Modeling Aβ42 accumulation in response to herpes simplex virus 1 infection: 2D or 3D?


1Department of Neurology, University of Pittsburgh School of Medicine, Pittsburgh, PA
2Geriatric Research Education and Clinical Center, Pittsburgh VA Healthcare System, Pittsburgh, PA, USA
3Department of Psychiatry, The Second Xiangya Hospital, Xiangya School of Medicine, Central South University, Changsha, China
4Department of Psychiatry, Western Psychiatric Institute and Clinic, University of Pittsburgh School of Medicine, Pittsburgh, PA
5Department of Molecular Genetics & Microbiology, University of Florida College of Medicine, Gainesville, FL.
6VISN 4 MIRECC, VA Pittsburgh Healthcare System, Pittsburgh, PA, USA

Running Title: HSV-1 and Aβ42 accumulation

*Address Correspondence to Leonardo D’Aiuto, Ph.D., daiutol@upmc.edu

Abstract Word Count: 141
Importance Word Count: 146
Text Word Count: 2,993
ABSTRACT

Alzheimer’s disease is a progressive neurodegenerative disease characterized neuropathologically by presence of extracellular amyloid plaques composed of fibrillar amyloid beta (Aβ) peptides and intracellular neurofibrillary tangles. Post-mortem and in vivo studies implicate HSV-1 infection in the brain as a precipitating factor in disease/pathology initiation. HSV-1 infection of two-dimensional (2D) neuronal cultures causes intracellular accumulation of Aβ42 peptide, but these 2D models do not recapitulate the three-dimensional (3D) architecture of brain tissue.

We employed human induced pluripotent stem cells (hiPSCs) to compare patterns of Aβ42 accumulation in HSV-1 infected 2D (neuronal monolayers) and 3D neuronal cultures (brain organoids). Akin to prior studies, HSV-1-infected 2D cultures showed Aβ42 immunoreactivity in cells expressing the HSV-1 antigen ICP4 (ICP4+). Conversely, accumulation of Aβ42 in ICP4+ cells in infected organoids was rarely observed. These results highlight the importance of considering 3D cultures to model host-pathogen interaction.

IMPORTANCE

The “pathogen” hypothesis of Alzheimer’s disease (AD) proposes that brain HSV-1 infection could be an initial source of amyloid beta (Aβ) peptide-containing amyloid plaque development. Aβ accumulation was reported in HSV-1-infected 2D neuronal cultures and neural stem cell cultures, as well as in HSV-1-infected 3D neuronal culture models.

The current study extends these findings by showing different patterns of Aβ42 accumulation following HSV-1 infection of 2D compared to 3D neuronal cultures (brain...
organoids). Specifically, 2D neuronal cultures showed Aβ42-immunoreactivity mainly in HSV-1-infected cells and only rarely in uninfected cells or infected cells exposed to antivirals. Conversely, 3D brain organoids showed accumulation of Aβ42 mainly in non-infected cells surrounding HSV-1-infected cells. We suggest that because brain organoids better recapitulate architectural features of a developing brain than 2D cultures, they may be a more suitable model to investigate the involvement of HSV-1 in the onset of AD pathology.

**INTRODUCTION**

Alzheimer's disease (AD) is a leading cause of dementia, yet its etiology is unknown and there is no effective therapy to prevent or arrest the disease. Primary neuropathological lesions of AD are extracellular plaques of fibrillized amyloid-β (Aβ) peptides and intracellular neurofibrillary tangles of hyperphosphorylated tau (p-tau) protein (1). Aβ plaques develop early in AD and can influence tau phosphorylation (2) and neuroinflammation (3), two processes more closely tied to cognitive impairment (4). This series of events was formalized as the amyloid cascade hypothesis of AD (5). Involvement of infective pathogens in AD pathogenesis was first proposed by Alois Alzheimer (6), and is supported by several recent studies which suggested that Aβ is an antimicrobial peptide (AMP) which may play a role in protecting neurons from infectious pathogens like bacteria and viruses (7, 8). In this regard, increased Aβ production could be a protective response to infection, but may lead to excessive Aβ accumulation and, possibly, fibrillization and deposition as amyloid plaques. Supporting this hypothesis, the neurotropic pathogen HSV-1 was detected in Aβ plaques in AD brains (9). In vitro studies using two-dimensional (2D) monolayer neuronal cultures reported that HSV-1 infection resulted in intracellular Aβ accumulation and up-regulation of β-secretase and the nicastrin component of γ-
secretase, which are required for generation of Aβ from its precursor protein\textsuperscript{(10)}. This phenomenon could be due to intermittent cycles of HSV-1 reactivation in the brain \textsuperscript{(11)}. A recent study using an \textit{in vitro} three-dimensional (3D) model of human brain also described extracellular accumulation of Aβ\textsubscript{42} following infection with HSV-1 \textsuperscript{(12)}, possibly due to HSV-1-induced Aβ overproduction/secretion and/or “seeding” of Aβ aggregation in the extracellular space by virus particles as an antimicrobial response \textsuperscript{(13)}. However, not well-understood are the mechanisms underlying altered Aβ production and accumulation during lytic HSV-1 infection or repeated cycles of viral reactivation, and how HSV-1-infected cells communicate with nearby cells to initiate this response.

