figure 1A) and the rectal temperatures (Figure 1B) were significantly increased the first 2 days following the days of injection, suggesting that an immune response was indeed induced and the change in body weight and temperature was not due to any other factor.

2. The analysis of the MMPI indicated that the STZ/Saline group exhibited learning deficits consistent with AD (significant difference versus ACSF/Saline), whereas the STZ/LPS did not exhibit these same deficits. STZ/Saline animals had significantly longer path lengths during hidden training than all other groups (Figure 2A). During the probe trial, all groups spent significantly more time in the target quadrants versus the other three quadrants (selective search) except for STZ/Saline animals (Figure 2B).

3. SDS PAGE/Western Blot

AP, pTaurine404

4. Figure 3 Western blot analysis of AP oligomers in hippocampus indicated that there was a significant increase in total AP oligomers as well as the larger oligomers in the STZ/Saline group (p < 0.05, Figure 3A). However, AP oligomers were elevated in both the STZ/Saline and STZ/LPS groups (Figure 3C, D). The STZ/LPS group did exhibit a significant increase in total oligomers versus the control group but significantly lower than the STZ/Saline group (p < 0.05, Figure 3A). Severe AP oligomers were elevated in both the STZ/Saline and STZ/LPS groups (Figure 3C, D), whereas Alpha AP oligomers were only significantly increased in the STZ/Saline group alone (Figure 3B). Representative images can be seen in Figure 3F.

5. Figure 5 Western blot analysis of NMDA receptor subunits. NMRA NR2B showed a significant decrease in the STZ/Saline group when compared to the control group, ACSF/Saline (p < 0.05, Figure 5A). Western blot analysis of NMDA NR2B (Figure 5A) showed no significant differences between groups. Representative images of NMRA NR2A and NMRA NR2B can be seen in Figure 4D, respectively.

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REFERENCES

Murtishaw, A. B., Bozak, K. M., & Wall, M., & Rozance, S. (2009). The role that acute brain inflammation plays in a diabetic model of AD. In order to examine this, we investigated if an acute exacerbation of pathological features, as well as if an acute inflammatory response may play a protective role in reducing the aggregation of Ap, and thus lowering the likelihood of cell loss. The purpose of this study was to investigate the role that brain inflammation plays in a diabetic model of AD. To examine this, we injected an immune response induced by (lypopoicysarlis, (LPS)) would alter AD-related behavioral or pathological features using the streptozotocin (STZ) diabetic model of AD.