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Anti-retroviral drugs do not facilitate hepatitis C virus (HCV) infection *in vitro*

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ABSTRACT

An estimated 4 to 5 million people are co-infected with HIV/HCV worldwide. Recently observed outbreaks of acute HCV infection among HIV-positive men who have sex with men (MSM) have been linked to behavioral factors such as high risk sexual practices and recreational drug use. However, at the molecular level, many drugs such as glucocorticoids or cyclosporine A have been found to modulate viral replication. Thus, it is conceivable that drugs used in highly active antiretroviral therapy (HAART) may heighten susceptibility to HCV infection and contribute to the recent outbreaks. We therefore performed a comprehensive screen of antiretroviral drugs covering all available drug classes both individually and in typical combinations used during HAART to probe for direct effects on HCV cell entry, replication, new particle assembly and release. Importantly, no significant enhancement or inhibition of HCV cell entry, replication or new particle production was detected. While raltegravir and ritonavir boosted atazanavir reduce HCV replication, a tenfold reduction of HCVcc entry by the CCR5 antagonist maraviroc was observed.

In conclusion, commonly used HAART agents do not specifically enhance HCV replication. Thus recent epidemic outbreaks of acute HCV in HIV-infected MSM are unlikely to be related to enhancing effects of HAART drugs.

1. Introduction

Worldwide more than 160 million people are chronically infected with Hepatitis C Virus (HCV). Liver cirrhosis and hepatocellular carcinoma due to chronic HCV infection are major indications for liver transplantation (Ciesek and Manns, 2011). HCV is a highly variable enveloped RNA virus belonging to the *Flaviviridae* family. The 9.6 kilobase-sized genome encodes for a single polyprotein cleaved by cellular and viral proteases into ten separated proteins: Core, E1 and E2 are structural proteins, P7 exhibits ion-channel activity and NS2, NS3A, NS3B, NS4A, NS4B, NS5A and NS5B are nonstructural proteins. The single open reading frame is flanked by untranslated regions at the 3' and 5' ends (von Hahn et al., 2011).

Due to similar modes of transmission, HCV is a common co-infection with Human Immunodeficiency Virus (HIV) in the industrialized countries. Approximately 4 to 5 million people are currently co-infected worldwide (Operskalski and Kovacs, 2011). Both viruses are transmitted parenterally via the sharing of contaminated needles by injection drug users. Sexual transmission is known to be a leading route for HIV, and seems to occur also for HCV albeit at a lower efficiency. In the western hemisphere the described co-infections are mostly due to intravenous drug abuse.

HIV infected HCV patients suffer from higher viral loads and weaker T-cell-specific HCV responses according to restricted immune system capacities (Danta et al., 2008; Capa et al., 2007). Faster development of fibrosis and end-stage liver disease has turned HCV infection into a leading cause of death in HIV/HCV co-infected individuals in the antiretroviral era (Vogel et al., 2011). Furthermore, hepatotoxicity of antiretroviral agents complicates HIV therapy in HCV co-infected patients (Sulkowski, 2008).

In the last ten years an increasing incidence of acute HCV infection among HIV-positive men who have sex with men (MSM) and who deny intravenous drug abuse has been observed. Reports from Europe (Vogel et al., 2010; Gambotti et al., 2005; Giraudon et al., 2008, the United States (Anon, 2011), and Australia (Matthews et al., 2007) describe this worldwide outbreak that especially seems to affect high-income countries. After introduction of HAART in 1996, HCV incidence in HIV-positive MSM has

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increased from 1–3 per 1,000 person years to more than 10 per 1,000 person years, whereas HCV incidence in HIV-negative MSM population remains low (van de Laar et al., 2010). Most affected men are infected with the difficult-to-treat genotype 1a and 4d (Urbanus et al., 2009; van de Laar et al., 2007). Further analysis showed the presence of a large European transmission network, linking outbreaks in different European cities (van de Laar et al., 2010). High risk sexual practices and recreational drug use have been proposed as causes for this phenomenon (Schmidt et al., 2011). Mucosal damage due to rough sexual techniques, ulcerative sexual transmitted diseases, the use of phosphodiesterase type 5 inhibitors and group sex promote bleeding and therefore HCV transmission. Drug abuse also lowers the personal inhibition threshold resulting in riskier sexual behavior. In addition to health risks due to the described co-infection, HCV-positive MSM suffer from stigmatization and discrimination by the MSM community (Owen, 2008). Several authors link the beginning of this epidemic of HIV/HCV co-infection to the introduction of highly active antiretroviral therapy (HAART) in 1996 reducing the threat of HIV infection and in this way re-opening ways for other (sexually transmitted) infections spreading via unprotected sexual intercourse (Stolte et al., 2004; MacKellar et al., 2011).

Besides the described behavioral factors, recent outbreaks could also be attributed to the HIV treatment regimes taken by most MSM that have contracted HCV infection during the recent outbreaks. Since replication of HCV is intricately linked to numerous cellular factors and processes, many drugs not primarily directed at HCV have been found to modulate viral replication. Prominent examples include glucocorticoids and cyclosporine A (Ciesek et al., 2010, 2009). Thus it is conceivable that drugs used as part of HAART might heighten susceptibility to HCV infection and contribute to the recent outbreaks.