We have recently reported on the use of induced pluripotent stem cells (iPSCs) to model HSV-1 acute and latent infection in 2D monolayer neuronal cultures and 3D brain organoids \textsuperscript{(14)}. These \textit{in vitro} models showed that iPSC-derived CNS neurons are permissive for HSV-1 infection, with reporter gene expression detected from both immediate early (ICP4) and strict late (gC) promoters being detected, and infectious virus being released into the medium during the acute infection period of both 2D and 3D cultures. In addition, we demonstrated that HSV-1 can establish latency in both culture systems. Perhaps the most novel finding of these studies was that 3D brain organoids, but not 2D monolayer neuronal cultures, have the ability to recapitulate the difficulty that HSV-1 has in reactivating from latency in CNS, as observed in animal models \textsuperscript{(14)}. This ability contributes to the potential superiority of 3D brain organoids to model host-pathogen interactions within the CNS.
In this study we employed iPSC-derived 2D monolayer neuronal cultures and 3D brain organoids to investigate the accumulation of Aβ42 in response to HSV-1 infection. This investigation compared the relative amount of HSV-1-induced Aβ42 in the two models following infection.

RESULTS

We initially investigated Aβ42 accumulation in 2D neuronal cultures derived from hiPSCs and acutely infected at multiplicity of infection (MOI) 1, 0.7, 0.5 and 0.3 of HSV-1, strain KOS. In order to block productive viral replication, neurons infected at MOI 0.3 were also maintained in the presence of antivirals ((E)-5-(2-bromovinyl)-2'-deoxyuridine and interferon alpha (IFN-α). Expression of an HSV-1 lytic gene, infected-cell polypeptide 4 (ICP4), and Aβ42 in uninfected and infected cells was analyzed by immunocytochemistry (ICC) at 48h post infection (p.i.) (Figure 1). Neurons in uninfected cultures showed faint, punctate Aβ42-immunoreactivity surrounding the nucleus (Figure 1a). When cultures were infected with MOI 0.3 with the antiviral 5BVdU and IFN-α, no increase in Aβ42-immunoreactivity was observed, even in the rarely observed ICP4+ cells (Figure 1b). When 2D cultures were infected with HSV-1 at MOIs of 1.0, 0.7, and 0.5 intense Aβ42 immunoreactivity was observed in approximately 19% of ICP4+ cells but not in any of uninfected cells in close proximity or distal to infected neurons (Figure 1c). Aβ42 immunoreactivity was observed surrounding and encapsulating the nucleus and consisted of dense puncta and larger globules of more dispersed Aβ42 immunoreactivity. Similar patterns were observed at MOI of 0.3 in approximately 8% of ICP4+ cells, though Aβ42 immunoreactivity was less intense (Figure 1d-f). These results, in line with other reports (10, 15), show that HSV-1 induces Aβ42 accumulation in infected monolayer cultures.
of neurons and that antiviral treatment prevents this accumulation during this experimental time frame (Figure 1b,f).

Next, we investigated the suitability of 3D neuronal cultures (i.e., brain cortical organoids) to model HSV-1 induced Aβ accumulation. Figure 2 illustrates immunohistochemical characterization of a representative non-infected 3D brain organoid at 9 weeks in vitro. Cortical brain organoids were generated from hiPSCs-derived neural rosettes and neural tube-like structures (for details regarding the differentiation procedure and characterization see the Material and Methods section).

