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Treatment of Severe Recurrent Laryngeal Papillomatosis By Local Injections of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (Cidofovir)

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Cidofovir (CDV) is an acyclic nucleotide analogue with broad spectrum antiviral activity against DNA viruses including herpes-, adeno-, papilloma- and polyomaviruses. Human papillomaviruses (HPV) are responsible for the proliferation of squamous epithelial cells, leading to the development of benign as well as malignant tumors. HPV types 6 and 11 have been mostly identified in recurrent laryngeal papillomatosis, a local disease with a high morbidity and the potential to spread distally into the bronchial tree. Here we report on 11 patients (6 females and 5 males with a mean age of 34 ranging from 11 to 54) with severe recurrent laryngeal papillomatosis that were treated locally with CDV. A 2.5 mg/ml solution of the compound was injected directly into the papillomatous lesions, under microscopic control, the patients being under general anesthesia. Patients were injected every other week. A total of 79 injections were performed, the number of injections per patient ranging from 2 to 13 and the mean volume of CDV solution injected per session varying from 3 to 8.3 ml. Of the eleven patients, 4 are disease-free with a mean follow-up of 7.5 months (3-13). One patient experienced a relapse 10 months after being in complete remission, and a second treatment again achieved remission (follow-up: 2 months). Three patients, still under treatment, showed a dramatic reduction in tumor size after 3, 3 and 10 injections, respectively. One patient showed a partial remission, and still needs treatment every 5 months while under laser therapy he had to be treated every other week. One patient, multitreated for 8 years, progressed under therapy after an initial dramatic response under CDV. Finally, one patient with known cardiac problems was dropped from the study, because of precardial complaints after CDV injections. Neither biological alterations nor local fibrosis or necrosis were noted in any of the patients. These preliminary results point to the potential usefulness of CDV for the treatment of severe recurrent laryngeal papillomatosis.

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Drug discovery assays for Hepatitis C Virus (HCV) NS3 ATPase/Helicase. E.M. August, L. Patnaude, D. L. Peterson[a], S.A.Siegel. Phytera, Inc., Worcester, MA, USA and [a]Virginia Commonwealth University, Richmond, VA, USA.

The NS3 protein of HCV possesses 3 enzymatic activities which are potential targets for antiviral drugs: a serine protease, nucleoside triphosphatase (ATPase) and an RNA helicase. A soluble truncated protein (NS3b) containing enzymatically active ATPase and helicase domains has been cloned and overexpressed in E. coli (L. Jin and D. Peterson, Arch. Biochem. Biophys., 323, 47-53, 1995). A high-throughput colorimetric assay measuring ATPase activity couples the hydrolysis of ATP to the oxidation of NADH using pyruvate kinase and lactate dehydrogenase. The assays are performed in 96-well plates and the resultant rate of decrease in absorbance at 340 nm is measured in a kinetic microtiter plate reader. The K_m for ATP was found to be 74 μM and the reaction is dependent on the presence of divalent cation (Mg^{2+}). The ATP analog β,γ-methylene ATP was a weak competitive inhibitor of ATP hydrolysis and was not a substrate. The reaction velocity was linear with increasing NS3b up to 50 μg/mL. We observed a 1.7- and 1.2-fold enhancement of activity by poly-adenylic acid and poly-uridylic acid, respectively. Rabbit muscle myosin is used as a secondary specificity assay with a similar platform. To date we have screened over 6000 extracts from Phytera's plant cell culture library: 90 extracts were found to be >50% inhibitory for a confirmed hit rate of 1.5%. We have also developed a gel-shift assay for the helicase, in which the release of a short [^{32}P]-labelled RNA from a larger RNA hybrid is detected, to further examine the activity of hits from the ATPase assay. A high-throughput helicase screening assay is also being developed. Thus, we have established a rapid and convenient assay system for this bifunctional protein construct and are currently screening Phytera's library of plant cell culture and marine microorganism natural product extracts for enzyme inhibitors with potential anti-HCV activity.

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Anti-Influenza Virus Activity of Plant Flavonoid, 5,7,4'-Trihydroxy-8-methoxyflavone, from the Roots of *Scutellaria baicalensis* and Enhancement of Its Activity by Drug Delivery System (DDS)

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Influenza virus sialidase has been considered to relate in the virus infection. Therefore, influenza virus sialidase inhibitors have a possibility to inhibit the virus infection. We screened influenza virus sialidase specific inhibitor from the plant flavonoids and found 5,7,4'-trihydroxy-8-methoxyflavone (F36) from the roots of *S. baicalensis* as a potent inhibitor (IC_{50}, 55 μM).[1)] F36 inhibited the replication of influenza virus A/PR/8/34 (H1N1 subtype) not only in MDCK cells,[1)] but also in mouse lung by *i.n.* (0.5 mg/kg) and *i.p.* (4 mg/kg) administrations.[2)] *I.n.* administration of F36 protected mice against a lethal A/PR8 virus infection.[2)] Studies on the mode of action suggested that F36 showed the anti-A/PR8 virus activity by inhibiting the fusion of the virus with endosome/lysosome membrane and budding from MDCK cell surface.[3)] Although F36 showed no antiviral activity against influenza virus A/Guizhou/54/89 (H3N2) in mice,[4)] when 0.4% hydroxypropyl cellulose (HPC) solution of F36 (0.5 mg/kg) was administered *i.n.* 5 min after the virus inoculation, proliferations of A/Guizhou virus both in nasal and broncho-alveolar cavities were inhibited significantly. These results indicate that DDS with HPC enhances the anti-influenza virus activity of F36 *in vivo*.

1) T. Nagai *et al.*, *Chem. Pharm. Bull.*, **38**, 1329-1332 (1990).
2) T. Nagai *et al.*, *Antiviral Res.*, **19**, 207-217 (1992).
3) T. Nagai *et al.*, *Antiviral Res.*, **26**, 11-25 (1994).
4) T. Nagai *et al.*, *Biol. Pharm. Bull.*, **18**, 295-299 (1995).

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Inhibition of influenza virus RNA polymerase and nucleoprotein of gene expression by antisense oligonucleotides

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Antisense RNA or antisense oligonucleotides within cells targeted toward the RNA transcript of a specific gene, can inhibit the expression or promote the degradation of the transcript, resulting in suppression of the function encoded for by the gene. However, the mechanism by which the antisense oligonucleotide inhibited viral protein synthesis, syncytia formation, and viral replication has not been fully elucidated.

We demonstrated that unmodified and modified (phosphorothioate) antisense oligo-nucleotides inhibit CAT (chloramphenicol acetyltransferase) protein expression in the clone 76 cell line.This cell line expresses the influenza virus RNA polymerase and nucleo-protein (NP) genes in response to dexamethasone. Antisense oligonucleotides with four target sites (PB1, PB2, PA, and NP) were synthesized and tested for the their inhibitory effects by a CAT-ELISA assay. Antisense phosphorothioate oligonucleotides (S-ODNs) complementary to the sites of the PB2-AUG and PA-AUG initiation codons showed a high inhibitory effect. On the other hand, the inhibitory effect of the S-ODNs targeted to PB1 was considerably decreased in comparison with the other three target sites.