Virology 424 (2012) 147–153

Contents lists available at SciVerse ScienceDirect

Virology

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

Mutagen-mediated enhancement of HIV-1 replication in persistently infected cells

Carmen Sánchez-Jiménez [a,1], Isabel Olivares [a,1], Ana Isabel de Ávila Lucas [b], Víctor Toledano [a], Mónica Gutiérrez-Rivas [a], Ramón Lorenzo-Redondo [a], Ana Grande-Pérez [c], Esteban Domingo [b,d], Cecilio López-Galíndez [a,*]

[a] Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain
[b] Centro de Biología Molecular "Severo Ochoa", (CSIC-UAM), Campus de Cantoblanco, Madrid, Spain
[c] Área de Genética, Facultad de Ciencias, Universidad de Málaga, Campus de Teatinos, Málaga, Spain
[d] Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Barcelona, Spain

ARTICLE INFO

Article history:
Received 13 October 2011
Returned to author for revision 28 November 2011
Accepted 22 December 2011
Available online 21 January 2012

Keywords:
HIV-1
Lethal mutagenesis
Viral replication
HIV-1 persistent cell line
Nucleoside analogs
5-hydroxy-2′-deoxycytidine (5-OHdC)
5-fluorouracil (5-FU)
2′,2′-difluoro-2′-deoxycytidine (gemcitabine)

ABSTRACT

Lethal mutagenesis, a new antiviral strategy to extinguish virus through elevated mutation rates, was explored in H61-D cells an HIV-1 persistently infected lymphoid cell line. Three mutagenic agents: 5-hydroxy-2′-deoxycytidine (5-OHdC), 5-fluorouracil (5-FU) and 2,2′-difluoro-2′-deoxycytidine (gemcitabine) were used. After 54 passages, treatments with 5-FU and gemcitabine reduced virus infectivity, p24 and RT activity. Treatment with the pyrimidine analog 5-OHdC resulted in increases of p24 production, RT activity and infectivity. Rise in viral replication by 5-OHdC during HIV-1 persistence is in contrast with its inhibitory effect in acute infections. Viral replication enhancement by 5-OHdC was associated with an increase in intracellular HIV-1 RNA mutations. Mechanisms of HIV-1 replication enhancement by 5-OHdC are unknown but some potential factors are discussed. Increase of HIV-1 replication by 5-OHdC cautions against the use, without previous analyses, of mutagenic nucleoside analogs for AIDS treatment.

Introduction

Lethal mutagenesis is a new antiviral approach consisting in virus extinction by enhanced mutagenesis. The concept behind this antiviral therapy is that the mutagenic drugs increase the already high mutation rate of RNA viruses beyond an error threshold incompatible with the maintenance of genetic information (Eigen, 2002). RNA viruses have been extinguished using different protocols with the administration of mutagenic nucleoside or base analogs such as ribavirin, 5-fluorouracil (5-FU), 5-azacytidine (5-AZC) alone or in combination with antiviral inhibitors (Agudo et al., 2010; Contreras et al., 2002; Crotty et al., 2001; Dapp et al., 2009; Day et al., 2005; Grande-Perez et al., 2002; Grande-Perez et al., 2005a; Grande-Perez et al., 2005b; Holland et al., 1990; Perales et al., 2009; Ruiz-Jarabo et al., 2003; Severson et al., 2003; Sierra et al., 2000; Sierra et al., 2007); reviewed in (Anderson et al., 2004; Domingo et al., 2005; Graci and Cameron, 2008). Regarding studies with human immunodeficiency virus type 1 (HIV-1), in a clinical trial, nucleoside analog KP-1461, an oral prodrug of 5-aza-5,6 dihidro-2′-deoxycytidine or KP-1212 (Harris et al., 2005) has been tested with HIV-1 infected patients and while it enhanced transiently the mutation spectrum of HIV-1, it did not lead to reductions in viral load (Mullins et al., 2011). The mutagenic pyrimidine analog 5-hydroxy-2′-deoxycytidine (5-OHdC) was successfully applied to extinguish HIV-1 in acute cell culture infections (Loeb et al., 1999; Tapia et al., 2005).

