Dakshinamurthy Sivakumar et al /J. Pharm. Sci. & Res. Vol.3(4), 2011,1141-1145

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

In Silico Rationalization for the Differential Bioavailability of ABT-737 and ABT-263 that Antagonise the Anti-Apoptotic Proteins

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Abstract
Designing BH3-mimitics, that are capable of displacing BH3-peptides from BH3-binding groove of anti-apoptotic proteins, is a promising strategy to develop therapeutics of cancer chemotherapy. ABT-737, a BH3-mimitic shown to act as potent inhibitor of anti-apoptotic proteins, failed to clear the clinical trials due to its poor bioavailability. ABT-263, an analog of ABT-737, is shown as potent inhibitor with appreciable bioavailability. We have herein shown that ABT-737 docks on CYP3A4, a metabolic enzyme present in intestine of human beings, with stronger binding affinity than the binding affinity of ABT-263 with the enzyme. Based on the binding affinities and mode of interactions between the ligands and the enzymes, the differential bioavailability of the ABT-737 and ABT-263 is attributed to their pre-systemic metabolic reactions that are presumably different from each other.
Keywords: ABT-263, ABT-737, Apoptosis, Bcl-X_L and CYP3A4.

INTRODUCTION

Apoptosis is a tightly regulated biological process by which cells die in a controlled manner in order to maintain the cell homeostasis. The intrinsic pathway of apoptosis in higher eukaryotes is mainly controlled by the Bcl-2 family of proteins, which are grouped into three distinct categories: the pro-survival proteins, pro-apoptotic proteins and BH3–only proteins. The fate of cells depends on a delicate balance of pro-survival and pro-apoptotic proteins [1,2,3]. Defects in apoptosis lead to disorders such as cancer, ischemia, neurodegeneration and AIDS etc. In these backgrounds, designing specific inhibitors to the anti-apoptotic proteins is becoming an attractive strategy for the development of therapeutics of cancer chemotherapy[4,5,6].

ABT-737, a BH3-mimitic chemical molecule from Abbott Laboratories, is shown as potent inhibitor to anti-apoptotic proteins such as Bcl-2, Bcl-X_L and Bcl-w [7]. The compound also exhibits stronger activity against various cancer cell lines including lymphoid malignancies, small cell lung cancer, lymphoma and chronic lymphocytic leukemia [8, 9]. However, due to its poor bioavailability, the compound failed to reach the market as a cancer drug. ABT-263, the structural analog of ABT-737, has been demonstrated as a promising anti-cancer compound vis-à-vis the ABT-737 in terms of bioactivity and bioavailability[10]. However, the differential bioavailability of these compounds has been left unaddressed to date, though there are many reports on the binding interactions of these compounds with anti-apoptotic proteins and on their capability of displacing the BH3-peptide from the BH3-binding groove of anti-apoptotic proteins [11, 12]. In the present studies, we have analyzed the binding interactions of ABT-737 and ABT-263 with Bcl-X_L and CYP3A4. The CYP3A4 is a metabolic enzyme belonging to CYP450 family of proteins. It has been reported that more than 90% drugs, particularly most anti-cancer drugs, are metabolized by CYP450 variants, especially CYP3A4, that are localized in mitochondria and endoplasmic reticulum of liver cells in human beings [13]. Interestingly, among the variants of CYP450, CYP3A4 is also present in enterocytes of intestine [14]. It implies that drugs showing stronger binding affinities with CYP3A4 may probably undergo pre-systemic metabolic reactions in intestine leading to poor bioavailability of the drugs to the system. Using computational methods, we have herein shown that the higher binding affinity of ABT-737 with CYP3A4 will make ABT-737 to be significantly metabolized in the intestine of human beings, which consequently lead to poor availability of the molecule to the system.

MATERIALS AND METHODS

Molecular docking an d structura l analysis of binding interfaces

Bcl-X_L (2YXJ) and CYP3A4 (3NXU) were retrieved from Protein Data Bank (http://www.rcsb.org) and both proteins were analyzed for any missing atoms using Prime (Schrodinger Inc, USA), which also adds

