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Short Communication

The helicase-primase inhibitor BAY 57–1293 reduces the Alzheimer's disease-related molecules induced by herpes simplex virus type 1

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ABSTRACT

Herpes simplex virus type 1 (HSV1) infection of cultured cells causes the formation of β-amyloid (Aβ) and abnormal tau (P-tau). These molecules comprise the main components of the abnormal protein deposits, amyloid plaques and neurofibrillary tangles, respectively, in Alzheimer's disease (AD) brains, and they have been implicated in disease development. The formation of P-tau, but not of Aβ, depends on viral DNA replication, but nonetheless, three antiviral agents that inhibit HSV1 DNA replication, including acyclovir (ACV), were found to reduce greatly the level of Aβ as well as P-tau, the former probably through prevention of viral spread. Previous studies showed that HSV1 DNA is present and is active in the brain of many elderly people, including AD patients, and that in combination with the type 4 allele of the apolipoprotein E gene, it is likely to play a role in the disease, perhaps via Aβ and P-tau production. With the aim of finding the most suitable antiviral for inhibiting Aβ and P-tau formation as well as HSV1 DNA replication, for future use in a clinical trial for treating AD, we compared the efficacy of ACV with that of another antiviral, BAY 57–1293, which acts by a different mechanism from ACV. We found that BAY 57–1293 is more efficient than ACV not only in inhibiting HSV1 replication, confirming previous studies, but also in decreasing Aβ and P-tau formation. Also, the cell clusters that are formed during infection are reduced in size much more efficiently by BAY 57–1293 than by ACV. These data suggest that BAY 57–1293 would be a more effective agent than ACV for treating AD.

Herpes simplex virus type 1 (HSV1) causes a variety of disorders, such as cold sores, genital herpes, and herpes simplex encephalitis. Alzheimer's disease (AD) might be another condition that could be added to this list since HSV1 has been implicated as a cause of this severe neuropsychiatric disorder. HSV1 is present (Jamieson et al., 1991) and is active (Wozniak et al., 2005) in the brains of a high proportion of elderly people and it is a risk factor for AD when present in the brains of people who possess a specific genetic factor, the type 4 allele of the apolipoprotein E gene (Itzhaki et al., 1997). Further, there is evidence that the virus might be responsible for the abnormal protein deposits – amyloid plaques and neurofibrillary tangles – thought to be central to disease pathogenesis, namely: (1), in brains of AD patients, the HSV1 DNA is located very specifically within amyloid plaques (Wozniak et al., 2009b); (2) in cell cultures HSV1 causes production of the main components of plaques and tangles – respectively β-amyloid (Aβ) (Piacentini et al., 2011; Santana et al., 2012; Wozniak et al., 2007) and abnormally phosphorylated tau (P-tau) (Lerchundi et al., 2011; Wozniak et al., 2009a; Zambrano et al., 2008); and (3) it causes Aβ accumulation in the brains of infected mice (Wozniak et al., 2007). These findings support a causative role for HSV1 in AD.

Acyclovir (ACV) is an inhibitor of HSV1 and is used for treatment of HSV1 infections in the clinic. Therefore, ACV might be effective at slowing the progression of AD. However, we initially considered that as ACV is an inhibitor of HSV1 DNA replication, it would be successful only if Aβ and P-tau accumulation were replication-dependent. We therefore investigated the stage of the virus replication cycle at which Aβ and P-tau were produced in infected cell cultures, using ACV and various recombinant strains of HSV1 (Wozniak et al., 2011). We found that P-tau production depended on HSV1 DNA replication, whereas that of Aβ depended on an earlier event in the virus replication cycle, after virus entry. Nonetheless, ACV reduced not only the HSV1-induced accumulation of P-tau but also that of Aβ, and we attributed the latter to the decrease in HSV1 DNA replication causing decreased viral production and hence decreased cell-to-cell spread of virus.

These data support the usage of ACV and other, even more effective antivirals for treating AD. One such agent is BAY 57–1293, which targets a different stage of viral DNA replication – the helicase-primase complex. This complex unwinds the double-stranded viral DNA and synthesises oligoribonucleotide primers

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for DNA synthesis by the viral DNA polymerase. Thus it functions at the viral replication fork. BAY 57–1293 was found to be active against HSV1 mutants, and to be nearly two orders of magnitude more potent than ACV *in vitro*, as judged by cytopathogenicity and plaque reduction assays, and even up to 6–10 h delayed addition of the drug post-infection scarcely affected its efficacy (Kleymann et al., 2002). As for *in vivo* assays, oral administration of BAY 57–1293 was found to be ten times more potent than valacyclovir (the prodrug of ACV) in mice in a lethal challenge model, both for HSV1 and for HSV2. In addition, BAY 57–1293 was effective in a model of cutaneous infection even if treatment was delayed after onset of disease (Betz et al., 2002; Kleymann et al., 2002). Further, BAY 57–1293 reduced the frequency and severity of recurrent disease (Kaufman et al., 2008; Kleymann et al.,

