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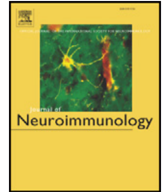

Intravenous immunoglobulin reduces beta amyloid and abnormal tau formation caused by herpes simplex virus type 1

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Intravenous immunoglobulin (IVIG) treatment of Alzheimer's disease (AD) has been encouraging. Its mechanism of action might be via anti-β-amyloid (Aβ) antibodies which facilitate Aβ clearance. However, IVIG's benefits might result from its antiviral activity, particularly against herpes simplex virus type 1 (HSV1), a virus implicated in AD. We investigated IVIG's effect on HSV1, specifically on the accumulation of Aβ and abnormally phosphorylated tau which it causes. We show that IVIG is effective at reducing the accumulation of these abnormal molecules and that it acts synergistically with the antiviral acyclovir, suggesting that their combined use would be beneficial for treating AD.

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1. Introduction

Intravenous immunoglobulin (IVIG) is a therapeutic product derived from the pooled plasma of thousands of people. It is used to treat immune-deficient patients who have reduced antibody levels. IVIG has also been tried as a treatment for Alzheimer's disease (AD) (Devi et al., 2008; Fillit et al., 2009; Relkin et al., 2009), the rationale being that it contains antibodies against the toxic protein β-amyloid (Aβ), the main component of amyloid plaques, which are a hallmark feature of AD. IVIG is thus thought to augment the presumed relatively low level of the patients' Aβ antibodies, thereby enhancing Aβ clearance. However, although treatment led to some encouraging preliminary effects on cognitive function (Devi et al., 2008; Relkin et al., 2009), the usage of monoclonal anti-Aβ antibodies for treatment has not yet done so. One explanation is that the treatment was given at too late a stage in the disease (therefore to investigate this, new trials are being set up which will initiate treatment at a much earlier stage). Alternatively, in the positive preliminary studies, IVIG might have affected some factor other than Aβ, perhaps through its antiviral properties. IVIG contains large amounts of neutralising antibodies to many microbes and so in AD patients it might have inhibited the action of an infectious agent.

Herpes simplex virus type 1 (HSV1), a microbe against which IVIG is known to have antiviral action (Erlich and Mills, 1986; Kohl and Loo, 1986; Masci et al., 1995), has been implicated as a cause of AD. The first relevant finding was our discovery that HSV1 DNA is present in a high proportion of AD patients and aged normals (Jamieson et al., 1991, 1992) and that when in brain of APOE-ε4 carriers it confers a strong risk for AD (Itzhaki et al., 1997; Lin et al., 1998). Later, we detected intrathecal antibodies to HSV in aged normals and AD patients (Wozniak et al., 2005), confirming HSV1 presence in brain and indicating that the virus had been actively replicating — and hence likely causing damage. We then found that HSV1-infection of cultured neural cells causes a large increase in Aβ, and that Aβ deposition occurs in brains of HSV1-infected mice (Wozniak et al., 2007); also, in HSV1-infected neural cell cultures, abnormally phosphorylated tau (P-tau), the main component of neurofibrillary tangles, is formed (Wozniak et al., 2009a). Recent data by others support these results in showing similar effects of HSV1 on Aβ and P-tau (see (Alvarez et al., 2012) and references within (Wozniak et al., 2011)). A further major discovery was that in AD brains, HSV1 DNA localises very specifically to amyloid plaques (Wozniak et al., 2009b). The latter result, together with the fact that HSV1 causes Aβ production, suggests that HSV1 is a major factor in the formation of toxic Aβ products and amyloid plaques.

These data point to the use of antiviral agents to stop the progression of AD. The main antiviral agent used to combat HSV1 is acyclovir (ACV); however, antivirals such as ACV that target HSV1 DNA replication might be successful only if Aβ and P-tau accumulations depend on viral DNA replication, prompting us to investigate ACV effects on Aβ and P-tau in HSV1-infected cells. We found that P-tau accumulation does depend on HSV1 DNA replication whereas Aβ accumulation does not, but treatment of HSV1-infected cell cultures with ACV greatly reduces both products (Wozniak et al., 2011),

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P-tau through the decreased replication and Aβ through the decreased production of new viruses and hence decreased viral spread. However, ACV did not reduce Aβ to control levels, suggesting that antivirals which act prior to viral DNA replication might be preferable for treating AD. IVIG is a possibility as it can neutralise extracellular virus and also help (in conjunction with lymphocytes) to destroy cells acutely infected with HSV1 (Kohl and Loo, 1986) — a useful feature as the virus can be transferred from cell to cell without the release of extracellular virus. In the present preliminary study we therefore aimed to find if IVIG reduces the production of Aβ and of P-tau, to compare any such reductions with those produced by ACV, and to find the combined effect of the two agents, using infected cell cultures.

2. Methods

2.1. Viruses, cells and antiviral agents

We used HSV1 strain SC16 prepared as previously described (Dobson et al., 2006). African Green Monkey kidney (Vero) cells were cultured as previously described (Wozniak et al., 2011). ACV was purchased from Sigma-Aldrich and IVIG (Privigen) was provided by CSL Behring.

Vero cells were cultured into wells of 24-well plates at 80% confluency and left overnight to settle. HSV1 infection was carried out either at a dose of 20 plaque-forming units (pfu/cell), incubating the cells for 7 h, or at a dose of 0.01 pfu/cell, incubating for 23 h. ACV or Privigen, or in the case of the low dose, long incubation experiments, ACV and Privigen together, were added concurrently with the virus suspension. After the incubation, the cells were fixed in PBS containing 4% formaldehyde and 10% acetic acid.

