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Molecular recognition pattern of cytotoxic alkaloid vinblastine with multiple targets

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ABSTRACT

Vinblastine (VLB), a cytotoxic alkaloid is used extensively against various cancer types and the crystal structure of its tubulin complex is already known. Multitarget affinity of vinblastine has been investigated and the nature of binding with biological receptors namely, duplex DNA and Human serum albumin (HSA) has been compared to the binding characteristics of its known complex with natural high affinity receptor tubulin using molecular docking and QM–MM calculations. VLB is found to interact with DNA as well as HSA protein, though, with weaker affinity as compared to tubulin. Analysis of various docked complexes revealed that the H-bonds and cation–pi bonds do not have significant contribution to the binding interactions and despite its large size, VLB remains in relaxed conformation and fits in the hydrophobic regions on the receptors.

1. Introduction

Vinblastine (VLB; Fig. 1) is a well-known anticancer agent effective against several types, such as Hodgkin's disease, lymphocytic lymphoma, histiocytic lymphoma, advance testicular cancer, advanced breast cancer, Kaposi's sarcoma and Letterer–Siwe disease [1–3]. Studies pertaining to VLB's physiological and therapeutic effects were initiated around the same time as that of the discovery of DNA double helix [4]. VLB is known to interact with a variety of biological macromolecules like proteins and nucleic acids [5–10]. The X-ray crystal structures of vinblastine sulfate [11] as well as VLB–tubulin complex [10] have already been reported. The interaction of VLB with human serum albumin (HSA) was investigated long ago on the leukemia cell line MOLT-3 using cell based assay technique [12], which had indicated for the first time that VLB binds weakly with human serum albumin protein. In addition, VLB's ability to interact with different types of molecules has also been identified in nanoparticle mediated drug delivery efforts. Effective delivery of VLB has been explored using various nanoparticles such as poly(lactide-co-glycolide) [13], liposomes [14], etc. Since albumin is a highly suitable material for use as nanoparticles for drug delivery mechanisms [15], a number of studies have been accomplished on the preparation of vinblastine loaded nanoparticles of serum albumin protein [16].

The information regarding the nature of binding with biomolecules, especially with proteins, nucleic acids, etc. is essential in order to understand the mechanism of their interactions and to identify similar possible ligands of potential medicinal importance. A survey of literature reveals that the exact nature of binding of vinblastine with multiple receptor targets and the detailed structural aspects of VLB-HSA interactions in particular has not been investigated thoroughly. Molecular docking is a fast and reliable tool for the study of inter-molecular interactions in the systems of biological and therapeutic significance. Although, the QM–MM approach, that combines the accuracy of quantum mechanics and speed of molecular mechanics, was introduced as early as 1970s by Warshel and Levitt [17] to obtain more realistic information about biomolecular interactions, most of the early stages of drug discovery process still involve the use of the molecular mechanics based docking programs to obtain dependable prediction toward the efficacy of the pharmaceutically important molecules [18–22]. Several docking programs with varied capabilities are available for calculating interactions with different biomolecules, some of them having their own limitations to explore specific regions on receptor molecule, e.g. major/minor grooves of DNA. Recently, a comparison between various computational docking methods suitable for studying minor groove DNA binders has been made [23]. The effect of the conformational preferences in several natural and

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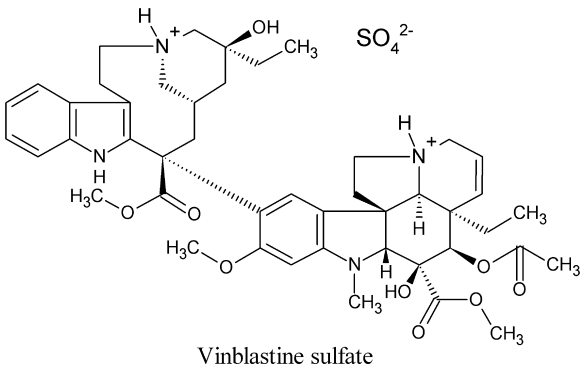

Fig. 1. Structure of vinblastine sulfate.

semi-synthetic vinca alkaloid molecules on their affinity to tubulin has been investigated previously by Kelly et al. [24] at semiempirical AM1 level using QM-MD simulations. In the present investigation, an attempt has been made to identify the nature of binding interactions of vinblastine with biological receptors such as duplex DNA and Human serum albumin using molecular docking program AutoDock-vina and applying QM–MM based ONIOM methodology. The binding characteristics of VLB–DNA/VLB–HSA complexes and their dependence on stable conformation have been compared with that of natural high affinity receptor tubulin.

2. Materials and methods

Molecular docking calculations were carried out using AutoDock-vina program from The Scripps Research Institute [25]. Rigid body docking program DNADock [26], used in an earlier study of VLB–DNA binding [5], had limitation to explore only the DNA minor groove binding space without any structural perturbations in DNA. The resulting docked structures were highly strained and no significant binding forces could be identified in the minor groove poses. AutoDock-vina program was used in present study because it has the option to explore both the minor and major groove binding modes and is reported to perform faster and do more accurate calculations than Autodock software [27–32].