Twelve-week old organoids (~2 mm size) were infected singularly with HSV-1 strain KOS, using 3000 PFU/organoid. The inocula were removed after 2h and organoids were analyzed at days 3 and 5 post infection. Aβ accumulation was assessed by immunohistochemistry (IHC) analysis of sections obtained from formalin-fixed, paraffin-embedded organoids. IHC showed ICP4+ neurons throughout and up to ~250 μm from the edge of the organoids (Figure 3a). HSV-1 infected organoids exposed to the antivirals 5BVdU + IFN-α had no detectable ICP4+ cells (Figure 3b) and Aβ42 immunoreactivity was comparable to uninfected organoids (Figure 3c). Contrary to what was observed in infected 2D neuronal cultures, Aβ42 immunoreactivity in ICP4+ cells was rarely observed in infected 3D organoids (Figure 3d, e). In these rare instances, Aβ42 immunoreactivity was observed mainly in areas that appeared to be between infected nuclei (Figure 3d), and perinuclear punctate Aβ42 immunoreactivity was not as robust as in 2D cultures. In rare instances, Aβ42 immunoreactivity overlapped ICP4+ particles (Figure 3f). Also, in contrast to 2D cultures, co-localization of ICP4
with large (15-20 µm in area) areas of Aβ42 immunoreactivity containing fragmented nuclei was also observed (Figure 3g-h). Overall, in infected organoids perinuclear Aβ42 immunoreactivity was predominantly detected in ICP4⁺ cells surrounding ICP4⁺ infected cells (Figure 3j-l). The robust perinuclear Aβ42 immunoreactivity in ICP4⁺ cells resembled those observed in ICP4⁺ neurons in 2D cultures and, in addition, Aβ42 was present in between nuclei, in extracellular spaces. These observations demonstrate that in the 3D brain organoid model (in contrast to 2D neuronal monolayers) Aβ42 accumulates primarily in ICP4⁺ cells.

**DISCUSSION**

The “pathogen” hypothesis of AD posits that Aβ peptides are produced as a form of antimicrobial protection (AMP) in response to pathogens invading the CNS (10, 16, 17). Specifically, HSV-1 infection could be a trigger for persistent Aβ over-production and Aβ plaque development (13), either alone or in combination with impaired Aβ clearance. Aβ exhibits antimicrobial properties (18) against a range of bacterial pathogens (19), influenza A virus (20), and herpesviruses (13), and its accumulation in AD may be a side-effect of a host innate response to microbial invasion. Among the number of neurotropic viruses that infect the brain, HSV-1 in particular has been proposed as an initial source of Aβ plaque development (13). Recent studies have shown Aβ accumulation in HSV-1-infected 2D neuronal cultures and neural stem cell cultures, which could be interpreted as a mechanism initiated to prevent virus spread (21-24). A recent study provided evidence that Aβ exerts its AMP role by mediating viral agglutination (13). Based on these observations, emerging questions are: how common is Aβ accumulation in HSV-1 infected cells and is the 2D cell monolayer model the optimal choice for studying this phenomenon? Our current immunohistochemical analysis of HSV-1 infected 2D monolayer
neuronal cultures showed high frequencies of perinuclear Aβ42 immunoreactivity in ICP4+ cells when compared to uninfected cells or infected cells exposed to antivirals 5BVdU + IFN-α. The frequency of ICP4+ cells exhibiting Aβ42 immunoreactivity was progressively lowered by reducing the inoculum to MOI 0.3. These results support the ability of HSV-1 to trigger Aβ42 accumulation. However, when we infected 3D brain organoids with HSV-1, Aβ42 immunoreactivity exhibited a pattern distinct from that observed in 2D neuronal monolayers. Specifically, Aβ42 immunoreactivity was observed primarily in ICP4− neurons, but rarely in ICP4+ neurons. A possible explanation for different patterns of Aβ42 immunoreactivity in 2D monolayers versus 3D brain organoids may be that spread of infection and Aβ42 production occur at slower rates in the latter model, providing more translational value in studies of brain pathology. It is important to consider the possibility that antiviral factors produced in infected monolayer 2D neuronal cultures are diluted out in the culture medium, whilst within brain organoids, these factors reach at least the minimal concentration required to exert their antiviral activity. Furthermore, cell-to-cell communication, which is known to be impaired in 2D cultures (25), may contribute to the differences observed between these two in vitro systems. It is also important to consider that the ICP4− cells which exhibit an increased Aβ42 immunofluorescence may represent abortively infected cells rather than uninfected cells (26). A recent study by Drayman et al on sorted cells population to investigate HSV-1 infection at single cell level showed that antiviral response is initiated in ICP4− cells (26). The antiviral genes IFIT1 and IFIT2 are specifically upregulated in the ICP4− cells. In general, interferon stimulated genes are more highly expressed in cells with low HSV-1 gene expression. Thus, it is possible that the increased Aβ42 immunofluorescence we observed in ICP4-negative cells may be the consequence of strong antiviral signaling elicited in this population of abortively infected cells. Though rarely
detected in uninfected organoids, it is possible that a fraction of cells expressing Aβ42 were present in organoids prior to HSV-1 infection and were ICP4+ due to the peptide’s AMP activity.