In order to investigate this, we screened a selection of the 12 mostly prescribed antiretroviral agents for HIV therapy to reveal possible effects on the replication cycle of HCV *in vitro*.

2. Material and methods

2.1. Drugs

Darunavir, efavirenz, lopinavir, maraviroc, raltegravir, rilpivirine and ritonavir were kindly provided by David Back. Abacavir, atazanavir, emtricitabine, nevirapine and tenofovir were purchased from Toronto Research Chemicals Inc (Canada). Mechanisms of action are displayed in Table 1. Effective *in vitro* concentrations were identified from the literature. Additionally, effective concentrations for non-nucleoside reverse-transcriptase inhibitors (NNRTIs), nucleoside reverse-transcriptase inhibitors (NRTIs) and integrase inhibitors were determined by inhibition of lentiviral based pseudoparticle transduction.

2.2. Cell culture and cell lines

Huh-7.5 cells were maintained in Dulbeccos modified eagle medium (DMEM; Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal bovine serum, L-glutamine, nonessential amino acids, penicillin and streptomycin (Invitrogen).

2.3. DNA constructs

The full length reporter virus genome Luc-Jc1, the replicon pFK-I3841PI-Luc/NS3-3/Con1/ET (Luc-Con1 ET) and expression plasmids for HCV E1/E2 proteins of the J6CF (genotype 2a) or the Con1 isolate (genotype 1b) or H77 (genotype 1a) have been described recently elsewhere (Ciesek et al., 2010, 2011a).

2.4. Pseudotyping of lentiviral particles and transduction of target cells

Pseudoparticles were generated as previously described (Ciesek et al., 2011b). Briefly, three plasmids were co-transfected into 293T cells with polyethylenimine. These encoded (1) a lentiviral backbone containing either a firefly luciferase reporter (CSFlucW2), (2) HIV gag-pol and (3) either the G protein of Vesicular Stomatitis virus (VSV-G) or the HCV glycoproteins E1 and E2 of strain H77, Con1 or J6 preceded by the core signal sequence. Supernatants were collected at 48 and 72 h post transfection, passed through a 0.45-μm-pore-size filter, and added to the target cells for 6 h in the presence of antiretroviral drugs. Luciferase activity was measured 72 h after transduction.

2.5. Cytotoxicity assay

Lentiviral VSV-G pseudoparticles were produced as described above and used to transduce Huh-7.5 cells. Transduced Huh-7.5 cells were passaged to establish a stable cell line expressing firefly luciferase (von Hahn et al., 2011). To test for cytotoxic or antiproliferative effects, drugs were added at different concentrations for at least 48 h. After 48 h, luciferase activity was measured.

2.6. Cell culture grown HCV (HCVcc) infection

Huh-7.5 cells were transfected with HCV genomes (5 μg Luc-Jc1 containing a firefly luciferase reporter gene or Luc-Con1 ET by electroporation as previously described (von Hahn et al., 2011). At 5 h post transfection, different concentrations of antiretroviral drugs were added and at 48 h post transfection HCV replication was quantified by measuring luciferase activity. To investigate HCV assembly and release of new HCVcc particles, supernatants were collected at 48 h post transfection with Luc-Jc1, filtered through 0.45 μm pore size filters, and used to infect target cells. Target cells were inoculated with 500 μl virus containing supernatant in

Table 1
Antiretroviral drugs.

Mode of action	Compound	Concentrations (μg/ml)	Toxic concentration (μg/ml)	Plasma in vivo concentration (therapeutic dosage) (μg/ml)
Protease inhibitors	Atazanavir	0–25	125	3.152
	Darunavir	0–50	250	6.5
	Lopinavir	0–50	10	9.4 ± 4,4
	Ritonavir	0–2	20	0.89
Non-nucleoside reverse-transcriptase inhibitors (NNRTIs)	Efavirenz	0–10	10	4.07
	Nevirapine	0–100	100	5.74
	Rilpivirine	0–2	100	0.204
Nucleoside reverse-transcriptase inhibitors (NRTIs)	Abacavir	0–50	500	4.26
	Emtricitabine	0–20	500	1.8
	Tenofovir	0–30	300	0.326
Integrase inhibitor	Raltegravir	0–50	>100	2.17
CCR5 receptor antagonist	Maraviroc	0–50	500	0.888

12-well-format. HCV infection was quantified by measuring luciferase activity. For HCVcc entry studies, HCVcc particles (Luc-Jc1) were produced in the absence of any drugs, filtered and used to infect naïve Huh-7.5 cells in the presence of antiretroviral drugs. After 5–6 h cells were washed with PBS and medium was replaced.

