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Research article

The effect of cyclin-dependent kinases inhibitor treatment on experimental herpes simplex encephalitis mice

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HIGHLIGHTS

- The effect of cyclin-dependent kinases inhibitor olomoucine treatment on experimental HSE mice is explored.
- Olomoucine can induce a blunted inflammatory response and maintain the blood vessel wall intact in HSE mice.
- Olomoucine can improve neurological function and increase survival in HSE mice.

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ABSTRACT

Herpes simplex encephalitis(HSE) is the most common and serious viral encephalitis in humans. There is a lack of effective medication to date for HSE. A better understanding of the mediators of tissue damage is essential for finding new targets for therapeutic intervention. In this project, we explored the effect of cyclin-dependent kinases inhibitor olomoucine treatment on experimental HSE mice. The following results were obtained: (1) olomoucine increased survival in HSE mice; (2) olomoucine inhibited microglial activation and reduced HSV-1-induced cytokines release; (3) olomoucine prevented neural cells apoptosis and attenuated brain tissue pathological changes following HSV-1 infection; (4) olomoucine reduced brain edema and improved neurological function in HSE. Overall, olomoucine can induce a blunted inflammatory response, maintain the blood vessel wall intact, improve neurological function and increase survival in HSE mice.

1. Introduction

Herpes simplex virus type 1(HSV-1) caused herpes simplex encephalitis(HSE) is the most common, sporadic and serious viral encephalitis in humans [1]. The limbic lobes system is the most affected area of the brain during HSE, which leads to the incidence of severe neurological deficits. Despite the introduction of standard acyclovir treatment during the acute phase, the morbidity and mortality remain significantly high for HSE. However, the pathological mechanism of HSE has not yet been elucidated.

The parenchymal damage is caused partially by virus-mediated lytic effects on neurons and glial cells. Patients with proven HSE present persistent progressive structural damage with abnormal signal intensity in cranial MRI despite early clinical recovery [2]. This supports that virus-independent mechanisms might be responsible for this devastating disease. Activated microglial cells have the potential to develop an immune response via induction of inflammatory mediators [3]. Treatment with conventional antiviral agents cannot influence the secondary mechanisms of structural damage. A better understanding of the mediators of tissue damage in HSE is essential for finding new targets for therapeutic intervention.

Cyclin-dependent kinases(CDKs) are a family of protein kinases that phosphorylate a serine or threonine followed by a proline. The effect of CDKs on the central nervous system has become a concern in recent years. Increasing evidence indisputably links CDKs to the pathogenesis of stroke. Cdk4/cyclin D1 levels and phosphorylation of retinoblastoma protein(pRb) increase after stroke. Administration of a CDKs inhibitor blocks pRb phosphorylation and dramatically reduces neuronal death, indicating that CDKs are an important therapeutic target for the treatment of ischemia-reperfusion injury [4,5]. Treatment with CDK inhibitors in rodent spinal cord injury model prevents neuronal cell death and reduces inflammation, resulting in markedly improved motor recovery [6]. CDK inhibitors enhance the resolution of established inflammation in the pleurisy model by promoting inflammatory cells apoptosis,

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thereby demonstrating a potential for the treatment of inflammatory disorders [7].

Previous study in our research group has found that CDKs inhibitor olomoucine induces microglial cells apoptosis at 24 h post-infection and inhibits the release of proinflammatory cytokine and chemokine [8]. In this project we further explore the effect of olomoucine treatment on experimental HSE mice.

2. Materials and methods

2.1. Mouse infection

The animal procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of Wuhan University Medical School, and complies with NIH Guide for the Use and Care of Laboratory Animals. 3–4 weeks female BALB/c mice were purchased from the Laboratory Animal Center of Hubei Province. Mice were anesthetized and then 20 μl of MEM (Gibco BRL) containing either no virus (vehicle only) or 10^5 plaque forming units (PFU) of HSV-1 (HSV-1 17syn$^+$ strain) were inoculated intracerebrally through midpoint of right canthus and external auditory canal opening (4 mm deep vertically into the brain using a Hamilton syringe fitted with a 30-gauge needle). For histological examination, hematoxylin and eosin staining and electron microscope were adopted. Mice were randomly divided into a normal group, HSV-1 control group (acyclovir-treated) and an olomoucine-treated group.

2.2. Cumulative survival of HSV encephalitis

The Kaplan-Meier method was used to generate survival curves for control and olomoucine-treated (Sigma; dissolved in 0.2% dimethyl sulfoxide, 3 mg/kg once daily for 7 days) mice (n = 20 per group) and the Logrank test was used to test for differences between groups. The mice of HSV-1 control group were treated with intraperitoneal acyclovir (100 mg/kg twice daily for 7 days) after HSV-1 infection.

