

Structural Insights of Nevirapine Interaction with HLA-B07 Supertype Alleles in Induction of Hypersensitivity among HIV Affected Population

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In modern drug therapy adverse drug reactions are the common life threatening problem among patients causes morbidity and mortality. Involvement of HLA in drug hypersensitivity well known underlying mechanism is unclear thus considered as thrust area for current research. Non nucleoside reverse transcriptase inhibitor Nevirapine (NVP) commonly used in HAART therapy. HLA-B07 alleles are highly polymorphic accounts 32% among Indian population and significantly associated with Nevirapine induced hypersensitivity. Insilico studies were used to dissect the molecular interaction of NVP with B alleles ability to alter the binding repertoire. Both conventional and non classical hydrogen bonding occurrence between NVP and HLA confers the stable interaction within the binding cavity of HLA B pocket and F pockets which is meant for self peptides. Current study thus open up a new prospective of the HLA-associated NVP hypersensitivity which would be helpful in improving the drug safety in future.

Key words: SJS syndrome, Nevirapine, Hypersensitivity, Human Leukocyte Antigen, HLA-B07 supertype alleles.

Adverse drugs reactions (ADR's) are potentially life threatening and are cause for uncertainty of certain drugs. These reactions involve immune mechanisms, and various host factor related studies revealed the drug hypersensitivity reactions and their Human Leukocyte Allele (HLA) allele specificity¹. The non-nucleoside reverse-transcriptase inhibitor nevirapine is widely used as a first-line treatment of human immunodeficiency virus (HIV) infection in developing countries like India, because of its low cost². Nevirapine is associated with hypersensitivity reactions (HSR) like fever, hepatitis, skin rash with clinical complications³. Genetic risk factors have been implicated in the development of both type A and type B ADRs.

The most significant associations that have been reported with ADRs are associated with the human leukocyte antigen genes present in the Major HistoCompatibility (MHC) complex situated on the short arm of chromosome 6⁴⁻⁹. HLA genes are known for their polymorphic character in the human genome, and HLA-B is the most polymorphic gene of the human genome. Three models have currently been proposed to explain the MHC-dependent T-cell stimulation by drugs, leading to an immune response like the hapten/prohapten model, the p-i model and the altered repertoire model. Here p-i model and altered repertoire model proposes that drugs can alter the repertoire of self-peptides presented to T-cells by occupying a specific site within the antigen binding cleft of the HLA molecule, and thus leading to the immune response¹⁰. The 'pharmacological interaction with immune receptors' (p-i) concept suggests that the interaction between drug-cell receptor and MHC

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molecule can be non-covalent and that direct stimulation of T cells can occur, independent of cellular processing¹¹. Bioinformatics approaches are also important to define the mechanisms of immune reactions that are associated with specific HLA types - this is now possible given that sequence-based HLA typing, which has resulted in increasing availability of individual and frequency data in public repositories^{12,13}. To date little is known regarding genetic risk factor for nevirapine induced hypersensitivity among Indian HIV infected populations. Therefore, we investigated the role of HLA on nevirapine-induced rash among the antiretroviral-treated HIV-1-infected individuals from India. Our aim was to identify the predictive human leukocyte antigen (HLA) markers that are associated with nevirapine hypersensitivity.

MATERIALS AND METHODS

HLA allele sequence retrieval and frequency assessment in Indian population

NVP Hypersensitivity related HLA alleles B*35, B*51, B*7 (supertype B07), sequences were fetched from the IMGT HLA sequence database¹⁴ <http://www.Ebi.Ac.Uk/Ipd/Imgt/Hla/>, a public repository for HLA sequences for Human Major Histo compatibility Complex includes official sequences for the WHO Nomenclature for factors of the HLA systems. Highly polymorphic specific HLA-B07 subtype alleles frequencies were evaluated based on frequency data from the Allele Frequency Net Database¹⁵ <http://www.allelefrequencies.net/> (AFND).

Ligand Nevirapine retrieval and preparation

Food and Drug Administration approved Nonnucleoside reverse inhibitor (NNRTI) Nevirapine Structure Data Format (SDF) structure was retrieved from Drug bank¹⁶, a repository for drugs including the information's like empirical formula and its compound structure, molecular weight, Xlogp, ADMET properties. "Prepare ligand" option in Discovery studio 2.0 was used to carry out energy optimization and hydrogen atom addition to Nevirapine to generate various confirmations based on different energy values.

