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Virtual screening of HIV-1 protease inhibitors against human cytomegalovirus protease using docking and molecular dynamics

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The clearance of cytomegalovirus viraemia in HIV-1-infected patients may partly result from the inhibition of cytomegalovirus protease by HIV-1 protease inhibitors contained in highly active antiretroviral therapy. We used a computational method to calculate the binding affinity of six HIV-1 protease inhibitors to cytomegalovirus protease based on its X-ray crystallography structure. The calculations showed that amprenavir and indinavir occupy the substrate-binding site of the cytomegalovirus protease with high affinity, and may be implicated in alleviating cytomegalovirus infection.

Cytomegalovirus is an AIDS-related opportunistic pathogen that usually infects HIV-1 patients with a high level of plasma HIV-1 RNA and low CD4 cell counts (< 200 cells/μl) [1–3]. Highly active antiretroviral therapy (HAART), consisting of HIV-1 protease and reverse transcriptase inhibitors, has been shown to lower plasma HIV-1-RNA levels and elevate CD4 cell counts, and is associated with a reduction in cytomegalovirus replication and the clearance of cytomegalovirus viraemia [4–11]. Reports from several groups have shown that immune recovery that results from HAART without any specific anti-cytomegalovirus therapy is able to suppress cytomegalovirus infection in HIV-1-infected patients [8–11]. However, it is unresolved as to whether HIV-1 protease inhibitors aid the clearance of cytomegalovirus viraemia by inhibiting cytomegalovirus protease activity.

In this study, we used an integrated molecular dynamics simulation and docking method to calculate the ability of six US Food and Drug Administration approved HIV-1 protease inhibitors to bind to the cytomegalovirus protease in terms of binding mode and binding energy. The X-ray crystallography structures of cytomegalovirus protease and HIV-1 protease inhibitors were retrieved from the Protein Data Bank (codes: 1NKM for cytomegalovirus protease, 1HPV for amprenavir, 1HSG for indinavir, 1MUI for lopinavir, 1OHR for nelfinavir, 1HXW for ritonavir and 1C6Z for saquinavir).

Docking calculations were carried out using AutoDock version 3.0.5 with a Lamarckian genetic algorithm [12]. We first performed preliminary docking experiments to identify the potential binding sites of the inhibitors by generating a grid box that is big enough to cover the entire surface of the protein. The protein-inhibitor complexes derived from the first ranked docking solution in the preliminary docking procedure were consequently solvated in a TIP3-water shell, and all atoms were allowed to relax using molecular dynamics simulation. The molecular dynamics simulation was carried out using the NAMD software version 2.5b18 [13]. The topology and parameters for each inhibitor were obtained from the PRODRG server [14]. One hundred steps of energy minimization of the protein–inhibitor–water complex were initially performed, followed by 0.1 picoseconds of molecular dynamics simulation at 300 K. The simulations were repeated with three different starting seeds. The trajectories at 0.1 picoseconds were recorded and processed in a second docking step using similar docking parameters as used in the preliminary docking procedure. The primary exception was in the creation of a three-dimensional affinity grid box, in which the C-α atom of Ser132 of the catalytic triad was set as a grid center, and the number of grid points in the x, y, z axes was set to $60 \times 60 \times 60$.

AutoDock generates three energy terms: intermolecular energy, internal energy of the ligand, and torsional free energy. The final docked energy was calculated from the sum of the intermolecular energy and the internal energy of the ligand. The free energy of binding was calculated from the sum of the intermolecular and the torsional free energies, and consequently converted into an inhibitory constant (K_i) according to Hess's law. The lowest-energy solution was accepted as the calculated binding energy and its K_i value was used to define the binding affinity of the inhibitors. Further details of the molecular dynamics simulation and docking protocols are given elsewhere [15,16].

Structural studies of the cytomegalovirus protease show that it belongs to the serine protease family, with a novel Ser132–His63–His157 catalytic triad, with His157 representing the third member instead of the typical Asp [17]. The substrate-binding site is composed of several subsites: the S_1 subsite is formed by residues Leu32, Ser132, Leu133, Arg165 and Arg166. The S_2 and S_4 subsites are fused together, forming a large pocket with residues His63 and Asp64 on one side, Ser135 on the other, and Lys156 in the middle. The S_3 portion of this pocket is formed by salt bridges between residues Glu31, Ser135, Arg137, Arg165 and Arg166 [17]. Theoretically, enzymatic activity would be significantly diminished if the catalytic triad, or part of the substrate-binding sites,

Table 1. Calculated energies and inhibitory constants (K_i) of six Food and Drug Administration approved HIV-1 protease inhibitors against the cytomegalovirus protease ranked in ascending order of calculated K_i.

PDB ID	Inhibitor	Intermolecular energy (kcal/mol)	Internal energy of ligand (kcal/mol)	Torsional free energy (kcal/mol)	Final docked energy (kcal/mol)	Calculated inhibitory constant (K_i)
1HPV	Amprenavir	−15.43	−0.27	4.36	−15.70	7.66×10^{-9}
1HSG	Indinavir	−15.31	1.05	4.36	−14.26	9.37×10^{-9}
1C6Z	Saquinavir	−15.02	0.80	4.98	−14.22	4.35×10^{-8}
1HXW	Ritonavir	−15.62	−0.37	6.85	−15.99	3.69×10^{-7}
1OHR	Nelfinavir	−12.23	−0.64	3.74	−12.87	5.93×10^{-7}
1MUI	Lopinavir	−13.45	0.02	5.60	−13.43	1.76×10^{-6}

PDB, Protein Data Bank.
Amprenavir and indinavir have a high affinity for the cytomegalovirus protease, with a final docked energy ≤ 14.00 kcal/mol and calculated $K_i < 1 \times 10^{-8}$.

were occupied by a small drug molecule or peptidomimetic inhibitor.

