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SV40 DNA Hybridization Assay: Use as a Primary Cell-Based Screen For Antiviral Inhibitors of Human Papovaviruses J.A. Barnes, W.C. Phelps. Burroughs Wellcome Co., Research Triangle Park, N.C. U.S.A.

SV40 virus, together with mouse polyomavirus, human JC and BK viruses, and the papillomaviruses are members of the Papovaviridae family. SV40 causes an inapparent infection in its natural host, the macaque monkey. Inapparent infections with human JC and BK viruses commonly occur during childhood, with JC and BK remaining dormant in the kidney until reactivation during immunosuppression at which time they can cause disease. Papillomaviruses infect the cutaneous or mucosal surface epithelium normally resulting in the induction of a benign wart that typically occurs on the hand, feet or genital area. Papillomaviruses are also strongly linked to premalignant intraepithelial neoplasia and cervical cancer. Current treatments for papillomavirus infections lack viral specificity and can be quite painful and ineffective. Available therapies include surgical excision, cryotherapy, CO_2 vaporization, electrocautery, topical treatment with cytotoxic agents such as podophyllin or 5-fluouro-uracil and chemical agents such as trichloroacetic acid or salicylic acid. Because papillomaviruses can not be cultivated *in vitro* using typical cell culture techniques, specific antiviral drug development has been hindered. We have chosen to use SV40 as a surrogate virus for the development of a high throughput *in vitro* DNA hybridization assay for testing compounds that might be effective against human papillomavirus, JC virus or BK virus replication. Polyomaviruses (SV40, BK and JC) and papillomaviruses share a number of features. Both goups of viruses are small, non-enveloped, double-stranded DNA tumor viruses that replicate exclusively in the nucleus of infected cells in association with host cellular histones. The Large T-Antigen of SV40, and E1, the dominant replication protein of papillomaviruses, each encode ATPase and helicase activities. In addition to their functional similarity, T-Ag and E1 share significant regions of amino acid sequence homology. We have tested a variety of known antivirals in the SV40 DNA hybridization assay. The anitiviral agents Zovirax®(acyclovir), Retrovir®(zidovudine), ARA-A, Ribavirin, and the dideoxynucleoside analogs ddA, ddC, ddG and ddT were inactive against SV40. FIAC, FIAU and ganciclovir were somewhat active with IC_{50}s of 40 μM, 90 μM and 130 μM respectively. Fifteen antitumor compounds that target a variety of host cell functions were also evaluated for activity in the SV40 assay. Camptothecin, a topoisomerse I inhibitor and VP-16, a topoisomerase II inhibitor had IC_{50}s of 0.54 μM and 12 μM respectively. Both however, showed toxicity. We will describe the SV40 DNA hybridization assay and the effects of these and other compounds on SV40 replication.

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The SCID-Ra Model For Evaluation Of Inhibitors Of Papillomaviruses.
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Kreider *et al*. reported (Cancer Research 39: 273-276, 1979) that rabbit skin which had been transplanted to immunodeficient nude mice, could be successfully infected with cottontail rabbit papillomavirus (CRPV). We have extended this observation in developing a rodent model for evaluation of compounds for activity against the PVs. In this model, rabbit ear skin is transplanted to the dorsum of SCID mice and after 3 weeks for healing, scratch infected with CRPV. Warty lesions develop within 2-3 weeks in >95% of infected animals. Topical and/or systemic therapy delivered either in the drinking water or in osmotic pumps, is normally initiated 1 to 7 days post-infection and is continued for approximately 6 weeks. Weekly lesion scores are recorded (no evidence of infection = 0; large, highly keratinized wart = 6) and compounds are evaluated for their ability to suppress wart growth when compared to the untreated control mice. The model was evaluated by testing compounds with reported anti-wart activity. Ribavirin which was shown to have a suppressive effect on the growth of warts in the rabbit back model (Ostrow *et al*., Antiviral Research 17: 99-113, 1992) as well as in humans for the treatment of laryngeal papillomatosis (McGlennen *et al*., Head and Neck, Nov/Dec 1993), was evaluated and showed significant anti-proliferative activity (82% reduction) with oral dosing. Antitumor or antiproliferative compounds including podophyllin and 5-fluorouracil have been used clinically for the treatment of human PV infections. Due to the wide range of antitumor compounds available, we evaluated a spectrum of antitumor compounds including antimetabolites, antimitotic agents, and topoisomerase I and II inhibitors. Navelbine, an antimitotic compound currently in clinical trials for the treatment of breast cancer, was evaluated and resulted in significant suppression (74%) of wart growth when mice were treated with a combination of topical and oral therapy. Results of these studies and further characterization of the model by *in situ* hybridization as well as the potential advantages of the SCID-Ra model over currently available PV animal models will be discussed.