Off-label indication: acyclovir as an anti-HIV drug?

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“Accumulating data demonstrates that HSV-2 affects both HIV transmission...and HIV pathogenesis...”

Herpesviruses (HHVs) emerged from a common ancestral mammalian virus 200 million years ago and thus accompanied every step of human evolution [1]. Generally, in the course of evolution in human populations, microbes become less pathogenic. Some of the HHVs, such as HHV-6 and -7, have a high prevalence and are not associated with any adult human diseases. By contrast, infections with other HHVs (HSV-1 and -2, VZV, EBV, CMV and KSHV) have lower prevalences and cause well defined diseases. In the early 1980s, severe clinical expressions of HHV infections were among the first recognized manifestations of AIDS [2], and those clinical conditions, among others (sustained by different microbes), define AIDS [3].

Accumulating data demonstrates that HSV-2 affects both HIV transmission [4,5] and HIV pathogenesis [6,7]. However, other HHVs, even low-pathogenic HHV-6 and -7, affect HIV pathogenesis as well [8]. During coevolution with their hosts, these viruses have acquired several cellular genes (encoding chemokines and chemokine-like receptors), which affect HIV by modulating chemokine spectra and the expression of HIV receptors and coreceptors [8–10].

Multiple publications provide evidence that several HHVs play a role in HIV disease irrespective of their morbidity [6–12]. By targeting these HHVs, one can hope to indirectly affect HIV disease. Fortunately, unlike in the case of HIV, an efficient, selective and relatively non-toxic drug targeting HHVs has been developed. This drug is acyclovir (ACV).

Acyclovir

ACV is a guanosine analog that was synthesized in the late 1970s. It is the most commonly used antiviral drug, and it was seen as the start of a new era of designed antiviral medicines. ACV was designed by Gertrude B Elion and

colleagues, and for this accomplishment, among others, she was awarded the 1988 Nobel Prize in Physiology or Medicine [13].

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ACV is particularly active against HSV-1, HSV-2 and VZV [14]. It is also active against other HHVs (EBV, CMV, HHV-6, HHV-7 and KSHV), although with lower potency [15]. ACV's specificity in suppressing HHVs is based on viral metabolism. ACV is converted into acyclovir monophosphate (ACV-MP), specifically by herpesviral kinases; cellular kinases are far less efficient in phosphorylating ACV into ACV-MP than viral enzymes. Subsequently, ACV-MP is further phosphorylated by cellular kinases into the active triphosphate form, acyclovir triphosphate (ACV-TP), which is a very potent inhibitor of herpesviral DNA polymerase. Its incorporation into viral DNA results in chain termination and viral DNA polymerase inactivation [16]. The affinity of ACV-TP for herpesviral polymerase is approximately 100-times greater than its affinity for cellular DNA polymerase, thus ACV-TP specifically terminates viral rather than cellular DNA elongation. This difference in affinity, in addition to the already high selectivity of ACV's phosphorylation by viral kinases, explains ACV's efficiency and safety. Indeed, the dose of ACV that halts herpesviral replication does not interfere with the synthesis of cellular DNA. Only at high doses does such interference occur and result in cell death. This phenomenon was the basis for the use of ACV as an anticancer drug in experiments in which neoplastic cells were transfected with HSV thymidine kinases. Also, similar experiments were performed to selectively eliminate HIV-1-infected

cells transfected with HSV-thymidine kinases [17]. However, these experimental strategies using ACV to selectively kill neoplastic or HIV-infected cells were not developed further, leaving ACV exclusively as a selective anti-HHV drug.

ACV inhibits HHVs in HIV-1-infected individuals: disrupting the vicious circle of this troublesome duo

The molecular mechanism by which ACV suppresses HHVs, described above, explains why ACV does not affect viruses other than HHVs.

Nevertheless, since HSV-2 is a common HIV copathogen, several clinical trials evaluating the effects of HSV-2 suppression in HIV-1-co-infected patients were performed. These trials demonstrated that HSV-2 suppression with ACV or its prodrug valacyclovir reduces plasmatic, genital and rectal HIV-1 load in HSV-2-co-infected individuals [18–22]. These recent trials are in agreement with early reported survival benefits in cohorts of ACV treated HIV-1-infected patients who expressed a high incidence of clinically identifiable HHVs (HSV-1, HSV-2 and VZV) [23].

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Figure 1. Acyclovir is activated into a HIV-1 reverse transcriptase inhibitor in herpesvirus-infected human tissues.

In herpesvirus-infected lymphocytes, ACV is phosphorylated by herpesviral kinases. The resulting monophosphorylated ACV is converted into ACV-PPP by cellular kinases. HIV-1 reverse transcriptase incorporates ACV-PPP into the nascent viral DNA chain, causing its obligate termination. ACV: Acyclovir; ACV-PPP: Acyclovir triphosphate; RT: Reverse transcriptase.