In summary, we demonstrate that altered Aβ immunoreactivity due to HSV-1 infection differs in 3D brain organoids compared to 2D neuronal monolayers. In brain organoids, this response is associated with Aβ42 immunoreactivity in ICP4+ cells. This differs from 2D monolayer cultures where HSV-1 infection leads to Aβ42 accumulation primarily in infected ICP4+ cells. Because 3D brain organoids of human iPSC recapitulate the 3D architecture of brain, they may be a more suitable model to study the interaction of neuronal HSV-1 infection or repeated cycles of viral reactivation with Aβ alterations in the context of a human neurodegenerative disease. Furthermore, brain organoids may provide critical insight into the mechanisms underlying the communication between HSV-1-infected cells and other cells in the same neuronal network which can initiate Aβ pathology. Clarifying the role of HSV-1 in AD pathogenesis could lead to antiviral prophylactic or intervention trials, a novel approach supported by a recent report of lower dementia risk associated with anti-herpetic medication for HSV-1 (27).

METHODS

Cell lines

Two hiPSC lines, 73-56010-01 SA and 73–56010-02, were employed in this study. Both hiPSC lines were generated from fibroblasts derived from skin biopsy samples that were collected from a healthy volunteer via 4-mm full thickness punch biopsies under local anesthesia. All identifying information pertaining to the healthy volunteer was removed and the hiPSCs
were established at the National Institute of Mental Health (NIMH) Center for Collaborative Studies of Mental Disorders-funded Rutgers University Cell and DNA Repository (http://www.rucdr.org/mental-health) (RUCDR). All cells were cultured in standard conditions (37°C, 5% CO₂, and 100% humidity).

**Generation of 2D neuronal cultures.**

The NPCs were derived from iPSCs as previously described (28) and cultured in Neurobasal medium supplemented with 2% B27 and BDNF 10 ng/ml for 6 weeks. Half of the culture medium was changed every other day.

**Generation of 3D neuronal cultures (brain organoids).**

HiPSCs were cultured in neural progenitor selection medium including dual SMAD inhibitors SB431542 (10 µM) and LDN193189 (100 nM) (NPS/Dual-SMAD medium). After 4-5 days in NPS/Dual-SMAD, neural rosettes (250 to 500 µm in diameter) were dissected manually and cultured in NPS/Dual-SMAD medium in ultra-low attachment plates for another 2-3 days. Neural rosettes were then re-plated to Matrigel-coated plates. After overnight incubation, the aggregates containing 1-4 neural rosettes were then isolated manually, transferred into 10-cm² petri dishes and cultured in cortical organoid differentiation medium I (DMEM:F12/Neurobasal (1:1 v/v) supplemented with 1X Glutamax, 1X B-27 (VitA[-]), 0.5X Non-essential amino acids, 0.5X N-2, Insulin (2.5 µg) and 1X penicillin-streptomycin (P/S) on an orbital shaker. One week later, the organoids were transferred into cortical organoid differentiation medium II (DMEM:F12-Neurobasal (1:1 v/v) supplemented with 1X glutamax, 1X B-27 (VitA[+]), 0.5X Non-essential amino acids, 0.5X N-2, Insulin (2.5 µg), BDNF (10
Early-stage neocortical organoids (day 33) exhibited a mosaic of ventricular zone (VZ)-like structures most of them containing a single central ventricle-like cavity (Figure 2a). The forebrain identity of the developing organoids was supported by their expression of FOXG1 (Figure 2b). Neural progenitor cells in the VZ-like structures expressed sex determining region Y-box 2 (SOX2, Figure 2c). Immunostaining for vimentin (a marker of radial glial cells) in the VZ-like regions was also observed (Figure 2d). The intermediate progenitor cells in the VZ-subventricular zone (SVZ)-like regions were highlighted by the expression of T-box brain protein 2 (TBR2) (Figure 2e). Cut-like homeobox 2 (Cux2)-immunoreactive neural precursor cells (Figure 2f), which are found in the SVZ and the intermediate zone during early stages of brain development and differentiate into upper layer neurons, were observed (Fig. 2f). Neuronal differentiation in the cortical organoids is shown by the expression of the neuronal markers neuron-specific class III beta-tubulin (TUJ1) and microtubule-associated protein 2 (MAP2) (Figure 2f and 3a). Immunohistochemistry for deep cortical layers markers T-box brain protein 1 (TBR1), calbindin, and chicken ovalbumin upstream promoter transcription factor interacting protein 2 (CTIP2) (Figure 1g-i), and the superficial layers neurons expressing special AT-rich sequence-binding protein 2 (SATB2, Figure 1j) were observed. Overall, these results indicate that the organoids employed in this study exhibit architectural features of a developing brain.

