Antiviral Activity of Recombinant Cyanovirin-N against HSV-1*

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Abstract: In this study, a standard strain of HSV-1 (strain SM44) was used to investigate the antiviral activity of the recombinant Cyanovirin-N (CV-N) against Herpes simplex virus type 1 (HSV-1) in vitro and in vivo. Cytopathic effect (CPE) and MTT assays were used to evaluate the effect of CV-N on HSV-1 in Vero cells. The number of copies of HSV-DNA was detected by real-time fluorescence quantitative PCR (FQ-PCR). The results showed that CV-N had a low cytotoxicity on Vero cells with a CC50 of 359.03±0.56 µg/mL, and that it could not directly inactivate HSV-1 infectivity. CV-N not only reduced the CPE of HSV-1 when added before or after viral infection, with a 50% inhibitory concentration (IC50) with 2.26 and 30.16 µg/mL respectively, but it also decreased the copies of HSV-1 DNA in infected host cells. The encephalitis model for HSV-1 infection was conducted in Kunming mice, and treated with three dosages of CV-N (0.5, 5 & 10 mg/kg) which was administered intraperitoneally at 2h, 3d, 5d, 7d post infection. The duration for the appearance of symptoms of encephalitis and the survival days were recorded and brain tissue samples were obtained for pathological examination (HE staining). Compared with the untreated control group, the 5mg/kg CV-N and 10mg/kg CV-N treated groups, the mice suffered light symptoms and the number of survival days were more than 9d and 14d respectively. HE staining also showed that in 5mg/kg CV-N and 10mg/kg CV-N treated groups, the brain cells did not show visible changes, except for a slight inflammation. Our results demonstrated that CV-N has pronounced antiviral activity against HSV-1 both in vitro and in vivo, and it would be a promising new candidate for anti-HSV therapeutics.

Key words: Recombinant cyanovirin-N; Herpes simplex virus type 1 (HSV-1); Antiviral activity; Real-time FQ-PCR; Encephalitis

Herpes simplex virus type 1 (HSV-1), an enveloped DNA virus, causes a variety of infections in humans. Primary infection usually occurs during childhood and subsequent to the initial outbreak, the virus enters the peripheral nervous system, residing there permanently in a latent state of infection; it is reactivated by the proper stimulus and causes recurrence of symptoms. Serious infection with HSV-1 can also lead to life-threatening encephalitis and ocular infections that result in corneal inflammation and scarification [24,26].
Immunocompromised individuals and the recipients of organ transplantations are at high risk for increased severity of HSV-1 infection \[6,9\]. In addition, HSV-1 has been shown to be a factor for spreading human immunodeficiency virus and causing severe diseases in AIDS patients \[20,21\].

Currently, most of the treatments for HSV are based on nucleoside analogs of guanine, for example, acyclovir (ACV) is specifically phosphorylated by viral thymidine kinase in infected cells \[17,24\]. However, widespread use of ACV has shown HSV develops resistance to ACV through mutations in genes coding for thymidine kinase or for DNA polymerase \[4,10,11\]. Thus, some immunocompromised patients and organ transplant recipients with recurrent HSV lesions develop resistance to ACV after repeated treatments \[20,21\]. Therefore, it is important to develop new antiviral drugs with different mechanisms of action which can substitute for, or complement, acyclovir.

New types of antiviral agents from natural sources, especially those that possess high efficacy on resistant mutant viral strains and low toxicity to the host, are considered to be the most promising. One such candidate is cyanovirin-N (CV-N), a 101-amino acid protein (11 kDa) with known three-dimensional structure, that was originally isolated from an aqueous extract of the cyanobacterium *Nostoc ellipsosporum* \[4\] and later produced recombinantly in *Escherichia coli* \[12\] as an active agent against HIV. The recombinant CV-N is identical to natural CV-N in structure and bioactivity. CV-N contains two sequence repeats, 50 and 51 amino acids long, which exhibit significant similarity and equivalently positioned disulfide bonds. No similarity with any other proteins thus far deposited in published databases has been reported \[2,3,13\].

In our previous study, we have produced the purified and renatured recombinant CV-N protein in *Escherichia coli* with high efficiency \[15\]. In this report, we describe the activity of CV-N against HSV-1 *in vitro*, and further show the ability of CV-N to treat HSV-1 infection in mice.

**MATERIALS AND METHODS**

**Reagents**

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Chemical Co.. The HSV DNA real-time FQ-PCR detection kit was purchased from Shenzhen PG Biotech, China. DMEM and fetal bovine serum (FBS) were purchased from GIBCO. All other chemicals used were of analytical reagent grade.

**Preparation of recombinant CV-N**

Purified recombinant CV-N, a unique 11-kDa cyanobacterial protein, was produced in *Escherichia coli* as reported previously \[15\]. In brief, the DNA coding sequence for CV-N was synthesized and amplified by PCR, the resulting PCR product was cloned into pET30a(+) vector and sequenced. The confirmed recombinant clone pET30a(+) -CV-N was transformed into *E.coli* BL21(DE3) and was induced to express proteins by IPTG. The expression of the protein was analyzed by SDS-PAGE and Western blot, and subsequently purified by Ni Sepharose column. The purified protein was found to be 11KDa and renatured successfully by the dilution method. After production the protein was stored at -80°C until use.

**Cells and cell culture**

Vero cells were maintained in our laboratory and grown in DMEM medium supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin.