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Researching of resistance to etravirine in some HIV-1 low-level viremia strains by in-silico methods

https://doi.org/10.1515/tjb-2023-0166
Received July 31, 2023; accepted August 9, 2023; published online August 25, 2023

Abstract

Objectives: Human immunodeficiency virus (HIV) is a significant infection that attacks immune system cells and integrates its genetic material into host cells. Left untreated, it leads to acquired immunodeficiency syndrome (AIDS). Antiretroviral therapy (ART) is used to control HIV infection. Etravirine (ETR) is an important non-nucleoside reverse transcriptase inhibitor (NNRTI) utilized in the treatment of HIV. Low-level viremia (LLW) is a serious clinical condition, and the underlying mechanisms remain incompletely understood. The aim of our study is to analyze and elucidate the resistance status of Lys104Gln, Lys102Gln, Lys101Arg-Lys104Arg, Ser191Phe, Ile94Leu-Lys104Arg, Lys104Glu-His235Leu, Ala98Ser and Val179Ile mutations using in-silico methods, which are identified as low-level viremic strains, because their resistance status to ETR is unknown.

Methods: Homology modeling was performed using the Swiss Model program. Molecular docking of ETR with the reverse transcriptase (RT) enzyme was conducted using the CB-Dock program developed by AutoDock Vina. Protein-ligand interaction analysis was carried out using the protein-ligand interaction profiler (PLIP).

Results: A98S and V179I mutations altered the physicochemical properties of the region, resulting in changes to the conformational structure of the NNRTI hydrophobic pocket compared to the wild-type and consequently decreased docking scores.

Conclusions: Based on the evaluation of literature data and in-silico analyses, it is believed that A98S and V179I mutations may alter the conformational structure of the hydrophobic pocket where ETR hinds, potentially resulting in low-level resistance against ETR.

Keywords: protein structure; HIV; etravirine; in-silico; drug resistance; low-level viremia

Introduction

Human immunodeficiency virus (HIV) is a pathogen that targets the immune system of the human host, specifically the CD4+ T lymphocytes, which play a critical role in the body’s defense against infections. HIV primarily spreads through sexual transmission, but it can also be transmitted through blood-borne routes such as contaminated needles or blood transfusions. HIV is a chronic condition for which there is currently no cure, but it can be managed through antiretroviral therapy (ART). ART is a combination of medications that can suppress the viral load and reduce the risk of transmission to others. When administered consistently, ART can enable individuals living with HIV to lead long and healthy lives. If left unchecked, HIV can progress to acquired immunodeficiency syndrome (AIDS), the most advanced stage of HIV infection, characterized by severe damage to the immune system and increased susceptibility to opportunistic infections [1].

The clinical significance of low levels of viremia, also known as low-level viremia (LLV), remains a topic of debate and investigation within the medical community. A major contributing factor to the lack of consensus regarding the clinical implications of LLV is the inconsistent definitions and measurement methods employed across studies, as well as the discrepancies in the findings of these studies. Nonetheless, a relatively broad definition of LLV, encompassing viral loads between 200 and 1,000 copies/mL, has consistently been associated with virologic failure, viral
evolution, and the emergence of drug resistance in multiple investigations. As such, the clinical significance of LLV warrants further examination in order to better understand its potential impact on the management of HIV [2, 3].

The effectiveness of narrow-spectrum non-nucleoside reverse transcriptase inhibitors (NNRTIs) is limited due to the low genetic barrier to resistance, which allows for the easy selection of single mutations that often confer complete cross-resistance. Etravirine (ETR) is a modified NNRTI that exhibits conformational flexibility, allowing it to bind to HIV reverse transcriptase (RT) in the presence of most mutations that would normally confer resistance to other NNRTIs [4]. Mutations have the potential to alter the three-dimensional structure and biochemical properties of proteins. Notably, changes occurring in the ligand-binding region can trigger resistance to drugs. Alterations in amino acid residues involved in protein-ligand interactions can modify physicochemical properties, binding affinity, number, and type of bonds [5].