Viral infections. HSV-1 strain KOS (VR-1493; ATCC) was employed in this study.

Infection of two-dimensional (2D) neuronal cultures.
For lytic infections, cell-free virus was adsorbed onto monolayer cultures of hiPSC-derived neurons at a range of multiplicities of infections (MOIs) from 1-0.3. One hour after the infection, the inocula were then removed, cells washed twice with DMEM-F12 medium, and cultured with neurobasal medium for 48 hours. For latent infections, cells were preincubated with 5BVdU + IFN-α. After 24 hours, cells were infected at MOI 0.3 and cultured with neurobasal medium in the presence of 5BVdU + IFN-α for 48 hours.

Infection of brain organoids.

Brain organoids were transferred singularly in 1.5 ml Eppendorf tubes and washed 500 µl of DMEM-F12 medium. The medium was then discarded, 50 µl of neurobasal medium with or without 5BVdU + IFN-α containing 3000 pfu of HSV-1 were added. After perforating the cap Eppendorf tubes using a 20-gauge sterile needle, the organoids were cultured in an incubator under standard conditions (5% CO₂, 37 ºC, and 100% humidity). To inhibit viral replication, the organoids were pre-treated with 5BVdU + IFN-α for 24 hours. Two hours after the infection, the inoculum was removed, the organoids washed twice with 500 µl of DMEM-F12 medium, and cultured cortical medium II in the presence or the absence of 5BVdU + IFN-α in 6-cm petri dishes on an orbital shaker. Infected organoids were prepared for immunohistochemistry analysis at days 3 and 5 post-infection.

Immunofluorescence.

The 2D neuronal cultures were fixed with 4% paraformaldehyde and permeabilized with 0.2% Triton-X before immunostaining. The paraffin-embedded slices of organoids were prepared as follows: the organoids were rinsed in PBS, and fixed by immersing in at least ten
volumes of 10% formalin overnight at 4° C. The organoids were again rinsed, and then embedded
in blocks of low melting point agarose. The agarose blocks were embedded in paraffin wax
following a standard protocol for formalin-fixed tissue, then sectioned to 5 µm for subsequent
staining. Before staining, paraffin sections were incubated at 60° C, dewaxed in xylene, hydrated
in absolute ethanol, 95%, and 70% ethanol and rinsed in pure water. Antigen unmasking was
performed by exposing paraffin sections to Antigen retrieval Citra Solution (Biogenex) at 95° C.
Paraffin sections were incubated with SuperBlock™ Blocking Buffer (Thermo Scientific) before
immunostaining. Paraffin sections were also stained with hematoxylin and eosin reagents.

Samples were incubated with primary antibodies overnight at 4 °C. Primary antibodies
used were mouse monoclonal anti-β-tubulin III antibody (conjugated clone TUJ1, R and D
Systems Cat# NL1195V, 1:100 dilution), mouse monoclonal anti-MAP2 antibody (Millipore
Cat# AB5622, 1:500 dilution), rabbit polyclonal anti-calbindin antibody (Abcam Cat#
AB11426, 1:400 dilution), rabbit polyclonal anti-vimentin antibody (Abcam Cat# AB45939,
1:500 dilution), rabbit polyclonal anti-CUX-2 antibody (Abcam Cat# AB130395, dilution
1:200), mouse monoclonal anti-HSV-1 ICP4 antibody (Abcam Cat# AB6514, dilution 1:200),
rabbit polyclonal anti Aβ42 antibody (Millipore, Cat# AB5078P, 1:500 dilution), rabbit
polyclonal anti-FOXG1 antibody (Abcam, Cat# 18259, 1:1000 dilution), rat monoclonal anti-
CTIP2 antibody (Abcam, Cat# AB18465, 1:500 dilution), rabbit polyclonal anti-TBR1 antibody
(ABcam, Cat# AB31940, 1:500 dilution), chicken polyclonal anti-TBR2 antibody (Millipore,
Cat# AB15894, 1:500 dilution), and rabbit polyclonal anti-SATB2 antibody (Abcam, Cat#
AB34735, 1:1000 dilution).
The following fluorophor-conjugated secondary antibodies were used to detect bound primary antibodies: Alexa Fluor 488 goat anti-rabbit (Thermo Fisher Scientific Cat# 1:300 dilution), Alexa Fluor 488 goat anti-mouse (Thermo Fisher Scientific Cat# A-10680, 1:300 dilution), Alexa Fluor 594 goat anti-rabbit (Thermo Fisher Scientific Cat# A-11012, 1:300 dilution), Alexa Fluor 594 goat anti-mouse secondary antibody (Thermo Fisher Scientific Cat# A-11005, 1:300 dilution), Alexa Fluor 488 goat anti-chicken secondary antibody (Thermo Fisher Scientific Cat# A-11039, 1:300 dilution), and Alexa Fluor 488 goat anti-rat (Jackson ImmunoResearch Labs Cat# 711-545-152, 1:300 dilution). A Leica IL MD LED inverted fluorescence microscope was used for image acquisition.

