Biochemical Pharmacology 156 (2018) 248–264

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Evaluation of resistance to HIV-1 infection ex vivo of PBMCs isolated from patients with chronic myeloid leukemia treated with different tyrosine kinase inhibitors

Mercedes Bermejo[a], Juan Ambrosioni[b], Guiomar Bautista[c], Núria Climent[d], Elena Mateos[a], Cristina Rovira[d], Sara Rodríguez-Mora[a,e], María Rosa López-Huertas[a,f], Valentín García-Gutiérrez[g], Juan Luis Steegmann[h], Rafael Duarte[c], Francisco Cervantes[i], Montserrat Plana[d], José M. Miró[b], José Alcamí[a], Mayte Coiras[a,*]

[a] AIDS Immunopathology Unit, National Center of Microbiology, Instituto de Salud Carlos III, Madrid, Spain
[b] Infectious Diseases Service, AIDS Research Group, Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Hospital Clínic, University of Barcelona, Barcelona, Spain
[c] Clinical Hematology Service, Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain
[d] Retrovirology and Viral Immunopathology Laboratory, AIDS Research Group, IDIBAPS, Hospital Clínic, University of Barcelona, Barcelona, Spain
[e] Division of Infection and Immunity, University College of London, UK
[f] Infectious Diseases Service, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) – Hospital Universitario Ramón y Cajal, Madrid, Spain
[g] Hematology Service, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain
[h] Hematology Department, Instituto de Investigación Sanitaria Hospital Universitario de La Princesa (IIS-IP), Madrid, Spain
[i] Hematology Department, IDIBAPS, Hospital Clínic, University of Barcelona, Barcelona, Spain

ARTICLE INFO

Keywords:
HIV-1
Viral reservoir
SAMHD1
Src tyrosine kinases
Chronic myeloid leukemia

ABSTRACT

Current antiretroviral treatment (ART) may control HIV-1 replication but it cannot cure the infection due to the formation of a reservoir of latently infected cells. CD4+ T cell activation during HIV-1 infection eliminates the antiviral function of the restriction factor SAMHD1, allowing proviral integration and the reservoir establishment. The role of tyrosine kinases during T-cell activation is essential for these processes. Therefore, the inhibition of tyrosine kinases could control HIV-1 infection and restrict the formation of the reservoir. A family of tyrosine kinase inhibitors (TKIs) is successfully used in clinic for treating chronic myeloid leukemia (CML). The safety and efficacy against HIV-1 infection of five TKIs was assayed in PBMCs isolated from CML patients on prolonged treatment with these drugs that were infected ex vivo with HIV-1. We determined that the most potent and safe TKI against HIV-1 infection was dasatinib, which preserved SAMHD1 antiviral function and avoid T-cell activation through TCR engagement and homeostatic cytokines. Imatinib and nilotinib showed lower potency and bosutinib was quite toxic in vitro. Ponatinib presented similar profile to dasatinib but as it has been associated with higher incidence of arterial ischemic events, dasatinib would be the better choice of TKI to be used as adjuvant of ART in order to avoid the establishment and replenishment of HIV-1 reservoir and move forward towards an HIV cure.

1. Introduction

The infection by the human immunodeficiency virus type 1 (HIV-1) is currently incurable. The antiretroviral treatment (ART) is very efficient for controlling the infection and the progression to the acquired immunodeficiency syndrome (AIDS) [1,2], but patients must be taking the medication for life, with the consequent adverse effects and burden on Healthcare Systems [3]. Nowadays, clinical care guidelines recommend universal treatment of HIV infection, regardless the time of infection or CD4 cell count. However, the viral reservoir, which is a major obstacle for eradication, is established very early, well before ART initiation. This reservoir is mainly formed by a small subset of infected memory CD4+ T cells that return to a resting state and persist in the organism for a long time [4,5]. When these latently infected

