Antiviral Research 96 (2012) 51–58

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

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

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ARTICLE INFO

Article history:
Received 26 May 2012
Revised 3 July 2012
Accepted 12 July 2012
Available online 24 July 2012

Keywords:
Hepatitis C virus
HIV
Co-infection
Antiretroviral drugs
MSM
Acute hepatitis C virus infection

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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http://dx.doi.org/10.1016/j.antiviral.2012.07.005

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