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Docking studies of Mitoxantrone with DNA, Signal transducer and activator of transcription 4 and Human serum albumin

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Abstract

With growing advances in the field of science and research, extensive work is being carried out in the area of cancer. A lot of anti-cancer drugs have surfaced over the years. These drugs are evaluated on the basis of their effectivity to adverse effect ratio. In the present study, we have performed docking studies of an Anthraquinone antibiotic, Mitoxantrone (MTX) with its possible targets such as DNA, Human serum albumin (HAS) and Signal transducer and activator of transcription (STAT4) using Autodock 4.2 and Schrodinger software suit. The interaction of MTX with DNA and HSA reveal its affinitive binding to these targets in the nucleus and blood respectively. It was found that partial intercalation of MTX occurs in the major as well as minor groove of the DNA with high preference for the former. Binding energy and docking score of Mitoxantrone to DNA was found to be -2.19 and -9.773 respectively. Docking studies reveal that MTX binds with HSA to a non-classical high affinity site in the domain 1, site III via hydrophobic, electrostatic and hydrogen bonds with a docking score and binding energy of -7.179 and -1.84 respectively. Mitoxantrone binds STAT4 with a high docking score of -5.373 and binding energy of -3.38.

***Keywords*: Cancer, Docking, Mitoxantrone, Signal transducer**

INTRODUCTION

Mitoxantrone (MTX), an anti-tumour antibiotic is a synthetic Anthracenedione derivative exploited clinically for its antineoplastic and immunomodulatory properties. It resulted in an attempt to develop a less cardiotoxic anti-cancer drug as compared to Daunomycin and Ddriamycin. Marketed as NOVANTRONE, this chemotherapeutic drug finds use in the treatment of a range of human malignancies, mostly metastatic breast cancer, acute myeloid leukemia and Non Hodgkins lymphoma, [1-2] lung carcinoma and hormone refractory advanced prostate cancer [3]. MTX is also used to treat multiple sclerosis [4]. It is administered intravenously alone or in combination with other drugs. In contrast to other Anthracyclines, Mitoxantrone produces less side-effect such as nausea, vomiting, alopecia and cardiac toxicity [5-6], which facilitates its wide applicability. The absence of an amino-sugar moiety in MTX is attributed for its less cardiotoxicity. Other effects may include diarrhea, constipation, upper respiratory tracts infection, and amenorrhea.

Under controlled conditions of laboratory Mitoxantrone can inhibit B cell, T cell, and macrophage proliferation and impair antigen presentation, as well as the secretion of interferon gamma and Interleukin-2. In chemotherapy, MTX kills cancerous cells by blocking enzyme Topoisomerase- II (an enzyme involved in DNA repair and relieving supercoiling) resulting in persistent double strand breaks in the DNA. It is also known to intercalate between DNA strands through hydrogen bonding and cause crosslinks and strand breaks. It acts in an immunomodulatory fashion in MS patients. Intravenous Mitoxantrone treatment improves neurological disability and delayed progression of MS in patients with worsening relapsing-remitting (RR) [also termed progressive-relapsing (PR) MS] or secondary-progressive (SP) disease [7].

Interaction of Mitoxantrone with DNA

Mode of interaction: Anticancer drugs act on the DNA of cells to prevent their growth or to kill them by intercalating and aggregating the DNA preventing its replication and RNA transcription [8-12]. Generally, Anthracyclines such as Adriamycin and Daunomycin bind the less condensed form of chromatin in the preference order of DNA to chromatin. Contrarily, Mitoxantrone shows increased affinity towards chromatin as compared to free DNA [8] which indicates

