Non-Thermal Plasma as a Therapy Alternative for Oral Herpes Simplex Virus Type 1 Infection

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Abstract: HSV-1 is a lifelong pathogen causing recurrent outbreaks of cold sores with no cure. NTP treatment of mice with oral HSV-1 infection reduced HSV-1 titers in acutely (lip) and latently (TG) infected tissues, the latter suggesting a lower potential for recurrent outbreaks due to reactivated infection. The safety of the NTP treatment was apparent in minimal skin damage and evidence of an innate immune response.

1. Introduction

Herpes simplex virus type 1 (HSV-1) is a contagious virus that causes infection in ~70% of the world's population. Its high infection rate is due to the lifelong persistence of HSV-1, caused by the establishment of latent infection in sensory neurons. For oral HSV-1 infection, this occurs in the trigeminal ganglia (TG). Latent infection allows the virus to become dormant and escape clearance by the host. Over time, HSV-1 periodically reactivates to cause outbreaks of oral cold sore lesions, a symptom of acute infection characterized by virus replication and production in keratinocytes. Current antiviral treatments are effective in managing symptoms during outbreaks but do not address latent infection or prevent recurrent outbreaks of cold sores [1].

Non-thermal plasma (NTP) effectively inactivates many types of viruses [2]. As a potential therapeutic, NTP inactivates cell-free HSV-1, disrupts HSV-1 replication in infected keratinocytes, and prevents HSV-1 infection of uninfected keratinocytes *in vitro* [3]. However, the effect of NTP treatment on latent infection and reactivation has not been investigated.

2. Methods

The safety and efficacy of NTP against oral HSV-1 infection was studied using the lip scarification model of infection [4] in BALB/c mice. Following anesthesia, 10 vertical strokes were created on the lower lip of mice using a 25-gauge needle followed by application of 10^5 PFU of McKrae HSV-1. At 3-hours post-infection (PI), the infected lower lip was treated with a single application of NTP for 3-minutes. Lip and TG tissues were collected at 4- and 11-days post-NTP. HSV-1 titer at the lip and the TG were measured by quantitative polymerase chain reaction (qPCR) using primers against the viral thymidine kinase gene. Reactivation potential of latent HSV-1 in the TG was measured using an explant reactivation model, with reactivated infectious virus measured via plaque assays. Tissues were also stained for H&E to examine histopathology.

3. Results and Discussion

NTP treatment of mice reduced the severity of oral HSV-1 infection. This observation correlated with reduced HSV-1 titers in lip tissues 4- and 11-days PI, suggesting less viral replication and virus production after NTP compared to untreated animals. Additionally, HSV-1 titers in the TG were reduced in NTP-treated mice compared to the infected control, suggesting reduced establishment of latent infection (Fig. 1). NTP-treated mice also exhibited lower reactivation potential of latent infection. Lastly, histopathological analysis showed NTP treatment caused negligible tissue damage and promoted the infiltration of inflammatory immune cells into the infection site.

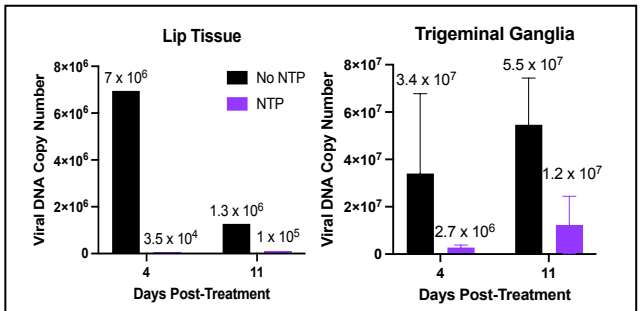

Fig. 1. HSV-1 titers were measured in lip and TG tissue by qPCR at 4- and 11-days post-NTP treatment in mice.

4. Conclusion

NTP treatment of oral HSV-1 lesions is safe and efficacious *in vivo*. Following NTP treatment, HSV-1-infected mice showed delayed cold sore development and enhanced resolution of lesions. NTP treated mice had reduced HSV-1 titers in both lip and TG tissues with lower TG reactivation potential.

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