JNK pathway. In this study we investigated whether p-KG03 could inhibit influenza A virus replication and examined the key steps in the viral replication cycle inducing antiviral activity. Cytopathic effect and plaque assays showed that co- or post-treatment of p-KG03 with influenza A/PR/8/34 (H1N1) into MDCK cells can result in a significant reduction in viral titer ($EC_{50}$, <1 μg/ml), but not pre-treatment of the polysaccharide. It means that the mode of the antiviral action could be involved in the inhibition of viral entry into cells or the inhibition of early viral RNA replication. Fluorescence microscopy using an NP-specific antibody proved that p-KG03 interferes with nuclear localizaton of viral NP protein at 3 and 5 h postinfection in a dose-dependent manner. However, it was not observed that p-KG03 affects viral RNA polymerase activity in 293T cells where negative GFP RNA flanked by NS 5′ and 3′ UTR is amplified by viral polymerases/NP proteins and expresses GFP protein as a reporter. Taken together, we suggest that p-KG03 has anti-influenza activity by blocking viral entry into cells or by making virus particles non-infectious. Thus it might be worthy of further investigation as a potential anti-influenza compound.

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## Excision of AZT and d4T Modulated by Deletions in the β3–β4 Hairpin Loop of HIV-1 Reverse Transcriptase

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Single-amino-acid deletions affecting residues 67 or 69 in the β3–β4 hairpin loop of HIV-1 reverse transcriptase (RT) have been identified in heavily treated patients. The deletion of Asp67 together with mutations T69G and K70R (Δ67 complex) are usually associated with thymidine analogue resistance mutations (TAMs) (*e.g.* M41L, T215Y, etc.) while the deletion of Thr69 (Δ69) is frequently associated with mutations of the Q151M complex, and rarely found together with TAMs. Biochemical assays showed that in the presence or absence of TAMs, Δ67/T69G/K70R enhances ATP-dependent phosphorolytic activity on primers terminated with 3′-azido-3′-deoxythymidine (AZT, zidovudine) or with 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T, stavudine). However, Δ69 (or the complex S68G/Δ69/K70G) antagonize the effects of TAMs in ATP-mediated excision activity assays. These results were consistent with AZT susceptibility data obtained with recombinant HIV-1 bearing the relevant RTs. Molecular dynamics studies based on models of wild-type HIV-1 RT and mutant RTs Δ69, Δ67/T69G/K70R and D67N/K70R support a relevant role for Lys/Arg70 in the excision reaction. The ε-amino of Lys70 is located >10 Å away from the putative pyrophosphate (PPi) binding site in the Δ69 RT. The loss of interactions between Lys70 and the incoming PPi in Δ69 RT could explain the lower excision activity of this enzyme. These studies also suggested that the substitution K219E could have an effect on thymidine analogue excision/discrimination. Pre-steady-state kinetics revealed minor differences in selectivity between AZT-triphosphate and dTTP, when the deletion-containing RTs (*i.e.* Δ67/T69G/K70R, Δ69 and S68G/Δ69/K70G) were compared with their homologous enzymes having the K219E mutation. However, K219E reduced both ATP- and PPi-mediated excision of primers terminated with AZT or d4T, only when introduced in RTs bearing Δ69 or S68G/Δ69/K70G, providing further biochemical evidence explaining the lack of association of Δ69 and TAMs in HIV-1 isolates.

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## Doxycycline in Tick-borne Encephalitis Virus Infection

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Tick-borne encephalitis (TBE) is endemic flavivirus in many parts of Europe and Asia. It is caused by TBE virus, which is transmitted into humans by tick bites. Ticks are vectors for various human pathogens, bacteria and protozoa. Bacteria includes Borrelia, Anaplasma, and Ehrlichia. One of the most useful drugs for prophylaxis bacterial infections after the tick bite is doxycycline. Mixt-infections of TBE virus and bacteria are very common (10–40%). Previously, structural analysis of Dengue virus E protein showed that doxycycline could have suppressing effect on DV infection in vitro by inhibiting low-pH induced conformational switch into fusogenic state during the entry process. The structure of E proteins is very common for all flaviviruses. Although there are some differences between mosquito- and tick-borne flaviviruses.

In the present study, we evaluated the doxycycline effect on TBE virus infection *in vitro* and *in vivo*.

*In vitro* experiments on PEK cells revealed that doxycycline addition prior or along with the TBE virus caused increased titers in plaque assay.

*In vivo* experiments in BALB/c mice showed that doxycycline caused slight positive effect on infectious process post high virus dose inoculation. Possibly, the effect was due to inhibiting of the secondary bacterial infection. There were no differences in mice mortality after small virus dose inoculation in treated and untreated groups. Although the virus titers in CNS of doxycycline treated mice were higher and were fixed more frequently than in infected control group. Analogous picture was observed with Amoksiklav(Amoxicillin + Clavulanic acid), but the effect was less expressed.

Thus, the doxycycline effect on TBE virus infection needs more investigations. Nevertheless, the presented data shows that doxycycline should be used very carefully in prophylaxis after tick bite due to high percent of mixt-infections.

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## Antiarboviral Efficacy of Combined Application of Interferon Inducers and Proteolysis Inhibitor

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Earlier, we have shown the possibility to increase production of endogenous interferon owing to the combined application of their inducers (amixin and new phytoremedy SK-19) with proteolysis inhibitor E-aminocaproic acid (E-ACA). This work presents the results of testing the same application method of these