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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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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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.