Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

P89 Combined anti-influenza virus effect of a plant polyphenol-rich extract and rimantadine

J. Serkedjieva, A. Nikolova-Teodosieva. *Institute of Microbiology, Bulgarian Academy of Sciences, 26, Acad. Georgy Bonchev St., 1113 Sofia, Bulgaria*

Background: The anti-influenza virus activity of the polyphenol-rich extract from *Geranium sanguineum* L (PC) has been studied intensively. Its in vitro virus-inhibitory effect was strain-dependent; PC inhibited the virus-induced cytopathogenic effect and plaque formation, the production of infectious virus and haemagglutinin, the synthesis of virus-specific RNA and proteins (Serkedjieva and Hay, 1998). PC protected mice from mortality in the experimental influenza A/Aichi/2/68 (H3N2) virus infection (EIVI) (Serkedjieva and Manolova, 1992).

Results: The in vitro combined use of PC with Rimantadine hydrochloride (Rim) resulted in synergystic inhibition of the A/chicken/Germany/34, strain Rostock (H7N1) virus replication in MDCK cells. The cooperative effects were defined on the base of infectious viral yields by two complementary methods. The joint application of PC and three other amantadine derivatives also resulted in marked enhancement of inhibition. Administration of PC in combination with Rim in the EIVI in mice produced a synergistic protective effect: mortality rates were significantly decreased (index of protection = 77.8%), mean survival times were markedly prolonged (+5.2 days). A pronounced reduction of the lung lesions due to infection and of lung infectious virus titres was achieved ($\Delta \log_{10} TCID_{50}$/ml = 1.6–2.4).

Discussion: While the need for novel potent antiviral agents continues to exist, the strategy of combined antiviral therapy with available antiviral drugs has proved its usefulness. The presented results suggest that the combined use of natural and synthetic viral inhibitors may be used successfully to potentate the antiviral efficacy of the plant preparations and may enable dose reduction of their toxic components.

P90 Inhibition of HCoV-NL63 infection at early stages of the replication cycle

L. van der Hoek[1], B.J. Bosch[2], B. Berkhout[1], M.F. Jebbink[1], R. Dijkman[1], P.J.M. Rottier[2], K. Pyrc[1]. [1]*Department of Human Retrovirology, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands,* [2]*Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, and Institute of Biomembranes, Utrecht University, 3584 CL Utrecht, The Netherlands*

Background: HCoV-NL63, a recently discovered member of the Coronaviridae family, has spread worldwide and is associated with acute respiratory illness in young children, elderly and immunocompromised persons. Further analysis of HCoV-NL63 pathogenicity seems warranted, in particular because the virus uses the same cellular receptor as SARS-CoV.

Methods: As there is currently no HCoV-NL63 specific and effective vaccine or drug therapy available, we evaluated several existing antiviral drugs and new synthetic compounds as inhibitors of HCoV-NL63, targeting multiple stages of the replication cycle.

Results and Conclusions: Of the 27 compounds that we tested, five potently inhibited HCoV-NL63 at early steps of the replication cycle: Intravenous immunoglobulins (IVIG), heptad repeat peptides, siRNAs, beta-D-N4-hydroxycytidine and 6-azauridine. These compounds showed low IC50 values and low cytotoxicity and are candidates to be developed further for mono- or combination therapy.

P91 Prevalence of two recently described HBV mutations and their effect on adefovir therapy

P.M. Cook[1], N. Price[1], J. Workman[1], D. Mutimer[2], E. Smit[1], H.K. Osman[1]. [1]*Birmingham Public Health Laboratory, Birmingham Heartlands Hospital, Birmingham, UK,* [2]*The Liver Unit, Queen Elizabeth Hospital, Birmingham, UK*

Background: Adefovir dipivoxil is licensed for the treatment of patients with chronic hepatitis B infection particularly those with lamivudine-resistant HBV infection. A minority of HBV infected patients respond poorly to Adefovir treatment while others develop resistance. There are two known mutations, A181V and N236T, that mediate resistance but recently two additional primary mutations L217R and I233V have been reported to be associated with Adefovir resistance.