the involvement of histone proteins in DNA-Mitoxantrone complex formation. MTX- DNA adduct is a sandwiched structure of MTX within terminal base pairs of DNA. Computational data and NMR studies show that this binding occurs preferentially in the major groove, although minor groove also show to be site for interaction. Belinda et al conclude that binding in major groove is much favored for non-methylated sequences. Methylated sites affect binding by shifting the equilibrium of bound drug to minor groove resulting in more adduct formation at methylated guanine residues from proximity with N2 exocyclic amino of guanine; suggested to be an absolute requirement for MTX-DNA adduct formation [3]. N2 and N7 position on guanine at intercalation site are required for MTX-DNA stable complex formation [13]. The reason for less affinity towards the minor groove is attributed to the lack of appropriate hydrogen bonding arising from MTX towards core of groove. Experimental studies confirm MTX biological activity by intercalating at the 5'-CpG-3' step preceded by electrostatic crosslinks with DNA backbone [13-14]. Mitoxantrone structure is characterized by the presence of a planar tricyclic Anthraquinone ring instead of the typical tetracycline ring of Anthracyclines [8-9, 13-15] which accounts for the easy formation of hydrogen bond between chromophore ring of the drug and DNA base pairs. The Daunosamine sugar of common Anthracyclines like Adriamycin is replaced by two identical amino alkyl side chains which stabilize the ring between base pairs by intercalating with the negatively charged phosphate backbone of DNA [3, 15]. Experiments conducted by Ritu et al denote that intercalation of Mitoxantrone ring into the DNA is in a parallel mode as a much stable conformation is achieved in that fashion. Belinda et al propose that only partial intercalation of chromophore ring occurs in the DNA while side chains at 5th and 8th position interact with phosphate backbone to stabilize the complex. This is in consent with the Raman data generated by Nan Li et al, which shows only particular vibrations get affected in Raman suggesting partial insertion of chromophore ring. The NH and OH functional groups form hydrogen bonds with the DNA and stabilize the interaction [13-14].

Site of interaction in the DNA

An attempt was made by Kapuscinski et al, to explain any preferred sequence of MTX for binding to DNA. Based on spectroscopic studies he concluded that there was no clear base specificity. However, contradictory results produced by exhibited greater binding of MTX-DNA at GpC compared to ApT highlighting the specificity and selectivity of base sequence exhibited by MTX. Further, experimental data suggested 5'-pyrimide -purine-3' step to be the intercalating site for the drug. Action of MTX on calf thymus double stranded and single stranded DNA in an experiment conducted by Ling et al, showed the disruption of DNA into DNA double strand segments. They concluded that MTX intercalation in AT plane resulted in disruption of hydrogen bonds between the base pairs.
In another study, Hajihassan and Chadeghani concluded, based on UV/Vis, fluorescence and CD spectroscopy that MTX and full form (EDTA) soluble DNA interaction proceeded to compaction and aggregation of DNA revealing reduced absorbencies at 608 nm and 260 nm. This was further proved by the disappearance of DNA on gel. The results suggested that in vitro MTX intercalates into DNA and causes it to precipitate in the reaction mixture. However, the absorbance study also revealed that much higher concentration of MTX was required to precipitate DNA as compared to chromatin validating higher affinity of Mitoxantrone towards chromatin compared to free nuclear DNA.

Effect of Mitoxantrone on DNA repair enzyme Topoisomerase –II is also of special significance in its anticancer activity. Topoisomerase - II is an enzyme critical for DNA repair. MTX accumulates in the cell nucleus and acts as a topoisomerase - II poison. It performs sequence selective cleavage of the topoisomerase- II DNA rendering it inactive. [19] Inactivation of this enzyme by MTX contributes to the cytotoxic effect of the drug [3]. Smith et al, propose cytotoxic action of Mitoxantrone is dependent upon a persistent and restricted way of binding to DNA which promotes progressive trapping of topoisomerase- II complexes [18].

Interaction of Mitoxantrone with Human serum albumin (HSA)

Human serum albumin is most abundant protein in the plasma. It is a carrier protein for steroids, fatty acids, and drugs. Designated as the principle extracellular protein ,HSA transports a broad range of endogenous and exogenous molecules including 70 per cent drugs and ligands, at a relative constant level of 3.4 -4.5 % (w/v)[20-21].It is a monomeric polypeptide of 585 amino acids and molecular weight of about 66,437 Da. Although the amino acid sequence of the protein is

known and its secondary and tertiary structures remain largely unknown [22]. On the basis of amino acid sequence and modelling experiments, it has been deduced that HSA is a largely helical structure having three homologous yet un-identical domains that assemble to form a heart shaped molecule [22-24]. Each domain, believed to be comprise of six helices form hydrophobic channels with basic and hydrophobic amino acid residues positioned at the ends. Each domain is further subdivided to subdomains which possess ligand binding sites.
Three well know sites for drug binding include site I, also known as Azapropazone Warfarin site, present in sub domain II A [25]. Site II present in subdomain III A also known as Indole Benzodiazepine site [26].
Site III and site IV are Digitoxin and Tamoxifen sites, respectively. [27]

