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GLUCOSIDASE INHIBITORS INDUCE HCV GLYCOPROTEINS MISFOLDING, IMPAIR VIRAL ASSEMBLY AND RELEASE, AND REDUCE THE INFECTIVITY OF RESIDUALLY SECRETED HCV PARTICLES

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Background and Aims: Novel anti-HCV molecules are still needed to better combat HCV infection. Molecules targeting specifically viral activities are the most attractive in terms of drug development and are therefore the most studied. However, an antiviral strategy based uniquely on the utilisation of this type of molecules is expected to encounter problems caused by the emergence of viral escape mutants. HCV morphogenesis is a key step of the viral cycle and represents an interesting alternative target for the developpement of new antivirals. It was shown previously using a HCV surrogate virus, the bovine viral diarrhoea virus (BVDV), that glucosidase inhibitors could inhibit viral morphogenesis as a consequence of the perturbation of N-glycosylation and protein folding pathways. Provided that N-glycosylation plays an important role for the correct folding of HCV glycoproteins, it was hypothesized that such inhibitors could also affect HCV assembly, but also infectivity. Methods: The antiviral properties of glucosidase inhibitors were established using the most relevant models currently available: 1) cell culture model enabling the complete replication of the HCV JFH-1 strain in Huh7.5 cells and the release of HCV particles (HCVcc) in cell culture medium, as well as 2) infectious HCV pseudotyped particles (HCVpp) produced in HEK-293T that display functional E1E2 glycoprotein complexes. The latter system was shown to be particularly relevant to study HCV entry and therefore measure infectivity. Results: First we measured the antiviral effect of various glucosidase inhibitors in both systems, and found that the N-nonyl-deoxinojirimycin (NN-DNJ) was the most potent with an inhibitory concentration 50% (IC50) comprised between 5 and 10μM and no toxicity (CC50>> 100μM). Studies of the mechanism of action showed that, upon glucosidase inhibitor treatment, HCV glycoproteins 1) contained unprocessed triglycosylated N-glycans, 2) were impaired in their interaction with calnexin and 3) were misfolded. As the consequence of this inhibition we found 1) that the assembly of HCVpp was impaired, 2) that residually secreted HCVpp contained misfolded viral glycoproteins, and consequently 3) that residually secreted HCVpp had a reduced infectivity leading to the inhibition of viral entry. Conclusions: Altogether, the data obtained using both HCVcc and HCVpp models demonstrate that the antiviral effect of iminosugars is due to both an inhibition of viral assembly and release, as well as a reduction of viral infectivity. These properties further demonstrate the potential usefulness of glucosidase inhibitors in combating HCV infection.

Disclosures:
The following people have nothing to disclose: Cynthia Chapel, Celine Garcia, Birke Bartosch, Philippe Roingeard, Nicole Zitzmann, François-Loic Cosset, Jean Dubuisson, Raymond Dwek, Christian Trepo, Fabien Zoulim, David Durantel

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CD8 T CELLS ARE RECRUITED TO THE LIVER IN ACUTE HEPATITIS C BY HCV RNA-INDUCED CXCR3- AND CCR5-LIGANDS

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In acute hepatitis C, T cell recruitment to the liver coincides not only with liver injury, but also with complete or partial clearance of HCV. How T cells are recruited to the liver has not been studied in acute hepatitis C. To identify the mechanisms of T cell recruitment, we studied serial biweekly liver biopsies and blood samples from six chimpanzees during acute HCV infection (genotype 1a). First, we analyzed liver biopsies for mRNA levels of T cell markers and chemokine receptors by real-time PCR. In all chimpanzees, an increase in CD8beta but not CD4 mRNA levels coincided with intrahepatic IFN-gamma expression and with the serum ALT peak and decrease in HCV RNA titer. Concomitant to the increase in CD8beta mRNA levels, which represents CD8 T cell recruitment, we observed an increase in CXCR3 and CCR5 mRNA levels in the liver, and expression of the corresponding chemokines (CXCL10 and CXCL11 as CXCR3-ligands, and CCL4 and CCL5 as CCR5-ligands) in liver and blood. In contrast, CXCL16, which is a ligand for CXCR6 known to play a role in chronic hepatitis C, was not increased. These data suggest that the acutely HCV-infected liver produces CXCR3- and CCR5-ligands, and that CD8 T cells are recruited to the liver by CXCR3 and CCR5. To study the mechanism of chemokine induction, Huh-7 cells were transfected with p90 HCV RNA or artificial double-stranded RNA, poly(I-C). Transfection with either HCV RNA or poly(I-C) increased the expression of CXCL10, CXCL11, CCL4 and CCL5. Double-stranded RNA-induced chemokine expression was abrogated by neutralization of type I IFN, suggesting that intracellular dsRNA induces the production of chemokines via the action of type I IFN. In summary, the presence of intracellular double-stranded RNA in HCV-infected hepatocytes induces the chemokines CXCL10, CXCL11, CCL4 and CCL5 via the secretion of type I IFN, and the secreted chemokines then recruit CXCR3+ or CCR5+ CD8 T cells to the HCV-infected liver, resulting in liver injury and decrease of HCV RNA titer. Therefore, CXCR3, CCR5 and their ligands might be targeted to increase T cell recruitment and HCV clearance.

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PRODUCTION OF INFECTIOUS HEPATITIS C VIRUS OF VARIOUS GENOTYPES IN CELL CULTURE

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Efforts to understand the viral life cycle of Hepatitis C virus (HCV) have been hampered by the lack of an efficient cell culture system. A subgenomic HCV RNA replicon system had been developed to assess HCV replication in cultured cells and to evaluate therapeutic targets. However this system does not afford production of viral particles nor the early infectious process, and therefore cannot be used to characterize these stages of viral life cycle. We recently developed a unique HCV strain, JFH-1, that in cell culture can generate infectious HCV in vitro and in vivo. In this study, we produce infectious HCV particles by using a DNA expression plasmid containing full-length HCV cDNA flanked by self-cleaving ribozymes with various strains of genotypes 1 and 2. This construct also contains the secreted alkaline phosphatase gene to monitor the expression from this construct and to normalize transfection efficiency and to control for effects of culture conditions, such as anti-viral testing. Various strains of HCV genotype 1a (H77), 1b (CG1b) and 2a (J6 and JFH1) were constructed and tested. After transfection into Huh7 derived cell line Huh7.5.1, continuous HCV replication and secretion were confirmed by detection of HCV RNA and core antigen in culture medium. HCV replication levels of strains H77, CG1b and J6 were comparable. However, HCV replication level of JFH1 was substantially higher as compared with other clones. Iodixanol density gradient centrifugation of the culture medium revealed co-localization of HCV RNA and structural proteins, and presence of HCV particles around the peak fraction was confirmed by electron microscope. The HCV generated by this system was infectious in naive Huh7.5.1 cells; HCV core and NS3 proteins were detected by immuno-fluorescence study 3 days after inoculation. The culture medium from CG1b (HCV genotype 1b)-transfected cells caused a typical course of HCV infection in chimpanzee. The HCV 1b propagated in vitro and in vivo had identical sequences as the HCV genomic cDNA used for cell culture transfection. Conclusion. The development of culture system for production of infec-