Herpes simplex virus type 1 and *Chlamydia pneumoniae* infection of astrocytes: The effects of co-infection on pathogen replication

Syed Jaffery, Fiora Zoga, Christine Hammond, Brian Balin, Denah Appelt and Susan Hingley

Center for Chronic Disorders of Aging, Philadelphia College of Osteopathic Medicine

Abstract

Background and Significance: *Chlamydia pneumoniae* (Cpn) and Herpes simplex virus type 1 (HSV-1) have been studied as pathogens contributing to neurodegenerative diseases. Since Cpn and HSV-1 are both ubiquitous, many individuals presumably are exposed to both pathogens during their life time. We speculate that the two pathogens act synergistically in promoting neuropathology.

Objectives: Determine whether Cpn and HSV-1 can co-infect cells and whether HSV-1 induces persistence of Cpn, thereby favoring chronic infection by Cpn.

Methods: The ability of Cpn and HSV-1 to co-infect an astrocyte cell line was analyzed by immunofluorescent (IF) labeling of infected cells. The relative levels of Cpn or HSV in different samples was quantified by RT-PCR; expression of various Cpn genes associated with stages of Cpn development was quantified by RT^2-PCR. DNA or RNA was derived from cells infected with Cpn alone, HSV alone, or infected with Cpn and HSV-1.

Results: IF revealed that cells could be infected with both Cpn and HSV-1. When infection with HSV-1 is established prior to addition of Cpn, cells demonstrated punctate labeling for Cpn but the frequency of larger aggregates of labeling, consistent with chlamydial inclusions, was diminished. Data reveal that replication of HSV-1 is lessened but not prevented by Cpn, while Cpn replication does not appear to be inhibited by HSV-1.

Conclusions: Inhibition of HSV-1 by Cpn may reflect competition for the same cellular receptor. Whereas disruption of host cell transcription by HSV-1 may modify normal Cpn development, Cpn is able to replicate in the presence of HSV-1.

Introduction

Our ongoing studies investigate the potential role of herpes simplex virus type 1 (HSV-1) and *Chlamydia pneumoniae* (Cpn) in the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease. Since both HSV-1 and Cpn are ubiquitous and have the ability to persist in a latent state, with the potential for reactivation to a productive infection, it is reasonable to postulate that infection by either or both pathogens might contribute to the gradual accumulation of pathology associated with neurodegeneration. This current study examines whether an astrocyte cell line can be simultaneously infected by these two pathogens and if co-infection alters pathogen replication and/or cellular pathology associated with infection by either pathogen individually.

It is known that both HSV-1 and Cpn can utilize heparin sulfate as receptors, thus the presence of one pathogen might interfere with attachment and internalization of the second pathogen. Furthermore, Cpn enters a state of persistence in response to changes in the host cell environment and we speculate that productive HSV-1 infection might set up an intracellular environment that induces persistence in Cpn. It is possible that both Cpn and HSV-1 might cycle through periods of productive infection and latency, thereby contributing to progressive neuropathology.

To assess whether the presence of HSV-1 alters Cpn gene expression, the relative levels of 6 different Cpn genes involved in different stages of the replication cycle were examined in RNA from cells infected with Cpn alone or dually infected with Cpn and HSV-1. The genes examined are listed in Table 1; L-29, TUF, and ABCX have been designated as potential chlamydial housekeeping genes whose expression remains relatively constant throughout the replication cycle (1). In this study, we use L-29 as an endogenous control to normalize quantity of Cpn in the different samples within an experiment. Immunofluorescence and determination of viral titers provide additional methodology to further evaluate Cpn or HSV-1 infection of astrocytes.

Methods

Cell Culture: An astrocyte cell line (STTG-1; ATCC) was cultured in RPMI-1640 (RPMI) with 10% heat-inactivated FBS. Cells were maintained at 37°C in 5% CO_2. Infections were performed in 12 well plates seeded with 2 x 10^5 cells per well; astrocytes were infected when monolayers were 50-70% confluent (5-8 x 10^6 cells per well).