hydrogens to missing side chains in the proteins. Water molecules occupying beyond 5Å of protein contacts were removed from the structures. Both protein structures were energy minimized using OPLS 2005 force field. The chemical molecules ABT-737 and ABT-263 were retrieved from pubchem database (http://pubchem.ncbi.nlm.nih.gov) and their ADME properties were calculated using Qikprop (Schrodinger Inc, USA). For molecular docking, using Ionizer in the Ligprep (Schrodinger Inc, USA), all possible chemical structures of ABT-737 and ABT-263 were generated in the pH range of 5.0 to 9.0 and the structures were energy minimized using OPLS 2005 force field. All the generated structures were docked with the Bcl-X_L and CYP3A4 at identical conditions using Glide-XP [15]. The maximum number of rotatable bonds was set to be 35. The van der Waals radii scaling factor of 0.8 and partial charge cut-off of 0.15 were maintained, throughout the docking processes. Grid dimensions of 88 X 88 X 88 Å was used to cover the entire BH3-binding groove of Bcl-X_L and Grid dimensions of 80 X 80 X 80 Å was set to cover the entire ritonavir binding groove of CYP3A4. The binding affinities of the ligands on the proteins were compared on the basis of glide scores of the docking complexes. The glide scores account all principle terms that favour as well as hinder interactions between the ligand and the protein. The various structural interactions of the complexes were viewed and analysed using PyMol (http://www.pymol.org).

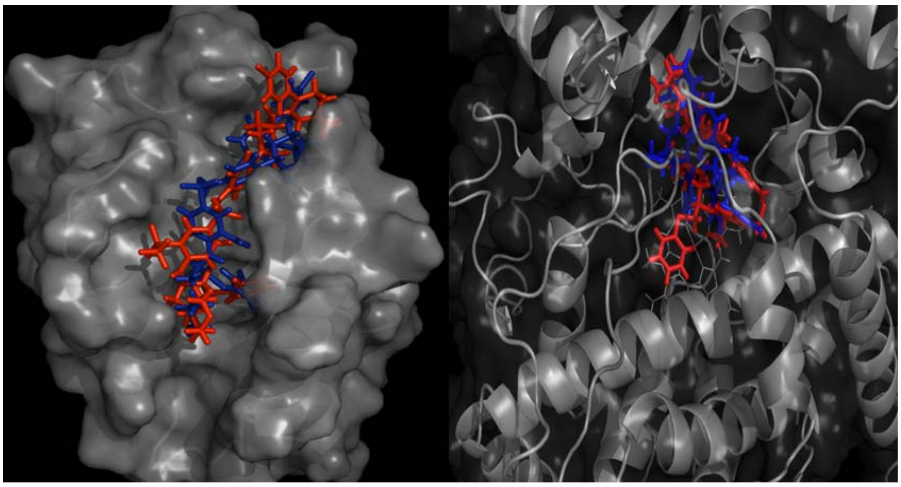
RESULTS AND DISCUSSION

To date, six structurally characterized anti-apoptotic proteins from human beings have been reported in literature [16]. They are all α-helical proteins having two well-defined binding grooves: BH-groove and BH3-binding groove [17]. The BH3-binding groove of the protein is constituted by BH1 & BH3 domains of the proteins which are capable of accommodating BH3-domain of the pro-apoptotic proteins [18]. By these interactions, anti-apoptotic proteins sequester pro-apoptotic proteins and consequently prevent their oligomerization through which the death signal cytochrome C is released from the inter-membrane surface of mitochondria [19]. Of the six anti-apoptotic proteins, the binding interactions of ABT-737 (K_d = 0.08 nM) and ABT-263 (K_d = 0.4 nM) have been experimentally well-characterized with Bcl-X_L, B-cell lymphoma extra large [10]. In order to determine the binding affinities of these ligands with the protein at identical conditions, we generated docking models for the ligands on the protein using Glide-XP docking tool. The glide scores for ABT-737 and ABT-263 were found to be -12.29 and -11.46, respectively (Table 1). The residues of Bcl-X_L that are interacting within 4Å close proximity of the ligands are similar in both cases. Moreover, the mode of interactions (geometrical orientations) of both ligands in the BH3-binding groove of the proteins is similar, if not identical (Fig. 1A). These data suggest that both ligands may act as potent inhibitors to the protein with equal binding affinities and the docking data herein reported for the two complexes are also consistent with binding affinities reported for the complexes using experimental methods.

Despite the similar inhibitor activities of these ligands to Bcl-X_L, they are drastically differed in their bioavailability: ABT-737 failed to clear clinical trials due to its poor bioavailability, whereas ABT-263 is undergoing clinical trials with appreciable bioavailability [10]. In order to understand the differential bioavailability of structurally similar ligands, we first predicted the ADME properties of both ligands using Qikprop (Schrodinger Inc, USA) and the data are shown in Table 2. The data imply that the overall percentage of oral absorption of ABT-737 and ABT-263 are 40% and 56%, respectively. To this extent, the data on the bioavailability of these drugs from the experimental and theoretical methods are in good agreement. Second, to understand the molecular mechanisms by which the metabolic fates of the two ligands are governed, we generated docking models for the ligands with the CYP3A4 using Glide-XP. The CYP3A4 is one of the variants of CYP450 metabolic enzyme and the enzyme has been reported to present in liver and enterocytes of intestine, as well [14]. Strikingly, glide scores of the docking models for the

Table 1: Glide scores and structura l interactions of docking m odels generated for ABT-737 and ABT-263 with Bcl-X_L (2YXJ) and CYP3A4 (3NXU).

