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Insilico study of HLA-DRB1*0101 allele association in Nevirapine (NVP) induced hypersensitivity among Indian HIV-Infected Population

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Abstract : Idiosyncratic adverse drug reaction (ADR's) are the common and a potentially life threatening problem in drug therapy and thus patient's experience unnecessary morbidity and mortality. Recent studies have revealed that immunogenetic factors involvement for drug hypersensitivity but the insights of underlying molecular mechanism is unknown. Nevirapine (NVP) a non-nucleoside reverse transcriptase inhibitor (NNRTIs) used for treatment of human immunodeficiency virus (HIV) in India. Human HLA-DRB1*0101 allele accounts for 5.8% among Indian population has been significantly associated with nevirapine-induced hypersensitivity.Current study based identification the genetic relationship between Nevirapine hypersensitivity and HLA-DRB1*01.01 allele, would lay the groundwork for biochemical and structural studies. *In silica* docking studies was used to assess mechanism of NVP binding within the antigen binding cleft of HLA-DRB1*0101 alleles. We used the DS 2.0: Ligand fit module to dock NVP on the HLA into the Binding pocket, and we observed that seven favourable bonding is observed for NVP with DRB1*0101 binding groove residues. The binding is stronger without the presence of peptide.pi-pi, pi-alkyl, p-sulfur bonds along with conventional Hydrogen bonds further accelerating stability of NVP and HLA allete interaction and thereby altering HLA allele's repertoire for self-peptides presentation to T cells thus self-peptides may bind at the terminal pockets of the binding cleft of HLS allele and loop over NVP to interact with the TCR and causing alloreactive T cell response. This study not only provides new perspectives of the mechanisms of HLA-associated drug hypersensitivity but also for preclinical screening and sheds insight on the improvement of drug safety.
Keywords: Nevirapine, Stevens Johnson syndrome, Human Leukocyte Antigen, Hypersensitivity, DRB1*0101 Allele.

Introduction:

Adverse drug reactions related to mobidity and mortality is having serious impact in patients and drug productions that are caused by drug specific T cell responses. Nevirapine(NVP) an approved Nonnucleoside reverse inhibitor (NNRTI), structurally nevirapine belongs to then dipyridodiazepinone chemical class is a cornerstone drug for treating the HIV patients in many low income countries like India[1].Nevirapine treated individuals develop allergenic reactions ranging from NVP induced rash to severe blistering skin reactions namely Stevens-Johnson Syndrome as well as toxic epidermal necrolysis[2]. Current studies on these altered drug responses revealed that toxicity of the drug is neither dose-independent nor associated with alterations of the cytochrome P450 metabolic enzyme but is more likely due to a hypersensitivity reactions in which drugs act as antigen and induce T cell mediated immune response in genetically susceptible individuals[3]. Host genetic factors like Human Leukocyte Antigen (HLA) play a significant role in the development of both type A and type B ADRs. HLAs are encoded in the major histocompatibility complex (MHC) on chromosome 6, a highly polymorphic region with considerable linkage disequilibrium [4-7]. Both Class I and II HLA allele's involvement

in antigen presentation to T cells and activation of immune response have been extensively studied[8].Currently there are three models have been proposed for MHC-dependent T –cell stimulation by drugs which would leads to an immune response.Oneof the model proposes that drug can alter the repertoire of self –peptides presented to T-cells by occupying a specific site within antigen binding clefts of the HLA molecule,and thus leading to the immune response[9].Immunological factors like HLA alleles relationship to drug hypersensitivity studies demonstrated for abacivir[10] and many other class of drugs like carbamazine[11] and allopurinol[12].HLA class I allele A,B,C and class II alleles DR,DP,DQ are considered as a key factor studying their association with nevirapine rash and hepatitis conditions among various populations[13-16].HLA-DRB1*0101 allele's role in nevirapine hypersensitivity was confirmed in Western Australian population[17].Current research emphasized on immunogenetic risk factors for nevirapine induced hypersensitivity and their relationship to HLA-DRB1*01:01 allelel in whites population [18-20].

Methodology:

Retrieval of HLA allele and analyzing the allele frequency among Indian population:

DRB1*01 allele sequence was fetched from the IMGT HLA sequence database[21] http:// www.Ebi.Ac.Uk/Ipd/Imgt/Hla/, a public repository containing HLA sequences for Human Major Histo compatability Complex and contents based on the official sequences for the WHO Nomenclature for factors of the HLA systems[22]. DRB1*01 allele frequency was evaluated based on frequency data from the Allele Frequency Net Database http://www.allelefrequencies.net/ (AFND). DRB1*01 allele frequencies for Indian population is about 5.8% from sample size 298 (n = 298)[23].

