in cross-resistance to both foscarnet and cidofovir (Chou *et al*., 1995; Chou *et al*., 2003). Treatment with aciclovir does not appear to induce GCV resistance (Drew *et al*., 1995). HSV-1 develops resistance to GCV more slowly than to aciclovir, requiring continuous exposure *in vitro* to GCV at a concentration of 30 μM for 70 days compared with 14 days of aciclovir for resistant variants to emerge (van der Horst *et al*., 1987).

2c. *In vitro* synergy and antagonism

Although GCV does synergize with other antivirals, none of the combinations has been established in humans and their use in humans is rare.

Foscarnet and GCV are synergistic against human and murine CMV and murine HSV *in vitro* (Smith *et al*., 1982b; Manischewitz *et al*., 1990) with up to 27-fold improvement in efficacy of GCV when the two drugs are used in combination and additive in an *in vivo* murine model (Freitas *et al*., 1989). The combination of GCV and nalidixic acid was more effective than either drug alone in inhibiting the replication of duck hepatitis B virus in the livers of infected ducks (Wang *et al*., 1995). Recombinant human interferon-beta and GCV are strongly synergistic in their activity against simian VZV infection in monkeys, with up to a 100-fold decrease in interferon-beta and 10-fold less GCV required to achieve an effective dose compared with monotherapy (Soike *et al*., 1987). Recombinant human interferon-alpha and GCV were found to be highly synergistic against HSV-1 and HSV-2 in human fibroblast cultures (Moran *et al*., 1985). Synergy has also been demonstrated between recombinant murine interferon-alpha and GCV both *in vitro* (Eppstein and Marsh, 1984) and when administered to mice infected with HSV-2, the effective dose of each drug being reduced 10-fold when given in combination (Fraser-Smith *et al*., 1985).

GCV has been found to antagonize the antiretroviral activity of both zidovudine and didanosine *in vitro*, increasing the EC_{50} of these drugs by 3- to 6-fold; antagonism occurred at drug concentrations well below cytotoxic levels (Medina *et al*., 1992). Furthermore, some investigators have found that zidovudine antagonizes the effects of GCV against human CMV *in vitro*; in this study, zidovudine also reduced the efficacy of GCV in a guinea pig model (Feng *et al*., 1993). However, this finding remains controversial because a number of other investigators found the opposite: Zidovudine has been reported to have an additive to synergistic effect with GCV against laboratory-adapted strains and clinical isolates of CMV *in vitro* (Snoeck *et al*., 1992; Freitas *et al*., 1993a; Roche Laboratories, data on file). There is no antagonism of the antiviral activity of GCV by its use in combination with amphotericin B, ketoconazole, dapsone, or co-trimoxazole (Pecyk *et al*., 1989; Freitas *et al*., 1993b).

3. MECHANISM OF DRUG ACTION

GCV's structure and mechanism of action are similar to aciclovir. The antiviral activity of GCV depends on its intracellular triphosphorylation (Cheng *et al*., 1983b; Field *et al*., 1983). Initial phosphorylation to GCV monophosphate is rate limiting and is mediated by the CMV UL97 gene product, a protein kinase (phosphorylating serine and threonine residues) that efficiently monophosphorylates GCV but not aciclovir (Littler *et al*., 1992; Sullivan *et al*., 1992a; Sullivan *et al*., 1992b; He *et al*., 1997). There are only minimal levels of the triphosphorylated drug within uninfected cells, indicating that infected cells preferentially phosphorylate GCV by the CMV-encoded protein kinase. After this step, cellular kinases complete the phosphorylation to the di- and triphosphorylated GCV, the active compound.

Cellular kinases are responsible for the subsequent di- and triphosphorylation of GCV. These cellular kinases include guanylate kinase, deoxyguanosine kinase, and phosphoglycerate kinase and are induced in cells infected with human CMV, further accelerating the production of the antiviral form of GCV (Boehme, 1984; Meijer *et al*., 1984; Matthews and Boehme, 1988). Levels of GCV triphosphate are 10- to 100-fold higher in CMV-infected versus uninfected cells (Biron *et al*., 1985; Freitas *et al*., 1985). *In vitro* experiments suggest that GCV triphosphate is slowly dephosphorylated in CMV- and HSV-infected cells, with 40–70% of original drug concentrations being detected 18–24 hours after the drug is removed and an intracellular half-life of > 6 hours (Biron *et al*., 1985; Smee *et al*., 1985a). This accumulation of GCV triphosphate within infected cells and, perhaps more important, the capacity of the CMV UL97 gene product to initiate GCV, but not acyclovir, phosphorylation explain GCV's superiority over aciclovir against CMV.

GCV triphosphate blocks the replication of CMV by inhibiting viral DNA synthesis by several mechanisms, competing with deoxyguanosine triphosphate (dGTP) for incorporation into DNA and thereby inhibiting the viral DNA polymerase as well as incorporating into the growing chain of viral DNA and markedly inhibiting its elongation (Frank *et al*., 1984; St Clair *et al*., 1987). Because GCV contains hydroxyl groups similar to the 3′ and 5′ hydroxyl groups of endogenous nucleotides, chain elongation is not completely terminated but can continue very slowly after GCV triphosphate incorporation (Frank *et al*., 1984; Reardon, 1989). This is in contrast to aciclovir, which only has the 3′ hydroxyl group and, when incorporated, causes chain termination. The continuation of viral DNA synthesis by incorporated GCV results in the production of short fragments of CMV DNA that accumulate within the nucleus but are not packaged or released as infectious virions (Hamzeh and Lietman, 1991). Chain termination is apparently reversible because chain elongation continues when the drug is removed (Matthews and Boehme, 1988).

GCV triphosphate is an excellent substrate for CMV DNA polymerase, competitively inhibiting this enzyme with respect to dGTP with an inhibitory constant (K_i) of 1.7 μM; cellular DNA polymerases are inhibited with a significantly higher K_i of 17 μM (Freitas *et al*., 1985).

In the case of cells infected by HSV or VZV, viral TK is responsible for the initial phosphorylation of GCV (Cheng *et al*., 1983a; Smee *et al*., 1983; Ashton *et al*., 1982). GCV triphosphate also selectively inhibits the DNA polymerases of