



2018.09.28

## ANTIVIRAL EFFECTS OF CETYLPYRIDINIUM CHLORIDE OVER THE REPLICATION CYCLE OF HERPES SIMPLEX VIRUSES

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Herpes simplex viruses type 1 and type 2 (HSV-1 and HSV-2) are pathogens that produce a wide spectrum of clinical diseases. Particularly, in the orofacial area, HSV-1 infects the mouth and the eye eliciting gingivostomatitis, lesions around the lips, conjunctivitis and keratitis, among others. Although most of these infections can be treated with acyclovir, its effectiveness is somewhat limited when applied locally. Hence, better topical antivirals against this virus would be desirable. Cetylpyridinium chloride (CPC) is a cationic quaternary ammonium compound that is added to some types of mouthwashes and toothpastes as an antiseptic and has a broad antimicrobial spectrum. While bactericidal and fungicidal effects have been widely reported for CPC, an antiviral effect for this compound has only been recently described over influenza. The aim of this study was to evaluate the antiviral effect of CPC over the replication of HSV in vitro and identify a possible mechanism of action. In our experiments, human gingival fibroblasts and epithelial cells treated with CPC displayed reduced infection with HSV strains that encode structural and non-structural versions of the green fluorescent protein (GFP). Our results suggest that CPC inhibits HSV replication and blockade occurs after the virus enters the cell, yet before viral genome replication takes place. Thus, CPC in topical formulations may act as an antiviral against HSV infection.

## CHARACTERIZATION OF POST-TRANSLATIONAL MODIFICATIONS DURING HUMAN CYTOMEGALOVIRUS INFECTION: IMPLICATIONS FOR NOVEL ANTIVIRALS

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Human Cytomegalovirus (HCMV), a widespread  $\beta$ -Herpesvirus, establishes a lifelong latency in the myeloid lineage, with reactivation often driven by inflammation. Autoimmune diseases (AD) are characterized by chronic inflammation due to an abnormal immune response against the body's own tissues. In genetically predisposed patients, HCMV has been associated with AD, but whether it initiates or supports the development of AD is still not known. Citrullination is a post-translational modification (PTM) catalyzed by peptidylarginine deaminases (PAD) that convert peptidylarginine into peptidylcitrulline, whose dysregulation has been linked to a spectrum of ADs, cancer, and neurodegenerative disorders. Against this background, the goal of this project is to characterize citrullination during infection with HCMV, that may be relevant in the etiopathogenesis of AD. Here, we demonstrate that HCMV infection upregulates the overall pattern of citrullination in HFF (Human Foreskin Fibroblasts) using two different approaches: a specific antibody recognizing citrullinated residues and a citrulline-specific rhodamine phenylglyoxal (RhPG)-based probe. Consistently, PAD2 expression increased both at the mRNA and protein levels, suggesting a predominant role for this isoform in HCMV-induced citrullination. Surprisingly, viral replication rate of the HCMV is strongly impaired in the presence of a specific pan PAD-inhibitor, indicating that citrullination is required for HCMV replication. By mass- spectrometry based-analysis, we observed antiviral proteins of the IFIT and Mx1 family to be significantly citrullinated at 48 hpi, suggesting that HCMV has evolved a strategy to evade the host's immune system by their citrullination-mediated inactivation. Based on our preliminary results, we hypothesize that altering HCMV-induced viral and /or cellular protein PTMs, which we show for the first time to be essential for HCMV replication, might represent an alternative