Binding of MTX to HSA

HSA exhibits excellent drug delivery system as it enhances the blood half-life of molecules and makes them multivalent. HSA bound drug are known to display increased solubility in plasma, and decreased toxicity [28]. It is reversible and covalent binding of drug molecules and significant influence on their pharmacokinetics makes is a potent candidate as a drug delivery vehicle [29].
MTX - HSA complex formation is marked by one high affinity binding site with an association constant of the order 10(5) [30]. The complex formation occurs in 1:1 mole ratio of MTX: HSA. Molecular docking reveals that MTX binds HSA to a non-classical drug binding site, on domain I, site III. This is the typical Anthracycline binding site in HSA [32]. In an experiment conducted by khan et al, to determine the HSA binding site of Mitoxantrone, site specific probes of each site were selected and allowed to bind HSA. These were site I specific warfarin, site II specific Diazepin, classical site III binder hemin and non-classical characteristic Anthracycline marker of site III, Daunomycin. Results from the experiments showed MTX -HSA adduct fluorescence was most affected by Daunomycin validating the non-classical fashion of MTX binding to HSA at site III. In another experiment, quenching trend between Daunomycin and Mitoxantrone were found to be most similar, confirming sharing of same binding site. The binding is termed non classical as MTX does not fit completely in the site III as Digitoxin, rather makes a partial hydrophobic interaction. The binding is sensitive to the presence of other molecules. The resence of Cu2+ ions in the vicinity of adduct is known to increase binding affinity.

The MTX-HSA adduct is stabilized largely by hydrophobic interactions. This is proved by a thermodynamic analysis of the MTX - HSA complex formation, carried out by Khan et al, showing a positive entropy change. HSA is a flexible protein and changes shape to accommodate ligand [24]. Upon ligand interaction the molecular conformation of the protein changes and khan et al propose that the water molecules arranged in an orderly fashion around the ligand and target are forced into a more random configuration as a result of hydrophobic interaction which explains the positive entropy. However, electrostatic interaction and hydrogen bonding also play a role in establishing the interaction. [28, 34] Synchronous fluorescence aimed at understanding the induced alteration in the microenvironment of the protein as a result of MTX -HSA adduct indicate closest residue to the interaction, tryptophan; was located in the hydrophobic cavity and disagglomerated on exposure to water loosening the HSA structure. This suggests MTX binds HSA in a hydrophobic cavity [28].

Interaction of Mitoxantrone with STAT4

STAT4 is a latent cytoplasmic protein of 748 amino acids involved in transcription of genes. It is a member of the signal transducer and activator (STAT) family and located on the long arm of chromosome 2 between positions 32.2 -32.3. It is believed to have arisen from STAT1 by gene duplication. STAT4 mediates interleukin 12 (IL12) response in lymphocytes regulating differentiation of mature T cells to special T helper cells , Th1. Th1 cells are critical to fighting cancerous cells and infectious agents. Cytokines released by these cells encourage activation of cytotoxic T lymphocytes which mediate tumor immunity.

STAT4 activation and action

Various cytokines and growth factors including IL -12 are responsible for the activation of STAT4. Cytokine exposure and subsequent engagement of cell surface cytokine receptors activate receptor associated Janus (JAK) kinases which trigger phosphorylation of the tyrosine site present in the Trans-activation domain of the protein [34]. STAT4 protein contains 4 regions, DNA binding domain, linker region, SH2 domain and a transactivation domain possessing tyrosine and serine phosphorylation sites critical for activation of STAT4.

Upon phosphorylation of transactivation domain sites latent STAT4 dimerizes via homology domain into an active or functional STAT4 dimer capable of translocating to the nucleus where it induces transcription of specific target genes. IL12 activation and JAK 2 mediated phosphorylation of stat4 is known to be the major cause of activation of the protein . Some other activators of stat4 include src family (hck, src), Erb B1, Erb B2, protein kinase C, gp 130, anaplastic lymphoma kinase and epithelium growth factor receptor . IL17 also capable of activating STAT4 in human monocytic leukemia cell lines. Activated STAT4 mediates IL12 signaling pathway which is critical for the development and function of Th1 cells. Th1 cells are known to bring about anti cancerous and infection clearing activity by activating and recruiting cytotoxic T lymphocytes and other immune cells at the target location. However, STAT4 is fundamental for the development of Th1 cells.

