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Dasatinib inhibits HIV-1 replication through the interference of SAMHD1 phosphorylation in CD4+ T cells

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ABSTRACT

Massive activation of infected CD4+ T cells during acute HIV-1 infection leads to reservoir seeding and T-cell destruction. During T-cell activation, the antiviral effect of the innate factor SAMHD1 is neutralized through phosphorylation at T592, allowing HIV-1 infection. Dasatinib, a tyrosine kinase inhibitor currently used for treating chronic myeloid leukemia, has been described to control HIV-1 replication through its negative effect on T-cell proliferation and viral entry. We demonstrate that Dasatinib can actually interfere with SAMHD1 phosphorylation in human peripheral blood lymphocytes, preserving its antiviral activity against HIV-1. Dasatinib prevented SAMHD1 phosphorylation in vitro and ex vivo, impairing HIV-1 reverse transcription and proviral integration. This was the major mechanism of action because the presence of Vpx, which degrades SAMHD1, in HIV-1 virions impeded the inhibitory effect of Dasatinib on HIV-1 replication. In fact, infection with VSV-pseudotyped HIV-1 virions and fusion of BlaM-Vpr-containing HIV-1 viruses with activated PBMCs in the presence of Dasatinib suggested that Dasatinib was not acting at fusion level. Finally, PBMCs from patients on chronic treatment with Dasatinib showed a lower level of SAMHD1 phosphorylation in response to activating stimuli and low susceptibility to HIV-1 infection ex vivo. Consequently, Dasatinib is a compound currently used in clinic that preserves the antiviral function of SAMHD1. Using Dasatinib as adjuvant of antiretroviral therapy during early primary HIV-1 infection would contribute to reduce viral replication and spread, prevent reservoir seeding, and preserve CD4 counts and CTL responses. These events would create a more favorable virologic and immunologic environment for future interventional studies aiming at HIV-1 eradication.

1. Introduction

Infection by the human immunodeficiency virus (HIV) remains a major public health concern all over the world. The start of combination antiretroviral therapy (cART) accomplished the goal of reducing AIDS mortality [1,2], but it cannot completely eliminate the virus from the organism. HIV infection is now a chronic disease that needs life-long treatment, with the subsequent emergence of co-morbidities related to side effects of cART, drug resistances, and the burden on health-care systems that were initially designed for acute care [3]. As cART is not enough to completely eliminate the virus from the organism, new strategies are being developed to eradicate the infection. This aim can only be achieved if the viral reservoirs, responsible for viral rebound during cART interruption, are completely eradicated [4,5], or a significant reduction achieved [6]. Therefore, a strategy of "kick and kill" has been initiated by several laboratories, testing different compounds to reactivate and destroy latent reservoirs [7], but none have proved to be effective so far [8]. However, early cART in primary infection results in smaller viral reservoirs and the increased possibility of viral control by the immune system [9,10]. As the smaller the size of

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the reservoir the easier could be its elimination, we proposed a concomitant strategy to reduce the size of the reservoir from the beginning of the infection by interfering with T-cell activation. In particular, we targeted the activity of protein kinase C (PKC) theta (θ) [11,12] that is one of the key regulators of signal transduction during T-cell activation [13]. Blocking PKCθ reduces HIV-1 replication in CD4+ T cells, providing a refractory state to HIV-1 infection [12]. However, none of the selective PKCθ inhibitors currently under development have begun clinical testing yet. Consequently, we searched for other cellular targets upstream of the PKCθ signaling pathway, trying to find other suitable candidates.

PKCθ activation occurs by phosphorylation at different residues and subsequent translocation to the plasma membrane [14], which is mediated by a tripartite interaction with CD28 and the tyrosine-protein kinase $p56^{lck}$ [15]. $p56^{lck}$ is an Src family tyrosine kinase (SFK), predominantly – but not exclusively – expressed in T cells [16], that is essential for transmitting signals from the TCR signaling complex [17]. $p56^{lck}$ is activated after TCR engagement, which leads to the phosphorylation of tyrosine 394 (Y394) in the catalytic domain and induces its kinase activity [18]. $p56^{lck}$ activation causes the phosphorylation of tyrosine residues present within immunoreceptor tyrosine-based activation motifs (ITAMs) [19], followed by downstream calcium flux and expression of T-cell activation markers such as CD69 and CD40 ligand/CD154 [20]. $p56^{lck}$ induces the phosphorylation of PKCθ at threonine 538 (T538) by MAP4K3/GLK [14], which is widely used as a surrogate marker for PKCθ kinase activation [21], and at tyrosine 90 (Y90), which leads to the activation of downstream effectors essential for T-cell function and HIV-1 transcription such as NF-κB, NFAT, and AP-1 [21–26]. As the activity of PKCθ is greatly dependent on $p56^{lck}$, we reasoned that $p56^{lck}$ inhibitors could exert a blocking effect on HIV-1 replication similar to the inhibition of PKCθ. In this regard, the influence on HIV-1 replication of the ATP-competitor Dasatinib has already been analyzed by some groups [27,28]. Dasatinib is an oral small molecule inhibitor of non-receptor tyrosine kinases such as Abl and SFK – including $p56^{lck}$ [29,30], that is currently used in clinic for treating patients with chronic myeloid leukemia (CML) [31–34]. Dasatinib impairs TCR-mediated signal transduction, cellular proliferation, cytokine production, and in vivo T-cell responses [35,36], although chronic treatment with Dasatinib does not increase susceptibility to fungal or viral infections [37].

