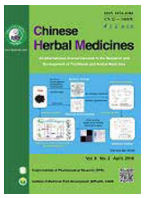

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Original article

Molecular Simulation Study on Bentysrepinine Metabolites Improving Binding Affinity of HBV DNA Polymerase

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ABSTRACT

Objective To study the effect of bentysrepinine (Y101) metabolites on improving binding affinity of HBV DNA polymerase. **Methods** The binding mode of Y101 and its metabolites with DNA polymerase has been driven by hydrophobic interaction. **Results** Two compounds, T2 and T4, exhibited the improvement of the binding affinity to HBV DNA polymerase protein, which suggests that the inhibitory activity against HBV DNA polymerase protein can be enhanced. **Conclusion** The variant docking poses of T2 and T4 might imply the novel recognition of inhibitory effects of T2 and T4, in comparison with Y101.

Key words

bentysrepinine; hepatitis B virus; molecular docking; polymerase; repensine

1. Introduction

The hepatitis B virus (HBV) remains a serious global public health problem even through the initiation of vaccine treatment in 1982 (Lee, 1997). Worldwide, 400 million people are currently infected with chronic HBV and 600 000 people die each year from HBV-related liver disease or hepatocellular carcinoma. Infection with HBV could induce serious consequences, such as end-stage liver disease and hepatocellular carcinoma upon hepatocyte destruction (Lee, 1997; Lin et al, 2004). While an efficient prophylactic vaccine is available, the treatments for chronic hepatitis B in clinical are still limited to type 1 interferon and five approved nucleos(t)ide analogues (NAs), which targets the viral polymerase, P protein, a multifunctional reverse transcriptase (Langley et al, 2007).

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However, only partial patients are eligible for interferon therapy showing sustained biological response due to the severe side effects. In comparison, the NAs are much better tolerable and with low rates of viral resistance development [eg. entecavir and tenofovir] (Chang et al, 2014). Although various treatment options exist for chronic HBV infection, none is entirely satisfactory. The obstacle in the treatment of HBV infection is that hepatitis B surface antigen (HBsAg) clearance is very rare (0–5%) even after prolonged treatment, and the frequent viral rebound upon therapy withdrawal indicates a need for lifelong treatment (Ellan et al, 1998). Also, reactivation can even occur, upon immunosuppression, in patients who suffered HBV infection even decades ago, indicating that the virus can be controlled but not eliminated (Ellan et al, 1998).

Dichondra repens Forst. (*Matijin* in Chinese), a dipeptide isolated from *D. repens*, exhibited anti-HBV activity in clinical utility. The anti-HBV activity of repensine and its derivatives had been reported in our previous studies in details (Liu et al, 2002; Xu et al, 2009). In our finding, some derivatives showed the remarkablely inhibiting activity on the replication of HBV DNA. Among over 200 derivatives, bentysrepinine (Y101) exhibited distinguished antiviral activity upon obstructing the replication of HBV DNA and cccDNA (Liu et al, 2015).

In State Key Laboratory of Drug Delivery Technology and Pharmacokinetics, Tianjin Institute of Pharmaceutical Research, Fan et al (Fan et al, 2012) found that Y101 has wide-range metabolism features in preclinical pharmacokinetic studies, initially confirmed the existence of more than 20 kinds of metabolites in animals, which were mainly M6, M7, M8, and M9 (T2, T4, T1, and T3 in Figure 1). In the Key Laboratory of Chemistry for Natural Products of Guizhou province, Chinese Academy of Sciences, Liang et al (Qiu et al, 2011) also synthesized T1, T2, T3, and T4 for clinical pharmacokinetics trials. It was also the existence of four metabolites in human blood in the clinical trial Phase I. To obtain the information about the anti-HBV activity from the mechanism, we use molecular docking technology to carry out this study. According to our previous study, it is still worth optimizing the function of Y101 upon structural modifications from metabolites. In this study, computational simulations were performed to investigate the structural activity relationships by docking with HBV DNA polymerase.

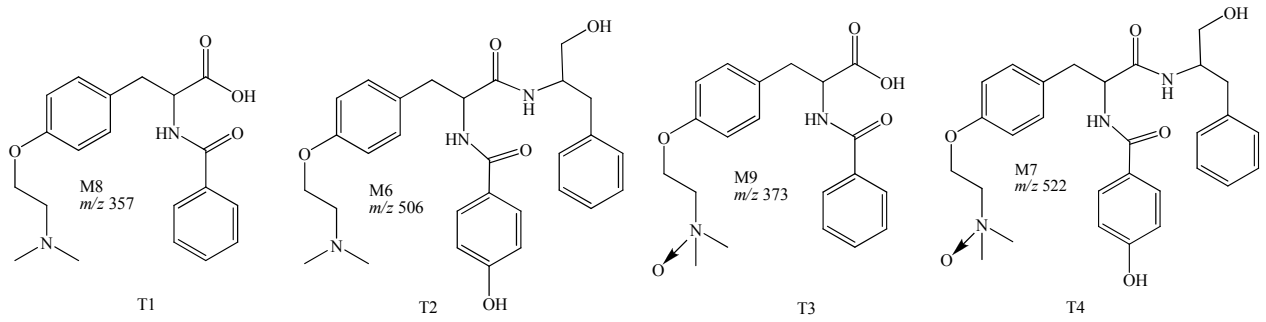

Figure 1 Chemical structures of Y101 derivatives T1, T2, T3, and T4

2. Methods

2.1 HBV DNA polymerase structure

The structure of HBV DNA polymerase was still not available so far, so the homology model previously built (Meng et al, 2015) was used as receptor. This model was built using the known HIV-1 RT structure with PDB code 1RTD as template. Ramachandran plot suggested that the structure had a good quality and most of the residues were in favored region and additional allowed region.

Molecular dynamics simulation was further performed on the homology model to refine the structure using Gromacs 4.5.4 (Hess et al, 2008) software with AMBER99SB force filed. The RMSD values of backbone atoms are well equilibrated during the MD simulation. Most of the residues have a RMSF less than 0.3 nm. All these indicated that the built HBV DNA polymerase structure had acceptable stability and could be used for molecular docking calculations.

2.2 Molecular docking

The structure of HBV DNA polymerase from homology modeling was adopted as receptor, and processed using Protein Prepare wizard in Schrödinger program. The original ligand thymidine-5′-triphosphate (TTP) was used as the docking center with a box size of 10 Å. Except for TTP, Y101 and its metabolites were also selected as docking ligands as they showed the good inhibitory activities against HBV DNA polymerase. The structures of ligands were prepared using LigPrep module: using Epik to determine possible ionization state at pH 7.0 ± 2.0 and adding metal binding states.

The OPLS-2005 forcefield was used to produce the low-energy conformer of ligands. Molecular docking computation was carried out using Glide module with default parameters. All these calculations were carried out in Schrödinger software (Friesner et al, 2004).

3. Results

The binding modes between receptor and ligands were predicted by using Glide module. The result indicated that structures of four Y101 metabolites shown in Figure 1 possessed similar glide scores with Y101 (Table 1). In further, T2 and T4 molecules showed better interaction with polymerase than T1 and T3. For T2, Van der Waals and