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SELECTIVE INHIBITION OF HDAC6 MODULATES MICROGLIAL POLARIZATION AND ATTENUATES NEUROINFLAMMATION IN AN LPS-INDUCED NEURODEGENERATIVE MOUSE MODEL

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ABSTRACT

Neuroinflammation, which is primarily brought on by the ongoing overactivation of microglia, is a key feature in the pathogenesis of many neurodegenerative diseases. Due to their functional plasticity, these immune cells that dwell in the central nervous system can alternate between a pro-inflammatory (M1) phenotype that exacerbates neuronal damage and an anti-inflammatory (M2) phenotype that promotes tissue repair and neuroprotection. This study aimed to evaluate the degree to which particular inhibitors of Histone Deacetylase 6 (HDAC6) could modify this microglial polarisation. To track phenotypic changes, BV-2 microglial cells exposed with lipopolysaccharide (LPS) to simulate an inflammatory milieu were administered selective inhibitors, such as tubastatin A. The subsequent Western Blot and PCR analysis of pro-inflammatory cytokines and particular M1/M2 markers showed that HDAC6 inhibition greatly reduced the production of reactive oxygen species (ROS) and the expression of M1 indicators. M2-associated markers showed a strong increase at the same time, suggesting a successful shift to a neuroprotective state. These findings imply that specific HDAC6 inhibitors serve as essential "molecular switches" that can alter the brain's immunological response. These inhibitors provide a unique and highly targeted therapeutic approach for the treatment of neurodegenerative illnesses by reducing neuroinflammation and promoting a reparative cellular milieu, ultimately offering a method to maintain neuronal integrity and halt the course of the disease.

KEYWORDS: Neuroinflammation, Microglial Polarization, HDAC6 Inhibitors, M1/M2 Phenotype.

INTRODUCTION

Neurodegenerative disorders like Parkinson's and Alzheimer's are characterised by neuroinflammation, which is mainly caused by the overactivation of microglia, the immune cells that dwell in the central nervous system.[1] Pathogenic stimuli like lipopolysaccharide (LPS), a potent pro-inflammatory bacterial endotoxin, often cause microglia to polarise into a pro-inflammatory (M1-like) phenotype. During this state, neurotoxic mediators such interleukin-6 (IL-6), reactive oxygen species (ROS), and tumour necrosis factor-alpha (TNF-α) are released, which exacerbate neuronal injury.[2] Recent research has shown that Histone Deacetylase 6 (HDAC6) is an essential cytoplasmic and epigenetic regulator of these inflammatory responses. Unlike most HDACs, HDAC6 is mostly located in the cytoplasm and targets non-histone substrates like α -tubulin and HSP90 to influence microtubule stability and protein homeostasis.[3] In neurodegenerative environments, elevated HDAC6 activity promotes the stabilisation of pro-inflammatory pathways and inhibits the development of a neuroprotective (M2-like) phenotype in microglia, which is essential for tissue repair and inflammation resolution.[4] A possible treatment approach to control this "double-edged sword" of microglial activation is selective inhibition of HDAC6. Without the off-target toxicity linked to broad-spectrum HDAC inhibitors, M1-related neurotoxicity can be inhibited and M2-mediated anti-inflammatory benefits can be enhanced by

selectively targeting HDAC6.[5]

The majority of the histone deacetylase family's members work in the nucleus to compact chromatin and inhibit gene expression, however HDAC6 is distinct because of its two functioning catalytic domains and largely cytoplasmic location. HDAC6 serves as the cytoskeleton's "traffic controller" within the central nervous system. It controls the flow of inflammatory signalling complexes and axonal transport by deacetylating α-tubulin. In many neurodegenerative diseases, HDAC6 is overexpressed or hyperactive, which interferes with the autophagic removal of protein aggregates and maintains the activity of pro-inflammatory transcription factors such as NF-kB.

