istered in a digital form by Olympus 8080 camera, introduced into computer Pentium IV port and processed in a few minutes. The addition of the anti-influenza immunoglobulin to the virus-containing preparations has led to the significant changes of *D*. These structural changes of the virus-cell system are, most probably, due to the reaction of antigen–antibody type that takes place in the system. We have shown also that the proposed FM allows to detect the virus-cell interaction without any coloring techniques used in regular luminescent microscopy. It operates at the minimal virus-containing concentrations in some minutes after the start of the infection process. The application of FM method could be successfully performed even in the case of the enveloped viral particles detection. It was demonstrated experimentally that *D* value could serve as the reliable quantitative measure of the real state of the virus-state system and the rate of its progress either to recovery under the influence of the antivirals or to cell death without antivirals application.

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65

Sublingual Delivery of SB 9000—An Anti-HBV Dinucleoside Phosphorothioate Analog

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SB 9000 is a novel dinucleotide anti-HBV agent. Studies in rats and mice suggest that SB 9000 is not orally bioavailable. The lack of oral bioavailability may be due to either: (a) its rapid degradation in gastric fluid and/or (b) the negatively charged backbone that inhibit its diffusion through the mucosal barrier.

The present study was undertaken to explore the feasibility of sublingual delivery of SB 9000 for systemic effect. The anticipated advantages in sublingual delivery include: (a) avoiding degradation of the nucleotide in GI tract, (b) overcoming first pass metabolism and (c) preventing the pre-systemic elimination of the nucleotide from the GI tract. Additionally, sublingual delivery is expected to have a high degree of patient compliance.

Bioavailability studies were carried out in fasted, albino rats following sublingual administration of SB 9000 in a penetration enhancer at a dose of 20 mg/kg. In parallel experiments, aqueous solution of SB 9000 was administered at the same dose intravenously. The observed plasma concentrations of SB 9000 were 44 μM, 3.5 μM and 2 μM at 30 (peak plasma level), 60 and 120 min respectively, sufficient to achieve significant antiviral effect against HBV [EC_{50} of SB 9000, 0.5 μM]. In contrast, intravenous administration of SB 9000 resulted in more rapid peak plasma levels within 5 min, which then dropped to near baseline values in 2 h.

Hence, our studies suggest that sublingual delivery, being a non-invasive, patient-compliant route, can be exploited for the systemic delivery of nucleotides for anti-HBV therapy. This may be particularly useful for pediatric patients and adults who have difficulty swallowing medicine.

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66

Initial Pharmacodynamic Evaluation of Orally Bioavailable Prodrugs of SB-9000, a Novel Anti-HBV Agent

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SB-9000 is a new dinucleotide class of anti-HBV agent. Previously, it has been demonstrated that SB 9000 had a very potent antiviral activity in the transgenic mouse HBV model with an EC_{50} of 1 mg/kg when administered intraperitoneally. However, bioavailability studies in mice and rats revealed that SB-9000 is not orally bioavailable. Therefore, a series of prodrugs for SB-9000 were synthesized and evaluated in vitro including: (a) cytotoxicity in a panel of cell lines including HFF, MDBK and Vero cells, (b) the bioconversion to SB 9000 using serum and (c) The stability in presence of simulated gastric and intestinal fluids. Bioavailability studies in mice showed that a few prodrugs were orally bioavailable based upon plasma analysis and disposition in liver. No acute toxicity was seen in mice up to 800 mg/kg.

Two prodrug analogs, SB-9001 and SB-9002-1 were chosen for pharmacodynamic evaluation in transgenic HBV mose model. In this initial dose-finding study, a high-dose of the two prodrugs was administered by oral gavage at 300 and 400 mg/kg/day in citric acid buffer. Adefovir dipivoxil (ADV) was used as a positive control. HBV DNA in liver and plasma was quantitated using Southern blot and PCR.