Protein	Compound	Glide Score	H-Bonds	Close Contacts (within 4Å)
Bcl-X_L	ABT-737	-12.29	ABT737:H47-GLU129:OE2	ALA93, GLU96, PHE97, ARG100, TYR101, ALA104, PHE105, LEU108, VAL126, GLU129, LEU130, ASN136, TRP137, GLY138, ARG139, VAL141, ALA142, SER145, PHE146, ALA149, PHE191, TYR195, ASN197.
	ABT-263	-11.46	ABT263:H56-GLU129:OE2 ABT263:O5-ARG139:HE ABT263:O5-ARG139:HH21	GLU92, ALA93, GLU96, PHE97, ARG100, TYR101, ALA104, PHE105, LEU108, VAL126, GLU129, LEU130, ASP133, GLY138, ARG139, VAL141, ALA142, SER145, PHE146, ALA149, TYR195, ASN197.
CYP3A4	ABT-737	-11.12	NIL	TYR53, PHE57, ASP76, ARG105, ARG106, PHE108, MET114 ,SER119 ,ILE120 ,LEU210, LEU211, PHE213, PHE215, THR224, PHE241, ILE300, ILE301, PHE304, ALA305, THR309, ILE369, ALA370, MET371, ARG372, LEU373, GLU374, GLY481, LEU482, LEU483, HEM508.
	ABT-263	-4.89	NIL	TYR53, PHE57, ASP76, ARG105, ARG106, PHE108, MET114 ,SER119 ,ILE120 ,LEU210, LEU211, PHE213, PHE215, THR224, PHE241, ILE300, ILE301, PHE304, ALA305, GLU308, THR309, SER312, PHE316, ILE369, ALA370, MET371, ARG372 ,LEU373, GLU374, ARG375, GLY481, LEU482, LEU483, GLN484, HEM508.



A **B**

Figure 1: Binding modes of ABT-737 (Blue) and ABT-263 (Red) **A**) in the BH3-binding groove of Bcl-X_L (2YXJ) and **B**) in the ritonavir binding cavity of CYP3A4 (3NXU).

ABT-737 and ABT-263 with the enzyme are -11.12 and -4.89, respectively (Table-1). It is obvious that ligands those are showing stronger binding affinities with the enzyme will have higher metabolic rates than that of ligands depicting weaker binding affinities with the enzyme. Thus, the docking data of the ligands with the CYP3A4 suggest that pre-systemic metabolic rate of ABT-737 in the intestine must be probably several folds higher than that of ABT-263.

The structures of ABT-737 and ABT-263 are depicted in Figure 2 and the two structures are differing from each other at three spots. The nitro group (spot1), dimethyl amino group (spot2) and benzene ring (spot3) in the ABT-737 have been substituted by trifluoro methyl group, morphilino ring and dimethyl cyclohexane ring, respectively, to generate the ABT-263. Overall, the binding site residues of CYP3A4 that are in the close contacts of the two ligands in their corresponding complexes

are very similar to each other (Fig. 1B & Table 1). However, the differences noted in the mode of interactions of the ligands on the enzyme are subtle but significant in terms of the structural interactions of the three chemical moieties of the ligands with the enzyme. For instance, the nitro group, a strong electron withdrawing group, in ABT-737 is surrounded by residues such as Arg 105, Arg 106, Phe 108 and Glu 374. Contrary to this observation, the trifluoro methyl group of ABT-263 is in close contact with the residues Ser 119, Ile 301, Phe 304 and Thr 309. These observations clearly reveal that the binding locations of the two ligands at these sites on the protein are different from each other.

