Computational Design and Biological Evaluation of Novel HIV-1 Entry Inhibitors Based on Glycosphingolipids

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In spite of disappointing results for over more than a decade, studies on the design of anti-HIV-1 drugs and vaccines to protect against viral infection have still been focused on the gp120 third variable (V3) loop, which is a 35-residue long sequence that is frequently glycosylated, highly variable, and contains a disulfide bond (reviewed in [1]). The V3 loop plays a central role in the biology of the HIV-1 envelope glycoprotein gp120 as a principal target for neutralizing antibodies for some HIV-1 isolates, and as a major determinant in the switch from the non-syncytium-inducing to the syncytium-inducing form of HIV-1 that is associated with accelerated disease progression [1]. The findings of a study [2] show that, despite the high sequence mutability, the HIV-1 V3 loop contains three structurally inflexible portions, which include residues crucial for cell tropism. These sites, therefore, represent potential HIV-1 vulnerable spots for therapeutic intervention.

In this work, novel anti-HIV-1 agents targeting the V3 loop of envelope protein gp120 were designed by computer modeling based on glycosphingolipid β-galactosylceramide (β-GalCer), which is an alternative receptor allowing HIV-1 entry into CD4-negative cells of neural and colonic origin. Models of these β-GalCer analogs bound to the V3 loops from five

various HIV-1 variants were generated by molecular docking and their stability was estimated by molecular dynamics (MD) simulations and binding free energy calculations. One of the designed glycosphingolipids presenting deacetylated β-GalCer analog was obtained and its HIV-1 inhibitory properties were validated by testing for antiviral activity.

The crystal structure of β-GalCer (http://www.rcsb.org/pdb/home/home.do) was used to generate the starting models of the β-GalCer analogs, which were designed by substitution of its fatty acid residue by different soluble acids. Quantum chemical calculations of the 3D structures for the β-GalCer analogs were performed by the GAUSSIAN 09 program package (http://gaussian.com/g09citation/). To calculate the molecular electronic configuration, the self-consistent field method of restricted Hartree-Fock with 6-31G* basis set was engaged in the studies (http://gaussian.com/g09citation/). The 3D structures of the V3 loops from five different HIV-1 modifications [3] were used as static receptors for flexible ligand docking of the β-GalCer analogs by the AutoDock VINA program (http://autodock.scripps.edu/resources/adt). The MD simulations that were carried out by the Amber 11 computer package using the Amber ff10 force field generated 30 ns trajectories for each V3 assembly using a Langevin thermostat with collision frequency 2.0 ps^{-1}, a non-bonded cut-off distance of 8 Å, and a simple leapfrog integrator with a 2.0 fs time step and hydrogen atoms constrained by the SHAKE algorithm (http://ambermd.org/). The free energy of binding was used as a measure of conformational stability of the complexes of interest and was calculated by the MM-PB/SA procedure in AMBER 11 (http://ambermd.org/).

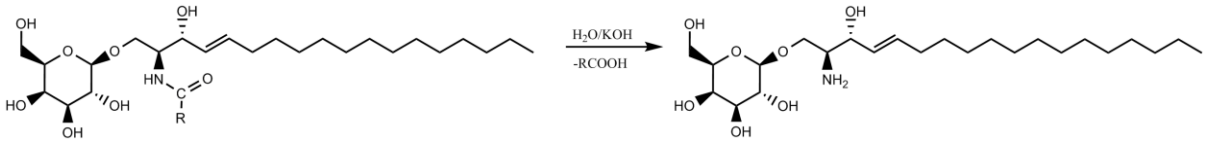
Analysis of the obtained data shows that the galactose ring of the designed glycolipids forms non-conventional XH···π H-bonds with π-conjugated side chains of the conserved Phe-20, Tyr-21 or His-34 residues of the V3 loop. This interaction mode is most likely to determine the specificity of the glycolipid binding to the envelope gp120 protein. This supposition is supported by the following rationale: the fatty acid of β-GalCer is buried in the cellular membrane, and the sugar residue is the most accessible element of the glycolipid structure [4]. Along with XH···π H-bonds, the standard H-bonds and π-π interactions involving the functionally important residues of the V3 loop greatly contribute to forming the complexes of the designed β-GalCer analogs with this site of gp120. In the list of these residues, it is necessary to note Arg-3 that is critical for binding to coreceptor CCR5 and

heparan sulfate proteoglycans as well as asparagines in positions 5–7 that make one of the potential sites of the gp120 N-linked glycosylation used by the virus for defense against neutralizing antibodies and for maintaining its infectivity [1].

According to the docking calculations, formation of the complexes of the β-GalCer analogs with V3 results in masking of the immunogenic tip Gly–Pro–Gly–Arg/Gln–Ala–Phe of the V3 loop or/and its base, which comprises the N- and C-terminal residues close to disulfide bridge Cys-1–Cys-35. These data deserve a specia attention because the above regions of the HIV-1 V3 loop form conserved structural motifs that contain residues critical for regulation of the molecular anatomy of CCR5 utilization [1, 2]. With the empirical data 4], specific binding of glycolipids to the HIV-1 V3 loop results in masking its central region, residues 15-20. The above docking data obtained for the V3 loops from diverse HIV-1 strains agree with these experimental observations and disclose one more potential site of V3 for specific binding to β-GalCer analogs, which may be accomplished primarily by XH···π interactions between the conserved His-34 residue of the V3 loop and the glycolipid galactose ring.

The MD simulations support the docking results. The majority of the MD trajectories show the structures keeping the intermolecular interactions that appear in the docked models. The complexes generated by molecular docking are energetically stable within the 25 ns time domain, which is validated by the low averages of binding free energies and their standard deviations.

In this way, the in silico data suggest that the designed soluble analogs of β-GalCer may be able to neutralize different HIV-1 modifications. One of these compounds, namely β-galactosylsphingosin, was synthesized (scheme 1) and its HIV-inhibitory properties were evaluated by medical trials.



Scheme 1

This β-GalCer analog was shown to exhibit anti-HIV-1 activity *in vitro*. Cellular protection rating is 51-53 % for the glycolipid concentrations varying from 1.0 to 0.2 μg/ml (as for azidothymidine, this index ranges from 56 to 100%). At the same time, maximal tolerant concentration is 8.0 μg/ml for MT-4 cells, and chemotherapeutic index of the molecule is 40.0, which testifies to its anti-HIV-1 activity.

Thus, examining the static and dynamic models for the complexes of the designed β-GalCer analogs with the HIV-1 V3 loops from various viral modifications shows that non-conventional XH···π interactions between the galactose ring and the V3 conserved residues with π-conjugated side chains play a key role in specific binding of the glycolipids to this functionally important site of gp120. Formation of the complexes results in blocking the immunogenic tip of the V3 loop and/or its base, which form conserved structural motifs that contain residues critical for cell tropism. MD calculations have shown that the docked models of the complexes of interest are energetically stable and exhibit the low values of free energy of their formation. Based on these findings, the designed β-GalCer analogs present promising basic structures for the development of novel, potent and broad anti-HIV-1 drugs.

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