The antiviral effect of non-thermal plasma against herpes simplex virus type 1

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Abstract: The proposed antiviral activity of non-thermal plasma (NTP) against herpes simplex virus type 1 (HSV-1) infection is through the generation and subsequent delivery of reactive oxygen and nitrogen species (RONS). Our results suggest that NTP-generated RONS can directly impact the HSV-1 virion and cells susceptible to infection, thereby reducing the number of HSV-1-infected cells. These initial studies are the basis of developing NTP as an alternative therapy for HSV-1 infection.

Keywords: Reactive Species, Oxidative Stress, Redox homeostasis, Therapy, Viral Infection

1. Introduction

The therapeutic properties of non-thermal plasma (NTP) in biomedicine are largely attributed to its ability to produce various reactive oxygen and nitrogen species (RONS). Thus, there is direct a relationship between NTP and redox biology in cells and in disease [1].

RONS are generated during cell metabolism and neutralized by cellular antioxidants to maintain redox homeostasis of the cell. Their activity in cellular signaling pathways and as an effector of innate immune cells is widely studied [2, 3]. Due to their short half-lives, RONS are typically found at low concentrations but, in some cases, can increase through secondary reactions and enzymatic processes resulting in oxidative stress. Such stress conditions can occur during viral infections as a mechanism of cellular hijacking and immune evasion that enhances viral pathogenesis. In other words, there is a strong correlation between virus infection and induction of oxidative stress in infected cells [4, 5].

Herpes simplex virus type 1 (HSV-1) infections are known to cause oxidative stress in infected cells [6, 7]. HSV-1 typically infects mucosal epithelial cells around the mouth, eyes, and genitalia. Active virus production results in appearance of clinical symptoms in patients, including cold sores that are hallmarks of oral HSV-1 infection [8]. To avoid clearance by the cell, HSV-1 manipulates the redox environment by increasing RONS production inside the cell. This, in turn, downregulates innate cellular immune responses, allowing the virus to persist [9].

HSV-1 can also leave the mucosal epithelium and travel into the nervous system to infect neurons where, instead of replicating, HSV-1 enters a state of latency that modulates the redox environment to promote neuron survival and hide the virus from immune surveillance [10]. This may lead to oxidative damage to neurons over time and cause diseases like encephalitis [11] and the neurodegenerative disorder Alzheimer's disease [12]. HSV-1 persists in this state for extended periods of time until viral replication in latently infected neurons is reactivated through stress stimuli [13].

Although there are a small number of drugs available to treat HSV-1 infections, they are limited in their effectiveness. Specifically, currently available drugs have no effect on the virus in latently infected neurons. Thus, the failure of antiviral drugs to eliminate HSV-1 from the patient, combined with the evasion strategies elicited by HSV-1 that include the alteration of redox homeostasis [9, 14], contribute to its lifelong persistence in people infected with HSV-1 [15]. Thus, there is a need for alternative therapies that address these limitations.

NTP inactivation cell-free virus has been demonstrated against many types of viruses [16]. However, the effect of NTP on HSV-1 infectivity, especially in an aqueous environment, has not been previously studied. Furthermore, the effects of cells on NTP-associated changes in redox chemistry is an aspect of plasma medicine that is understudied and not fully appreciated. Our studies examined both effects and demonstrated changes in both virus infectivity and media chemistry subsequent to NTP application.

2. Methods

NTP was generated using a microsecond dielectric barrier discharge (DBD) system operated at 7 kV and frequencies of 500 Hz, 1000 Hz, or 1500 Hz for 10 seconds.

Vero cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C and in 5% carbon dioxide (CO_2). Cells were seeded in 12-well plates with DMEM at a $2x10^5$ cells/mL and maintained for 24 hours prior to NTP exposure. NTP exposure was conducted by removing the medium from the cells and then positioning the electrode 1 mm above the cell monolayer.

A cell-free preparation of KOS-GFP-HSV-1 was prepared in serum-free DMEM at a virus concentration that

would result in a multiplicity of infection (MOI) of 1 when applied to cells at a volume of 400 µL. This volume was placed in a 12-well plate and exposed to NTP with a 2 mm gap between the electrode and the surface of the liquid.

3.Results

Experiments were conducted to (i) examine the effect of cells on NTP-induced changes in liquid chemistry, (ii) investigate NTP-associated changes in cellular redox, and (iii) examine the effect of NTP on HSV-1 infectivity.

3.1.Vero cells influence the composition of NTP-generated RONS in media

RONS were quantified in phenol red-free, serum-free DMEM in the presence or absence of Vero cells. Hydrogen peroxide and nitrite were measured in the supernatant immediately (0 hour) or 24 hours following NTP exposure using the Spectroquant hydrogen peroxide test and Invitrogen Griess reagent kit, respectively, in accordance with the manufacturer's instructions.