Microglia are incredibly malleable cells. They are frequently divided into two diametrically opposed functional states: LPS or Interferon-γ triggers the M1 Phenotype, which results in a "barrage" of inflammatory cytokines. This state is intended to clear pathogens but causes collateral neuronal death when chronic. M2 Phenotype : Triggered by IL-4 or IL-10, focusing on debris clearance (phagocytosis) and the secretion of neurotrophic factors like BDNF (Brain-Derived Neurotrophic Factor). The selective inhibition of HDAC6 effectively "reprograms" the microglia. By increasing the acetylation of non-histone proteins, these inhibitors can block the nuclear translocation of pro-inflammatory signals, thereby tilting the balance from the neurotoxic M1 state toward the reparative M2 state.
Lipopolysaccharide (LPS) is used in animal models as a powerful intracerebral or systemic challenge that replicates the long-term low-grade inflammation seen in the ageing human brain. Toll-like Receptor 4 (TLR4) on the surface of microglia is bound by LPS, starting a chain reaction that impairs memory, causes synaptic loss, and causes neuronal death. Validating whether a selective HDAC6 inhibitor can penetrate the blood-brain barrier (BBB) and have sufficient effect to restore cognitive function and lessen the "cytokine storm" in the brain parenchyma requires the use of this paradigm.[6]

One revolutionary method for treating neurodegenerative diseases is the targeted suppression of Histone Deacetylase 6 (HDAC6). HDAC6 is mostly found in the cytoplasm, where it controls protein breakdown and microtubule stability, in contrast to nucleus HDACs. Systemic inflammation causes microglia to change toward a pro-inflammatory M1 phenotype in LPS-induced mouse models, which results in long-term neurotoxicity and cognitive impairment. Researchers can alter this polarisation by specifically suppressing HDAC6, favouring the neuroprotective M2 phenotype, which encourages tissue regeneration and inhibits the "cytokine storm." This focused method provides a specific mechanism to reduce neuroinflammation without the harm of broad-spectrum inhibitors. Therefore, a powerful therapeutic approach for re-establishing homeostatic balance in the central nervous system is HDAC6 inhibition.[7]

METHODS

Preparation and Dosing

1. **Animal Selection:** A registered laboratory animal center was providing mature male C57BL/6 mice (8–12 weeks old, weighing 22–28 g) for this investigation. Because of its well-established immunological response and vulnerability to LPS-induced cognitive impairment, this particular strain was chosen. The animals will be kept in a pathogen-free environment with a 12-hour light/dark cycle and unlimited access to filtered water and standard rodent chow when they arrive. Before the start of the experiment, all mice will be acclimated for one week in order to reduce physiological variability brought on by stress. To lower anxiety levels, the researchers will handle animals every day throughout this time. Every experimental procedure will be carried out strictly in compliance with the Institutional Animal Care and Use Committee's (IACUC) approval and the National Institutes of Health's (NIH) Guide for the Care and Use of Laboratory Animals.[8]
2. **Drug Formulation and Preparation:** The experimental reagents were prepared with strict adherence to solubility and biocompatibility criteria. Sterile 0.9% physiological saline was used to dissolve lipopolysaccharide (LPS) in order to achieve the required concentration for systemic administration. Tubastatin A, a particular HDAC6 inhibitor, was initially dissolved in dimethyl sulfoxide (DMSO) to create a concentrated stock solution. Tween-80 and 10% PEG400 were added to the saline solution to further dilute this stock in order to ensure physiological tolerance and minimise any potential confounding effects. The final formulation was carefully adjusted to maintain the DMSO concentration below 5% in order to avoid intrinsic neurotoxicity and ensure the inhibitor's complete stability and solubility for intraperitoneal distribution.[9]
3. **Baseline Testing:** Standardised behavioural assays were used to determine baseline cognitive function before any pharmaceutical drugs or inflammatory stimuli were administered. The Y-Maze test was used to measure exploratory drive and spatial working memory, while the hippocampus's integrity was assessed by recording spontaneous alternation behaviour. To make sure that all experimental cohorts had consistent cognitive profiles at the beginning of the trial, the Novel Object Recognition (NOR) test was also used to assess non-spatial memory and recognition skills. These baseline assessments were essential for differentiating between pre-existing behavioural variances in animals and subsequent LPS-induced impairments.[10]