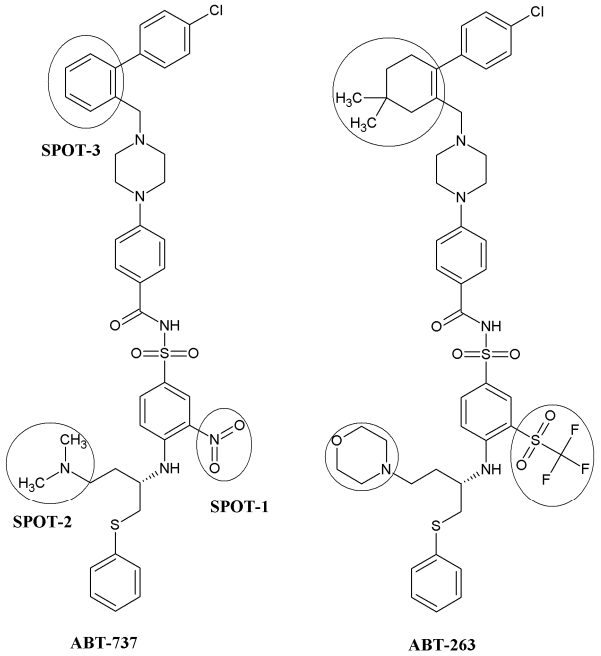


Figure 2: Two-dimensional structures of ABT-737 and ABT-263. The two ligands are differing from each other in three different positions that are encircled.

Table 2: ADME properties of of ABT-737 and ABT-263 as predicted using Qikprop.

Molecular Property	ABT-737	ABT-263
Molecular Weight (Da)	813.4	974.6
SASA (A^2)	1046.8	1332.1
Molecular volume (A^3)	2215.6	2666.5
Rotatable bonds	15	14
HB donor	2	1
HB acceptor	12	16
LogP(Octanol/water)	6.1	7.9
% human oral absorption	40	56

In general, nitro groups are highly prone to get reduced to yield amine through hydroxylamine intermediate. The nitro group of ABT-737 is well stabilized by tight network interactions with charged amino acids and aromatic ring of Phe 108, whereas the trifluoro methyl group of ABT-263 has weak interaction with the polar residues surrounding the group. Similarly, dimethyl amine of ABT-737 is surrounded by hydrophobic amino acids flanked by an arginine residue, whereas the morphilino ring of ABT-263 is in close contact with heme and residues such as Arg 105, Ile 369, Ala 370, Met 371, and Arg 372, which are located on the surface of the enzyme. It indicates that the morphilino ring of ABT-263 makes the molecule to be very labile to interact with the enzyme. Moreover, dimethyl amine of ABT-737 will readily undergo dealkylation due to its tertiary nitrogen and its higher lipophilic property. The docking data unambiguously map the lipophilic binding environment (Phe 57, Ile 369, Ala 370, Met 371, Arg 372, Gly 481, Leu 482 & Leu 483) of the enzyme to the dimethyl amine moiety. The benzene ring and cyclohexane ring at the spot 3 of ABT-737 and ABT-263, respectively, are also in the different binding locations. The benzene ring of ABT-737 is enveloped by residues Ser 119, Ile 301, Phe 304, Ala 305 & Thr 309 constituting a cavity of the enzyme, whereas the cyclohexane ring of ABT-263 is lifted to the exterior surface of the enzyme and stabilized by polar environments networked by Tyr 53, Phe 57, Asp 76, Arg 106, Phe 213, Thr 224, Arg 372 and Glu 374. On the basis of these structural interactions, we herein propose that the three groups, which are differentiating the ABT-737 and ABT-263 from each other, are responsible for the differential interactions of the ligands with the CYP3A4 enzyme as inferred from the docking models of the enzyme-ligands complexes. The stronger binding affinity of the ABT-737 with the CYP3A4 presumably promotes the pre-systemic metabolic reactions of the ligand in the intestine of human beings, which would lead to poor bioavailability of the ligand to the system.

CONCLUSIONS

The ABT-737 and ABT-263 are structurally similar BH3-mimtics and highly potent inhibitors to the anti-apoptotic proteins. Despite their structural similarity and bioactivity, the two ligands are remarkably differing in their bioavailability. A comprehensive analysis of structural interactions of docking models of ABT-737 and ABT-263 with CYP3A4 metabolic enzyme reveal that nitro group, dimethyl amino group and benzene ring of the ABT-737 facilitate the ligand to strongly interact with the enzyme, whereas trifluoro methyl group, morphilino ring and dimethyl cyclohexane ring of ABT-263 are greatly weakening the binding interactions of the ligand on the enzyme. In these backgrounds, it has been herein proposed that the stronger binding affinity of the ABT-737 with the CYP3A4 presumably promotes the pre-systemic metabolic reactions of the ligand in the intestine of human beings, which would lead to poor bioavailability of the ligand to the system. The mode of structural interactions brought into fore between the ligands and the enzyme in the present studies may pave a way of designing ligands that are capable of avoiding the pre-systemic metabolic reactions in the intestine of human beings.

ACKNOWLEDGEMENT

This work is partly supported by research grants from the Department of Biotechnology, India (BT/PR13378/GBD/27/262/2009) and TRR Memorial research grant (TRR/13/Aug/2009), SASTRA University, India.

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