2.7. Luciferase assay

Cells were washed with phosphate-buffered saline (PBS), lysed directly on the plate with either 1 ml or 350 μl lysate buffer (0.1% Triton X-100, 25 mmol/L glycylglycine, 15 mmol/L MgSO4, 4 mmol/L EGTA, and 1 mmol/L DTT; pH 7.8) and frozen to −20 °C. For quantifying luciferase activity, 100 μl defrosted lysate was mixed with 360 μl assay buffer (25 mmol/L glycylglycine, 15 mmol/L MgSO4, 4 mmol/L EGTA, 1 mmol/L DTT, 2 mmol/L ATP, 15 mmol/L K2PO4, pH 7.8) and combined with 200 μl luciferin solution (200 μmol/L luciferin, 25 mmol/L glycylglycine, pH 8.0) in a luminometer (Lumat LB9508; Berthold, Freiburg, Germany) measuring luciferase activity for 20 s. All measurements were performed in duplicates for HCV replication and in quadruplicates for HCV infection.

2.8. Western Blot Analysis

Cells were lysed in sample buffer (400 mmol/L Tris, ph 8.8, 10 mmol/L EDTA, 0.2% bromophenol blue, 20% sucrose, 3% sodium dodecyl sulfate). Proteins resolved by electrophoresis and blotted onto a polyvinylidene difluoride membrane were detected using SR-BI (anti-rabbit; Novus Biologicals), claudin-1 (anti-mouse, clone 2H10010; Zymed; Invitrogen), occludin (anti-mouse, clone OC-3F10, Zymed; Invitrogen), or Nieman Pick C1L1-specific antibodies (Cell Signaling/ New England Biolabs, Frankfurt am Main, Germany) and the ECL Plus Western Blotting Detection System (GE Healthcare Europe, Freiburg, Germany).

2.9. Flow cytometry

Huh-7.5 were stained with anti-CD81 (JS-81; BD Pharmingen, Heidelberg, Germany) followed by Alexa-Fluor 488 goat anti-mouse secondary antibody (Life Technology; Darmstadt, Germany). A BD FACSCalibur flow cytometer (Becton Dickinson, Heidelberg, Germany) was used for acquisition and FlowJo software (Tree Star; Ashland, OR) for data analysis.

2.10. Statistical analyses

Numerical data were analyzed using Excel (Microsoft, Redmond, WA, USA). All *in vitro* experiments were repeated on separate occasions and each repetition was done in multiple replicates. Unless stated otherwise the mean ± standard deviation of the replicates from one representative experiment is shown with the number of replicates indicated. For *in vitro* data, significant differences were identified using the unpaired two-sided student's T-test. Throughout *p*-values below 0.05 were considered significant and are indicated by an asterisk.

3. Results

3.1. Antiretroviral drugs do not enhance HCV cell entry

We evaluated the effect of commonly used antiretroviral drugs on HCV cell entry by HCV pseudoparticles (Fig. 1A) as well as infection assays with Luc-Jc1 virus (Fig. 1B). Antiretroviral drugs were added simultaneously with the viruses and were present for 4–5 h. To assess the influence of protease inhibitors and the CCR5 receptor antagonist maraviroc on HCV entry we used HCV pseudoparticles (HCVpp). Because these are lentiviral cores carrying HCV glycoproteins in their envelope, only the early steps of virus entry are HCV dependent, i.e., virus binding, uptake, and virus-membrane fusion, whereas all later steps are dependent on lentiviral proteins. The effect of reverse-transcriptase and integrase inhibitors cannot be tested using the pseudoparticle system which requires these enzymes to be functional.

Protease inhibitors and the CCR5 receptor antagonist maraviroc did not influence HCV entry in the pseudoparticle system and showed no effect on cell entry of HCVcc. Additionally, reverse