Several in vitro studies have demonstrated that multiple mutations are required for the development of high-level resistance to etravirine (ETR) [6, 7]. Clinical trials have shown that ETR is effective in rapidly reducing plasma viral loads in both treatment-naive patients [8] and those with NNRTI-resistant viruses. Studies have identified several ETR resistance-associated mutations (RAMs), including V90I, A98G, L100I, K101E/P, K101H, E138A, V179T, V106I, V179D/F, Y181C/I/V, M230L, and G190A/S (http://hivdb.stanford.edu) [9].

The clinical implications of LLV remain unclear and have been a focus of interest in clinical research. One of the difficulties in understanding the significance of LLV is the discrepancy between the results of various studies, as well as the lack of consistency in the definitions of LLV and virologic failure used in these studies. While some research has consistently shown that LLV in the range of 200–1,000 copies/mL is associated with virologic failure, viral evolution, and the development of drug resistance [2, 3], it is not yet clear whether LLV in the range of 50–199 copies/mL (below the current threshold defined by the Department of Health and Human Services as persistent viremia ≥200 copies/mL) is associated with virologic failure [10, 11]. Some studies have found that LLV in this range is not associated with virologic failure, while others have found that it is associated with virologic failure and the emergence of drug resistance [12].

In our study, mutations that are located close to the binding pocket of etravirine (ETR) and that have the potential to alter the location of amino acids within the binding pocket were analyzed for their effect on ETR resistance using in silico methods. The mutations (S191F, L228R, K101R-K104R, K104E – H235L, K102Q, K104Q, A98S, V179I) analyzed in this study were obtained from the National Reference Laboratory of HIV/AIDS and Viral Hepatitis, Ministry of Health, Turkey.

Materials and methods

Homology modeling

Amino acids that can change the location of amino acids associated with etravirine (ETR) by altering the alpha helix and beta sheet folding of proteins when considering the three-dimensional structure of the ETR pocket are those in the ranges of 107–109, 177–191, 225–240, and 316–321. Homology modeling studies were performed using the Swiss Model. All models were based on the three-dimensional model of ETR-HIV-RT interaction, ISMQA, by Das K. and colleagues [13]. The resolution of the ISMQA model is 3.00 Å, with a sequence identity of ± 96.06 and a sequence similarity of 0.62. The generated homology models were analyzed using the UCSF Chimera (1.15rc) program (Resource for Biocomputing, Visualization, and Informatics, University of California, San Francisco, America). The wild type and mutant models were superimposed separately, and the conformational differences and changes in the mutation region were visually depicted at the atomic level. The distances between the mutant amino acid C-α atoms, and ETR were measured in all models. The atom closest to the mutant amino acid was used as the reference for ETR.

Molecular docking

Molecular docking studies were performed using the CB-Dock bioinformatics tool developed by AutoDock Vina (Resource for The Scripps Research Institute, California, America). The ligand and homology models were prepared in pdb format. The data from the molecular docking were analyzed using the BIOVIA Discovery Studio Visualizer (DS) program (Dassault Systèmes, Deutschland, Germany). The 2D ligand-amino acid regions were visualized using the DS program.

Protein-ligand interaction analysis

The statement describes an analysis conducted on homology models of etravirine-HIV RT generated using CB-Dock. The analysis focused on identifying the amino acid residues involved in the interaction between ETR and the homology model using various molecular interactions such as hydrophobic interactions, hydrogen bonding, π-stacking, π-cation halogen bonding, salt bridges, and metal interactions. The analysis was performed using the Protein-Ligand Interaction Profiler program (Resource for Biotechnology Center TU Dresden, Dresden, Germany) [14].

Results

Homology modeling

The homology models of the wild-type (Figure 1A) and mutant strains were compared, and conformational changes
were analyzed. When considering the homology model of the wild-type strain, it was observed that there were changes in physicochemical properties (such as hydrophobicity, electrical charge, polarity, etc.) at the atomic and amino acid level due to the amino acid substitutions in the mutation regions of the mutant strains. However, the alpha helices and/or beta sheets did not change, or only minor conformational changes occurred. The analysis revealed that the wild-type and K10Q, K101R-K104Q, S191F, K104Q, L228R, I94L-K104Q, and K104E-H235L mutant strains all have a similar hydrophobic NNRTI pocket. However, in the V179I and A98S mutant strains, there were changes in the hydrophobic pocket due to the mutations, and in the A98S mutant, there was also a change in hydrophobicity. A distance analysis was performed to identify the amino acid locations associated with ETR in the hydrophobic ETR pocket. An average change of 0.3 Å was observed in the A98S mutation (Table 1), while the V179I (Figure 1B) mutation was found to affect the entry point of ETR into the pocket by increasing the volume at the pocket’s entrance.