The first ranked docking solution derived from the preliminary docking procedure showed that all inhibitors bound to the substrate-binding site of the cytomegalovirus protease. The binding energy and the calculated K_i obtained after molecular dynamics simulation and second round docking showed that amprenavir and indinavir had high affinity for the cytomegalovirus protease (as indicated by calculated $K_i < 10^{-8}$ and final docked energy ≤ 14.00 kcal/mol) with the inhibitor occupying subsites S_1, S_2 and S_3 (Table 1). The other four inhibitors, lopinavir, nelfinavir, ritonavir and saquinavir, only partly fit into one or two subsites. The docked energy, the calculated K_i and the binding modes of nelfinavir and lopinavir indicated that these two inhibitors bound the cytomegalovirus protease more weakly than the other inhibitors. Identical results were obtained for all the three starting seeds used.

A number of studies have suggested that protease inhibitors included in the HAART regimen have a significant impact on cytomegalovirus infection by decreasing the incidence, changing the clinical course, and altering the clinical presentation [4–11]. However, none of them have identified the inhibitory activity of individual HIV-1 protease inhibitors against cytomegalovirus.

This computational study provides evidence for the inhibitory activity of two approved inhibitors, amprenavir and indinavir, against the cytomegalovirus protease. Including either of these two inhibitors in a HAART regimen should help to control the cytomegalovirus viral load in HIV-1-infected patients. The activity of the cytomegalovirus protease would be inhibited soon after starting HAART, in contrast to inhibition by promoting immune system restoration, which may take several weeks.

This study also provides a list of candidate inhibitors that may be experimentally tested for cytomegalovirus protease inhibitory activity, and for the further design of broad-spectrum inhibitors, to control both HIV-1 and cytomegalovirus infection. Structural studies of human herpes proteases (of which cytomegalovirus is one) indicates homology among several subtypes [18–20]. Further studies to investigate the interaction and activity of these inhibitors, including approved drugs, against proteases from human herpesviruses may thus be fruitful in combating opportunistic infections originating in HIV-1 patients.

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Do oestrogen receptors play a role in the pathogenesis of HIV-associated lipodystrophy?

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Epidemiological data show an increased risk of HIV-associated lipodystrophy in women, and sex hormone abnormalities have been reported with highly active antiretroviral therapy (HAART). This study, which demonstrates that oestrogen receptor β expression is significantly reduced in the subcutaneous adipose tissue of HIV-infected lipodystrophic patients, downregulated by HAART regimens including protease inhibitors (PI), and restored after switching from PI, opens perspectives for the investigation of selective oestrogen receptor modifiers for the management of this syndrome.

Highly active antiretroviral therapy (HAART), a treatment consisting of a combination of protease inhibitors (PI), nucleoside analogue reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors, has dramatically improved the long-term survival of HIV-infected patients. However, such a therapy is associated with a lipodystrophy syndrome, characterized by changes in body fat distribution with several adverse metabolic effects including insulin resistance, glucose intolerance and dyslipidemia, which affects patients' adherence to therapy and impairs their prognosis by increasing the risk of cardiovascular disease. Epidemiological data have demonstrated an increased risk of HIV-associated lipodystrophy in women [1], and despite abnormalities of sex steroid hormone levels being reported in HIV-positive patients receiving HAART [2], their impact on the development of lipodystrophy has never been evaluated. Oestrogens and androgens play a critical role in adipose cell differentiation and fat distribution by acting through their specific receptors [3]. The aim of this study was to investigate whether HAART treatment could modulate the expression of sex steroid hormone receptors in the adipose tissue of HIV-infected patients.

We enrolled 14 male HIV-positive patients (median age 39 years, range 29–56), including six naive patients who were starting a HAART protocol (group 1) and eight who were switching from a HAART regimen including PI to a PI-free treatment (group 2). Five patients of group 2 had lipodystrophy. All patients gave their informed consent according to the guidelines of the local ethics committee. Patients were evaluated before starting HAART therapy (group 1) or before switching from PI (group 2; time 0 months) and 6 months after the first evaluation (time 6 months). Endocrine and metabolic examinations consisted of a thorough evaluation of the hypothalamic–pituitary–adrenal, gonadal and thyroid axes, a determination of the serum lipid profile, glucose, and insulin, anthropometric measurements, an evaluation of body composition by dual energy X-ray absorptiometry, an assessment of abdominal fat distribution, total and subcutaneous fat at the level of the thigh by computed tomography scan. In order to obtain information on the adipose tissue gene expression profile, all patients were submitted to a needle biopsy of abdominal subcutaneous fat at times 0 and 6 months. The expression of the following genes, measured by real-time reverse transcriptase–polymerase chain reaction, was investigated: genes encoding for sex steroid hormone receptors (*ERα*, *ERβ*, *AR*, *PGR*); aromatase (CYP19), the enzyme that converts testosterone to oestrogen; LRH-1, a nuclear orphan receptor that activates *CYP19* expression; factors produced by adipose tissue and involved in adipogenesis [TNF-α, peroxisome proliferator-activated receptor gamma (PPAR-γ) 2, vascular endothelial growth factor