* Corresponding author at: AIDS Immunopathology Unit, National Center of Microbiology, Instituto de Salud Carlos III, Ctra. Majadahonda-Pozuelo km2, 28220 Madrid, Spain.
E-mail address: mcoiras@isciii.es (M. Coiras).

https://doi.org/10.1016/j.bcp.2018.08.031
Received 25 June 2018; Accepted 20 August 2018
Available online 22 August 2018
0006-2952/

lymphocytes become activated in the context of a normal immune response, a massive viral replication occurs, causing T-cell destruction, peaks of viremia, and the reservoir replenishment [6]. The reseeding of the reservoir may also occur by homeostatic proliferation of the infected CD4+ T cells after low level stimulation induced by cytokines such as interleukine-2 (IL-2) or -7 (IL-7) [7,8].

Several strategies are actively being developed to tackle and destroy the viral reservoir [9,10] such as the use of latency reversal agents (LRAs) to reactivate the latent proviruses without causing global T-cell activation [11]. However, none of them has been fully successful so far and more efforts are necessary to design new strategies that may really facilitate the reservoir destruction. In this context, the lower size of the reservoir, the better disease prognosis and the longer control of viremia after treatment interruption [12,13]. Due to its early development, it is really difficult to completely prevent the reservoir establishment [14,15]. Early treatment has proven quite successful to control the size of the reservoir [15] but it cannot avoid its formation, even when ART is initiated very soon after the infection [16,17]. Moreover, a very small quantity of infected CD4+ T cells is enough to replenish the reservoir, once the chance is given to the virus [16,18,19]. Therefore, additional strategies aimed at interfering with the formation of the viral reservoir or its replenishment should complement the eradication approaches that are currently being developed.

The idea of using immune-based therapies against HIV-1 infection is lately gaining strength. Whereas ART is only aimed at the control of HIV-1 replication, the concomitant use of immune-based agents could preserve and enhance the immune system of the patient to promote the elimination of the virus. Several attempts are being performed to restore the number and function of specialized CD4+ and CD8+ T cells able to direct a successful response against HIV-1 infection [20–23]. One potential approach would be increasing cellular restriction mechanisms in order to inhibit the reservoir formation or its replenishment. Therefore, it is worth exploring whether cancer immunotherapy could be useful to restore HIV-specific immunity and avoid the formation of the reservoir. In this regard, we described previously that the tyrosine kinase inhibitor (TKI) dasatinib, which is successfully used in clinic for the treatment of chronic myeloid leukemia (CML) and has an immunomodulator profile [24,25], significantly interferes with HIV-1 proviral integration in CD4+ T cells isolated from patients with CML on chronic treatment with it [26]. We determined two mechanisms of action for the inhibition of HIV-1 infection by dasatinib: first, it preserves the antiviral function of the innate immune factor SAMHD1 by impeding its deactivation through the phosphorylation at T592 residue; and second, dasatinib interferes with the activation and proliferation of CD4+ T cells in response to stimulation with PHA and IL-2. As HIV-1 may infect both quiescent and activated CD4+ T cells once SAMHD1 is phosphorylated [27] but only replicates in activated cells [28,29], the use of drugs that interfere with the activation of infected CD4+ T cells in combination with ART could provide an additional mechanism to avoid the establishment of the reservoir. Moreover, preventing the massive replication of HIV-1 that occurs during the acute phase of the disease would not only avoid the formation of the reservoir, but it would preserve the CD4+ T cell count and the immune response at normal levels, providing an efficient immune system to control the infection.