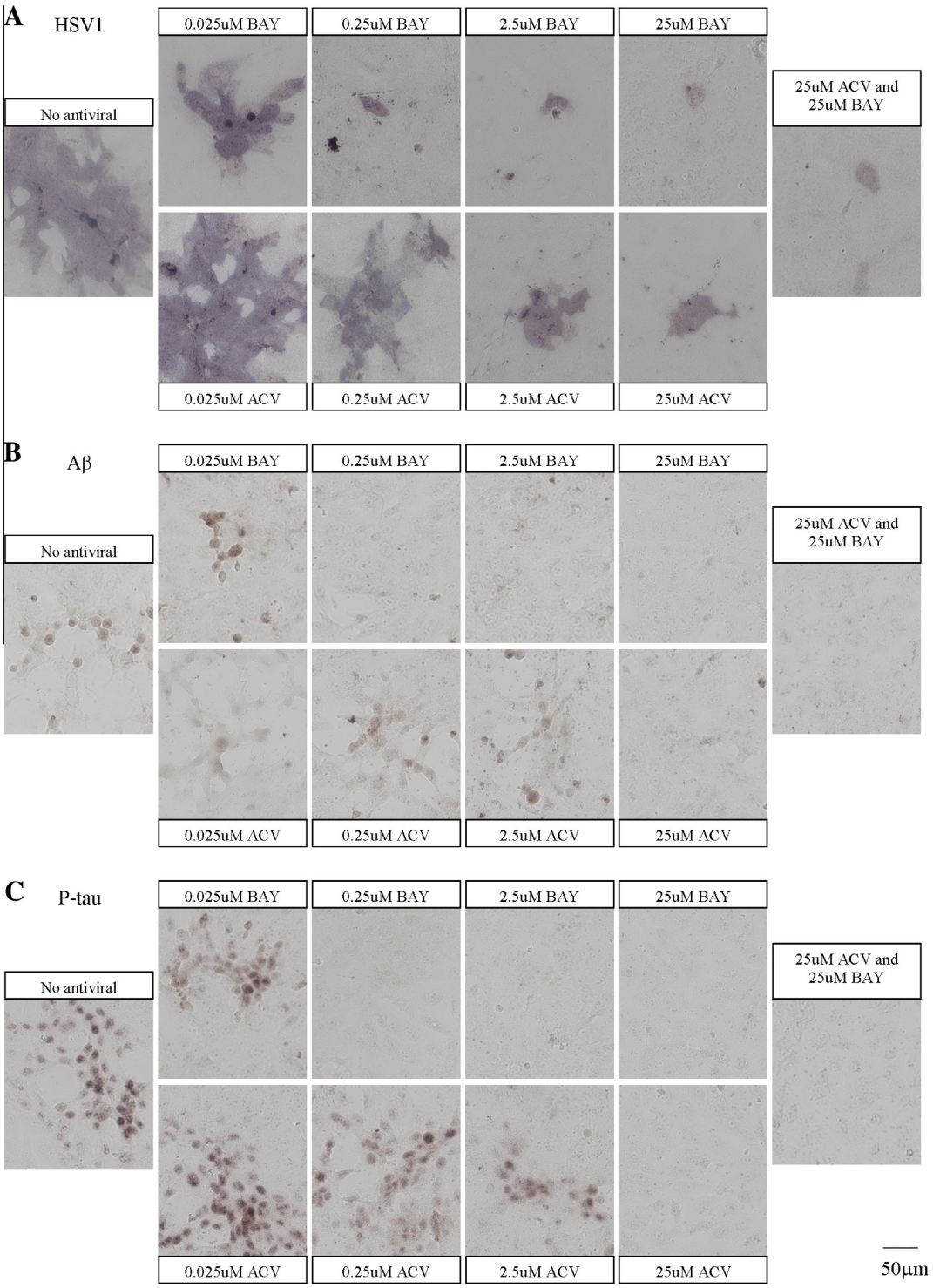

Fig. 1. BAY 57–1293 is more effective than ACV at reducing the Aβ and P-tau produced during low-level HSV1 infection. Vero cells were infected with HSV1 at 0.01 pfu/cell for 24 h and treated with varying concentrations of ACV and/or BAY 57–1293. Subsequently, cells were fixed and examined for (A) HSV1 proteins, (B) Aβ1–42 and (C) P-tau (using the AT100 antibody), as previously described (PLoS One). The results clearly show that BAY-1293 and ACV are effective at reducing all staining and that the concentrations required for the reduction are lower for BAY 57–1293 compared with ACV.