Homology modeling of DRB1 *01 HLA allele, model evaluation and binding pocket analysis:

Experimentally resolved structure for DRB1*01 is not available in public protein structure repositories, so the three dimensional structure was modeled using I-Tasser[24]. I Tasser explores template search based on locally implemented meta server LOMETS, and TM-align allows fragment assembly simulation and finally function predictions are concluded from the consus hits among the top structural matches along with function scores calculated based on the confidence score of I –TASSER structural models .TM-Score and sequence identity in the structurally aligned regions were used to evaluate structural similarity between target and template models.To access the PROCHECK we used PDBsum[25] gateway which assigned an query idenfier for our predicted model with Z948 and the models stereo chemical quality was assessed by PROCHECK[26] server ,which indicates the amino acids with unusual backbone conformation. PROSA[27] analysis compares Z scores of target and template structure based on Z score compatibility evaluates the modeled structure quality.The binding pockets of the DRB1*01 receptor were also determined by using Accelrys Discovery Studio 2.0 Binding site analysis module [28] and CHARMM[29] forcefield applied to Modeled allele.

Nevirapine retrieval and ligand preparation:

An approved Nonnucleoside reverse inhibitor (NNRTI) Nevirapine SDF format structure was retrieved from Drug bank[30], 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 was used to carry out energy optimization and hydrogen atom addition to Nevirapine to generate various confirmations based on different energy values.

HLA and Drug Interaction analysis using Molecular docking:

Molecular docking is a method to evaluate the feasible binding geometries of a putative ligand with a target whose target site is known. To assess the HLA allele binding affinity for Nevirapine Discovery studio "Ligand fit" module of Discovery studio2.0[31], based on cavity detection algorithm to assess the active sites and Monte Carlo conformational search,then to evaluate protein-ligand interaction energies candidate poses are minimized in the context of the active site using a grid based method. An inbuilt Broyden-Fletcher-Goldfarb-Shanno (BFGS) method was used for pose optimization.Thus docking analysis of Nevirapine for HLA * DRB1 allele was carried out to predict the binding affinities based on various scoring functions like LigScore ,PLP, JAIN, PMF, and dock score and their relative stabilities.Based on dock score of best conformation ligand binding energy calculated.

Results and Discussion:

HLA allele sequence from IMGT HLA allele.

HLA allele DRB1*0101 sequence protein sequence retrieved from HLA sequence database , its population frequency of among Indians considered as a key factor allele selection and Fasta formatted sequence was submitted to I-Tasser prediction for 3D structure prediction.

Homology modeling of HLA allele and Model Evaluation:

A protein biological function cannot be inferred only with mere knowledge of its primary and secondary sequence,so it is mandatory to predict the 3D structure of DRB1 allele for assessing its affinity for Nevirapine,and prediction was done using I-Tasser. 5 models were reported for I-Tasser and based on Z score and model evaluation parameters most accurate one is used in our study. PDBsum's PROCHECK module was used to assess stereochemical quality of the structure and its Ramachandran plot **(Figure3)** revealed that 87.3% of residues are in the favorable region and average G-factor score -0.42 indicates the model quality. PROSA web tool provided Z-score (signify overall model quality) that determined whether the structure is within the range of scores found in native proteins of comparable size. Modeled DRB1*0101 allele verified with its Z score of -5.66 (**Figure2)**determined by NMR and X-ray generated Z score plot using PROSA program and finally binding pocket analysis done using PDBsum ,and visualization of modeled DRB1*010 carried out using Accelrys DS 2.0 software**(Figure1**) (Accelrys Inc., San Diego, CA, USA).

