Antiviral Research 92 (2011) 7–14

Contents lists available at ScienceDirect

Antiviral Research

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

Molecular strategies to design an escape-proof antiviral therapy

Ben Berkhout *, Rogier W. Sanders

Laboratory of Experimental Virology, Department of Medical Microbiology, Academic Medical Center, University of Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 14 January 2011
Revised 21 March 2011
Accepted 4 April 2011
Available online 12 April 2011

Keywords:
HIV-1
Virus evolution
Drug resistance
Fusion inhibitor
RNA interference

ABSTRACT

Two antiviral approaches against the human immunodeficiency virus type 1 (HIV-1) were presented at the Antivirals Congress in Amsterdam. The common theme among these two separate therapeutic research lines is the wish to develop a durable therapy that prevents viral escape. We will present a brief overview of these two research lines and focus on our efforts to design an escape-proof anti-HIV therapy. The first topic concerns the class of HIV-1 fusion inhibitors, including the prototype T20 peptide and the improved versions T1249 and T2635, which were all developed by Trimeris–Roche. The selection of T20-resistant HIV-1 strains is a fairly easy evolutionary process that requires a single amino acid substitution in the peptide binding site of the viral envelope glycoprotein (Env) target. The selection of T1249-resistant HIV-1 strains was shown to require a more dramatic amino acid substitution in the viral Env protein, in particular the introduction of charged amino acid residues that cause resistance by charge-repulsion of the antiviral peptide. The third generation peptide T2635 remains active against all these HIV-1 escape variants because the charged residues within this peptide are "masked" by an introduced intra-helical salt bridge. This charge masking concept could facilitate the future design of escape-proof antiviral peptides. The second topic concerns the mechanism of RNA interference (RNAi) that we are currently employing to develop an antiviral gene therapy. One can make human T cells resistant to HIV-1 infection by a stable RNAi-inducing gene transfer, but the virus escapes under therapeutic pressure of a single inhibitor. Several options for a combinatorial RNAi attack to prevent viral escape will be discussed. The simultaneous use of multiple RNAi inhibitors turns out to be the most effective and durable strategy.

Contents

1. HIV-1 and AIDS

Over 33 million persons are currently infected with HIV-1. This virus causes a chronic infection that ultimately leads to AIDS and death. Much progress has been made in the past 25 years to develop an effective antiviral therapy. Disease progression can be halted effectively with antiviral drugs, and in particular a combinatorial approach can avoid the evolution of drug-resistant HIV-1 variants. Problems associated with such drug regimens include serious toxicity during long-term follow-up. In the absence of any breakthrough at the anti-HIV vaccine front, one should continue to think

* Corresponding author.
E-mail address: b.berkhout@amc.uva.nl (B. Berkhout).

0166-3542/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2011.04.002

about the design of more durable therapeutic measures. We will discuss the concept of novel entry blockers of the fusion inhibitor peptide class that are specifically designed to prevent viral escape. In addition, we will discuss attempts to develop an RNAi-based gene therapy against HIV-1, which should durably protect cells of the immune system that are susceptible to virus infection.

2. HIV-1 entry into the cell

The original antiviral drugs target the HIV-1 reverse transcriptase and protease enzymes, but more recently drugs have been developed that target the process of virus entry into the cell. Maraviroc is an entry inhibitor that acts as CCR5 receptor antagonist. Another subclass of entry inhibitors consists of the fusion inhibitors that prevent fusion of the viral and cellular membranes, a process that is required for the intracellular release of the viral RNA genome and the enzymes needed for genome replication. We and others studied whether HIV-1 can become resistant to these fusion inhibitors, and the causal molecular mechanisms provided useful insight for the design of improved drugs with anti-escape properties.

A more detailed introduction on the entry mechanism is important to understand the mechanism of action of fusion inhibitors. The viral protein that is targeted is the envelope glycoprotein (Env) trimer on the outside of the virion particle that consists of three globular surface gp120 subunits and three trans-membrane gp41 subunits. Upon binding of Env to the CD4 receptor on the host cell, the conformation of Env changes such that it can bind to a second receptor, either CCR5 or CXCR4. This interaction triggers additional conformational changes in both the gp120 and gp41 subunits that activate the fusion machinery of gp41. A trimeric core is formed by the first helical repeat domains (HR1) of three gp41 subunits and the hydrophobic N-terminal fusion peptide of gp41 inserts itself into the membrane of the target cell. Next, the second helical repeat domains (HR2) fold onto the grooves of the HR1 core to form a very stable six-helix bundle structure, and the energy released in this process triggers the fusion of the juxtaposed viral and cellular membranes.