HLA allele structures retrieval and receptor preparation and binding Pocket analysis

Experimentally resolved HLA-B allele structures HLA-B*51-PDB:1E27¹⁷, HLA-B*35-

PDB: 1ZHL¹⁸, HLA-B*07- PDB: 3VCL¹⁹, were retrieved from Protein Data Bank (PDB)²⁰. In Receptor HLA alleles Heteroatom's were removed and hydrogen atoms were added using CHARMm force field and Energy minimization was performed using conjugant gradient method on Accelrys Discovery studio client (version 2.0) software. Binding pocket residues of HLA-B alleles assessed using based on conservation among B pocket and F pocket residues of HLA-B alleles Discovery Studio 2.0²¹, Conservation among the B and F binding pocket residues of HLA-B alleles and were grouped into supertype B07²². B and F binding pockets are large binding groove generally accommodate self peptides.

Molecular docking of Nevirapine and HLA -B07 (Supertype)

In general to evaluate the feasible binding of a putative ligand with a target protein Molecular Docking studies were used. "Ligand fit" module of Discovery studio2.0²³ a cavity detection algorithm which quantify the active sites and Monte Carlo conformational search was used to assess the HLA allele binding affinity for Nevirapine. Protocol implements an evaluation for protein-ligand interaction energies and candidate poses are minimized in the context of the active site using a grid based method. Broyden-Fletcher-Goldfarb-Shanno (BFGS) method embedded in ligandfit module was used for pose optimization. Systematic docking analysis of Nevirapine on HLA-B07 supertype alleles were carried out in order to predict the binding affinities based on various scoring functions like LigScore, Piecewise Linear Potential (PLP), JAIN, Potential of Mean Force (PMF), and dock score and their relative stabilities. Finally dock score and binding energy values were analyzed to find out best conformation.

Endogenous peptide prediction and 3D structure modeling

Binding affinity of HLA-B alleles for endogenous peptides of Ribosomal Binding Protein were estimated using the Immune Epitope Database²⁴ (IEDB, www.iedb.org) consensus method. Low percentile ranked self peptides nonamers like IPKHLTDAY (B*35), KPHCSRNPV (B*07), FVIATSTKI (B*51) were selected to evaluate their interaction pattern with HLA-B alleles loaded with NVP. Three dimensional structure of self peptides were modeled using

PEPFOLD²⁵, *de novo* based approach for peptide structure server accessible via RPBS Mobyle Portal. Top listed model provided by the server was chosen for the docking study based on avg, gdt, max, q, and tm the scores.

Self peptides interaction with NVP loaded HLA-B alleles and HLA-B alleles without NVP

Structural insights of HLA-B allele interaction with self peptide analyzed using ClusPro²⁶, an automated, fast rigid-body docking and discrimination algorithm that rapidly filters docked conformations and ranks the conformations using clustering of computed pairwise RMSD values. Docking of the HLA and B07 supertype alleles was performed using two established FFT-based docking programs like DOT and ZDOCK²⁷, further filtering and discrimination was performed Based on the binding energy values of each cluster docked structures were ranked. Similarly the ClusPro resulted model was again docked with NVP loaded HLA allele to find out insights of binding groove occupancy between NVP and self peptide.

RESULTS AND DISCUSSION

HLA allele sequence retrieval and frequency assessment in Indian population

HLA alleles B*35, B*51, B*7 (B-07 supertypes) Fasta formatted protein sequences were retrieved and its population frequency of among Indians analyzed according to Allele frequency dataset, about 32% of B-07 supertype alleles distribution seen among Indian population. Since NVP induced hypersensitivity is

common problem in HAART therapy regiment frequency need to be quantify for B-07 supertype allele.

Ligand (NVP) and Receptor (HLA-B7 supertype) preparation

Nevirapine's SDF strcture retrieved and basic physiochemical properties and analogues analyzed for their variation, then for interaction study ligand need to be minimized to attain energy optimization. Crystal structures of HLA-B*35-PDB: 1ZHL, HLA-B*07- PDB: 3VCL, HLA-B*35-PDB: 1E27 are the receptor for current study and then heteroatom's were removed, hydrogen atoms were added using CHARMM force field and Energy minimization was performed using conjugant gradient method. The binding pocket residues of HLA-B alleles and their distribution among B pocket and F pocket were assessed, and the Conservancy level were estimated by means of multiple sequence alignment. Thus signifies the identification of marker residues which involved in NVP interaction.