2002). The efficacy against HSV1 in mice was confirmed by Biswas et al., who showed also that BAY 57–1293 was superior to famciclovir (the prodrug of another nucleosidic antiviral, penciclovir) and was potent even in athymic mice, which are severely immunocompromised (Biswas et al., 2007).

In the present study, we have compared the efficacy of BAY 57–1293 with that of ACV in respect to Aβ and P-tau production. Initially we examined the effect of BAY 57–1293 on viral replication and spread by infecting African green monkey kidney (Vero) cells with HSV1 at 0.01 plaque forming units (pfu)/cell for 24 h. These conditions led to clusters of infected cells which were clearly visible when stained by immunocytochemistry for HSV1 proteins. Treatment with BAY 57–1293 reduced the cluster size, i.e., number of stained cells in clusters: at 0.025 μM, clusters were slightly smaller but at higher BAY 57–1293 concentrations (0.25 μM, 2.5 μM and 25 μM), they were much smaller, containing only one or two infected cells (Fig. 1A). Consistently, staining for Aβ and for P-tau was reduced at all concentrations of BAY 57–1293

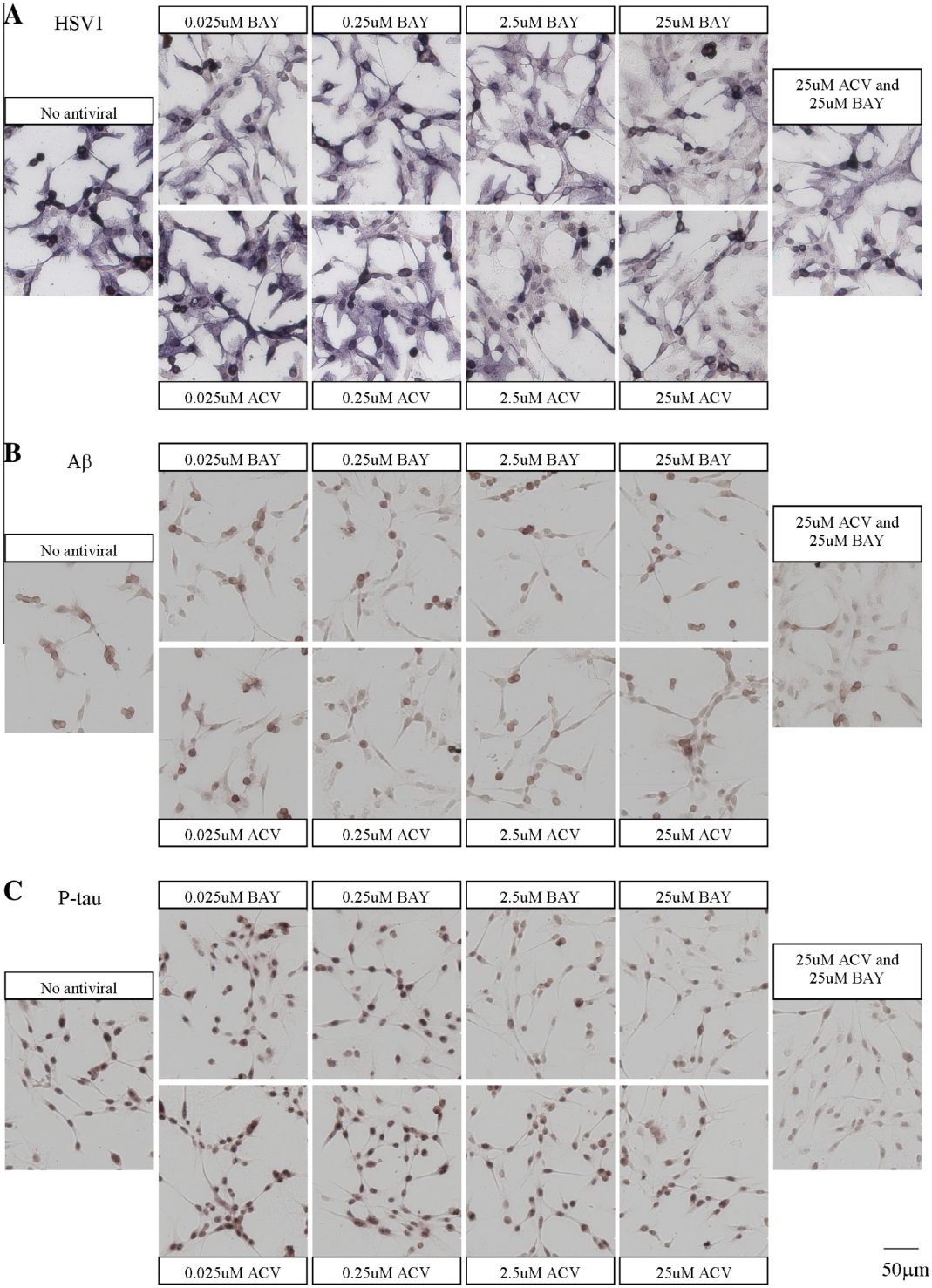

Fig. 2. BAY 57–1293 and ACV have little effect on HSV1 proteins, Aβ and P-tau produced during high-level HSV1 infection. Vero cells were infected with HSV1 at 10 pfu/cell for 8 h and treated with varying concentrations of ACV and/or BAY 57–1293. Subsequently, cells were fixed and examined for (A) HSV1 proteins, (B) Aβ1–42 and (C) P-tau (using the AT100 antibody), as previously described (PLoS One).

(Fig. 1B and C). No staining was observed in mock-infected cells (data not shown). Treatment with equimolar concentrations of ACV also resulted in smaller clusters of infected cells but the effect was not as great as with BAY 57–1293: 0.025 μM ACV had little effect on cluster sizes whereas higher ACV concentrations (0.25 μM, 2.5 μM and 25 μM) reduced their sizes but less so than did equimolar BAY 57–1293. As for Aβ and P-tau, at low concentrations of ACV, staining of these molecules was still visible, i.e., their level was reduced far less than by similar concentrations of BAY 57–129; however both of the antivirals at 25 μM reduced Aβ and P-tau levels totally. These results are consistent with our previous data which showed that ACV reduced Aβ and P-tau staining in Vero cells infected with HSV1 at 1pfu/cell for 24 h (Wozniak et al., 2011).

