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Antiviral Activity of a Thioglycoside Derivative Mimicking Tunicamycin Structure

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Tunicamycin has an antiviral effect against a broad spectrum of viruses affecting the first step of N-glycosylation process. However, the therapeutic use of tunicamycin has been limited by its toxicity in animals. Recently, using classical swine fever virus (CSFV), which can cause an acute, highly infectious and economically damaging disease in swine and wild boars, we reported that tunicamycin analogues – uridine derivatives of 2-deoxy sugars, possess significant antiviral properties affecting late steps of glycosylation process. Another group of compounds mimicking tunicamycin structure was synthesized in order to investigate whether these compounds also exhibit antiviral properties against CSFV. A lead compound – a thioglycoside derivative – designed GP6 was identified as the most selective inhibitor. The antiviral activity analysis of GP6 included the study of the inhibitor on penetration and propagation of CSF virus (using plaque reduction and virus yield assay), and on maturation of viral envelope glycoproteins by immunoblotting. GP6 effectively arrested viral growth in swine kidney cells (SK6) at a 50% inhibitory concentration (IC_{50}) of 5 ± 0.12 mg/ml without significant toxicity for mammalian cells. Moreover, it reduced the formation of viral glycoproteins E2 and E^{rns} in a dose-dependent manner. We have excluded the possibility that the inhibitor acts at the replication step of virus life cycle using real time RT-PCR method. Time of drug addition studies demonstrated that virus adsorption is the main target of GP6 inhibitor. Further experiments are needed for investigating whether this compound can be used as a safe antiviral agent against other members of Flaviviridae family and other RNA viruses.

doi:10.1016/j.antiviral.2011.03.107

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Hepatitis E Virus (HEV) Proteome and RNA Silencing Suppressors (RSS): A Search

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Background/Aims: HEV is an emerging pathogen responsible for sporadic and epidemic hepatitis. Etiological agent is a single-stranded positive sense RNA encapsidated in capsid. Viral genome replicates via negative sense intermediate. The dsRNA intermediates can trigger RNAi response against HEV. Thus, presence of RSS in the HEV genome cannot be ruled out. Here we report the absence of RSS activity in the HEV proteome.

Methods: We have designed a RNAi-RSS system. siRNAs against Firefly luciferase (Fluc) gene were designed, synthesized, converted into shRNA cassette and cloned. HEV genes encoding Methyltransferase, Helicase, Replicase, ORF2 and ORF3 proteins were cloned individually in eukaryotic expression vector. Fluc shRNAs were validated by co-transfection with target (pcDNA3-Fluc) in Huh7 cells. Inhibition was determined by dual luciferase assay at 48 h post-transfection. Suppressor assay was performed by co-transfecting Fluc shRNA along with target and individual viral protein expressing constructs into Huh7 cells. shRNA against Enterovirus 70 was used as unrelated control. Empty vector transfected along with target was used as reference control. Flock house Virus (FHV) protein B2 was used as a positive control for silencing suppression. HEV protein which shows any RSS activity was further evaluated as enhancer of HEV replication in the presence of pre-validated anti-HEV shRNA. HEV RNA was co-transfected with individual HEV protein expressing vector and shRNA designed against 3′ NCR of the virus. Using Real Time PCR, post 48 h transfection, RSS activity of viral protein was determined.

Results: Methyltransferase, Helicase, Replicase and pORF2 proteins did not show any RSS activity in dual luciferase RSS assay. pORF3 gave a strong RSS activity comparable with that of FHV B2 protein. But when HEV genome was targeted with pre-tested shRNA against HEV along with pSG1-ORF3, no enhancement effect was observed as demonstrated by Real Time PCR.

doi:10.1016/j.antiviral.2011.03.108

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Investigation of the Effects on Early Secretory Pathway in Cultured Cells and Potential Application of Antiviral Substances

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Early secretory pathway is a dynamic place where microtubule motor proteins play key role in the transport of cargo. Previous study characterised the activity of two main microtubule motors dynein and kinesin within the distance between endoplasmic reticulum (ER) and Golgi apparatus using fluorescence microscopy. Our present study is focused on the anterograde transport from ER towards Golgi membranes. We have described dynein association to membraned along microtubule filaments and we are now interested to explorer further its associated traffic events along microtubules. Using fluorescent antibodies we labelled membranes from the early secretory pathway to characterise p115 ERGIC membrane activator in Hela cells which were treated or nontreated with *E. coli* O157:H-. At the first step we detected alterations in p115 labelling using CLSM. We want to introduce as a next step some antiviral substances routinely used in our laboratory to elucidate possible effects on membrane movement in the anterograde transport and characterize Golgi/ERGIC membrane morphology in tissue culture cells. This research will provide prospectives to apply our approach in investigation of viral infections and other pathogen causative agents.

doi:10.1016/j.antiviral.2011.03.109

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Virucidal Activity of Calluses' Extracts from Tobacco-Plants

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Background: Plants are a rich source of substances with antiviral and/or virucidal activity. We have previously founded antiviral effect of protease inhibitors from soy beans, haricot, potatoes and of powder from wheat germ.

Objectives: We have studied virucidal activity of calluses' extracts from tobacco-plants in this research.

Methods: Freeze-dried powder were obtained from calluses. Extracts of them were prepared at the rate of 100 mg to 9.9 ml