Effect of Ganciclovir [9-(1,3-Dihydroxy-2-Propoxymethyl)Guanine] on Viral DNA and Protein Synthesis in Cells Infected with Herpes Simplex Virus

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The effect of ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)guanine] on herpes simplex virus type 1 DNA and protein synthesis was studied. Ganciclovir markedly inhibited the synthesis of viral DNA and gamma proteins in a dose-dependent manner. However, the synthesis of viral beta proteins was significantly increased by ganciclovir in the later stage of infection.

Ganciclovir, also known as 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) or 2'-nor-2'-deoxyguanosine, is an analog of acyclovir (ACV) and has been shown to be a potent inhibitor of herpes simplex virus (HSV) and cytomegalovirus (3, 9, 14). As with ACV, herpesvirus-specific thymidine kinase phosphorylates ganciclovir to its monophosphate, which is further phosphorylated to its di- and triphosphate by cellular guanylate kinase and other cellular enzymes, respectively (1, 3, 10, 14). Ganciclovir is more rapidly converted to its corresponding triphosphate form in virus-infected cells than is ACV (1). Ganciclovir-triphosphate inhibits the synthesis of viral DNA by suppressing DNA polymerase (4). Some laboratory animal studies indicate the superiority of ganciclovir to ACV in the treatment of HSV infections in vivo (14, 15).

HSV contains a DNA genome from which nearly 50 virus-specific infected cell polypeptides (ICPs) are expressed (6). These ICPs are classified into three groups as alpha, beta, and gamma proteins, whose syntheses are coordinately regulated and sequentially ordered in a cascade manner (7, 8). Although ganciclovir is known to inhibit the activity of herpesviral DNA polymerase and subsequently suppress the synthesis of viral DNA (4), the effect of ganciclovir on HSV protein synthesis is unknown. Because the synthesis of viral alpha and beta proteins precedes viral DNA synthesis, a study of the effect of ganciclovir on viral protein synthesis may provide insight to the mechanism behind its antiviral action.

To study the effect of ganciclovir on viral DNA synthesis, Vero cell monolayers were infected with HSV type 1 (HSV-1) F strain at a multiplicity of infection of 10. Then, medium containing methyl-[3H]thymidine (10 μCi/ml, 23Ci/mM; New England Nuclear Corp.) and ganciclovir (1, 5, or 10 μM) was added to the cultures. After incubation for 12 h, the cells were collected and digested with proteinase. The cell digestion was mixed with NaI and ethidium bromide, and isopycnic equilibrium NaI density gradient centrifugation was performed (16). Viral and cellular DNA bands were visualized and photographed in UV light. The gradient fractions were then spotted onto a GF/C filter disk (Whatman Inc.), and the radioactivity of the spotted GF/C disk was measured.

To examine the effect of ganciclovir on viral protein synthesis, Vero cells were infected with HSV-1 at a multiplicity of infection of 20 and cultured in medium containing ganciclovir (5, 10, 20, or 50 μM) for 1.5, 4, 7, or 11 h. The cells were then radiolabeled with [35S]methionine (20 μCi/ml, 900 mCi/mM; New England Nuclear) for 1 h. Finally, the cell protein was extracted in sodium dodecyl sulfate for subsequent electrophoresis on polyacrylamide gel and autoradiogram (11). Absorbance measurements of the autoradiographic images were made in a Bio-Rad video densitometer (model 620; Bio-Rad Laboratories), and each peak of the scanned autoradiogram was converted to percentage of the total absorbance. As a representative of alpha proteins, the high-molecular-weight nonstructural polypeptide ICP4 was chosen. ICP6 and ICP5 were selected to represent beta and gamma proteins, respectively.

In the presence of 5 μM ganciclovir, viral DNA synthesis was barely detectable, whereas the amount of cellular DNA remained the same as that of the control (Fig. 1). Synthesized viral DNA was not noticeable with a great reduction in the amount of cellular DNA in the presence of 10 μM ganciclovir (Fig. 1). Synthesis of viral DNA, which was monitored by determining the radioactivity of the DNA, was inhibited by 73, 98, or 99% in the presence of 1, 5, or 10 μM ganciclovir, respectively. Cellular DNA synthesis in virus-infected cells was also significantly inhibited by ganciclovir.

FIG. 1. Effect of ganciclovir on synthesis of viral and cellular DNA (vDNA and cDNA, respectively). After the isopycnic NaI density gradient, the DNA bands were visualized in UV light (see the text for details). (A) DNAs from the infection control. (B) DNAs from infected cells in the presence of 5 μM ganciclovir. (C) DNAs from infected cells in the presence of 10 μM ganciclovir.
The levels of HSV-1.

Although the synthesis of ICP6 was not changed by ganciclovir at 2.5 and 5 h postinfection, a significant increase in the synthesis rate was noticed at 8 and 12 h after viral infection as compared with that of the infection control. As shown in Fig. 3, there was a significantly noticeable synthesis of ICP8, another beta protein, at 12 h after viral infection in the presence of ganciclovir, whereas the synthesis of ICP8 was mostly shut off in the infection control. However, the synthesis of ICP5 and other gamma proteins, such as ICPs 15, 19, 25, 32, 33, 43, and 44, was significantly reduced by ganciclovir (Fig. 3).

The inhibitory effect of ganciclovir on viral and cellular DNA synthesis was similar to that reported by Sme et al. (14). Because the synthesis of viral DNA begins at 3 h postinfection and coincides with the appearance of beta proteins (12, 13), the increased expression of beta proteins by ganciclovir in the later stage of infection may be related to the inhibition of viral DNA synthesis. Furthermore, because parental DNA can serve as a template for mRNAs of all proteins (6), the stimulation of beta protein synthesis by ganciclovir may be a result of more parental DNA being available. Alpha proteins are made first and are required for the synthesis of beta proteins, which in turn shut off the synthesis of alpha proteins and induce the synthesis of gamma proteins. The gamma proteins shut off the synthesis of beta proteins (2). Therefore, the decrease in the synthesis of gamma proteins by ganciclovir may allow the continuous synthesis of beta proteins in virus-infected cells. Moreover, the decrease in the synthesis of gamma proteins by ganciclovir might result in increased relative densities of alpha and beta proteins in autoradiographic images of electrophoretically separated proteins. Because the synthesis of viral DNA occurs before the expression of most gamma proteins (2) and a greater proportion of viral gamma proteins are made from the newly synthesized progeny viral DNA (5, 11), the inhibition of viral progeny DNA synthesis might be responsible for the decrease in the synthesis of gamma proteins. The reduction in gamma protein synthesis may play a role in the antiviral activity of ganciclovir.

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