Molecular docking

This statement describes the results of molecular docking using CB-Dock, developed by AutoDock Vina, with homology models and the ETR ligand. The results are presented in Table 2, with each mutation and wild-type model examined separately. The resulting ETR-HIV RT models obtained from molecular docking were analyzed using the Discovery Studio Visualizer program. It was observed that the A98S and V179I mutant types had a lower binding score compared to the wild-type, indicating weaker binding affinity between the ETR ligand and the mutant RT protein.

A98S mutant type

According to the molecular docking analysis results, when the wild type (Figure 2A) and A98S (Figure 2B) models are compared, hydrogen bonding interaction between Lys101 and ETR has increased, the \( \pi \)-interaction regions of Leu100 with ETR have changed and increased, the \( \pi \)-interaction regions of Val179 with ETR have changed and increased, the \( \pi \)-interaction region of Val106 with ETR has changed, and the interaction between Tyr188 and ETR has been lost.

V179I mutant type

According to the molecular docking analysis results, when the wild type (Figure 3A) and V179I (Figure 3B) models are compared, the interaction region of Ile179 amino acid with ETR has changed and decreased. A new donor-donor interaction has formed, the interaction region of Val106 with ETR

Table 1: This statement refers to the distances between the 98th amino acid and the amino acids ETR (average distance), L100, K101, and Y318 in both wild-type and mutant models.

<table>
<thead>
<tr>
<th>Distance, Å</th>
<th>ETR</th>
<th>L100</th>
<th>K101</th>
<th>Y318</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 98</td>
<td>9.8</td>
<td>5.8</td>
<td>8.7</td>
<td>5.7</td>
</tr>
<tr>
<td>S 98</td>
<td>9.5</td>
<td>5.7</td>
<td>8.4</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Table 2: Molecular docking score table.

<table>
<thead>
<tr>
<th>Mutation name</th>
<th>Docking score</th>
<th>Mutation name</th>
<th>Docking score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>−10.6</td>
<td>A98S</td>
<td>−9.6</td>
</tr>
<tr>
<td>V179I</td>
<td>−9.6</td>
<td>K104Q</td>
<td>−10.5</td>
</tr>
<tr>
<td>K102Q</td>
<td>−10.6</td>
<td>L228R</td>
<td>−10.6</td>
</tr>
<tr>
<td>K101R-K104Q</td>
<td>−10.6</td>
<td>I94L-K104Q</td>
<td>−10.5</td>
</tr>
<tr>
<td>S191F</td>
<td>−10.7</td>
<td>K104E-H235L</td>
<td>−10.6</td>
</tr>
</tbody>
</table>
has changed, the interaction region of Leu100 with ETR has changed, the interaction region of Trp229 with ETR has increased, the interaction region of Lys103 with ETR has changed, the hydrogen bond is not broken, and Ile101 replaced Val179, the π-interaction between Tyr181. ETR has formed, the π-interaction between Pro95 and ETR has formed, the interaction between Tyr318 and ETR has disappeared, the interaction between Leu234 and ETR has disappeared, and the interaction between Tyr318 and ETR has disappeared.

**Protein-ligand interaction analysis results**

The molecular binding score analysis and protein-ligand interaction analysis were evaluated separately. The PLIP analysis of the A98S and V179I mutant types, which have a lower molecular binding score than the wild type, and the wild type, are given in Table 3.