Subsequently, we compared the effect of BAY 57–1293 and ACV on a higher dose of HSV1, namely, 10 pfu/cell for 8 h. We chose these conditions specifically in order to reduce viral spread after HSV1 replication which, during this period, would be only a single round, so that the antivirals would interfere only with the initial viral DNA replication stage and not with viral spread. Thus, if at this high viral dose the levels of Aβ were unaffected by BAY 57–1293 or ACV, it would support the supposition that at low HSV1 doses, the ACV-induced decrease in Aβ occurs through reduced viral spread (Wozniak et al., 2011).

We found that most cells showed strong staining for HSV1 proteins, indicating that most were infected. However, treatment with BAY 57–1293 at 25 μM did not reduce staining for HSV1 proteins or Aβ although there was a small effect on P-tau staining (Fig. 2). ACV too had no effect on staining for HSV1 proteins, Aβ or P-tau, even at 25 μM. The combination of BAY 57–1293 and ACV had no effect on staining for HSV1 proteins or Aβ but there was a small effect on P-tau staining, although it was not greater than the effect of BAY 57–1293 alone. Again no staining was observed in mock-infected cells (data not shown). The lack of effect of the antiviral agents could be explained if the proteins we detect (Aβ, P-tau and some HSV1 proteins) were replication-independent. However, our previous study on ACV showed that P-tau is replication-dependent, and consistently, BAY 57–1293 causes a decrease – even though slight – in HSV1-induced P-tau. We cannot discount the possibility that at a very high HSV1 dose, the lack of a decrease in HSV1 proteins and in Aβ reflects respectively a partial or total independence of HSV1 replication, but a much more likely explanation is that BAY 57–1293 like ACV (Harmemberg et al., 1980, 1985), is unable to inhibit HSV1 at such artificially high infectious doses.

We have suggested that HSV1 periodically reactivates in human brain and that in people with the type 4 allele of the apolipoprotein E gene this leads to AD (Wozniak and Itzhaki, 2010). We proposed also that reactivation probably results in low levels of HSV1 infection, much closer to 0.01 pfu/cell than to 10 pfu/cell, and so our results with the former dose of HSV1 are more likely to be relevant to AD. Therefore, provided that the passage of BAY 57–1293 across the blood–brain barrier is at least equal to that of ACV, our current findings with HSV1 infection at 0.01 pfu/cell suggest that BAY 57–1293 might be more effective than ACV if used as a treatment for AD.

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