Aims: To investigated the prevalence of the L217R and I233V mutations in a cohort of patients chronically infected with HBV and treated at the Liver Unit, Queen Elizabeth Hospital, Birmingham, UK.

Methods: HBV sequences from samples sent to our laboratory between January 2003 and December 2005 from patients on antiviral therapy were examined.

Results: Samples from 36 patients were reviewed. The majority were genotype A (47%) and genotype D (31%). Lamivudine associated mutations were detected in 25 samples. L217R mutation was detected in 10 patients (28%), all infected with genotype A and in 9 cases Lamivudine associated mutations were also detected. 6 of the patients received Adefovir therapy and all responded virologically, some up to 2 years. To date no other Adefovir associated mutations have been detected in any of the patients. None of the 36 patients had the I233V mutation.

Conclusion: The L217R mutation was detected in 59% of our genotype A samples and does not seem to be associated with a poor response to Adefovir.

P92 Naturally-occurring hepatitis C virus protease variants: implications for resistance to new antivirals

F.X. López-Labrador[1,2], A. Moya[2], F. González-Candelas[2]. [1]*Public Health Department, Conselleria de Sanitat,* [2]*Evolution Genetics, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, University of Valencia, Spain*

Background: Recent publications have identified, in the replicon system, amino acid mutations in the HCV protease associated with resistance to new HCV protease inhibitors. However, there is little information in the literature on the natural variability of proteases from the different HCV subtypes.

Aims: To determine the natural variability on HCV proteases both within and between different viral subtypes and to compare it with known mutations conferring resistance to protease inhibitors.

Methods: Data mining on the EuHCVdb and Los Alamos HCV databases. A total of 211 non-redundant full-length HCV NS3 sequences were selected (159 genotype 1, 35 genotype 2, 17 genotypes 3, 4, 5 or 6). Amino acid frequencies were calculated to each position using VESPA and variation in sites potentially associated with resistance to protease inhibitors BILN-2061, VX-950, SCH-503034, and SCH6 were then compared to the HCV Con-1 protease sequence.

Results: Sequence heterogeneity was frequent in compensatory-mutation sites, such as T72, P89 and specially Q86 (Q86P in all non-genotype 1, and in 30% of genotype 1 sequences). BILN-2061 resistance-associated D79E was present in almost all genotype 2 sequences, thus validating our analysis. T54A, R109K, A156T/V/S, D168V/A/Y, or V170A (associated to BILN-2061, VX-950, or SCH-503034 resistance) were not observed, except for one genotype 6 isolate carrying V170A. New variations with unknown effect in resistance were common, such as D168E (in genotype 1 isolates) and V170I (in 45% of genotype 1 and in almost all non-genotype 1 sequences). Furthermore, 14% of subtype 1b isolates carried I153V, potentially implicated in low-level resistance to SCH6.

Conclusions and Discussion: There is relevant sequence heterogeneity in the NS3 protease of natural HCV isolates from different viral subtypes, with potential implications in HCV resistance to protease inhibitors. These data may be very useful for future genotypic testing before and during new antiviral treatments.

P93 Amantadine resistance among influenza A isolates in Sweden 2001–2006

M. Brytting, E. Qamrul, P. Petersson, M. Stivers. *Swedish Institute for Infectious Disease Control, Solna, Sweden*

Adamantanes (amantadine and rimantadine) can be used as prophylaxis or treatment of Influenza A infections. These matrix protein 2 blockers prevent release of the viral RNA in the cell. Recent

studies have shown a high prevalence of resistance to adamantanes among circulating influenza viruses in Asia and USA. In Sweden, amantadine has not been used for several years.