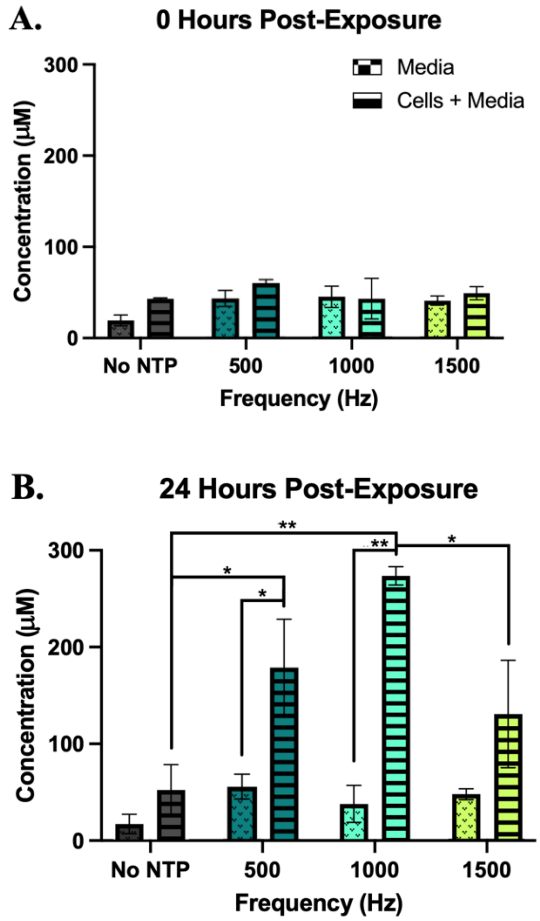

Fig. 1. Concentration of hydrogen peroxide in media with and without Vero cells immediately after exposure to NTP (0 hour) (A) and 24 hours after NTP exposure (B). (*$p < 0.05$, **$p < 0.005$)

Hydrogen peroxide was generally present at higher concentrations in the presence of Vero cells, compared to media alone 24 hours after NTP exposure at all doses tested. In contrast, there was very little difference between media alone and media over cells immediately after NTP exposure (0-hour time point). However, in the absence of NTP, hydrogen peroxide was measured in the media of Vero cells, indicating that these cells may naturally produce RONS as a consequence of their normal metabolism (Fig. 1A). At 24 hours, there was a 3-fold increase in hydrogen peroxide in unexposed Vero cell media. A significant increase in hydrogen peroxide concentration after 24 hours post-exposure to NTP was also measured in Vero cell media. These results suggest that Vero cells can influence the hydrogen peroxide content measured in media in response to NTP (Fig. 1B). We will also present the results for nitrite concentration in media with and without Vero cells.

3.2.NTP induces intracellular oxidative stress in cells

RONS can also react with and alter the structures of cellular macromolecules. At high concentrations RONS can lead to the damage of organelles and proteins involved in cellular homeostasis, leading to cell death. However, at lower concentrations, RONS can influence signal transduction pathways and stimulate innate immune responses [17, 18]. NTP provides a controlled method to generate and deliver RONS to induce specific oxidative stress responses in cells.

To examine this biological effect in cells exposed to NTP, we measured oxidative stress through live cell staining of mitochondrial superoxide. Our ongoing studies show superoxide concentrations within the mitochondria to increase with higher NTP frequencies following NTP exposure. These results will be shared in the presentation. While oxidative stress is proposed to increase with HSV-1 infection, we hypothesize that NTP exposure will further modify this stress response, contributing to its antiviral activity against HSV-1 and through stimulation of pathways that modulate immune responses against HSV-1.

3.3.NTP reduces HSV-1 infectivity

RONS also cause oxidative damage to macromolecules that are structural components of the virus. The HSV-1 virion is composed of a DNA genome surrounded by a protein capsid, a tegument protein layer, and a lipid bilayer envelope containing glycoproteins [8], all of which are susceptible to oxidative damage by RONS. We postulate that NTP-generated RONS can directly impact the structure and integrity of the HSV-1 virion, disrupting its ability to mediate attachment and entry into target cells for as the first steps in infection of host cells.

We hypothesized that a direct application of NTP to a suspension of HSV-1 viral particles would adversely affect the ability of viral particles to infect cells susceptible to infection (defined as infectivity). To demonstrate the effect of NTP on virus infectivity, we exposed cell-free KOS-GFP-HSV-1 to NTP and quantified its infectivity using Vero cells as target cells. In these experiments, there was no effect of NTP on virus infectivity at 500 Hz and 1000

Hz when compared to the negative control. However, NTP generated at 1500 Hz resulted in a 59% decrease in HSV-1 infectivity (Fig. 2). Given the reactive nature of NTP-generated RONS, our results suggest a direct antiviral effect of NTP on the virus. In other words, RONS generated by NTP may be impacting the integrity of the macromolecules in HSV-1 virion that are involved in the initial stages of virus replication.