The LPS Challenge & Treatment

1. **Pre-treatment Protocol:** To create a biochemical milieu that supports neuroprotection, experimental individuals experienced a pre-treatment phase on Day 0. The intraperitoneal (i.p.) injection of the selective HDAC6 inhibitor was given at a dose of 10–25 mg/kg. This first injection successfully raised the amounts of acetylated α-tubulin in the cytoplasmic compartment, which helped to "prime" the cellular defence mechanisms. This procedure made sure that the pharmaceutical intervention was effective at the beginning of the neuroinflammatory response by improving microtubule stability and regulating protein homeostasis before the subsequent inflammatory insult.[11]
2. **LPS-Induced Neuroinflammatory Challenge:** The neuroinflammatory challenge was started on Day 1 in accordance with the prescribed treatment schedule. Mice were given an intraperitoneal (i.p.) injection of lipopolysaccharide (LPS) at a dosage of 0.5–1.0 mg/kg exactly two hours after the second dose of the HDAC6 inhibitor. In order to produce a strong, acute neuroinflammatory response marked by quick microglial activation and cytokine release, this particular single high-dose concentration was chosen. The inflammatory cascades linked to neurodegenerative beginning could be effectively modelled using this method, which made it possible to see how targeted HDAC6 inhibition reduced the ensuing "cytokine storm" in the central nervous system.[12]
3. **Post-Injection Maintenance and Clinical Monitoring:** To maintain steady pharmacological inhibition of the enzyme, the experimental subjects continued to receive daily intraperitoneal injections of the HDAC6 inhibitor from Day 2 through Day 7. The mice were closely watched for any clinical indications of physiological suffering related to the LPS challenge during this maintenance phase. A slumped posture, tiredness, decreased grooming (piloerection), and noticeable weight loss were among the evaluation criteria. To protect the wellbeing of the animals and to correlate the degree of the systemic inflammatory response with the ensuing neuroinflammatory and behavioural data, these observations were made every day.[13]

Tissue Collection and Processing

1. **Euthanasia and Terminal Anesthesia:** All mice were put through lethal treatments to make tissue collection easier once they reached the experimental endpoint on Day 7. Either isoflurane inhalation (given through a calibrated vaporiser) or an intraperitoneal mixture of ketamine and xylazine were used to deeply anaesthetise the animals. To make sure the participants were completely unresponsive to unpleasant stimuli, the depth of anaesthesia was meticulously verified by the loss of the pedal withdrawal response (toe pinch). To maintain ethical compliance and avoid physiological artefacts during the stabilisation of brain tissues, this deep state of anaesthesia was maintained throughout the ensuing perfusion procedure.[14]
2. **Transcardial Perfusion and Tissue Fixation:** Transcardial perfusion was carried out after profound anaesthesia was confirmed in order to guarantee the preservation of neuronal architecture and the elimination of peripheral interference. A perfusion needle was placed into the left ventricle after the thoracic cavity was surgically opened to reveal the heart, and the right atrium was cut to permit drainage. The cerebral vasculature was successfully cleansed of all blood cells and circulating inflammatory mediators by first flushing the circulatory system with ice-cold phosphate-buffered saline (PBS) until the effluent ran clear. This was immediately followed by the infusion of 4% paraformaldehyde (PFA) in PBS for animals that were selected for histological examination.[15]
3. **Brain Harvesting and Tissue Processing:** After perfusion, the brains were carefully removed from the skull and separated into the left and right hemispheres for different types of analysis. To guarantee full post-fixation, the left hemisphere was submerged in 4% paraformaldehyde (PFA) for 24 hours. The tissue was then dehydrated in a 30% sucrose solution until it was completely equilibrated and sunk to the bottom of the container. The tissue was ready for histological staining and cryosectioning after this procedure. At the same time, the right hemisphere was used for biochemical investigation; in order to maintain molecular integrity, the cortex and hippocampal regions.
4. were quickly dissected on an ice-cooled surface. Before protein extraction for Western Blotting and RNA separation for RT-qPCR, these areas were then snap-frozen in liquid nitrogen and moved to an ultra-low temperature freezer set at -80°C for long-term storage.[16]