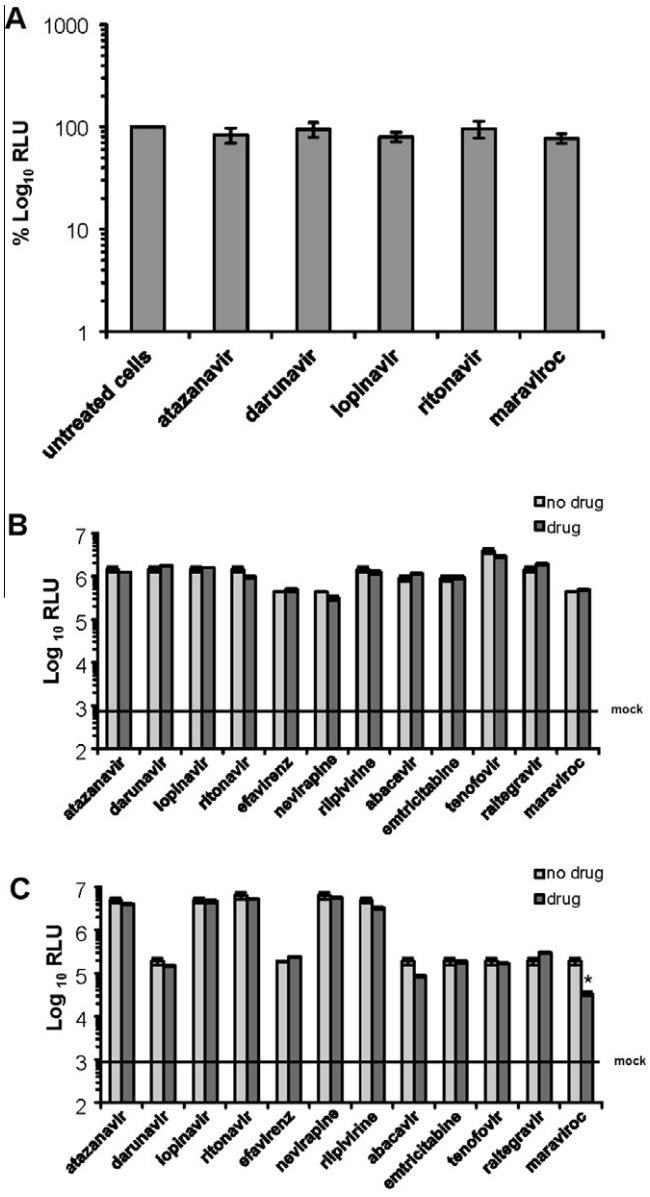

Fig. 1. Hepatitis C virus entry is not facilitated by antiretroviral drugs. Highest non-cytotoxic drug concentrations are presented (compare Table 2). (A) Lentiviral based HCV pseudoparticles with H77 derived glycoproteins were used to transduce Huh-7.5 cells in the presence or absence of antiretroviral drugs. Mean values of two independent repetitions and the standard deviation are shown. (B) Huh-7.5 cells were inoculated for 4 h with antiretroviral drugs together with Luc-Jc1 particles prepared in the absence of the drugs. A representative experiment of two independent repetitions with mean values and ranges is given. (C) Huh-7.5 cells were inoculated with antiretroviral drugs. After 24 h Luc-Jc1 particles prepared in the absence of the drugs were added. A representative experiment of two independent repetitions with mean values and standard deviations is presented.

transcriptase inhibitors as well as the integrase inhibitor raltegravir do not promote HCVcc entry (Fig. 1A and B).

We next administered antiretroviral drugs for 24 h before infection with Luc-Jc1 viruses to see if elongated presence changes infection efficiency. While most of the drugs did not have an effect on HCV entry even after 24 h pre-treatment, pre-treatment with the CCR5 receptor antagonist maraviroc showed an 8 to 10-fold reduction of HCVcc entry (Fig. 1C).

3.2. Maraviroc moderately reduces HCVcc entry but has no effect on HCVpp

To further characterize the HCV entry inhibition by maraviroc, we next wanted to know if this effect is (i) dose dependent, (ii) independent of HCV genotype and (iii) caused by reduced expression levels of HCV entry factors. As shown in Fig. 2A, pre-treatment of Huh-7.5 cells with increasing concentrations of maraviroc showed a dose dependent inhibition of HCVcc entry. By using HCV pseudoparticles with glycoproteins of different HCV genotypes we were able to show that HCVpp entry was not affected by maraviroc (Fig. 2B). In addition, expression levels of SR-BI, claudin-1, occludin and Nieman Pick C1L1 (NPC1L1) were not influenced by maraviroc pretreatment (Fig. 2C and D).

Importantly, the cellular target of maraviroc, the CC chemokine receptor type 5 is known to be present on a subpopulation of Huh-7.5.1 cells (El-Hage et al., 2011). In line with these findings, we were also able to detect CCR5 by FACS analysis on a subpopulation of the Huh-7.5 cells used in this study (data not shown).

3.3. Antiretroviral drugs do not promote HCV replication

Since none of the antiretroviral drugs enhance HCV entry, we next investigated if any of these compounds promote viral replication. For this we used full length firefly luciferase reporter viruses based on the intragenotypic genotype 2a chimera Jc1 or subgenomic replicons based on Con1 (genotype 1b).

As shown in Fig. 3A, replication of genotype 2a HCV construct Luc-Jc1 was not significantly enhanced by the drugs in comparison to the control. Atazanavir induced the most prominent inhibitory effect at a concentration of 25 μg/ml. Usage of higher concentrations was not possible due to cytotoxic effects.

To analyze influence on other HCV genotypes, we examined replication of a Con1 subgenomic replicon. The Con1 replicon is known to replicate with lower efficiency in cell culture than Luc-Jc1. In this way possible enhancing effects may appear more clearly because complete capacity utilization of replication enzymes is improbable. As presented in Fig. 3B, no enhancing effects on replication of HCV genotype 1b by singly applied antiretroviral agents were observed. On the contrary, the integrase inhibitor raltegravir reduced replication of the Con1 replicon by ca. 10-fold.

3.4. Impact of antiretroviral agents on HCV particle release

Thus, even though virus entry and replication are unlikely to be enhanced, it is important to analyze effects of antiretroviral drugs on new viral particle release in the final step of viral replication cycle. For that reason, Huh-7.5 cells were transfected with RNA of Luc-Jc1and treated with the drugs during replication. After 48 h, supernatant of these cells was collected and applied on naïve Huh-7.5 cells.

Apart from moderate changes lower than 1-fold, neither inhibition nor, more importantly, promotion of viral particle release was observed (Fig. 3C). For lopinavir and emtricitabine we noted diminutive reduction in viral replication whereas particle release is either marginally increased or remains unchanged in comparison to the control (Fig. 3C).