Infection: Astrocytes were mock infected with RPMI alone, or inoculated with *Chlamydia pneumoniae* AR-39 (Cpn; ATCC) and/or Herpes simplex virus 1 (HSV-1; ATTC) for 24 or 48 hours. The HSV inoculum was an approximate MOI of 0.2, while the Cpn inoculum was 0.7. Cpn and HSV-1 were allowed to attach to astrocyte monolayers in 0.3 ml RPMI for 1 hour, after which time the volume was maintained at 1 ml for the duration of the infection. Co-infections included the simultaneous inoculation of HSV-1 and Cpn (HSV/Cpn 48/48) or addition of the second pathogen at 24 hours post infection (hpi), which allowed one pathogen to establish infection before addition of the second one (HSV/Cpn 48/24 and HSV/Cpn 24/48). Cells from co-infected monolayers were harvested at 48 hpi. A qualitative evaluation of infection by Cpn and HSV-1 was assessed by immunofluorescent labeling for the two pathogens using antibodies specific for Cpn (Fitzgerald; 61C75-A) and HSV-1 (Abcam; ab9533). In addition, HSV-1 present in culture supernates of single and dual infections was titered by plaque assay.

Analysis of gene expression: RNA or DNA was isolated from infected and uninfected cell monolayers using Qiagen's RNeasy Mini Kit or DNA Blood & Tissue Kit, respectively, following the manufacturer's directions. RNA was reverse transcribed (Retroscript kit; Ambion), and the expression of Cpn genes and cellular actin and GAPDH was quantified from RNA by RT^2-PCR using TaqMan gene expression assays (Invitrogen/Life Technologies). The Cpn gene L-29 was used as an endogenous control to assess relative expression of Cpn genes; this gene was designated as a potential housekeeping gene for Cpn in a prior investigation (1). HSV-1 or Cpn sequences in DNA samples were quantified by RT-PCR using primers specific for HSV-1 or Cpn (PrimerDesign) following the manufacturer's recommended protocol.

Results

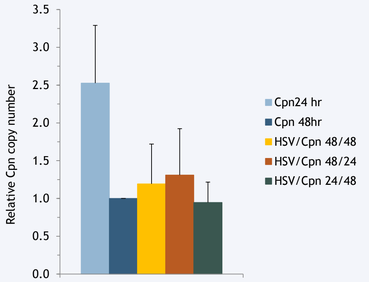

Figure 1. Quantification of Cpn from infected astrocytes in the presence or absence of HSV-1. Astrocyte monolayers were infected with Cpn alone or co-infected with Cpn and HSV-1. Cpn DNA was quantified by RT-PCR using a Cpn 16S rRNA-specific primer; copy number was calculated from a standard curve derived from amplification of a known concentration of primer-specific sequences and expressed relative to that of the 48 hour single Cpn infection. Results represent the mean and standard deviation from 4 separate experiments.

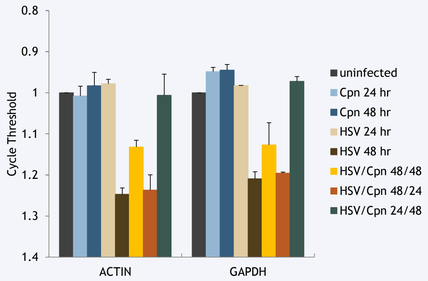

Figure 2. Amplification of host genes. RNA derived from single or dually infected astrocytes was reverse transcribed and equal amounts of cDNA were amplified with primers specific for GAPDH and actin. Levels of actin and GAPDH is inversely related to cycle thresholds; expression of these host genes is shown relative to that of uninfected samples. The longer the bar in the above plot, the greater the copy number of host gene sequences present in RNA. Results represent the mean and standard deviation from 2 separate experiments; actin data was pooled following amplification using 2 different actin-specific primers.

Figure 3. Expression of Cpn genes from infected astrocytes in the presence or absence of HSV-1. Astrocyte monolayers were infected with Cpn alone or co-infected with Cpn and HSV-1. Relative expression of Cpn genes was obtained following RT^2-PCR amplification of RNA from infected monolayers. To assess differential expression of Cpn genes in infected astrocytes, L-29 was used as an endogenous Cpn control.

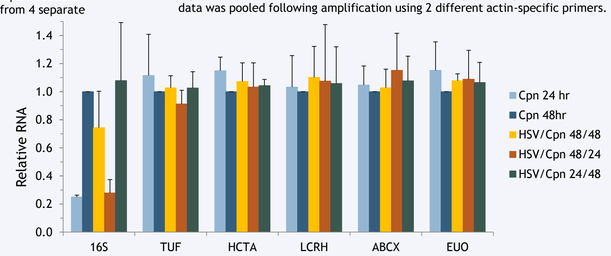

Table 1. Identification of select chlamydial genes analyzed in this study.