2.2. Immunocytochemistry

Cells were washed twice in Tris-buffered saline (TBS) for 5 min and then twice in TBS containing 0.025% Triton X-100 (TBS-Tx) (each wash lasting 5 min), before being blocked for 1 h at room temperature in TBS containing 10% goat serum. After two five-minute washes in TBS-Tx, primary antibody was applied. Slides were incubated overnight at 4 °C, washed in TBS-Tx and biotinylated secondary antibody was added for 1 h. Subsequently, the slides were washed in TBS-Tx and then treated with an extravidin–alkaline phosphatase conjugate for 30 min before being washed in TBS. Substrate (BCIP/NBT) was then added until sufficient staining had developed. The primary and secondary antibodies have been described previously (Wozniak et al., 2011).

2.3. Quantification of results

We quantified the ICC staining using the image analysis software Image J, which we previously validated for HSV1 using standard virological methods — HSV1 ELISA and plaque reduction assays (Wozniak et al., 2011). For each antibody we analysed three fields of view, measuring the total staining in the field. With Aβ, presence of Privigen resulted in background staining; to compensate for the latter, values obtained for mock-infected cells were subtracted.

To examine the effects on viral spread, we used low HSV1 doses for relatively long duration; this resulted in the formation of clusters of cells of size up to a few hundred cells per area. To assess the effect of ACV and Privigen on the numbers of cluster, numbers were counted in four fields of view, and the average number was determined. Student's *T*-test was used to determine statistically significant differences between the quantitative ICC values. Student's *T*-test was used also to determine difference in the number of clusters. In each case,

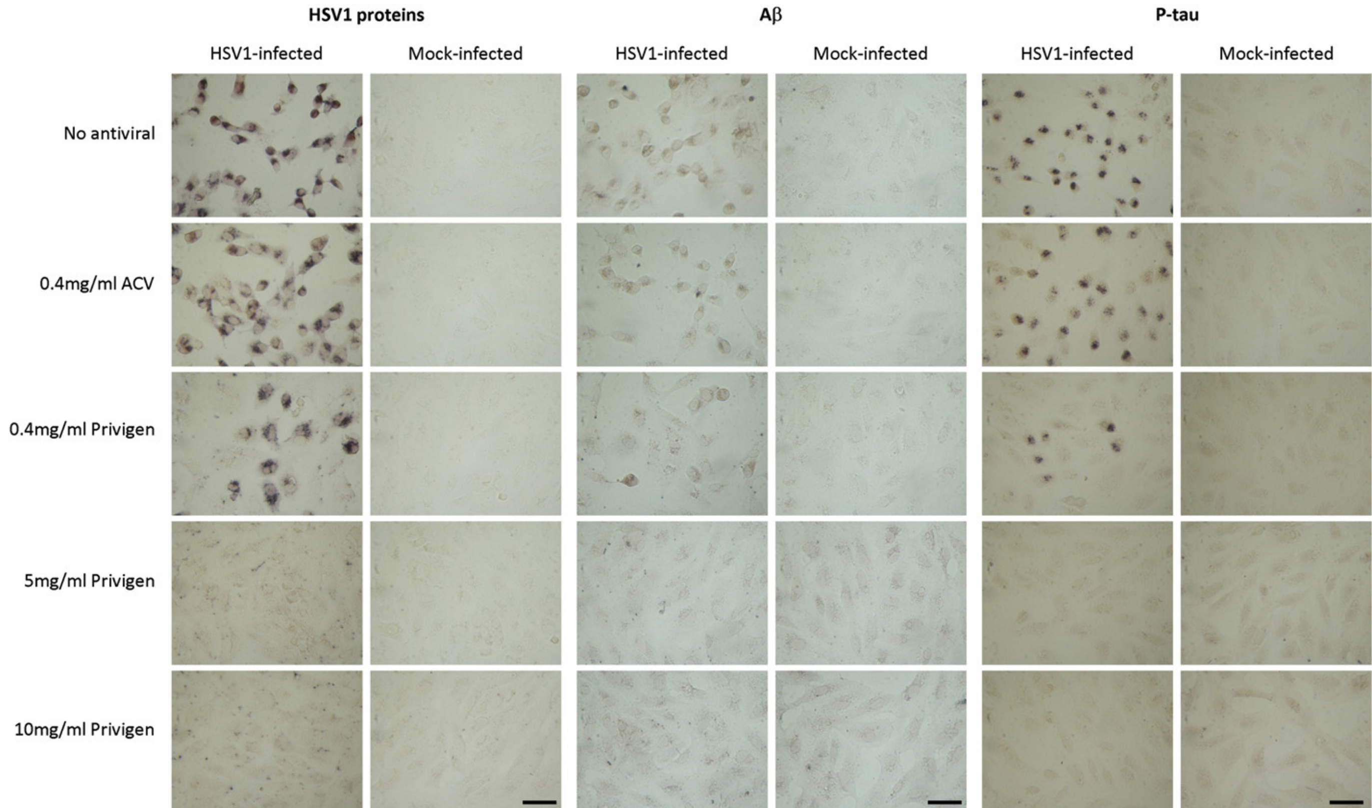

Fig. 1. The effect of Privigen (and acyclovir) on HSV1 proteins, β-amyloid and abnormal tau phosphorylation (high HSV1 dose, short duration). Vero cells were infected with HSV1 SC16 (20 pfu/cell) for 7 h, which allowed only 1 cycle of viral replication to occur. Cells were treated either with 0.4 mg/ml acyclovir (ACV), or with 0.4 mg/ml, 5 mg/ml or 10 mg/ml Privigen, or were untreated; the antivirals were present throughout infection. After fixation the cells were tested for HSV1 proteins, β-amyloid accumulation and abnormal tau phosphorylation (AT100) using immunocytochemistry. Scale bars: 50 μm.

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