CD experiments were conducted on a Jasco J-815 spectropolarimeter in the spectral region 210–400 nm to monitor the drug induced conformational changes of the DNA structure [30]. VLB is an optically active drug having active CD pattern in the same region of DNA peaks, making difficult assessment of structural changes on complexation. Hence, two separate titrations between VLB-DNA and VLB-Buffer were performed. Circular dichroism (CD) experiments performed with VLB and DNA indicated that no significant perturbation occurs in DNA structure, therefore, the flexibility option for the receptor biomolecule was not included.

For molecular docking calculations, the receptor and drug coordinate files were converted into PDBQT format using MGLTools (version 1.5.4). 3D structure of vinblastine dication was prepared in PDB format from its X-ray geometry [11] using Discovery Studio Visualizer 3.0. Four B-form DNA sequences (Table 1), as used in the previous study [5], were utilized to study the possibility of sequence specific interaction with any of the chosen four complementary central base pair combinations.

Table 1
Four B-DNA decamer sequences used for docking calculations.

	DNA sequence
S1	5′-d(GATGGCCATC)$_2$
S2	5′-d(GATCCGGATC)$_2$
S3	5′-d(GGCAATTGCC)$_2$
S4	5′-d(GGCTTAAGCC)$_2$

Docking of VLB with four DNA sequences was performed using vina program with a large grid box (size $28 \times 26 \times 36$ with grid center at $x = -0.668$, $y = 0.358$, $z = 15.0$) so as to accommodate both the major and the minor grooves of DNA. DNA docking was carried out with increased exhaustiveness of 32 which is four times the default value in Autodock-vina program. This helped in evaluating the preference of VLB toward one or the other grooves. HSA structure file was obtained from Protein Data Bank (PDB) [HSA PDB ID 1E7A] and for docking calculations, a three-dimensional grid box of size $44 \times 48 \times 40$ centered on coordinates $x = -1.101$, $y = -10.483$, $z = -0.224$ was prepared with a grid spacing of 1.0 Å. Due to the larger size of the grid box, in this case also the calculations were performed at the exhaustiveness of 32 in Autodock-vina program. For each docking calculation, 20 different poses were requested within the energy range of 2 kcal mol^{-1} to sample greater variation in poses. All other parameters were kept at their default values. The choice of docking with a single program (vina) enabled effective comparison of VLB's binding with different receptors.

2.1. Docking standardization procedure

In order to obtain reliable docking results, standardization was done by reproducing the crystal structure of the vinblastine–tubulin complex using Autodock-vina program as described [32]. Vinblastine–tubulin complexes (PDB ID 1Z2B and 4EB6) were studied for their binding motifs. The PDB file of Vinblastine–tubulin complex was taken from the PDB (ID 1Z2B) and VLB molecule was removed from the complex. Vinblastine was then allowed to dock onto the tubulin protein at the original binding site to obtain binding poses with minimum deviation from the crystal structure. Interestingly, the docking calculation produced only one pose in the defined pocket, which was laid over the original crystal structure pose by superimposing the indole rings to assess any deviations in conformation. The binding pose of VLB was found to be almost same as in the original VLB–Tubulin complex crystal structure (Fig. 2a and Table 2) validating the choice of docking program. The analysis of docking results was carried out using Discovery Studio Visualizer, UCSF-Chimera (version 1.8) and MOE software.

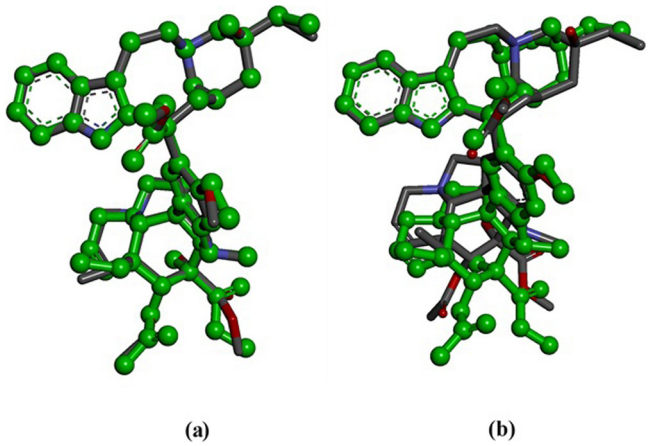

Fig. 2. Superimposition of crystal geometry (green ball and stick model) of VLB in VLB–tubulin complex extracted from PDB 1Z2B – (a) with vina-docked pose (stick model) of VLB in VLB–tubulin complex; and (b) with crystal structure of isolated VLB molecule (stick model) [11]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)