Molecular docking of Nevirapine and HLA -B07 (Supertype)

Three stage protocol of ligandfit module processed the HLA and NVP interaction and series of scoring functions like LigScore, PLP, JAIN, PMF, and dock score and their relative stabilities of docked complexes were analyzed and listed in Table 1. The score value returned may be the negative of the selected score to provide a consistent interpretation of that data, but generally a more positive value indicates a better score. Identification and optimization of molecular

Table 2. Endogenous peptide prediction using IEDB and scores

Allele	Self Peptides	Percentile Rank	ANN Ic50	Smm Ic50	Comblib Score
HLA-B*35:01	IPKHLTDAY	0.4	7	16.28	0.000197
HLA-B*51	FVIATSTKI	1.4	4889	1408.09	0.000182
HLA-B*07	KPHCSRNPV	0.2	8	15.1	0.000166

Table 1. Molecular docking of Nevirapine and HLA -B07 (Supertype)

ALLELE	RBP self Peptides	-PLP 1	-PLP 2	PMF	DOCK SCORE
HLA-B*35	IPKHLTDAY	77.46	76.54	127.3	72.657
HLA-B*51	FVIATSTKI	77.58	73.68	93.29	75.213
HLA-B*07	KPHCSRNPV	63.86	62.37	113.69	44.344

interactions between a NVP and a HLA is the goal in drug induced hypersensitivity study. Molecular interaction between NVP and HLA including traditional interactions that have been well understood for decades (e.g., classic hydrogen bonds) as well as more recently discovered

interactions such as weak hydrogen bonds with carbon donors. The different types of interaction vary in strength, but imply significant effect. Based on distance, angle constraints interactions between HLA and NVP assessed.

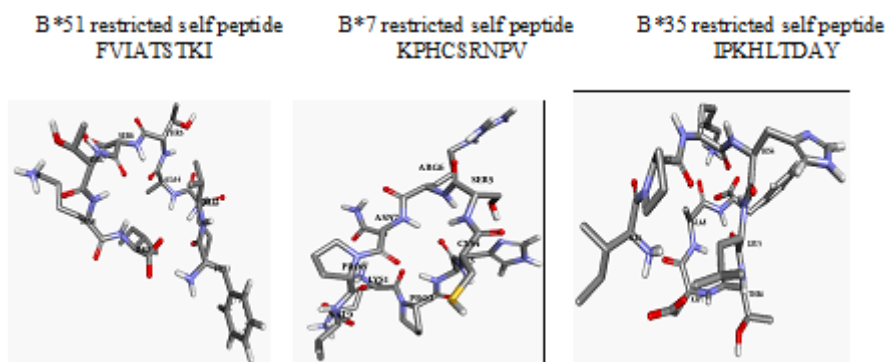
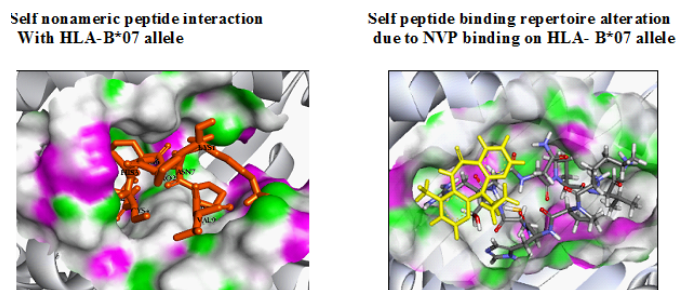


Fig 1. Self nonameric peptides of Ribosomal Binding Proteins



P-I Concept of NVP interaction with HLA-B*07 allele

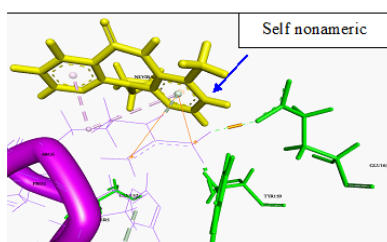
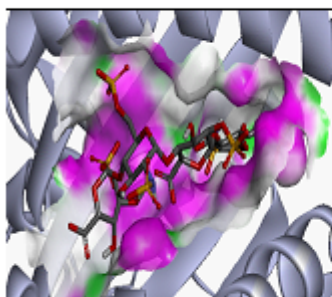


Fig 2.