Based upon Southern blot and quantitative PCR analysis, SB-9001 and SB-9002-1 were found to significantly reduce HBV DNA in the liver. Also, there was no apparent toxicity or mortality observed in the SB 9001 and SB 9002-1 treatment groups. Based on these initial results, a dose-ranging study is planned using appropriately formulated form of the prodrugs.

In conclusion, prodrugs SB 9001 and SB 9002-1 have been developed as orally bioavailable analogs of SB 9000, a novel anti-HBV agent.

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67

Characterization of Influenza Virus Clinical Isolates Obtained During Clinical Study of Arbidol

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An antiviral drug arbidol has been widely used in Russia now. Clinical trials and experience of using of this drug in the clinic have shown arbidol to be effective in preventing and treating influenzas A and B and well tolerated by patients. Our aim was to monitor the arbidol susceptibility of clinical isolates obtained in group of patients treated for influenza. Arbidol-resistant mutants were obtained by 15 passages of virus in MDCK cells in the presence of increasing from 5 to 20 μg/ml drug concentrations. Resistance of mutants was confirmed in cell ELISA and plaque activity assays and by haemolysis tests. To determine the molecular basis of arbidolresistance, the HA genes of the wild-type and arbidol-resistant mutants were sequenced. All mutants had amino acid substitutions only in the HA2 subunit, but at different positions. Paired isolates ($n = 25$) obtained from patients before and during therapy with arbidol (3×200 mg for 5 days) were studied for susceptibility to arbidol using ELISA-cell assay in MDCK cells. All isolates were equally sensitive to arbidol with IC50 falling in the range of 7.0–12.5 μg/ml and similar to IC_{50} previously observed for laboratory and clinical isolates. Two matched pairs of isolates of two patients from whom we were able to obtain days 4 and 5 samples were chosen for sequence analysis. No amino acid changes that had previously been identified in vitro as being involved with reduction of susceptibility to arbidol were observed. In our clinical study, it was shown that no arbidol resistance had emerged during 5 days of therapy of acute influenza infection.

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68

Preclinical Development of A New Class of Orally Active Drug Candidates for the Treatment of RSV Infections

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Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis and pneumonia in children under 1 year of age and is a leading cause of severe lower respiratory infections in infants and young children. It has been estimated in some U.S. communities that between 50% and 80% of bronchiolitis hospitalizations from November through April are due to RSV disease.

Prophylactic antibodies such as Synagis® (palivizumab) effectively reduce the incidence and severity of RSV disease in high-risk pediatric populations but the only antiviral treatment available for patients with RSV disease is ribavirin, a nucleoside analog with suboptimal clinical efficacy and safety profile.

We have developed a novel, potent class of small-molecule, orally available candidates that specifically target the RSV fusion glycoprotein. Representatives of this imidazoisoindolone class of fusion inhibitors are orally bioavailable in multiple species and have demonstrated efficacy in rodent models. They represent promising candidates for advancement into clinical trials for RSV.

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69

Carbohydrate-Binding Agents (CBAs) Potently Inhibit HIV Infection In Human Primary Monocytes/Macrophages and Efficiently Prevent Viral Capture and Subsequent Transmission to CD+4 T Lymphocytes

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Macrophages (M/M) are recognized as an important cellular target of HIV, and a crucial virus reservoir, producing and releasing large amounts of infectious viral particles for a long period of time. Moreover, productively infected M/M can interact with $CD4^+$ T-lymphocytes and transfer the virus to these cells. Carbohydrate-binding agents (CBAs) have been recently proposed as innovative anti-HIV compounds selectively targeting the glycans of the HIV-1 envelope glycoprotein gp120. Short pre-exposure of HIV-1 to CBAs prevents the DC-SIGN-expressing B-lymphoblast Raji cells (Raji/DC-SIGN) to efficiently bind HIV-1 and no syncytia formation occurs upon subsequent co-cultivation with CD4+ T-lymphocyte C8166 cells. Thus, the mannose-specific (i.e. the plant lectins HHA,