We have investigated the frequency of amantadine resistance by sequencing the matrix protein 2 of influenza A circulating in Sweden during 2001–2006. We found no resistance among subtype H1 isolates. The first resistant H3 isolate was collected from a traveller from Asia during season 2002–2003. The following season, 2003–2004, 3 out of 48 (6%) H3 isolates were resistant (no travelling history for any of these patients). In season 2004–2005, 4 out of 37 (11%) isolates were resistant (2 of the resistant isolates were from travellers from Asia). During season 2005–2006 the frequency of resistant isolates increased dramatically to 42%, or 8 out of 19 isolates. Three out of the 8 resistant isolates were collected from patients with travelling history from Asia. The resistant isolates during the last two seasons (2004–2005 and 2005–2006) were all collected during May 2005 until Feb 2006, and they all clustered together phylogenetically.

In all the 16 resistant isolates the S31N mutation was found, one of the isolates also had an additional V27A mutation.

P94 Changing epidemiology of hepatitis D virus (HDV) infection in Lebanon in the last two decades

S. Ramia[1], M. Zaatari[2], A.I. Sharara[3], F. Ramlawi[1], B. Farhat[4]. *[1]Faculty of Health Sciences, Department of Medical Laboratory Technology, American University of Beirut, Beirut, Lebanon, [2]Department of Laboratory Medicine, Hamoud Hospital, Saida, Lebanon, [3]Faculty of Medicine, Department of Gastroenterology, American University of Beirut, Beirut, Lebanon, [4]Department of Gastroenterology, Rasoul Al-Azam Hospital, Beirut, Lebanon*

Objective: Recently and in a national study we investigated the prevalence of Hepatitis B virus (HBV) genotypes and the association between these genotypes and the clinical status of HBV-infected patients in the Lebanese population. Hepatitis delta virus (HDV) is a unique single – stranded RNA virus that requires the helper function of HBV for infection. The aim of this study therefore was to determine the prevalence of HDV infection and the significance of HDV genotypes in the Lebanese population.

Patients and Methods: Two hundred and fifty eight HBsAg-positive patients (107 asymptomatic blood donors, 92 with chronic hepatitis, 24 with cirrhosis, 15 with hepatocellular carcinoma, 20 patients on hemodialysis) from ten medical centers in Lebanon were tested for antibody to hepatitis D virus (anti-HDV). Those testing positive were analyzed further for HDV-RNA and for genotyping by reverse transcriptase-polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP).

Results: Three samples (1.2%) were anti-HDV-positive and out of these, only one was HDV-RNA-positive (0.6%) and was analyzed as HDV genotype I.

Conclusion: Our results point to a low endemicity of HDV in the Lebanese population which is in sharp contrast to data reported from Lebanon 20 years ago and to the situation in neighboring Arab and non-Arab countries in the Mediterranean region. HDV genotype I seems to be the predominant genotype in Lebanon and the Middle East.

P95 Discordant outcome of perinatal transmission of hepatitis C in twin pregnancies

E. Boxall[1,2], K. Baumann[1], N. Price[2], J. Sira[1], D. Kelly[1]. *[1]Liver Unit, Children's Hospital, Birmingham B4 6NH, UK, [2]Health Protection Agency, Public Health Laboratory, Heart of England NHS Trust, Birmingham B9 5SS, UK*

Background: Risk factors for perinatal transmission of hepatitis C virus (HCV) from mothers to their babies include viraemia and co-infection with HIV. We describe the follow-up of 4 sets of twins born to HCV positive mothers. Factors influencing the risk of transmission (birth order, method of delivery, obstetric interventions, identical/non-identical twins, breast feeding, maternal viraemia) were investigated.

Methods: Out of a cohort of 70 HCV infected children referred to a specialist paediatric liver unit, 4 sets of twins were identified. Children were tested for antiHCV by EIA (Ortho Clinical Diagnostics) and HCV RNA (Roche Amplicor Monitor).