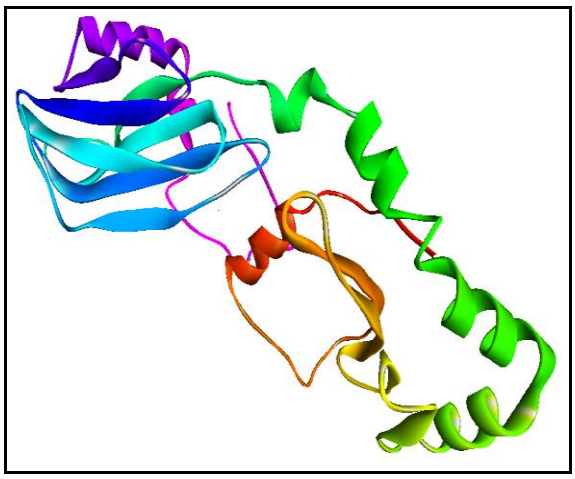

Figure 1: Modeled HLA-DRB1*0101 allele Structure

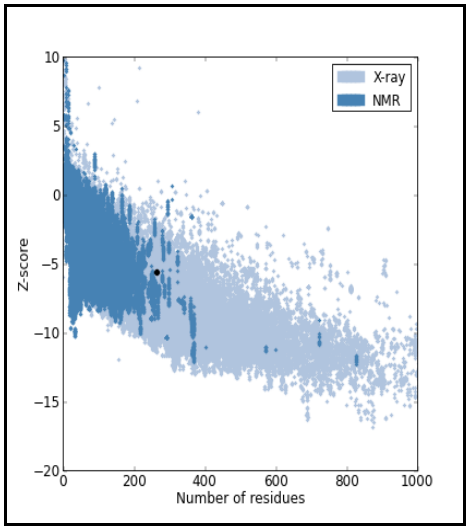

Figure 2. HLA-DRB1*0101 allele Evaluation using Prosa Server

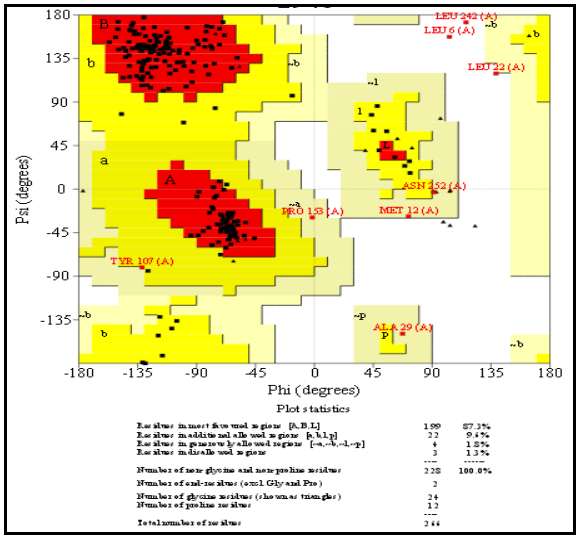

Figure 3.HLA-DRB1*0101 allele using Ramachandran plot

Insilico HLA and NVP interaction analysis using Docking:

HLA allele and NVP associated hypersensitivity were reported for various alleles among Europeans,but there is a lag for Indian HLA alleles studies .Molecular docking simulation studies were carried out to understand the binding affinity of HLA allele DRB1*0101 for NVP using Discovery Studio 2.0's "Ligandfit" module .The best docking score of 43.868 (kcal/mol) was identified for first ligand pose **(Figure 4**). Amino acid residues TRP38, GLU57, TYR76, ASP86, LEU40, VAL67, MET1 of the DRBR*0101 protein assisting the functional conformation and ligand binding(**Figure5,6,7)**. Analysis of docking results showed that NVP are placed within the binding pocket of the self peptide binding groove of HLA-DRB1*0101 and changing the shape and chemistry of antigen binding clefts Figure.4 and 5, where NVP makes total of 7 different favorable bonds like carbon hydrogen bond between H29 atom of NVP and OE1 atom of GLU57 of DRB1*0101 allele with the bond distance of 2.20 A^0 ,pi-alkyl interaction includes DRB1*0101 binding groove residues LEU40,TYR76 and VAL67,pi-anion bond of electrostatic ligand protein bonding involves OD1 atom of ASP86 ,pi-pi T shaped hydrophobic interaction involves TRP38,pi-sulfur bond involves MET 1 to interact with Nevirapine and alter their binding repertoire thus leading to the immune response against self peptides. The dock score, Ligscore1, Ligscore 2, PLP1, PLP2, Jain scores for all NVP and DRB1*0101 were used to assess the highest dock score of 43.868 (kcal/mol) in docking analysis.