3.5. Influence of antiretroviral combinations on the HCV replication cycle

Clinical guidelines recommend triple combination therapy for HIV treatment. Individualized therapy schemes should comprise at least two components from different classes of drugs to prevent development of resistant HIV subspecies. In addition to that, it is common practice to boost protease inhibitors with ritonavir. To mimic real-life conditions as closely as possible it is important to test singly applied drugs as well as combinations.

We therefore examined typical commercially available drug combinations and mixed the components according to concentration ratios published at Hannover Medical School pharmacy database [MHH AiDKlinik] (Table 2).

In comparison to drugs applied alone, cytotoxicity appeared more frequently, especially for combinations of three compounds (data not shown). According to the data presented in Fig. 4A, virus entry was not influenced by combination treatment. There was no difference between directly applied antiretroviral drugs or pre-treatment of 24 h.

Also replication of genotype 1b replicon remained unaffected (Fig. 4B). In contrast the combination of ritonavir with atazanavir reduced Luc-Jc1replication by more than 10-fold at the highest tested doses (Fig. 4C). Likely as a consequence of reduced RNA replication, the de novo production of infectious viral progeny was also impaired by ca. 10-fold (Fig. 4C left). However, none of the tested drugs, applied alone or in combination, show any promoting effects on the hepatitis C replication cycle.

4. Discussion

The data presented in this study indicate that antiretroviral drugs do not enhance HCV replication or infectivity *in vitro*. Except for moderate virus entry reduction by the CCR5 receptor antagonist maraviroc, and decreased virus replication and particle release by singly applied raltegravir or the combination of atazanavir and ritonavir, no antiviral activity against hepatitis C *in vitro* was found.

Interestingly, the CCR5 receptor antagonist maraviroc showed a significant reduction with HCVcc but not HCVpp entry. This reduction is only visible if cells are pretreated with the drug for 24 h indicating that the observed entry inhibition may be due to effects on the host cell rather than direct effects on the virus itself. The CCR5 receptor is present on the surface of Huh-7.5 cells (El-Hage et al., 2011), but it is has not been reported that hepatitis C virus entry is linked to this receptor. The entry inhibition by maraviroc is small and in this way makes a direct involvement of CCR5 in the HCV entry pathway rather unlikely. Additionally we could show that the entry inhibition is not caused by down-regulation of the known HCV entry factors by maraviroc pretreatment. Nevertheless, further studies will be required to obtain more precise information.

Atazanavir is reported to be well tolerated and safe in HCV/HIV co-infected patients (Rockstroh et al., 2008) and ritonavir is used to boost effects of newly developed direct-acting-antiviral agents against HCV. Moreover, Trimoulet et al. reported a reduction of intrahepatic HCV RNA and liver enzyme levels in the presence of antiretroviral drugs, suggesting benefits for HCV therapy (Trimoulet et al., 2002).

Interestingly, HCV genotype 1 NS5B RNA dependent RNA polymerase shares an active site fold with the HIV integrase (Chen et al., 2012). Inhibitors containing diketo acid (two keto groups in their molecular structure) chelating functionality are capable of interacting directly with metal ions present in the enzyme's active site (Summa et al., 2004). Originally derived from the evolution of diketo acids against HCV NS5B polymerase, raltegravir is