It has been described that the mechanism of action of Dasatinib to thwart HIV-1 replication in CD4+ T cells was to blockade the viral entry [27], as well as by its anti-proliferative effect [38]. However, Pogliaghi et al. [28] observed that Dasatinib was only effective to inhibit HIV-1 replication in peripheral blood lymphocytes (PBMCs) from infected patients when they were activated with phytohemagglutinin (PHA) and interleukin-2 (IL-2) for less than 3 days before treatment with Dasatinib because after this time Dasatinib could not completely shut down viral replication. Accordingly, mitogenic T-cell activation might be triggering some mechanism able to block Dasatinib-mediated inhibition of HIV-1 replication. In this work, we demonstrate by fusion assays of BlaM-Vpr-containing HIV-1 viruses that Dasatinib was ineffective to stop HIV fusion and that its main mechanism of action was actually the interference of the phosphorylation of SAMHD1 (sterile alpha motif domain and HD domain-containing protein 1), which was induced after T-cell activation with PHA and IL-2 for at least 2 days. SAMHD1 is a key regulator of cell cycle progression and a major viral restriction factor that blocks early reverse transcription of HIV-1 genome by depleting the intracellular dNTP pool [39,40] and by degrading viral RNA (vRNA) through its RNase activity [41]. The function of SAMHD1 is regulated through the phosphorylation of threonine 592 (T592) by cyclin A2/Cdk1, an event that is induced by T-cell activation and that renders the cells susceptible to HIV-1 infection [42]. The accessory protein Vpx of HIV-2 and the simian immunodeficiency virus (SIV) targets SAMHD1 for ubiquitination and proteasomal degradation [43]. As HIV-1 does not encode Vpx, it remains sensitive to SAMHD1-mediated restriction until the T cell receives an activation signal. Evidence that the major mechanism of action of Dasatinib for inhibiting HIV-1 replication was through the protection of SAMHD1 activity was that Dasatinib did not significantly affect HIV-1 infection in CD4+ T cells when the virions carried Vpx.

Accordingly, through the preservation of SAMHD1 antiviral activity, Dasatinib impaired HIV-1 reverse transcription and, consequently, strongly affected proviral integration. The effect of Dasatinib on SAMHD1 phosphorylation in vivo was confirmed by low levels of phosphorylated SAMHD1 observed in CD4+ T lymphocytes from CML patients on chronic treatment with Dasatinib that were activated ex vivo. Moreover, PBMCs from these CML patients were also resistant to HIV-1 infection.

2. Materials and methods

2.1. Cells' and patients' samples

Peripheral blood lymphocytes (PBMCs) were isolated from blood of untreated healthy donors by centrifugation through a Ficoll–Hypaque gradient (Pharmacia Corporation, North Peapack, NJ). Human CD4+ T lymphocytes were isolated from PBMCs using CD4+ T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the 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, 100 U/ml penicillin (Biowhittaker, Walkersville, MD). PBMCs were activated by treatment with 5 μg/ml PHA (Sigma–Aldrich, St. Louis, MO) and 300 units/ml IL-2 (Chiron, Emeryville, CA) for 48 h. Then, they were maintained in culture with IL-2. Jurkat E6-1, MT-2 and TZM-bl cells were obtained from the NIH AIDS Reagent Program [44]. Jurkat E6-1 and MT-2 cells were cultured in RPMI 1640 medium supplemented as described above. TZM-bl cells were grown in DMEM medium with 10% fetal bovine serum supplemented with penicillin/streptomycin and 4 mM L-glutamine.

PBMCs from three treated chronic asymptomatic HIV-1 individuals were obtained at the Hospital Clinic (Barcelona, Spain). These patients showed baseline CD4+ T lymphocyte counts >500 cells/mm^3 and plasma viral loads ranging from 50 to 10,000 HIV-1 RNA copies/ml. All individuals gave informed written consent and this study was approved by the Institutional Ethics Committee board of Hospital Clinic (Barcelona, Spain).

PBMCs from five HIV-negative, CML Phi Chromosome-positive patients receiving Dasatinib treatment were also obtained at the Hospital Clinic (Barcelona, Spain). All of them had more than 2 years of follow-up from CML diagnosis and were taking Dasatinib for at least two years. All patients were on hematological remission and none of them presented previous or ongoing serious adverse events related to Dasatinib use, neither infectious complication related to their hematological disease or to the treatment with Dasatinib. All of them had normal routine blood and biochemistry test at sampling. Table 1 summarizes the main clinical characteristics of CML patients. All individuals who participated in this study gave informed written consent.

2.2. Reagents and antibodies

Dasatinib (BMS-354825, Sprycel; Bristol-Meyers Squibb, New York, NY) was kindly provided by Dr. Stephen Mason (Discovery Virology, Bristol-Meyers Squibb) and Dr. Carey Hwang (Discovery Medicine – Virology, Bristol-Myers Squibb). Lck inhibitor II (Merck