**A98S mutant type**

According to the protein-ligand interaction analysis results, when compared to the wild-type, in the mutant model interaction of A98S mutation, it was analyzed that there are two new hydrophobic interactions with Leu100 amino acid, one of the two hydrophobic interactions with Tyr188 amino acid disappeared, hydrophobic interactions of Trp229 and Leu234 amino acids with ETR disappeared, a new hydrophobic interaction occurred with Val179 amino acid, a π-stacking interaction occurred with Tyr181 amino acid, and
the distances with other interacting amino acids changed at a value less than 0.5 Å. As a result of these changing interactions, it is considered that the A98S mutant type has a lower binding score (−9.6) than the wild-type (−10.6).

**V179I mutant type**

When the wild type was compared to the V179I mutation in the protein-ligand interaction analysis results, it was found that two new hydrophobic interactions formed with the Leu100 amino acid, and a hydrogen bond disappeared with Lys101 amino acid. These two new interactions change the interaction region between the ligand and the protein compared to the wild type. The distances between the interacting amino acids and the ligand were found to have changed by a value less than 0.5 Å. As a result of these changes in interactions, it is believed that the V179I mutant type has a lower binding score (−9.6) compared to the wild type (−10.6).

**Discussion**

According to the results of homology modeling, molecular docking, and protein-ligand interaction analysis, it is believed that the mutations S191F, K104E – H235L, K102Q, and K104Q identified in the HIV-RT protein of low-level viremic patients do not confer resistance to ETR. LLV is not caused by these mutations [2, 15, 16]. Instead, it is thought that LLV may be due to virological failure [11].

In the literature, it has been reported that the S191F mutation alone did not confer resistance [17], and the K104 polymorphisms were suggested to be associated with resistance in a study by Sushama et al. in 2016 [18]. The K102Q mutation was not found to be associated with resistance, but the K102R mutation was reported to be resistant in combination with other mutations in a study by Hachiya et al. in 2004. There is no study suggesting that the H235L mutation is associated with resistance. Based on the literature and our analysis, we believe that the mutations S191F, K104E – H235L, K102Q, and K104Q in the HIV-RT protein do not confer resistance to ETR and, therefore cannot cause LLV.

The A98S mutation is believed to lead to low-level resistance due to the formation of a new π-stacking interaction with the 181TYR amino acid and certain ETR-amino acid interactions, which affect the ETR-protein binding score. According to a study conducted by Vingerhoets et al. in 2010 [19], the A98S mutation can lead to resistance in combination with another NNRTI mutation in the same strain. Additionally, it has been reported that the A98G mutation can lead to resistance in combination with other mutations, according to the Stanford HIV Drug Resistance Database [19, 20].

It is believed that the V179I mutation leads to low-level resistance due to the disappearance of a hydrogen bond and the appearance of two new hydrophobic interactions, which cause changes in the ETR-protein binding region. According to the results of homology modeling, molecular docking, and protein-ligand interaction analysis, it is believed that the mutations S191F, K104E – H235L, K102Q, and K104Q identified in the HIV-RT protein of low-level viremic patients do not confer resistance to ETR. LLV is not caused by these mutations [2, 15, 16]. Instead, it is thought that LLV may be due to virological failure [11].

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to previous reports, the region of V179 is highly prone to mutations, and V179D/E/F/L/T mutations have been suggested to cause resistance [21]. In a cell culture study conducted by Dwivedi R. and colleagues in 2022, the V179I mutation was found to have drug susceptibility similar to the wild type, but it showed resistance to rilpivirine, one of the NNRT inhibitors when combined with Y181 mutations. Additionally, in a bioinformatics analysis conducted by Marcelin A. and colleagues in 2012, changes at the V179 position were found to be important for ETR resistance, and V179/L mutations were suggested to be associated with resistance with other mutations, reducing phenotypic susceptibility.

Our in-silico analyses, parallel to the evaluation of literature data, imply that A98S and V179I mutations may alter the conformational structure of the ETR bound hydrophobic pocket, potentially resulting in low-level resistance against ETR. Based on literature studies and our analysis results, it is considered that A98S and V179I mutations detected in HIV-RT protein can show low-level resistance to ETR alone and can lead to high resistance in a combined situation.

Research ethics: The local Institutional Review Board deemed the study exempt from review.

Informed consent: Not applicable.

Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: All other authors state no conflict of interest.

Research funding: No financial support has been received.

Data availability: The raw data can be obtained on request from the corresponding author.

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