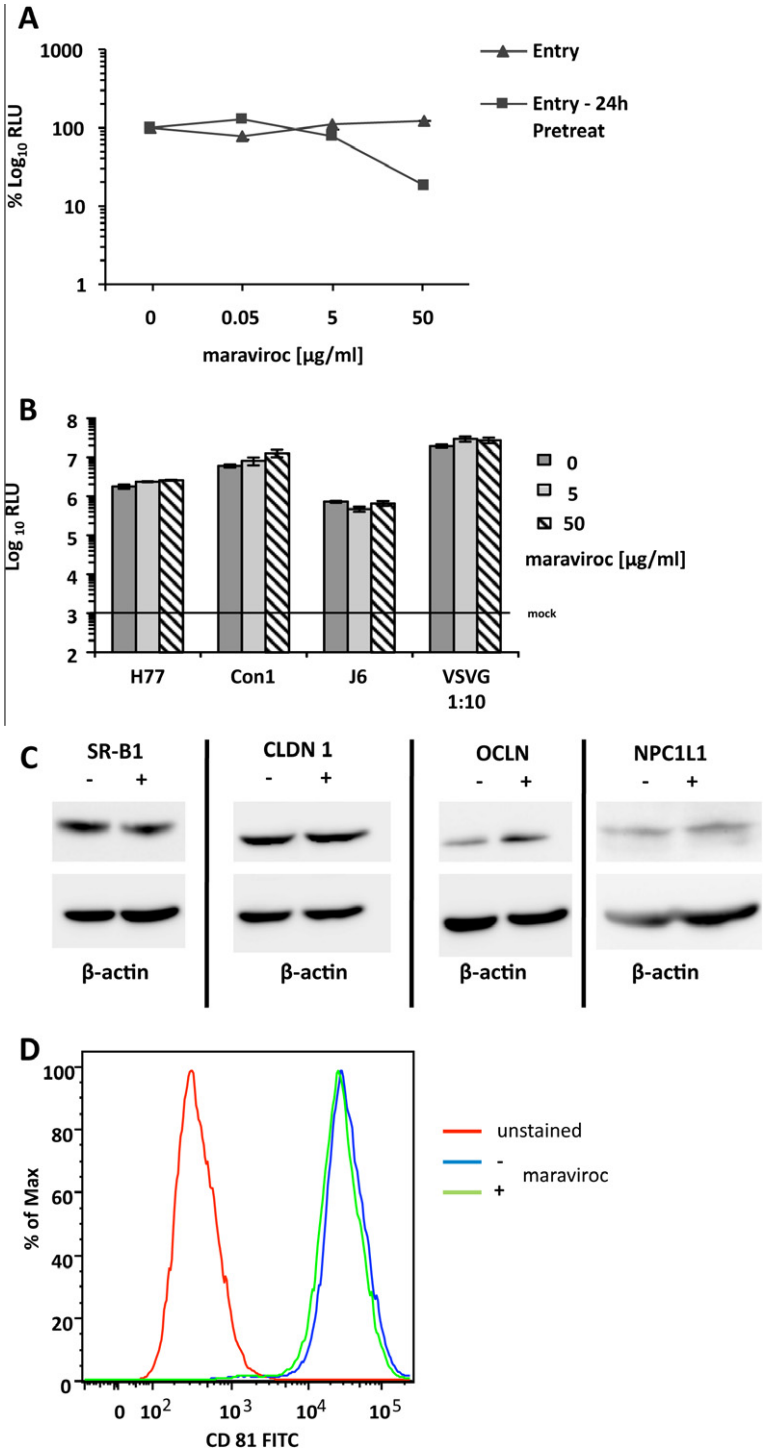

Fig. 2. Maraviroc inhibits HCVcc but not HCVpp entry. (A) Huh-7.5 cells were inoculated with Luc-Jc1. Increasing concentrations of maraviroc were added 24 h before or during inoculation. Luciferase signal was measured 48 h after inoculation. (B) Huh7.5 cells were treated for 24 h with increasing doses of maraviroc. Then lentiviral based HCV pseudoparticles with H77 (genotype 1a), Con 1 (genotype 1b) or J6 (genotype 2a) derived glycoproteins were used to transduce Huh-7.5 cells. Luciferase activity was measured after another 48 h. A representative experiment of three independent repetitions with mean values and standard deviations is presented. (C) Huh 7.5 cells were treated with maraviroc for 24 h and levels of SR-BI, claudin-1 (CLDN1), occluding (OCLN) and NPC1L1 were measured by western blotting. (D) CD81 surface expression of Huh-7.5 cells treated with maraviroc for 24 h was measured by FACS analysis.

the result of biochemical modifications to optimize metabolic stability, pharmacokinetic profile and antiviral activity against a panel of HIV-1 integrase mutations and to become inactive (IC50's > 22 µg/ml) against the HCV polymerase (Summa et al., 2008). Nonetheless, higher concentrations of raltegravir were used in our experiments and inhibitory effects on HCV replication due to inhibition of NS5B polymerase are conceivable. In addition to this, several studies name the integrase inhibitor raltegravir as an appropriate drug for treatment of HCV/HIV co-infected patients with less frequent liver enzyme elevations and the capability to reduce side effects associated with other antiretroviral agents (Rockstroh et al., 2012; Cao et al., 2010; Vispo et al., 2010).