CML is a neoplastic condition of hematopoietic stem cells characterized by unrestrained growth of myeloid cells in the bone marrow that accumulate in peripheral blood and spleen. It is caused by the formation of a chimeric fusion protein BCR-ABL with uncontrolled tyrosine kinase activity [30,31]. CML treatment is performed with several TKIs. Imatinib was the first TKI against BCR-ABL introduced in clinical practice in 2001 and it highly increased the survival of the patients [32]. The second-generation TKIs nilotinib, dasatinib, and bosutinib were introduced later for CML patients with resistance or intolerance to imatinib [33] and they were all more potent than imatinib against BCR-ABL [34,35]. Recently, the third-generation TKI ponatinib has been approved for CML patients with resistance to the second-generation TKIs, including the T315I mutation of the BCR-ABL kinase domain [36]. Although these drugs target mainly BCR-ABL, they also affect the activity of other kinases, which could be useful in other diseases. Dasatinib and bosutinib display a broader target spectrum than imatinib and nilotinib [37]. Dasatinib and bosutinib inhibit Src family of tyrosine kinases (SFK) such as the non-receptor tyrosine kinase SRC (C-Src proto-oncogene) and the SFK regulator CSK (C-Src tyrosine kinase) – which in turn activates SRC by phosphorylation [38,39]. It is known that dasatinib and bosutinib also target other SFKs such as LYN that is mostly expressed in T and B cells, and LCK that is essential for T-cell development and function [40]. Active LCK induces direct or indirect phosphorylation of many substrates [41], including SAMHD1 [8] and other downstream kinases such as the protein kinase C theta (PKCθ), which leads to full T-cell activation [41,42]. Consequently, in the context of HIV-1 infection, LCK activation enhances viral infection [26] and PKCθ activation is essential for HIV-1 transcription [43,44]. Therefore, using TKIs that could selectively suppress the activity of SFKs such as LCK and downstream kinases such as PKCθ could be useful to interfere with HIV-1 infection and the reservoir establishment.

We previously proposed the use of dasatinib as adjuvant of ART as an alternative to reduce the reservoir size. However, 30% of CML patients on treatment with dasatinib for the first year may develop pleural effusion, which is the most characteristic secondary effect of this TKI and requires dose reduction or even treatment interruption [45]. Therefore, first we analyzed whether a lower dose of dasatinib than the one currently used for treating CML could be effective against HIV-1 infection and second, we determined if other TKIs could also be useful as adjuvant of ART in a clinical setting and with less probability of adverse reactions. The susceptibility to HIV-1 infection ex vivo of PBMCs isolated from patients with CML on chronic treatment with imatinib, nilotinib, bosutinib and ponatinib was then evaluated and compared to dasatinib, and the mechanism of action for this restriction was also analyzed.

2. Materials and methods

2.1. Cells and patients' samples

Thirty seven patients with CML on chronic treatment with one of the assayed TKI or 42 untreated healthy donors were recruited for this study. Peripheral blood lymphocytes (PBMCs) were isolated by centrifugation through a Ficoll-Hypaque gradient (Pharmacia Corporation, North Peapack, NJ). Human CD4+ T lymphocytes were isolated with CD4+ T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany), according to manufacturer's instructions. Cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum (FCS), 2 mM L-glutamine, 100 µg/ml streptomycin, 100UI/ml penicillin (Biowhittaker, Walkersville, MD). PBMCs were activated with 1 µg/ml CD3 (OKT3) antibody (Miltenyi Biotec) and 300 units/ml IL-2 (Chiron, Emeryville, CA) for 72 h and then, they were maintained in culture only with IL-2.

HIV-negative, CML Phi Chromosome-positive patients receiving treatment with one TKI were obtained from the Hospital Clinic (Barcelona, Spain), Hospital Puerta de Hierro (Majadahonda, Madrid, Spain), Hospital La Princesa (Madrid, Spain) and Hospital Ramón y Cajal (Madrid, Spain). All of them had more than one year of follow-up from CML diagnosis and were taking the current TKI for at least 9 months. All patients were on hematological remission and none of them presented previous or ongoing serious adverse events related to the use of TKI, neither infectious complication related to their hematological disease or to the treatment with the TKI. All of them had normal routine blood and biochemistry test at sampling. Table 1 summarizes the main clinical characteristics of CML patients. Blood samples from the healthy donors were obtained from the Centro Regional de