HIV-1 natural infections are characterized by a massive replication during the acute primo-infection followed by a phase with continuous viral replication which explains the consideration of HIV infections as persistent infections. The study of HIV-1 persistence was approached in our laboratory by the establishment of two persistently infected lymphoid cell lines, designated H61 and M61 (Sanchez-Merino et al., 2007). These cell lines showed continuous viral production but at low levels. In contrast to the extensively studied cell lines with latent infections (like ACH-2 and U1 cells), viral production in H61 and M61 did not increase as a result of treatment with mitogens (Sanchez-Merino et al., 2007). A clonal cell population, termed H61-D, was obtained by limiting dilution from H61 cells. This clonal cell line had continuous viral production and evidence of new rounds of viral replication as shown by the decrease in viral production with AZT treatment, new provirus formation, presence of 2-LTR forms and proviral diversity (Olivares et al., unpublished results).

* Corresponding author at: Centro Nacional de Microbiología, Instituto de Salud Carlos III, Carretera de Pozuelo Km 2, Majadahonda, 28220 Madrid, Spain. Fax: +34 91 509 79 19.
E-mail address: clopez@isciii.es (C. López-Galíndez).
[1] Both authors contributed equally to the work.

0042-6822/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2011.12.016

The present study was undertaken to examine the effect of mutagenic pyrimidine analogs on the HIV-1 resident in the persistently infected H61-D cells. The three mutagenic drugs used were: 5-OHdC, 5-FU and gemcitabine. 5-OHdC is incorporated into DNA by DNA polymerases and HIV-1 reverse transcriptase (RT), yields predominantly G>A, A>G and C>T transitions and it was successfully applied to extinguish HIV-1 in acute infections in cell culture (Loeb et al., 1999; Tapia et al., 2005). 5-FU interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid, and is a potent mutagenic agent for RNA synthesis in several RNA viruses yielding mainly A>G and U>C transitions (Agudo et al., 2008; Grande-Perez et al., 2005a; Ruiz-Jarabo et al., 2003; Sierra et al., 2000). Gemcitabine inhibits ribonucleotide reductase, and it is used to treat patients with locally advanced or metastatic cancer of the pancreas (Burris et al., 1997). Recently, gemcitabine has been used as a lower toxicity substitute for hydroxyurea in antiretroviral therapy (Clouser et al., 2011).

In the present study, we have analyzed the effect of 5-OHdC, 5-FU and gemcitabine, alone or in combinations, on the HIV-1 resident in the persistently infected H61-D cells. Upon treatment with 5-FU and gemcitabine, a reduction in viral yield was obtained. Unexpectedly, the use of 5-OHdC resulted in an augmentation of HIV-1 replication. This result is in contrast with previous investigations which documented strong decreases and extinction of HIV-1 infectivity mediated by 5-OHdC in acute infections in cell culture (Loeb et al., 1999; Tapia et al., 2005). Several possible mechanisms of a mutagen-mediated enhancement of HIV-1 replication are considered and implications for a lethal mutagenesis approach for HIV-1 infections are discussed.

Results

Toxicity evaluation of mutagenic drugs in an HIV-1 persistently infected cell line

The persistently infected H61-D cell line was used for the exploration of lethal mutagenesis in HIV-1. Since H61-D cells displayed a continuous but limited viral production, we assumed that it would be necessary to maintain the cells in permanent presence of the mutagenic agents during long periods of time to attain a mutagenic effect. The prolonged application of the drugs in the cells could result, however, in toxic effects. The toxicity of 5-OHdC, 5-FU or gemcitabine in H61-D cells was quantified by counting cell viability upon mutagenic treatment. Based on previous studies (Loeb et al., 1999; Sierra et al., 2000; Tapia et al., 2005) a range of concentrations of 1–3 mM for 5-OHdC, 0.7–3 μM for 5-FU and 0.2–0.8 nM for gemcitabine was tested (Fig. 1). From these experiments the highest drug concentrations that left cell viability over 80% after serial passages were: 1 mM for 5-OHdC and 1.5 μM for 5-FU. The gemcitabine treatment could be applied only once a week due to its high toxicity, and the highest concentration tolerated was 0.6 nM (Fig. 1).