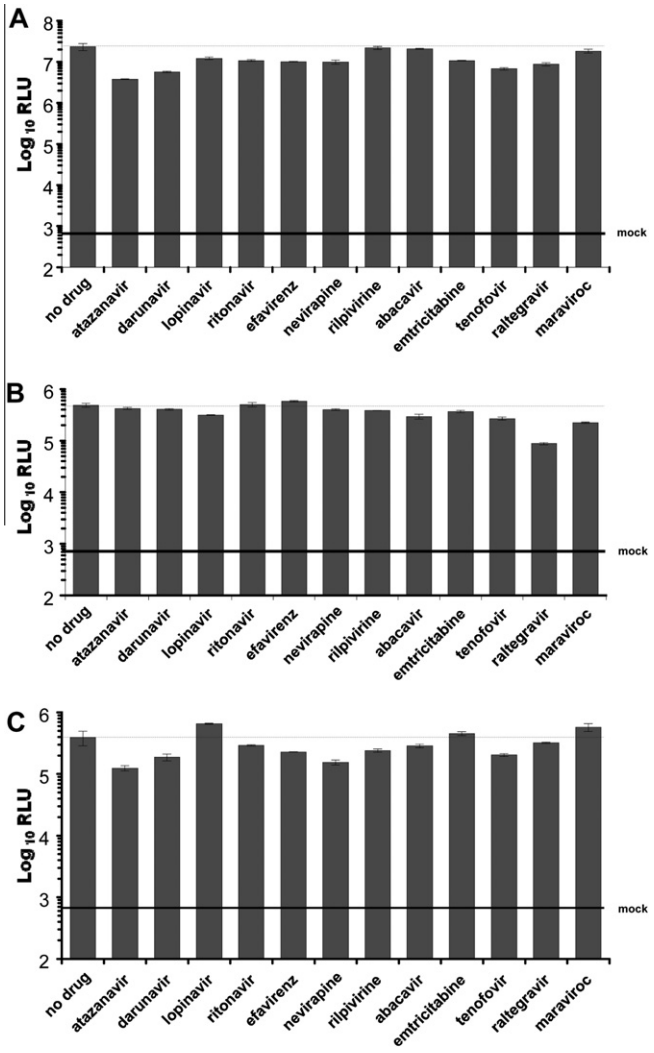

Fig. 3. Antiretroviral drugs show no promoting effect on Hepatitis C replication and release. Highest drug concentrations without cytotoxic effects are shown (see Table 2). (A) Huh-7.5 cells were electroporated with Luc-Jc1 and antiretroviral drugs were added 4 h later. Replication was measured 48 h post-transfection using luciferase assays. (B) Replication assays as described in part (A) were performed using the subgenomic RNA of genotype 1b HCV strain Con1. (C) Supernatant of electroporated cells from (A) was used to infect naïve Huh-7.5 cells. Mean values and standard deviations of one representative experiment of two independent repetitions are shown.

These studies are supported by our experimental results, which show that raltegravir, and ritonavir in combination with atazanavir, mildly reduce HCV replication, and could account for the positive effects of these antiretroviral drugs in co-infected patients. All of these aspects are attended to by our described experimental results and demonstrate the supportive effects of antiretroviral drugs in co-infected patients. Even if pharmacokinetics and possible drug-drug interactions cannot be faithfully mimicked in cell culture experiments, our findings exclude the possibility of direct promoting effects of antiretroviral agents on hepatitis C virus replication and infectivity *in vitro*. Thus, antiretroviral therapy is unlikely to be responsible for the increasing incidence of HCV infections in HIV positive MSM through a direct positive effect on HCV replication. According to our *in vitro* data antiretroviral regimes containing maraviroc, raltegravir or ritonavir boosted atazanavir may be useful for treatment of co-infected patients or persons at risk for acquiring HCV in addition to HIV. Even if therapeutic dosages differ from concentrations we used in our experiments inhibiting effects on HCV infection and replication are reliable and support the clinical use of these drugs.

These findings are important for affected patients, high-risk groups, and physicians as they may help to boost confidence in and compliance with antiretroviral regimes that are required to control HIV infection. Moreover, firm suppression of HIV infection is crucial for effective HCV treatment in co-infected patients given that stable CD4+ T-cells counts >350 cells/μl are a predictor of sustained virological response to HCV treatment with pegylated interferon plus ribavirin (Rockstroh et al., 2008). Co-infected patients suffer from faster progression to liver fibrosis and eradication of HCV to avoid end-stage liver disease has a high priority in this difficult to treat patient group.

Even if antiretroviral therapy does not influence HCV infection on a molecular level, the availability of effective HIV treatment and perceived protection from manifestation of acquired immune deficiency syndrome (AIDS) changes behavior of person at risk. Riskier sexual behavior, most notably unprotected sexual intercourse, promotes the spread of HIV and other sexual transmitted infections (STIs) that apparently include HCV (Dougan et al., 2007). Serosorting behavior, the practice of choosing sexual partners concordant with a person's own HIV status, limits HIV spread in HIV-negative individuals but promotes non-HIV-STIs in HIV positive individuals. In general, sexual transmission of hepatitis C is debated, but in terms of HCV propagation in HIV-positive MSM, this has turned out to be the leading cause. Traumatic sexual practices where bleeding occurs frequently permit HCV transmission, and mucosal damage due to sexually transmitted diseases facilitates infectious blood exchange (Danta and Rodger, 2011). Furthermore, nasal or rectally applied drugs play an important role thus leading to disinhibition and sensation seeking and sexual risky behavior (Colfax et al., 2004). Along these lines, newly acquired HCV infections in HIV positive MSM may be mostly linked to behavioral factors.

In addition to this, reduction of AIDS related mortality allows development of chronic diseases which lead to non-AIDS related deaths in HIV positive patients. Several studies indicate liver-related deaths as the most frequent cause of non-AIDS related death with hepatitis C playing a major role (Weber et al., 2006; Rosenthal et al., 2009; Chen and Ding, 2009). Co-infected patients suffer from reduced spontaneous clearance of HCV infection, higher viral RNA blood levels and are more likely to develop liver fibrosis and end-stage liver disease than HIV mono-infected patients.

Table 2
Highest non-toxic concentration of applied drugs.

Compound	Concentrations (μg/ml)
Atazanavir	25
Darunavir	50
Lopinavir	2
Ritonavir	2
Efavirenz	2
Nevirapine	10
Rilpivirine	2
Abacavir	50
Emtricitabine	20
Tenofovir	30
Raltegravir	50
Maraviroc	50
Emtricitabine/tenofovir	20/13.6
Emtricitabine/tenofovir/efavirenz	2/1.3 6/6
Lopinavir/ritonavir	2/0.5
Darunavir/ritonavir	10/1.25
Atazanavir/ritonavir	25/8.325

