In silico* analysis of interactions of flucloxacillin and its metabolites with *HLA-B*57:01

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Abstract

An antibiotic flucloxacillin (FX) which is widely used for the treatment of staphylococcal infection, is known to cause liver injury. A genome-wide association study has shown that FX induced idiosyncratic drug toxicity (IDT) is associated with *HLA-B*57:01*. FX is processed in the human body to produce several metabolites. Molecular interactions of FX or its metabolites with *HLA-B*57:01* should play a crucial role in the occurrence of the adverse drug reaction. In this study, we have undertaken docking simulations of interactions of FX and its metabolites with *HLA-B*57:01* to understand molecular mechanisms leading to the onset of IDT.

Key Words: flucloxacillin, idiosyncratic drug toxicity, *HLA-B*57:01*, docking simulations

Area of Interest: molecular recognition and molecular modeling

1. Introduction

Flucloxacillin (FX) is an antibiotic belonging to the penicillin class. FX has a broad range of uses in the treatment of Gram-positive bacterial infections and used widely for the treatment of staphylococcal infection. Several works have shown FX to be associated with liver injury at a frequency of ca.8 per 100,000 people [1].

Drug-induced liver injury (DILI) is a leading cause of attrition of compounds in drug development and also a major cause for drug withdrawals, restrictions and project terminations [2]. Therefore, it is important to understand the molecular mechanisms which cause DILI to minimize the attrition. Although the molecular mechanisms causing DILI are complex, a genome-wide association study has unequivocally shown the association of FX induced DILI with *HLA-B*57:01* (OR=80.6, $P=9.0 \times 10^{-19}$) [3].

There are several possible modes of interactions between drugs and HLA molecule leading to

an adaptive immune response. One possible mode is that drugs or their metabolites directly bind to the peptide-binding groove of HLA which trigger the following activation of T cells. Direct interaction between abacavir which causes idiosyncratic adverse drug reaction and a particular allele of *HLA-B*15:02* has been confirmed by X-ray analysis [4]. This study indicates that the direct binding of drugs or their metabolites to the corresponding HLA molecule should be an important step leading to an immune response.

In the present study, we have undertaken *in silico* docking studies of FX and its metabolites at the peptide-binding groove of *HLA-B*57:01* in order to identify the chemical species responsible for the FX-induced DILI and the binding mode.

A previous investigation on FX metabolism has shown that FX could be biotransformed to produce four major metabolites [5]: 5'-hydroxymethyl flucloxacillin(5-OH-FX), (5*R*)-flucloxacillin penicilloic acid (FX-PA), (5*S*)-flucloxacillin penicilloic acid ((*S*)-FX-PA), and 5'-hydroxymethyl-(5*R*)- flucloxacillin penicilloic acid (5-OH-PA) (Figure 1). The epimer of 5-OH-PA has not been discovered as a metabolite yet. As hydroxylation of (*S*)-FX-PA could occur, the epimer ((*S*)-5-OH-PA) was also considered in this study.

Figure 1. Chemical structures of flucloxacillin (FX) and its metabolites

2. Methods

A crystal structure of *HLA-B*57:01* (PDB ID: 3VH8) deposited at the Protein Data Bank [6] was used in this study. A software system MOE (molecular operating environment) [7] was used throughout this study. Possible binding sites of FX and its metabolites were identified by the ‘alpha finder’ function implemented in MOE and all possible binding sites at the protein-binding groove of the HLA molecule were taken into account in docking simulations. Docking simulations between *HLA-B*57:01* and the molecules shown in Figure 1 were performed by use of a docking software ASEDock [8]. The complex structures were optimized and the binding affinity was judged by a scoring function of GBVI/WSA_dG which is considered to express protein-ligand binding free energy [9]. Only the backbone heavy atoms of *HLA-B*57:01* were fixed during optimization.

3. Results and Discussion

The lowest GBVI/WSA_dG values (kcal/mol) of the complexes of FX and its metabolites with *HLA-B*57:01* are as follows: FX-PA -8.93, (*S*)-5-OH-PA -8.86, 5-OH-FX -8.76, (*S*)-FX-PA -8.41, FX -8.19, and 5-OH-PA -8.18.

The binding modes of these molecules at the antigenic-peptide binding groove of *HLA-B*57:01* are shown in Figure 2(a). The six molecules bound at the same site in the groove are not deeply buried into the peptide-binding groove and largely exposed on the surface of *HLA-B*57:01*. It indicates that recruiting novel antigenic peptides on top the bound FX and its metabolites as in the case of abacavir should not be possible. As carboxy groups of the bound FX and its metabolites stick out from the binding groove, it is highly possible that the bound FX and its metabolites would directly interact with T-cell receptors leading to an immune response.

Maier-Salamon *et al.* [10] have reported that the biliary concentration of 5-OH-FX is high and the liver toxicity of FX might be due to the continuously elevated 5-OH-FX. The present docking simulations have shown that the binding affinity of 5-OH-FX to *HLA-B*57:01* is significantly high. The predicted strong affinity to *HLA-B*57:01* and the observed high concentration in the body unequivocally indicate that 5-OH-FX should be the main risk compound of DILI. The binding mode of 5-OH-FX is shown in Figure 2 (b). The exposed carboxy group is depicted in the upper right.

Maier-Salamon *et al.* [10] also have monitored the plasma concentrations of FX and its metabolites, and found that the plasma concentrations of 5-OH-FX and 5-OH-PA, but not FX and FX-PA, increased steadily before reaching steady state after 40 hours. This indicates that FX-PA would not cause liver toxicity in spite of its highest binding affinity to *HLA-B*57:01*. However, Maier-Salamon *et al.* [10] observed the formation of FX-PA in some patients is predominant and it indicates that FX-PA could be a major cause of liver toxicity in these particular patients.

(*S*)-5-OH-PA has not been experimentally detected as a metabolite so far. However, the present docking simulations have shown (*S*)-5-OH-PA is a strong binder to *HLA-B*57:01* and it could be a metabolite to cause DILI in certain patients.

Figure 2. (a) FX and its five metabolites (drawn in ball-and stick model) bound to *HLA-B*57:01* (depicted in cartoon mode). (b) Binding mode of 5-OH-FX (drawn in space-filling model) to *HLA-B*57:01*.

Supplements

The atomic coordinates of the following structures in PDB format are available as supplemental data at <https://doi.org/10.1273/cbij.19.1>: FX_B5701, FX-PA_B5701, 5-OH-FX_B5701, (S)-FX-PA_B5701, 5-OH-PA_B5701 and (S)-5-OH-PA_B5701.

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