ACKNOWLEDGEMENTS

The work in this manuscript has been supported in part by the following: Stanley Medical Research Institute (07R-1712), NINDS (R01NS115082 and R21NS096405), NIA (P01AG014449, P01AG025204, R01AG052528) and the Veterans Health Administration (I01RX000952 and I01RX001778). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, the Department of Veterans Affairs, or the United States Government.
Figure 1. Immunofluorescence for amyloid-β42 and the HSV-1 antigen ICP4 in uninfected and HSV-1-infected hiPSC-derived neurons. hiPSC-derived neurons were infected at MOIs 1-0.3. Neurons were also infected at MOI 0.3 in the presence of antivirals 5BvdU + IFN-α. (a-f) Microphotographs depicting immunostaining of Aβ42 and the HSV-1 antigen ICP4 in uninfected neurons (a), neurons infected at MOI 0.3 in the presence of 5BvdU + IFN-α (b), and neurons acutely infected at MOIs 1-0.3 (c-f). Scale bar is 25 μm in (a) and (c, right panel), 10 μm in (b), (c, left panel), (d, left panel), (e) and (f) Quantification of Aβ42 immunofluorescence intensity using ImageJ. The increase in Aβ42 fluorescence intensity in infected neurons when compared to uninfected neurons was assessed using Student’s t-test. Error bars represent standard deviations.

Figure 2. Characterization of brain organoids (a) Hematoxylin and eosin staining of 9-weeks old organoid. (b-j) Immunostaining of 8 microns thick sections of organoids with antibodies recognizing FOXG1 (b), SOX2 (c), Vimentin (d), TBR2 (e), (TuJ/CUX2). TBR1 (g), calbindin (h), CTIP2 (i), CTIP2/SATB2 (j). Nuclei were counterstained with Hoechst 33342. Scale bar is 100 μm in (a), 75 μm in (b), (d), and (j), 50 μm in (c) (e) and 250 μm in (f), (g), (h) and (i).

Figure 3. Immunofluorescence for amyloid-β42 and the HSV-1 antigen ICP4 in uninfected and HSV-1-infected brain organoids. Twelve-week-old organoids were infected singularly with 3000 pfu in the presence or absence of antivirals 5BvdU + IFN-α. After 2 hours, the inocula were removed and the organoids were cultured in the presence or absence of antivirals. After 72 hours the organoids were processed for immunofluorescence. (a) Microphotographs of 8 microns thick sections of HSV-1 infected organoid immunostained with ICP4/MAP2, and (b) uninfected, and infected organoids in the presence (c) or absence of antivirals (d-l) with
ICP4/Aβ42. Nuclei were counterstained with Hoechst 33342. ICP4⁺ cells exhibiting intracellular Aβ42 immunoreactivity are indicated by arrows. The insets contain enlarged details. Scale bar is 250 µm in (a), 25 µm in (b), (c), (d), (e), (g), and (i), and 10 µm in (f), (i), (j), and (k).

REFERENCES


27. Tzeng NS, Chung CH, Lin FH, Chiang CP, Yeh CB, Huang SY, Lu RB, Chang HA, Kao YC, Yeh HW, Chiang WS, Chou YC, Tsao CH, Wu YF, Chien WC. 2018. Anti-herpetic Medications and Reduced Risk of Dementia in Patients with Herpes Simplex Virus