3. Three generations of HIV-1 fusion inhibitors

Peptides based on the HR2 domain of viral fusion proteins are effective inhibitors of virus entry (Bosch et al., 2004; Pyrc et al., 2006; Liu et al., 2004; Porotto et al., 2007; Wang et al., 2003; Zhu et al., 2005). Most HIV-1 fusion inhibitors are peptides that mimic the HR2 domain of the viral gp41 protein and that act by competitively binding to the HR1 core, thereby preventing the binding of HR2 and the formation of the six-helix bundle and fusion of the viral and cellular membranes (Fig. 1). The first generation of this class of peptide inhibitors is called T20 or enfuvirtide, which has been approved for clinical use (Kilby et al., 1998). In succession to T20 the second generation peptide fusion inhibitor T1249 was developed that contains an optimized amino acid sequence with increased antiviral potency (Lalezari et al., 2005). The third generation inhibitor T2635 contains several adjustments like introduced salt bridges that stabilize the α-helical structure of the peptide. These modifications led to a greatly improved half-life in serum and also boosted the antiviral activity that depends on the α-helical conformation of the peptide (Dwyer et al., 2007). These first, second and third generation peptides (T20, T1249 and T2635, respectively) were developed by Trimeris–Roche.

4. How HIV-1 escapes from fusion inhibitors

Resistance to T20 usually maps to the 36–45 region of the peptide binding site in the HR1 domain of the viral gp41 Env protein, with position 38 being the hotspot for resistance. This insight was obtained in both clinical studies and well-controlled laboratory settings (Rimsky et al., 1998; Greenberg and Cammack, 2004). A single amino acid substitution in this domain can cause a moderate to high level of resistance, although a combination of two or three mutations is frequently selected to yield >100-fold resistance (Mink et al., 2005). Mutations in HR2 of gp41 are also involved in the development of high-level resistance and/or restoration of proper Env folding and function. These HR2 mutations increase the affinity for the mutated HR1, thereby favouring the HR1–HR2 association over HR1-drug binding (Ray et al., 2009; Tolstrup et al., 2007). We demonstrated the evolution of a peculiar HIV-1 variant with a combination of HR1 and HR2 mutations that caused a T20 drug-dependent phenotype (Baldwin et al., 2004), and the underlying molecular mechanism was subsequently resolved for this individual patient. In particular, we demonstrated that this Env variant is hyperactive, which necessitates the presence of the antiviral drug to restrict Env of "firing" too early. Obviously, the drug has to dissociate eventually to allow the conformational switch to occur (Baldwin and Berkhout, 2007, 2008).

Initial data suggested that the selection of T1249-resistant HIV-1 variants is very difficult (Melby et al., 2007). The first T1249-resistant HIV-1 variants were selected by randomization of the

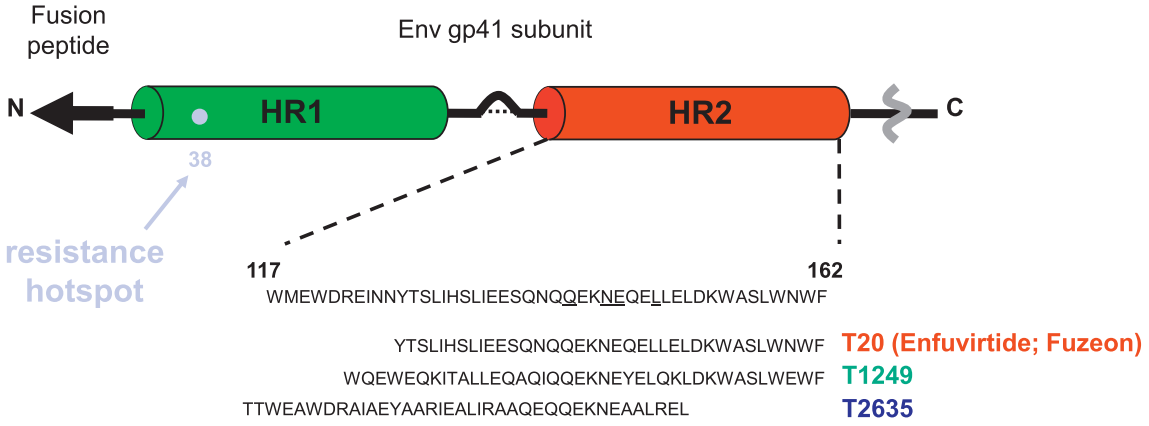

Fig. 1. Three generations of HIV-1 fusion inhibitors. Shown is the extracellular part of the gp41 subunit of the HIV-1 Env protein. The HR1 and HR2 domains are indicated. The HR2 amino acid sequence was copied in the three generations of antiviral peptides: T20, T1249 and T2635. The HR2 domain contains the position 38 residue that forms the hotspot for acquiring resistance against the HR2-based peptides. For further details, see (Eggink et al., 2008).