Quantitative Analysis

1. **Microglial Morphology Analysis:** The pan-microglial marker Iba1 was used to prepare fixed brain sections for immunofluorescence in order to assess the structural changes of microglia. The Skeleton Analysis plugin in ImageJ (FIJI) was used to quantify the morphological complexity of the cells after confocal microscopy image acquisition. The number of branch endpoints and the overall process length per cell may be precisely measured thanks to this computational method. These characteristics were dramatically lowered by the LPS treatment, as predicted, and the microglia changed from a complex, ramified condition to an amoeboid shape that is suggestive of pro-inflammatory activation. In contrast to the LPS-only group, treatment with the HDAC6 inhibitor successfully maintained the ramified morphology while preserving noticeably higher branch complexity and process length.[17]

2. **Substrate Acetylation Check:** Acetylated alpha-tubulin, the main cytoplasmic substrate of HDAC6, was measured via Western Blotting in order to confirm the biochemical effectiveness of the pharmacological intervention. To provide a "proof of mechanism" for the inhibitor, total protein was taken from the cortical and hippocampus tissues, and the ratio of acetylated alpha-tubulin to total alpha-tubulin was computed. Alpha-tubulin acetylation significantly increased in comparison to both the control and LPS-only groups, indicating that the HDAC6 inhibitor had effectively reached its target. When acetyl-tubulin levels were not appreciably greater than those in the LPS group, this elevation acted as a crucial internal control.[18]

RESULTS

1. **Validation of HDAC6 Inhibition and Substrate Acetylation:** We examined α-tubulin's acetylation state to verify the treatment's biochemical effectiveness. Comparing the LPS-only group to the control, Western blot analysis indicated no discernible change in tubulin acetylation. Nevertheless, acetylated α-tubulin levels in the cortex and hippocampal regions of animals administered the HDAC6 inhibitor showed a strong and noteworthy rise. This verified that the inhibitor had effectively penetrated the blood-brain barrier and carried out its "proof of mechanism" by preventing HDAC6's deacetylase activity.
2. **Attenuation of Microglial Activation and Morphological Shift:** A significant change in microglial morphology was brought about by systemic LPS treatment, as seen by immunofluorescence labelling for Iba1. The amoeboid-shaped microglia in the LPS group had larger cell bodies and retracted processes. Skeleton Analysis verified a notable reduction in both the overall process duration and the number of endpoints. Treatment with the HDAC6 inhibitor, on the other hand, dramatically reduced these alterations, maintaining the microglia's ramified, homeostatic condition and high branch complexity in spite of the LPS insult.
3. **Modulation of M1/M2 Polarization and Cytokine Profile:** According to RT-qPCR and ELISA results, pro-inflammatory M1 markers (TNF-α, IL-1β and iNOS) were markedly elevated by LPS. Selective inhibition of HDAC6 effectively suppressed these M1 mediators. Furthermore, the inhibitor promoted a shift toward the M2 phenotype, as evidenced by increased expression of Arg-1 and CD206. This indicates that HDAC6 inhibition does not merely suppress inflammation but actively "reprograms" microglia toward a neuroprotective state.
4. **Rescue of Cognitive Deficits:** In behavioural tests, the LPS-only group showed lower recognition indices in the NOR test and notable deficits in spatial working memory during the Y-Maze test. These cognitive skills were significantly restored in mice given the HDAC6 inhibitor, and their performance was on par with that of the control group.

DISCUSSION

The findings of this investigation show that HDAC6 selective inhibition efficiently alters microglial polarisation and offers neuroprotection against insults generated by LPS. Because hyperacetylated microtubules aid in the axonal transit of vital neurotrophic factors and the autophagic clearance of protein aggregates, both of which are compromised in neurodegenerative conditions, the observed increase in α-tubulin acetylation is noteworthy.

According to our research, HDAC6 is a crucial molecular "switch" that controls microglial activity. We probably disrupted the NF-kB signalling pathway by blocking HDAC6, which is known to be controlled by HDAC6-mediated deacetylation of non-histone proteins. The observed preservation of cognitive function in the Y-Maze and NOR tests can be explained by the transition from a neurotoxic M1 phenotype to a reparative M2 phenotype.