Results: In all cases, one of each set of twins had become infected and one remained negative on follow up. In 3 out of 4 cases, the second born child became infected. All children were non-identical girls delivered at 37 weeks gestation. Two families were delivered by Caesarean section and two by normal vaginal delivery, no invasive foetal monitoring was used. Infection was independent of HCV genotype and was not present in the infants at delivery.

Discussion and Conclusions: Maternal factors apply to both infants in twin pregnancies. Factors applying to only one infant are determined by individual genetic susceptibility and placental integrity. Maternal bleeding or placental leakage could apply to one infant. A longer labour could present the greater opportunity for placental leakage and hence exposure to the second infant. The investigation of the obstetric factors associated with twin pregnancies can give us more information about the factors associated with perinatal transmission of HCV.

P96 HCV-RNA typing by direct sequencing in clinical settings: method comparison

S. Gabella, T. Allice, S. Varetto, F. Pittaluga, V. Ghisetti. *Microbiology laboratory, Molinette Hospital, Turin, Italy*

Background and Aims: Genotyping and subtyping of hepatitis C virus (HCV) is clinically relevant to epidemiology, prognosis and therapeutical management of HCV infection. Aim was to study the feasibility of direct sequencing for HCV genotyping and subtyping of HCV strains from infected patients and comparing it with a commercial genotyping assay (InnoLiPA HCV II).

Methods: Seventy clinical samples cross-representing HCV genotypes 1 to 5 typed with InnoLiPA (LIPA) were subjected to a laboratory-developed 5′UTR direct sequencing protocol (5′UTR Seq). A NS5B direct sequencing protocol (NS5B Seq) was apply to 21/70 samples (12/21 negative by the 5′UTR). Two libraries of 5′UTR and NS5B HCV prototypes were constructed; BioEdit 7.0.0 and ClustalW were used for alignments and phylogenetic analysis.

Results: 5′UTR Seq typed 46/70 InnoLiPA positive samples (66%). Concordance between the two assay was 87% (40/46) and 80% (32/40) for HCV genotyping and subtyping, respectively. LIPA and 5′UTR discordant samples were typed by NS5b Seq. NS5b Seq typed 8/12 samples negative with 5′UTR Seq. All genotypes and subtypes detected with NS5b Seq were concordant with InnoLiPA. 5′UTR and NS5b direct sequencing pointed out a much wider HCV subtype determination than LiPA for genotype 1, 2 and 4. By the combination of 5′UTR and NS5b direct sequencing, 81% of InnoLiPA positive samples were typed. **Conclusion:** For clinical and epidemiologic purposes, a conclusive genotyping and subtyping of most HCV isolates by direct sequencing requires the combined analysis of at least two viral genomic regions with different level of genetic variability.

P97 Molecular characteristic and epidemiology of hepatitis B, C viruses in the Shiraz Province of Iran

S. Farzaneh[1], S.M. Reza[2], S.H. Reza[1]. *[1]Department of virology, Shafa hospital, Shiraz, Iran, [2]Department of virology, Bu Ali Sina University, P.O. Box 65176, Hamedan, Iran*

We carried out a molecular characteristic-based epidemiological survey of various hepatitis viruses, including hepatitis B virus (HBV), hepatitis C virus (HCV), in Shiraz. The study population of 185 subjects consisted of 181 individuals residing in the city of Shiraz, Shiraz province and the surrounding suburbs with the evidences of hepatitis, including: HCV Ab and/orHbs Ag and/or Ab HbcAg, Hbc Ab, Hbe Ag Hbe Ab, Abnormal LFT test, and some complications in physical exam. The infection rates of the viruses among the 185 subjects were as follows: for HBV 26.5% (49 patients) and 22.7% for HCV (42 patients). These results suggest that these hepatitis virus infections are widespread in Shiraz and have led to a high incidence of acute and chronic liver disease patients in the region.

1. Phone: (711) 2301164, fax: (711) 2270788; 2. Phone: (811) 4227350, fax: (811) 4227475.