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

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Abbreviations

AD - Alzheimer's disease
Aβ - Amyloid beta
AβOS - Oligomeric amyloid beta
AβAggs - Amyloid plaques
ACSF - Artificial cerebrospinal fluid
AβPP - Amyloid precursor protein
ACTD - Amyloid C-terminal domain
APT - Amyloid precursor protein
CSF - Cerebrospinal fluid
DPP-IV - Dipeptidyl peptidase IV
DRUG ADMINISTRATION

Fourty ANIMALS

ANIMALS

Fifty-four male Sprague-Dawley (~8 weeks old) were used throughout the studies. The animals were single-housed in a standard animal facility with a 12–12 hr 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 NIH guidelines for ethical treatment of research subjects.

DRUG ADMINISTRATION

Rats were randomly distributed into four groups. To induce DM, streptozotocin (STZ) was injected intraperitoneally at a dose of 65 mg/kg body weight (bw). Rats were then injected with streptozotocin two times (1.0 mg/kg bw each injection) and were 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 recorded for the first 72 hours and then once a 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 learning task two weeks after LPS or saline injection. The water maze was conducted using a white, circular polyethylene tank (1.5 m in diameter, 76 cm in height, 4.77 mm thick) filled with tap water to a depth of 30 cm and maintained at 27°C. A circular platform (10 cm in diameter) was submerged at a depth of 1 cm below the water surface. A white, non-reflective platform, and water divided into four quadrants for analysis. Four different large, colorful shapes and posters on the walls were used as visual spatial cues. Path length, swim speed, perimeter time (frigorometric), 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 10 cm below the water’s surface) located in one of four quadrants. If an animal located the platform after 60 seconds, they were guided to the platform. Once the platform was found 20 seconds to orient and lambaric 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 number of times the animal 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 to ensure motoric and visual function was equivalent between groups.

TISSUE COLLECTION

Rats were euthanized via CO2 anesthesia (Kinney et al., 2003; Sabagh et al., 2009). In two of the groups, bilateral injections of streptozotocin (STZ, 25 mg/kg, 8 ml/rat) was slowly administered on each side using stereotaxic coordinates: 0.7 mm posterior, 1.4 mm lateral to bregma, and 3.5 mm ventral to surface of the skull (Panoson & 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 either LPS (1.0 mg/kg bw) or saline (1.0 ml/kg bw of body weight) were then administered, resulting in the four groups: ACSF/Saline, STZ/Saline, ACSF/LPS, and STZ/LPS.

SDS PAGE/WESTERN BLOT

Samples (20 μg) from the hippocampal 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 (rabbit anti-Tau, 1:10,000 Cell Signaling; rabbit-anti-p-tau Ser202/Thr205, 1:5,000 Cell Signaling; mouse anti-Actin 1:10,000, Cell Signaling; rabbit anti-STEP, 1:1000, Cell Signaling; rabbit anti-NMDAR2B, 1:1000, Cell Signaling; rabbit anti-Giur, 1:1000, Cell Signaling; rabbit anti-IgG, 1:1000, Cell Signaling). Following incubation, membranes were washed three times with phosphate buffer saline and TWEEN (PBS)- for 5 minutes each, and then placed in secondary antibody (IR goat anti-rabbit, 1:500, Bio-Rad). The membranes were washed again in PBSH three times for each 5 minutes each and a final 10 minutes PBS wash prior to imaging. Membranes were imaged using Odyssey Q4 Infrared Imaging System (LI-COR) and intensity was examined by changing the wavelength intensifiers for the protein of interest and β-Actin for a comparison between groups.

RESULTS

LPS Effects

Figure 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.

Figure 2 The analysis of the NMM indicated that the STZ/Saline group exhibited learning deficits consistent with AD (higher proportion to Control) as compared to ACSF/Saline (the STZ/LPS group did not exhibit these same deficits) (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).

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

CONCLUSIONS

- STZ impaired learning and memory, consistent with AD.
- LPS rescued STZ-induced learning deficit.
- STZ increased oligomeric Aβ, like AD.
- Total Aβ oligomers were increased in STZ/LPS group compared to STZ group alone, indicating LPS may have reduced the Aβ oligomers.
- p-domain was significantly increased in the STZ/Saline and STZ/LPS groups.
- A significant deficit was observed in the absence of the NMDA receptor subunits NMDA in the STZ/Saline group, but not in STZ/LPS group. No significant differences were observed for the NR2B subunits.
- The changes in hippocampal subunits support the immune response may have rescued the reduction in NR2A, and may be related to the lack of impairment in spatial learning.

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REFERENCES