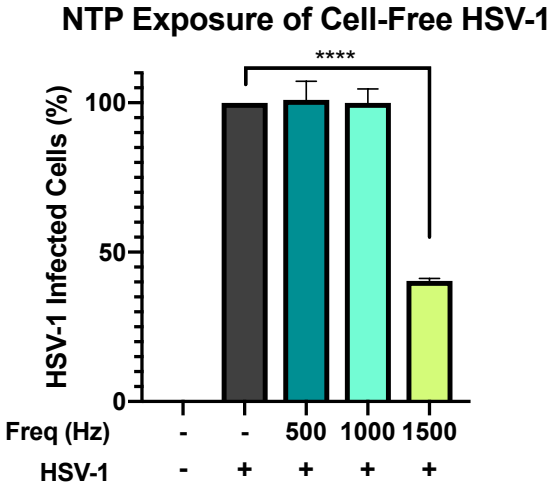

Fig. 2. HSV-1 infectivity as measured by Vero cell infection. Changes in infectivity (relative to virus not exposed to NTP) were measured after exposure to NTP generated at 500, 1000, and 1,500 Hz. (****$p < 0.0001$)

4. Conclusions

As a promising alternative therapy for HSV-1 infection, NTP can directly reduce virus infectivity while also stimulating a biological response in cells through the generation of RONS. Our studies showed that NTP-generated RONS can impact both the virus and the cell. By exposing cell-free HSV-1, we demonstrated a reduced ability of the virus to infect susceptible cells. We also demonstrated that NTP to alters cell oxidative stress in cell that are susceptible to HSV-1 infection.

The effects of NTP on HSV-1 infection likely extend beyond the impact of NTP on virus infectivity. Our preliminary data indicate that application of NTP shortly after infection adversely affects the ability to support HSV-1 replication. Given the well-defined roles of RONS in the innate immune responses mounted against virus infection, increases of mitochondrial superoxide as an indicator for oxidative stress in NTP-exposed cells may also correlate with the modulation of immune responses against HSV-1. Previous studies demonstrated that NTP led to increased emission of immunogenic markers on the surface of cells latently infected with HIV-1 [19], suggesting that similar changes will take place on HSV-1-susceptible and HSV-1-infected cells. Therefore, our future investigations will examine the effect of NTP on HSV-1 infection, the abilities of host cells to support HSV-1 infection, and immune responses stimulated by HSV-1 infection of host cells. These studies will be further progress in the development of NTP as a treatment option for HSV-1 infection that will address both acute and latent infection through antiviral effects and immunomodulation driven by NTP-associated changes in oxidative stress.

5.Acknowledgements

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6.References

[1] von Woedtke, T., et al., In Vivo, 33, 4 (2019).
[2] Zhang, J., et al., Oxidative medicine and cellular longevity, 2016 (2016).
[3] Dumas, A. and U.G. Knaus, Frontiers in immunology, 12 (2021).
[4] Rehman, Z.U., et al., Oxidative Medicine and Cellular Longevity, 2018 (2018).
[5] Komaravelli, N. and A. Casola, Journal of pharmacogenomics & pharmacoproteomics, 5 (2014).
[6] Mathew, S.S., P.W. Bryant, and A.D. Burch, Free radical biology & medicine, 49, 3 (2010).
[7] Kavouras, J.H., et al., Journal of NeuroVirology, 13, 5 (2007).
[8] Ibáñez, F.J., et al., Frontiers in microbiology, 9 (2018).
[9] Danastas, K., M. Miranda-Saksena, and A.L. Cunningham, International Journal of Molecular Sciences, 21,14 (2020).
[10] Nicoll, M.P., et al., PLoS pathogens, 12, 4 (2016).
[11] Stoeger, T. and H. Adler, Frontiers in microbiology, 9 (2019).
[12] Bradshaw, M.J. and A. Venkatesan, Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics, 13, 3 (2016).
[13] Santana, S., et al., PLOS ONE, 8, 10 (2013).
[14] Su, C., G. Zhan, and C. Zheng, Virology journal, 13 (2016).
[15] Cernik, C., K. Gallina, and R.T. Brodell, Archives of Internal Medicine, 168, 11 (2008).
[16] Mohamed, H., et al., Frontiers in Physics, 9, (2021).
[17] Lin, A., et al., Plasma Processes and Polymers, 12, 10 (2015).
[18] Graves, D.B., Journal of Physics D: Applied Physics, 45, 26 (2012).
[19] Mohamed, H., et al., PLOS ONE, 16, 3 (2021).