HIV and HHVs, in particular HSV-2, are a troublesome duo. It seems that they are engaged in a vicious circle in which one worsens the impact of the other. On one hand, people co-infected with HIV and HSV-2 have more frequent HSV-2 mucosal reactivations due to inefficient immune control [7]. On the other hand, HIV-1 genital and plasma load are markedly increased during HSV-2 reactivations [6,7], probably because of the recruitment of immune cells (which provide new targets for HIV) and HIV-1 long-terminal repeat transactivation by HSV proteins [24,25]. Also, this vicious cycle results in an increase in the risk of HIV-1 acquisition and transmission due to the HSV-induced disruption of genital mucosa, the recruitment of activated cells and the increase of HIV-1 genital load [4,5].

However, in two clinical trials, HSV-2 suppressive therapy with ACV failed to protect HSV-2-seropositive women or men who have sex with men from HIV-1 acquisition [26,27]. Whether this failure is due to the subjects' low adherence, to behavioral disinhibition, to low ACV dosage [28–31], or simply to the insufficiency of HSV-2 suppression for prevention of HIV-1 acquisition, remains to be understood. Despite the failure of efforts to prevent HIV-1 acquisition by suppressing HSV-2, there is no doubt that HSV-2-suppressive therapy with ACV in HIV-1-co-infected individuals results in a significant decrease of HIV-1 viral load [18–22]. This decrease has been attributed to the indirect suppression of HSV-2-mediated inflammation. However, the surprising discovery of the direct effect of ACV on HIV-1 reverse transcriptase (RT) makes a revision of this conclusion necessary.

ACV: a new nucleoside HIV-1 reverse transcriptase inhibitor

Recently, we reported on a surprising insight into the anti-HIV activity of ACV in patients co-infected with HIV and HSV-2. We found that ACV-TP, but not ACV itself, inhibits HIV-1 RT in a cell-free system [32]. This finding was recently confirmed by a group from Johns Hopkins University (MD, USA) [33]. As soon as ACV is transformed into an ACV-TP in HSV-2-infected cells, it suppresses both HSV-2 and HIV-1 (FIGURE 1). This provides a new explanation for the decrease of HIV load in HSV-2-co-infected patients treated with ACV.

In human lymphoid tissues *ex vivo* that are treated with ACV and are not infected with HSV-2, HIV replication was found to be suppressed as well: we found that these tissues

were infected with one or several other HHVs that activate ACV into an HIV-1 RT inhibitor (FIGURE 1). In HHV-free cell lines, where no such activation occurs, HIV-1 replication was not suppressed by ACV. However, when HHV-infected cells were added to these cell lines, ACV was able to suppress HIV-1 replication again. Thus paradoxically, ACV, designed as a specific antiherpetic drug, becomes an HIV inhibitor in the presence of HHVs [32].

These findings suggest that ACV may suppress HIV-1 in a wide range of patients infected by various HHVs. Moreover, for continuous HIV-1 suppression, such a strategy should be based on HHVs other than HSV-2, since this virus is readily suppressed by ACV and therefore will no longer activate ACV into ACV-TP. Other HHVs that can phosphorylate ACV but are not readily suppressed by this drug since their DNA polymerases are much less sensitive to it than that of HSV-2 [34] may thus provide a continuous supply of ACV-TP. Remarkably, the nonpathogenic and ubiquitous HHV-6 is among such viruses and has been shown *ex vivo* to activate ACV into an HIV suppressor [32].

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In light of these findings, new targeted clinical trials should evaluate the efficacy of ACV in reducing HIV-1 viral load in individuals co-infected with HHVs other than HSV-2 and who are not qualified for highly active antiretroviral therapy (HAART). In the new clinical trials aimed at preventing HIV-1 acquisition and reducing HIV-1 viral load, the dose of administered ACV should be adjusted to provide the adequate amount of ACV-TP for HIV-1 suppression, rather than for complete HSV-2 suppression. These new clinical trials should answer whether the strategy designed *ex vivo* would work *in vivo* and should indicate which HHVs are optimal for supplying ACV-TP and what level of HHV replication is sufficient to provide enough ACV-TP to suppress HIV. Moreover, since HIV eventually develops resistance against nucleoside RT inhibitors, it is important to evaluate whether ACV resistance is acquired *in vivo* and whether it could affect HIV-1 sensitivity to other anti-HIV drugs and *vice versa*.

Conclusion

ACV conversion by HHVs into an anti-HIV-1 drug provides the basis for a new principle of anti-HIV therapy: a ‘binary weapon’ in which

an inert compound is converted by an endogenous component into an active therapeutic drug. Generally, since the human body is populated by a large number of microbes, exploiting the metabolic activity of one microbe against another could be a valid strategy in therapy [35].

The use of ACV or its derivatives for HIV suppression is in line with a popular trend to identify new uses for old drugs with a known pharmacokinetic and safety profile [36]. Several such new applications of old drugs have been described in recent years [37]. As far as ACV and its derivatives are concerned, since HHVs are among the most common infections associated with AIDS, the ability of ACV to dual target both HHVs and

HIV, together with its history of 30 years of safe application, are of great advantage. Therefore, in the future, ACV or its derivatives may become a part of drug cocktails to suppress HIV.

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