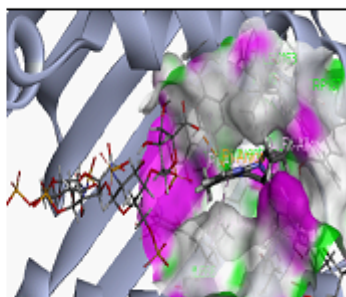
Table 3. Self peptides interaction with NVP loaded HLA-B alleles and HLA-B alleles without NVP

ALLELE	Self Peptides	-PLP 1	-PLP 2	PMF	DOCK SCORE
HLA-B*35	IPKHLTDAY	60.2	52	107.71	57.306
HLA-B*51	FVIATSTKI	76.74	70.61	124.38	75.418
HLA-B*07	KPHCSRNPV	38.08	34.08	33.38	27.993

**Self nonameric peptide interaction
With HLA-B*35 allele**



**Self peptide binding repertoire alteration
due to NVP binding on HLA- B*35 allele**



P-I Concept of NVP interaction with HLA-B*35 allele

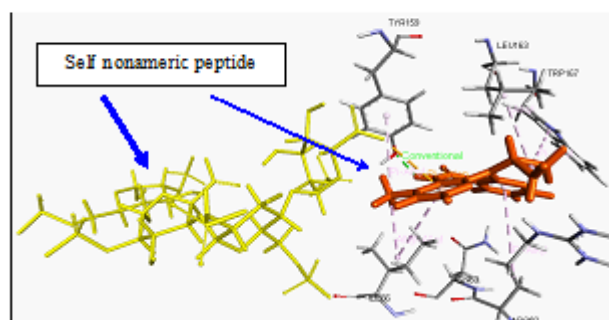
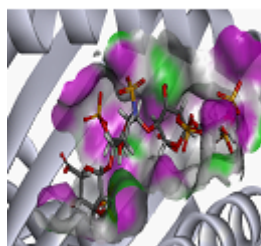
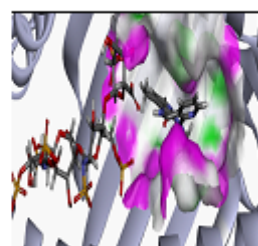


Fig 3 .

**Self nonameric peptide interaction
With HLA-B*51 allele**



**Self peptide binding repertoire alteration
due to NVP binding on HLA- B*51 allele**



P-I Concept of NVP interaction with HLA-B*51 allele

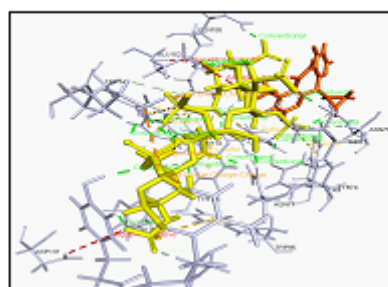


Fig 4.

Endogenous peptide prediction and 3D structure modeling

Endogenous peptides IPKHLTDAY (B*35), KPHCSRNPV (B*07), FVIATSTKI (B*51) were selected based on their low percentile rank, corresponding IC_{50} values and matrix score were listed in Table 2. Binding affinity assessment of these nonamer needs the three dimensional structure (Figure 1) and were modeled based on *de novo* based approach. Score values like avg, gdt, max, q, and tm the score list the models prediction accuracy and C score listed in Table 2.

Self peptides interaction with NVP loaded HLA-B alleles and HLA-B alleles without NVP

Molecular docking analysis of NVP loaded HLA allele and nonameric self peptide as well as HLA and self peptide were carried using ClusPro a protein protein docking tool to estimate the interacting residues of HLA allele for NVP and binding groove occupancy pattern self peptide variation between NVP loaded HLA allele and HLA alleles. ClusPro resulted with ten models ranked as the most probable prediction candidate for self peptide and HLA allele interaction based on the binding energy and models retrieved as a PDB file and finally interaction between HLA and self peptide was visualized using DS visualizer 4.0. Finally self peptides were docked with NVP loaded HLA allele to assess the binding pattern interaction on HLA alleles. Resulting clusters of docked complexes and their binding energy values were listed in Table 3. Conventional Hydrogen bonding and non classical carbon hydrogen bonding between these factors HLA-NVP-Self peptides implies the complex p-i concept of drug hypersensitivity and thus causes adverse off target effect by stimulating the T Cell results in hypersensitivity. Altered self repertoire of HLA allele due to NVP occupancy on binding groove meant for self peptides, thus T cell response would be induced against self peptides results in hypersensitivity (Figs. 2-4).

CONCLUSION

In conclusion our studies revealed the structural insights of Nevirapine induced adverse drug reactions. HLA Class I molecules are have conserved B and F pockets that accommodate the peptides termini but Nevirapine loaded HLA alleles

masking the peptide binding clefts by having stable conventional hydrogen bonding with the HLA B-07 supertype allele (B*35, B*51, B*7). Thus Nevirapine binding to HLA alleles and causes adverse off target effects by stimulating the T cell results in Hypersensitivity. Insight studies on HLA-Nevirapine structural basis interaction studies would be helpful in understanding the underlying mechanism of drug hypersensitivity as well as for improving the drug safety. Nevirapine binding at this HLA polymorphic site would alter the peptide binding repertoire.

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