Effect of mutagenic treatments on the persistently infected H61-D cells

The investigation of the mutagenic effect of 5-OHdC, 5-FU and gemcitabine was carried out in two parallel cultures of H61-D cells during 54 passages. Levels of p24 protein, RT activity and infectivity ($TCID_{50}$) of HIV-1 in the cell culture supernatant at passage 54 were compared with the corresponding values for the initial cell population (passage p0) for the different treatments. Passage of cells in the absence of drugs resulted in an increase of viral production and viral infectivity, presumably as a result of fitness optimization of the virus in the course of persistence (Fig. 2). At passage 54, reductions of viral production of 16% to 49% relative to the cells cultured in the absence of drugs were observed in all cases, except in the cells passaged only in the presence of 5-OHdC. Passages in the presence of 5-OHdC resulted, surprisingly, in increases of about 18% in p24 and RT activity, and more than 100% in infectivity. To confirm this unexpected effect of 5-OHdC, cells were subjected to 74 additional passages in the presence of 5-OHdC. At passage 128, the analysis confirmed the stimulation of HIV-1 expression by 5-OHdC (Fig. 2). Thus, in contrast to 5-FU, gemcitabine and their combinations, 5-OHdC stimulated HIV-1 expression from persistently infected cells.

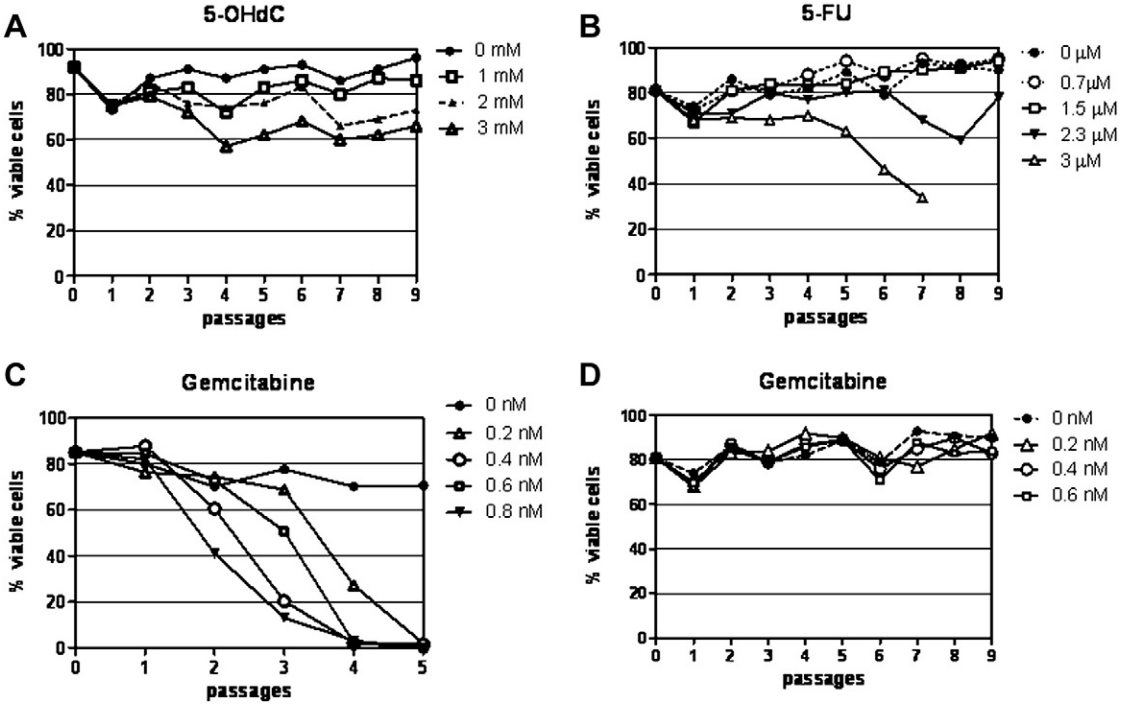

Fig. 1. Toxicity analysis of the mutagenic drugs. Graphics show viability of H61-D cells during five to nine passages in the presence of the indicated mutagens and concentrations. In panels A, B, C the drug was added at each cell passage, whereas in panel D, (gemcitabine) was added only once a week. Toxicity was evaluated by the quantification of cell viability with trypan blue following procedures in Materials and methods.