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Identification of an Antisense Oligonucleotide with *in vivo* Activity Against Human Papillomavirus. 2. Efficacy against benign human genital condyloma in a mouse xenograft model.
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We have studied the inhibitory action of antisense oligonucleotides that target expression of the E1 helicase of human papillomavirus (HPV) types 6 and 11. Activity of antisense oligonucleotides *in vitro* was measured in assays using mammalian cells transfected with an E1-luciferase reporter gene (see abstract by Roberts *et al.*). Selected compounds were further examined *in vivo* using a kidney xenograft model in which HPV-infected human foreskin fragments are implanted under the renal capsule of a nude mouse. A phosphorothioate 2'-O-methyl RNA hybrid oligonucleotide targeting the E1 AUG of HPV types 6 and 11 was dosed daily via the subcutaneous route for a 90 day period. This compound reduced the size of condylomas produced in the mouse model by up to 95% compared to the control - saline treated mice. Histological analysis of human condyloma tissue in this model suggests that HPV viral load has been decreased, with koilocytosis and thickness of the human epithelium greatly reduced. The optimal dose and treatment time for HPV antisense oligonucleotides in this model are currently under evaluation and will be reported.

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CIDOFOVIR: INDUCTION OF APOPTOSIS IN HUMAN PAPILLOMA VIRUS (HPV)-CONTAINING CELL LINES
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Cidofovir [HPMPC, (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine], an acyclic nucleoside phosphonate with broad spectrum of activity against DNA viruses, has proved effective in the treatment of human papilloma virus (HPV)-associated diseases. We have analyzed the effect of various concentrations of HPMPC on the proliferation of human cervical keratinocytes immortalized by HPV-33 (CK1 cells). Treatment of CK1 cells with HPMPC resulted in inhibition of cell proliferation in function of time. Thus, 50% cytotoxic concentration (CC_{50}) for HPMPC was 58 ± 38 µg/ml at day 3, and 0.7 ± 0.4 µg/ml at day 7. This phenomenon of increased cytotoxicity in function of time was not observed with acyclovir (CC_{50}: 200 µg/ml and 136 ± 42 µg/ml at day 3 and day 7, respectively), while AraC elicited a marked antiproliferative effect at both day 3 (CC_{50}: 0.098 ± 0.03 µg/ml) and day 7 (CC_{50}: 0.047 ± 0.021 µg/ml). Similar results were observed with established cervical epithelial tumor cell lines containing HPV-16 (CaSki and SiHa) or HPV-18 (HeLa). When the effect of HPMPC on the proliferation of keratinocytes isolated from normal human cervix was tested, its CC_{50} was 26.5 ± 32.8 µg/ml at day 7. We investigated whether the process leading to cell death was due to apoptosis (programmed cell death) by a cellular DNA fragmentation ELISA, a photometric enzyme immunoassay for the detection of BrdU-labeled DNA fragments, in the culture supernatant and cytoplasm of cell lysates. The results demonstrated that both AraC and HPMPC, but not acyclovir, were able to induce apoptosis in a dose-dependent manner in CK1, SiHA, CaSki and HeLa cells after 4 days of incubation. HPMPC also showed an increased cytotoxicity in function of time and induction of apoptosis in other tumor cell lines not containing HPV, i.e. human melanomas, lung carcinomas and brain tumor cells.

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PRELIMINARY RESULTS ON THE ACTIVITY OF CIDOFOVIR FOR THE TREATMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN) GRADE III
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(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC, cidofovir) is an acyclic phosphonate derivative with broad spectrum antiviral activity against DNA viruses including human papillomavirus (HPV). A strong association has been demonstrated between the development of CIN, but also vaginal and vulvar proliferative lesions and the presence of HPV. We describe here the preliminary results of a study performed to evaluate the potential efficacy of cidofovir in the treatment of CIN grade III lesions. The patients included in the study had a biopsy showing a CIN grade III lesion and were planned to have a conisation. After the diagnostic was confirmed by histology, the patients received three applications of cidofovir gel at 1%, every other day. Cidofovir was applied by the gynaecologist under view control using acetic acid to visualize the lesions. Within 8 to 10 days following the last application, the conisation was performed and the cone carefully analyzed for persistence of tumor cells. Eight patients were included and evaluated. For all of them the PCR performed on the biopsy pre-treatment showed the presence of high risk types of HPV. For three patients the pathology on the cone, after cidofovir treatment demonstrated a complete disappearance of the CIN III lesions, confirmed by a negative PCR for two of them. For two patients, there was persistence of CIN III lesions at the bottom of the glands, the most superficial layers of the lesions being replaced by an erosion; in one case, the PCR was positive while the other was negative. For the three remaining cases, there was an erosion on most of the surface together with the persistence of typical CIN III in two patients, while one patient had persistence of CIN III lesions on the whole surface. The treatment was well tolerated and only one patient mentioned a short episode of pruritus after the first application of cidofovir that was considered as probably related to the treatment. These preliminary results deserve further investigations and confirm the potential anti-HPV activity of cidofovir and the role that the molecule may play in the treatment of CIN grade III lesions.

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A Comparative Study of the Use of the Herpchek™ Assay vs Viral Culture for the Detection of Labial Herpes Simplex Virus (HSV) in a Clinical Drug Trial.
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Herpes simplex viral culture (VC) has been considered the standard method for evaluating viral positivity in antiviral clinical trials. Our objective was to compare the sensitivity and specificity of Dupont Herpchek™ (HC) vs VC as a measure of viral shedding in a HSV labialis topical drug trial. Fifty-seven patients (both prodromal and early lesional) were swabbed using a Herptran™ swab followed by a VC swab twice daily over a five day trial period unless lesion healed or formed a hard crust. A total 346 clinical specimens were assayed using HC and compared to VC in Vero cells for 7 days as a measure of viral shedding. 87.7% of labialis study patients were VC-confirmed positive for HSV (94% HSV-1). Comparing the assays, 18% of the total specimens had discordant results; 14.5% were HC positive-VC negative, 3.5% were HC negative-VC positive. Of the HC positive-VC negatives 82% occurred after VC positivity had ceased in individual patients (within 30 hours of VC positivity in 80% of these discordants). Discordant VC negatives corresponded to low absorbance values in positive HC assays, suggestive of decreased viral antigen levels in these specimens. Comparing assays using a 'combined HC-VC gold standard' in which HC positives are considered true positives from patients with at least one culture-confirmed positive during the treatment period; the sensitivity and specificity of HC is 94.4% and 100% respectively with a PPV of 100% and a NPV of 91.6%. Applying the same standard, VC had a sensitivity of 76.7%, specificity of 100%, PPV of 100%, and NPV of 72.4%. In conclusion, HC is a sensitive and specific assay for detection of labial HSV, although use as a measure of viral shedding in a clinical trial setting could result in extended periods of viral positivity compared to those measured by VC.