2.3. Clinical disease scoring of HSV encephalitis and brain water content

Symptoms were blindly scored by two independent observers according to the following: gait (0 = normal, 1 = ataxia), posture (0 = normal, 1 = hunching), paralysis (0 = none, 1 = paralysis in the hind limbs, mobile, 2 = paralysis in the hind limbs, not mobile), seizures (0 = none, 1 = present) [9]. Brain water content was measured with wet/dry method. Briefly, right hemisphere was separated and weighed, and then the tissues were put in an oven at 110 °C for 24 h and reweighed. Brain water content was calculated by: (wet weight − dry weight)/wet weight × 100%.

2.4. Flow cytometric detection microglial cells surface antigen CD11b

Approximately 0.2 g of the right hemisphere brain tissue was obtained, digested with 0.125% trypsin for 3–5 min into a single cell suspension. The cell suspension was centrifuged at 1000 rpm for 5 min then we removed the supernatant. 1×10^6 cells were collected with 100 μl PBS suspension. 20 μmol/L FITC-labeled rat anti-CD11b (1:100; Life technology, USA) were added, then incubated at 37 °C for 30 min, and then centrifuged at 1000 rpm for 5 min. Cells were suspended in 500 μl PBS, followed by detection of fluorescence intensity using flow cytometric.

2.5. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was isolated from brain sample centered on the injury site using a RNeasy Plus Mini Kit (QIAGEN Sciences, MD). RT-PCR was performed as described previously [8]. Briefly, one microgram of RNA from each sample was used for reverse transcription using a TaKaRa RNA PCR Kit Ver.3.0 (TaKaRa, Japan). cDNA amplification was performed with primers: *HSV glycoprotein D* (142 bp), 5′-ATC CGA ACG CAG CCC CGC TG-3′ (sense) and 5′-TCT CCG TCC AGT CGT TTA TCT TC-3′ (antisense); *β-actin* (348 bp), 5′-TGG AAT CCT GTG GCA TCC ATG AAAC-3′ (sense), 5′-TAA AAC GCA GCT CAG TAA CAG TCCG-3′ (antisense). Amplified product was electrophoresed in a 2% agarose gel containing 0.5 mg/ml of ethidium bromide and visualized under UV light.

2.6. Quantitative real-time PCR analysis of cytokines

Real-time PCR was carried out with the M63000P QPCR System (Stratagene, La Jolla, CA, USA) using previously published primers for *TNF*-α and *IL-6* [10]. β-actin was used as an endogenous control and relative quantifications were calculated by the standard curve method.

2.7. TUNEL assay

TUNEL staining for apoptotic nuclei was performed using a DAB coloration kit (Roche Diagnostic, IN). Briefly, paraffin-embedded tissue sections were dewaxed and dehydrated, then incubated in 3% H_2O_2 for 10 min. After being processed by proteinase K, digoxigenin-labeled dUTP was added to react with terminal transferase for 120 min. DAB showed color after being processed by avidin oxidase for 30 min. The percentage of apoptotic cells was calculated (TUNEL-positive cells/total cells) and averaged across at least five randomly chosen microscopic fields for each slide.

2.8. Statistical analysis

All data were expressed as means ± SD. Statistical analysis was evaluated by one-way analysis of variance. The difference was considered significant at $P < 0.05$

3. Results

3.1. CDK inhibitor olomoucine increases survival in HSE mice

First, we investigated the effect of olomoucine on mortality in HSE. We observed that olomoucine-treated mice had significantly lower mortality than acyclovir-treated mice as shown in Fig. 1 ($P < 0.05$). Specifically, the survival rate of olomoucine-treated mice was 80% compared with 50% of acyclovir-treated mice within two weeks following HSV-1 infection. In the next set of experiments, we intended to disclose the underlying mechanism for the protective effect of olomoucine in HSE.

3.2. Olomoucine inhibits microglial activation in HSE

Microglia-mediated host immune response plays a key role in the pathogenesis of HSE [11]. Hence, we studied microglial activation in the brain tissue following HSE. Flow cytometry was used to detect the expression of microglial cells surface antigen CD11b. We found the expression of CD11b was significantly increased compared with the normal group at 7 days post-infection and acyclovir treatment ($P < 0.01$). Olomoucine treatment significantly reduced CD11b expression compared with HSV-1 control group mice ($P < 0.01$) (Fig. 2).