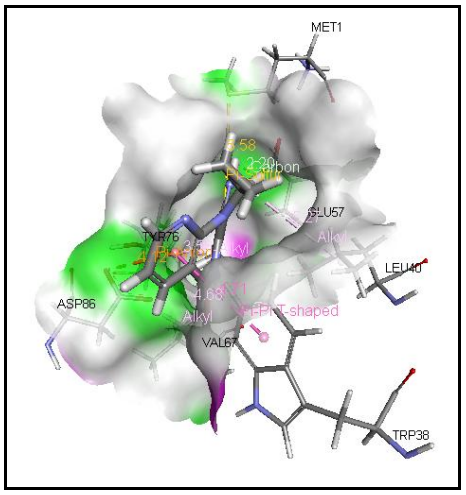

Figure 4: Binding pocket of HLA-DRB1*0101 with NVP

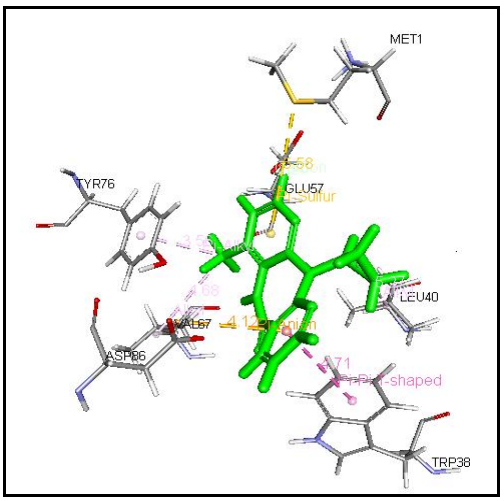

Figure 5: Interacting atoms of DRB1*0101 allele and NVP

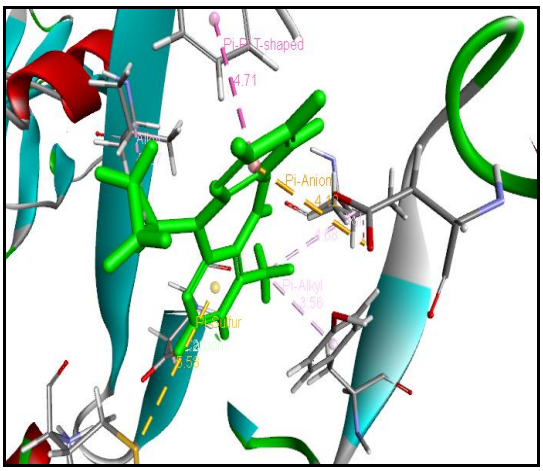

Figure 6: Interacting atoms of DRB1* 0101 receptor and NVP

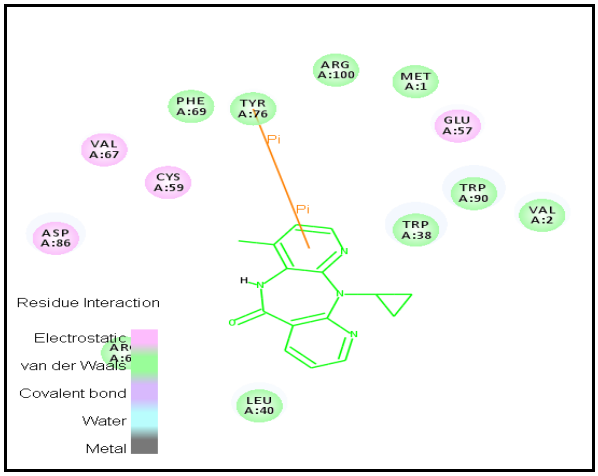

Figure 7: A schematic 2D plot of NVP and HLA-DRB1*0101 allele

Conclusion:

Systematic study of HLA –DRB1*0101 allele affinity for NVP suggests that unmodified Nevirapine binds noncovalently to the floor of the peptide binding groove of HLA-DRB1*0101 and changing the shape and chemistry of the antigen binding clefts of the HLA molecule, there by altering the repertoire for self peptides.

Among the proposed idiosyncratic ADR's mechanism, the p-i mechanism is involved in NVP drug interaction among HLA-DRB1*0101 individuals. Therefore seven different favorable interactions of the drug with the HLA molecule (p-i HLA) that alters the presented peptide-HLA complex on the surface is responsible for NVP specific T cell responses. Computational analysis of HLA-DRB1*0101 binding affinity assessment for NVP would be helpful in screening patients in HIV clinical care and reduce the occurrence of NVP hypersensitivity reactions and improving patient safety.

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