Gene	Gene description/function	Locus	Comment
16S	16S ribosomal RNA		Standard Curve quantification
lcrH_1	Low calcium response protein H	CPn0811	Marks transition from RB to EB
hctA	Histone-like developmental protein	CPn0886	Marks transition from RB to EB
rpmC	50S ribosomal protein L29	CPn0639	housekeeping gene candidate
tuf	Elongation factor Tu	CPn0074	housekeeping gene candidate
euo	Chlamydial repressor protein	CPj0561	Early chlamydial gene
abcX	ABC transporter ATPase	CPn0691	housekeeping gene candidate

Reference

1. Maurer AP, Mehlitz A, Mollenkopf HJ, Meyer TF: Gene expression profiles of *Chlamydophila pneumoniae* during the developmental cycle and iron depletion-mediated persistence. PLoS Pathog 2007, 3(6):e83.

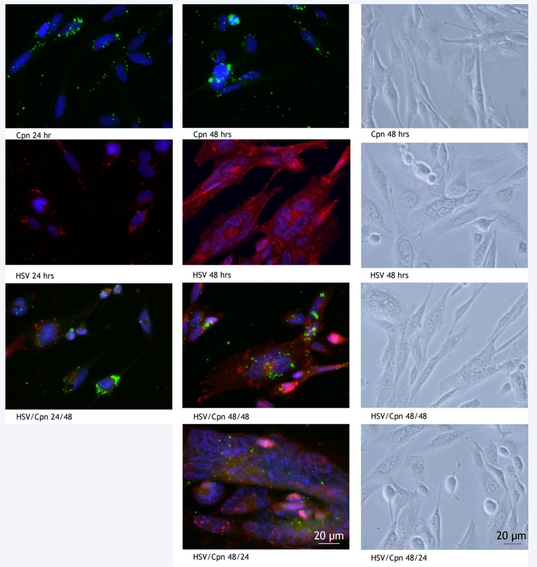

Figure 4. Immunofluorescent labeling and phase contrast microscopy of astrocytes infected with HSV-1 and/or Cpn. In the two left columns, infected astrocytes were labeled by immunofluoresence for HSV-1 (red) and Cpn (green); cell nuclei are in blue. Phase contrast microscopy (third column) revealed HSV-1-induced syncytia formation and cell rounding at 48 hpi in the presence or absence of Cpn; minimal HSV-1-induced cellular damage was noted at 24 hpi (data not shown).

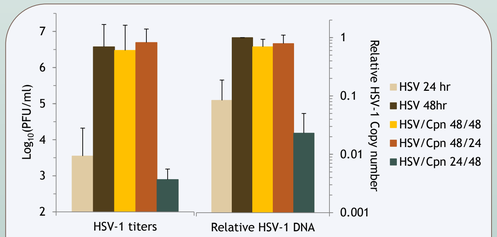

Figure 5. Quantification of HSV-1 from infected astrocytes in the presence or absence of Cpn. Astrocyte monolayers were infected with HSV-1 alone or co-infected with HSV-1 and Cpn. Viral titers in culture supernates were measured by plaque assay and expressed as the Log_{10} (Plaque Forming Units [PFU]/ml). Results represent the mean and standard deviation from 3 separate experiments. HSV DNA was quantified by RT-PCR using an HSV-1-specific primer; copy number was calculated from a standard curve derived from amplification of a known concentration of primer-specific sequences and expressed relative to that of the 48 hour single HSV-1 infection. Results represent the mean + SD from 4 separate experiments.

Results - summary

- Single or dual infections contained similar levels of HSV-1 at 48hrs post infection, however, at 24 hours post infection some inhibition of HSV-1 was observed in monolayers previously infected with Cpn
- HSV-1-induced syncytia formation was observed at 48hrs post infection in the presence or absence of Cpn
- HSV-1 inhibited host cell expression of GAPDH and Actin; extent of inhibition correlates with amount of HSV-1 present in the infected monolayers
- Immunofluorescent labeling revealed astrocytes can be simultaneously infected with both HSV-1 and Cpn
- Co-infected monolayers had similar levels of Cpn as the 48hr single infections
- Cpn genes showed similar levels of expression when normalized using a Cpn housekeeping gene

Conclusions

- The presence of Cpn may inhibit initial viral attachment and/or entry
- Cpn does not prevent HSV-1 induced cytopathology
- HSV-1 can alter gene expression in astrocytes
- The presence of one pathogen does not prevent intracellular replication of the other one
- Differential Cpn gene expression is not observed in single vs dual infections with the genes examined in this study

Funding

This work was funded by Division of Research at the Philadelphia College of Osteopathic Medicine.