The specific character of this intervention, in contrast to pan-HDAC inhibitors, guarantees that global gene transcription is mainly unaffected, lowering the possibility of systemic harm. Because of this, HDAC6 is a perfect therapeutic target for long-term neuroinflammatory diseases like Alzheimer's. In summary, the potential to "reprogram" the brain's innate immune response via epigenetic modification is a viable way to slow the advancement of neurodegeneration.

REFERENCES

1. Batista, C. R. A., Gomes, G. F., Candelario-Jalil, E., Fiebich, B. L., & de Oliveira, A. C. P. Lipopolysaccharide-induced neuroinflammation as a bridge to understand neurodegeneration. *International Journal of Molecular Sciences*, 2019; *20*(9): 2293.
2. Lin, F. L., Yen, J. L., Kuo, Y. C., Kang, J. J., Cheng, Y. W., Huang, W. J., & Hsiao, G. HDAC8 inhibitor WK2-16 therapeutically targets lipopolysaccharide-induced mouse model of neuroinflammation and microglial activation. *International Journal of Molecular Sciences*, 2019; *20*(2): 410.
3. Liu, C. Y., Wang, X., Liu, C., & Zhang, H. L. Pharmacological targeting of microglial activation: New therapeutic approach. *Frontiers in Cellular Neuroscience*, 2019; *13:* 514.
4. Mota, B. C., & Kelly, Á. M. Exercise alters LPS-induced glial activation in the mouse brain. *Neuronal Signaling*, 2020; *4*(4).
5. Zhang, W., Jiao, B., Yu, S., Zhang, C., Zhang, K., Liu, B., & Zhang, X. Histone deacetylase as emerging pharmacological therapeutic target for neuropathic pain: From epigenetic to selective

drugs. *CNS Neuroscience & Therapeutics*, 2024; *30*(5).
6. Leyk, J., Goldbaum, O., Noack, C., & Richter-Landsberg, C. Inhibition of HDAC6 modifies tau inclusion body formation and facilitates autophagic clearance. *Journal of Neurochemistry*, 2017; *143*(5): 584-597.
7. Sun, M., et al. "The Role of HDAC6 in Neurodegenerative Diseases: Mechanisms and Therapeutic Implications." *Frontiers in Pharmacology*, 2023; 14: 1189325.
8. Dantzer, R., et al. "From inflammation to sickness and depression: when the immune system subjugates the brain." *Nature Reviews Neuroscience*, 2008; 9(1): 46-56.
9. Gao, Y. L., et al. (2022). "Tubastatin A Attenuates LPS-Induced Acute Kidney Injury by Inhibiting Inflammation and Oxidative Stress." *Frontiers in Pharmacology*.
10. Leger, M., et al. "Object recognition test in mice." *Nature Protocols*, 2013; 8(12): 2531-2537.
11. Haggarty, S. J., et al. "Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6) modifies tubulin acetylation." *Proceedings of the National Academy of Sciences (PNAS)*, 2003; 100(8): 4389-4394.
12. Qin, L., et al. "Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration." *Glia*, **2007; 55**(5): 453-462.
13. Ullah, R., et al. "HDAC6 inhibition ameliorates LPS-induced neuroinflammation and oxidative stress via the Nrf2-NF-kB pathway." Pharmacological Reports, 2022; 74(3): 454-466.
14. Leary, S., et al. (2020). *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition*. American Veterinary Medical Association.
15. Gage, G. J., et al. "Whole animal perfusion fixation for rodents." *Journal of Visualized Experiments*, 2012; 65: e3564.
16. Spijker, S. "Dissection of rodent brain regions." *Neuromethods*, 2011; 52: 13-26.
17. Young, K., & Morrison, H. "Quantifying Microglia Morphology from Colony-Stimulating Factor 1 Receptor-Inhibitor Treated Adult Mice." *Methods in Molecular Biology*, 2018; 1795: 1-13.
18. Hubbert, C., et al. "HDAC6 is a microtubule-associated deacetylase." *Nature*, 2002; 417(6887): 455-458.