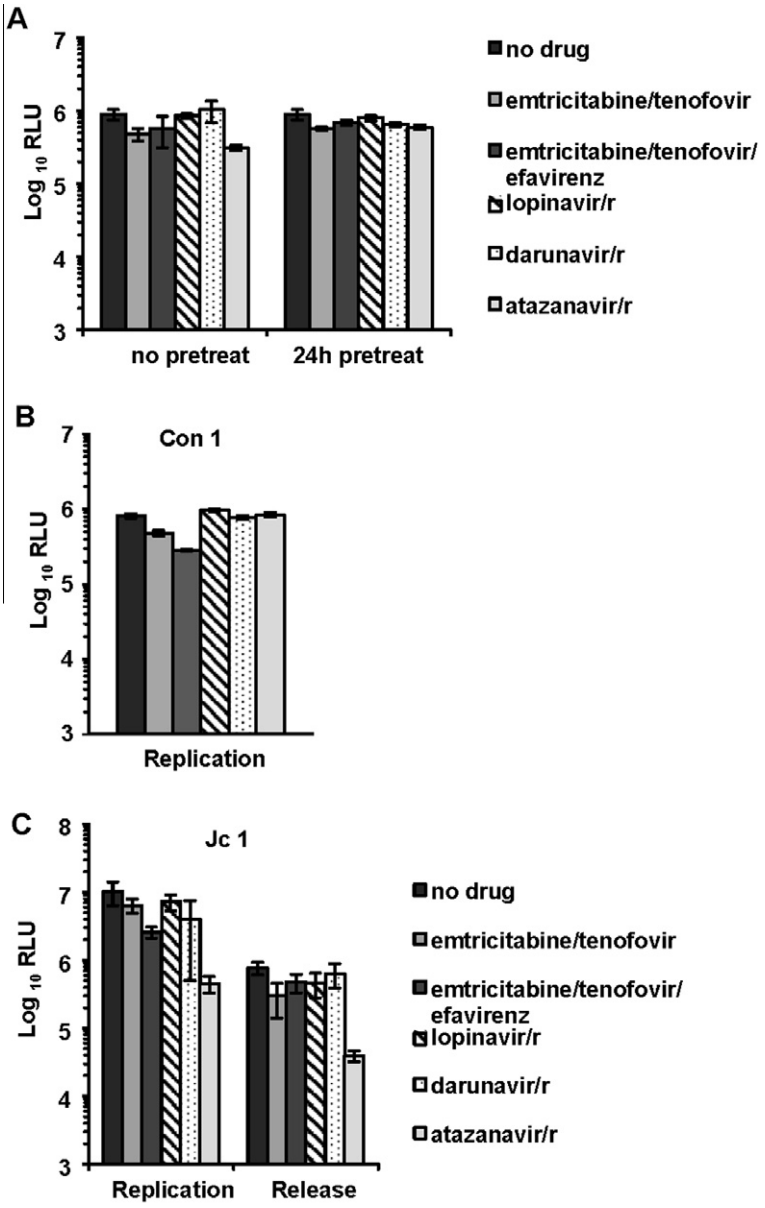

Fig. 4. Combination of antiretroviral drugs do not modulate the HCV replication cycle. Highest non-cytotoxic drug concentrations are shown (see Table 2). (A) Huh-7.5 cells were inoculated with Fluc-Jc1 virus particles and antiretroviral agents either simultaneously or after a drug pretreatment of 24 h. Mean values of two independent experiments including standard deviations are shown. (B) Replication experiments were performed by electroporating Huh-7.5 cells with Con1 (HCV genotype 1b) RNA. Drugs were added after 4 h and were present during replication for 48 h. Then cells were lysed and luciferase activity was measured. (C) Naïve Huh-7.5 target cells were infected with supernatant from the Fluc-Jc1 transfected cells. Drugs were added after 4 h and were present during replication for 48 h. Then cells were lysed and luciferase activity was measured. Mean values and standard deviations of two independent experiments are presented.

For this reason it is highly important to prevent HIV-positive patients from HCV infection, and to detect HCV infection as early as possible in order to start HCV treatment on time.

Once acquired, treatment of co-infected MSM demands a multidisciplinary model of health care delivery. Apart from internal medical care, intensive psychosocial support is needed. Stigmatization of HCV infected men in the MSM community and the burden of a second, severe disease may explain higher rates of depressive symptoms and mental health problems reported by Pantalone et al. (2011).

With HCV emerging as an STI among HIV-positive MSM, epidemiology of HCV has changed significantly and clinical and public health institutions have to face this new situation.

In 2011, Taylor et al. (2011) reported that 54% of HIV-positive MSM are unaware that traumatic sexual and drug practices put them at risk for HCV (El-Hage et al., 2011). In addition, one third of co-infected MSM are not aware of their HCV infection and infectivity (Urbanus et al., 2009) illustrating the need for education and intervention. Recently reported HCV re-infections in HIV-positive MSM following successful treatment demonstrate the present lack of sufficient prevention measurements needed to manage this growing problem (Lambers et al., 2011).

In this setting it is of great importance to know that antiretroviral drugs do not increase susceptibility to hepatitis C virus. HIV infected patients on HAART do not face a greater risk to become infected with HCV due to promoting effects of HAART on HCV infectivity. These findings may help to maintain treatment acceptance of HIV in the MSM community and in this way facilitate implementation of strategies to limit further spread of HCV infections.

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