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Phosphonoacetic Acid Inhibition of Channel Catfish Virus Replication

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The virus responsible for epizootic outbreaks of channel catfish disease was first isolated by Fijan et al. (1970) in cell cultures derived from ictalurid fish. Detailed characterization of this virus by Wolf and Darlington (1971) clearly indicated the assignment of channel catfish virus (CCV) to the herpesvirus group of DNA viruses. Members of this diverse family of viruses are found throughout the phylogeny of the species, although presently only three representatives have been associated with teleost fish (Wolf, 1973; Wolf et al., 1976). The extent of parasitism shown by the herpesviruses ranges from persistent subclinical infections to latent virus infections (Rapp and Jerkofsky, 1973) to neoplastic changes resulting in malignancy (Biggs et al., 1972). Although several herpesviruses are capable of these multiple modes of virus-host interaction, the herpesvirus of channel catfish disease is known primarily for its direct, devastating effects upon young populations of aquacultured catfish. The destruction of infected stock and decontamination of all facilities is currently the only means available to ensure complete eradication of channel catfish disease outbreaks (Plumb, 1972).

Few viruses to date, regardless of their taxonomic classification or that of their host, will respond to conventional chemotherapy. The herpesviruses are no exception. Recently, however, a chemical derivative of the simple molecule acetic acid has been shown to be effective in selectively inhibiting the replication in tissue culture of representative herpesviruses (Barahona et al., 1977; Duff and Overby, 1975; Huang, 1975; Lee et al., 1976; May et al., 1977; Overby et al., 1974; Summers and Klein, 1976; Yajima et al., 1976). This compound, termed phosphonoacetic acid (PAA), functions specifically by inhibiting the herpesvirus coded DNA dependent DNA polymerase in its process of replicating virus DNA (Mao and Robishaw, 1975). In tissue culture systems, PAA will not significantly inhibit cell polymerase activity, representatives of single-stranded RNA virus groups, or other double-stranded DNA virus groups (Duff and Overby, 1975; Leinbach et al., 1976; Mao and Robishaw, 1975). Vaccinia virus, a member of the poxvirus group, was apparently an exception to this rule (Duff and Overby, 1975). In all cases, the effective tissue culture dose of PAA which inhibited herpesvirus replication has been 100 μg PAA/ml or less.

On the basis of this information, it was of interest to determine if CCV would show similar sensitivity to PAA. Increasing concentrations of PAA were prepared in overlay medium, and these were added to brown bullhead catfish (BB) cell cultures infected with a known quantity of CCV. In all experiments, the results indicated that greater than 95 percent inhibition of CCV replication was obtained in the presence of 1,000 μg PAA/ml. Viable cell counts as determined by trypan blue dye exclusion at the beginning and end (72 hours postinoculation) of each experiment indicated there was no cell death due to drug toxicity.

Dose dependency experiments indicated a direct relationship between the number of infectious virus particles infecting one cell and the amount of PAA required to inhibit virus replication. This concentration ranged from greater than 500 μg PAA/ml for cultures with an input multiplicity of 0.01 plaque forming unit per cell to 2,000 μg PAA/ml for cultures with an input multiplicity of 6.0 plaque forming units per cell. The toxicity level of PAA in BB cells was evident at 3,000 μg PAA/ml of culture medium.

These results indicate that PAA is an effective and selective agent in the inhibition of channel catfish virus replication. The fact that 10 times the amount of drug required for other herpesvirus systems is necessary to inhibit CCV should not be surprising. To date, all herpesvirus-PAA systems reported have been of homeothermic mammals and birds. This report is the first concerning a herpesvirus of a poikilothermic species. One major implication of this work, therefore, is that the action of PAA may be dependent upon either temperature or, relatedly, the physiology and metabolic rate of the host cell. This is not unusual, as the mode of action of this drug is interference with the enzymes of viral DNA replication. Rate kinetics of the enzyme-substrate reaction are directly related to temperature (Lehninger, 1970), and temperature as such a catalytic mechanism has been shown to regulate many life functions in poikilothermic species (Swan, 1974).

Direct application of such tissue culture data has been investigated for PAA and two human viruses—herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2)

(Shipkowitz et al., 1973). When HSV-2 is inoculated into the denuded skin on the backs of CF strain mice, observable virus lesions are evident in 3-5 days. In 11-15 days, virus-inoculated mice develop a flaccid, posterior paralysis resulting in death within 24 hours. Topical application of PAA in ointment or aqueous form in a minimum concentration of 0.5 percent significantly reduced virus-induced mortality. Equally efficacious, oral administration of PAA at a minimum dosage of 800 mg/kg/day for 6 days resulted in 100 percent reduction of virus-induced mortality. Using HSV-1 in a rabbit keratitis model system, these same authors demonstrated that topical application of 0.5 percent PAA into infected eyes reduced virus-induced corneal lesions from 5 to 9 days after infection (Shipkowitz et al., 1973).

PAA has been demonstrated to selectively inhibit the replication in tissue culture of many different herpesviruses of homeothermic hosts. In two reported instances, this compound has proved effective against herpesvirus-induced morbidity and mortality in animal model systems. Results herein reported indicate that the replication of a poikilothermic herpesvirus, CCV, can also be inhibited in tissue culture systems. Logic dictates that oral preparations of PAA in catfish feed next be tested to determine if this will significantly reduce the incidence of mortalities resulting from experimental infection of fry with CCV. If successful, these data would suggest that PAA may be efficacious as a chemotherapeutic agent in the control of channel catfish virus disease.

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