In normal cells, STAT4 regulates cell proliferation, differentiation and functional capacity of lymphocytes. However, growing evidences indicate a number of STAT4 target genes involved in tumor formation. Sustained stat activity has been described in a variety of tumors including leukemia. The cause is not understood but could be attributed to probable mutation of stat regulating proteins or deregulation of signaling pathway. There is also a considerable involvement of certain stat members in the up regulation of Cyclin D, myc and non-apoptotic protein, bcl xl. There is evidence that, cytokines, such as IL-3 and IL-6, stimulate Cyclin D1 promoter activity via STAT3 and STAT5. STAT directly regulate the up regulation of bcl xl resulting in prolonged expression. It is believed that blocking of STAT4 protein or STAT4 signaling pathway could suppress cancer cell growth. Evidence suggests that phosphorylation of SHP2 domain of STAT4 associated with JAK1 and JAK2 inhibits the initiation of JAK-STAT pathway. Blockers of STAT4 can be oligonucleotides, small peptides and small molecules. Previously studied blocker include Curcumin, Resvertrol, Cucurbitacin, Piceatannol, Indirubin etc. They block STAT4 mediated DNA binding, gene regulation and cell transformation [35]. Deepika et al. attempted molecular docking of various drugs including Mitoxantrone with STAT4. Their results suggest Flurbiprofen docks with STAT4 in the best fit. However, the mode of interaction of these drugs with STAT4 could not be predicted. Although STAT4 is an essential transcription modulatory protein, and have been a focus of research, yet its interaction with Anthracyclines has not been studied. In fact, the complete tertiary structure of the protein is also not available on PDB. In current work, 3D structure of STAT4 was modeled using homology modeling and docking studies between STAT4 and Mitoxantrone was performed.

METHODOLOGY

Ligand and receptor preparation

The 3D structure of Mitoxantrone (ligand) was downloaded from Drugbank (accession no. DB1204) in pdb and sdf format. We chose three receptors for docking, DNA, Human serum albumin (HSA) and human STAT4. The PDB structure of DNA (PDB ID: 1BNA) was downloaded from RCSB protein databank. PDB structure of human serum albumin complexed with stearic acid (PDB ID: 1E7I) was downloaded from RCSB protein databank and the bound ligand was removed using Discovery studio. The 3D structure of human STAT4 was modeled as it was not available on PDB. For this, the complete amino acid sequence of human STAT4 was taken from NCBI and the structure was modeled using homology modeling tool Glide on Schrödinger.

Docking of ligand with receptors

Docking of each receptor was done with Mitoxantrone separately, using Autodock 4.2 and Schrondingër Glide. The docking results of Autodock 4.2 were analyzed for the docked complex with the least binding energy (i.e. most stable binding) and best docking. The best docking interactions were selected and visualized using Discovery studio. The docking results of Schrodinger were analyzed for the least docking score (best docking) and complexes selected.

RESULTS

The receptors DNA, HSA and stat4 each were docked separately with Mitoxantrone using Autodock 4.2 and Schrodinger. The results were visualized using discovery studio.

Docking studies of MTX with DNA using Autodock 4.2:

Mitoxantrone binding was observed in the major groove of double helix DNA. The binding energy of the interaction was found to be -2.19. (Table 1)

Docking studies of MTX with DNA using Schrodinger:

Docking results show that Mitoxantrone binds in the terminal minor groove of the double helix DNA. The docking score of the interaction is -9.773. (Table 2)

Docking studies of MTX with HAS

Using Autodock 4.2:

The binding energy of the interaction is recorded as -1.84. The amino acid residues involved in the interaction with Mitoxantrone are CYS (278), GLU (277), LYS (276), LEU (275), LYS (274), SER (273), GLN (268) and ASP (269). (Table 1)

Using Schrodinger:

Mitoxantrone binds human serum albumin with a docking score of -7.719. The OH and NH_2 of the MTX side chain bind to GLU (153). MTX is fit in a pocket lined by amino acid residues - VAL (241), ARG (257), LEU (260), LYS (199), PHE (223), LYS (195). The interaction is stabilized by the presence of LEU (238), ILE (264), ALA (261), GLN (196), SER (287), PHE (211), LEU (219), ALA (215) and ARG (218). (Table 2)

Docking studies of MTX with stat4

Using Autodock 4.2:

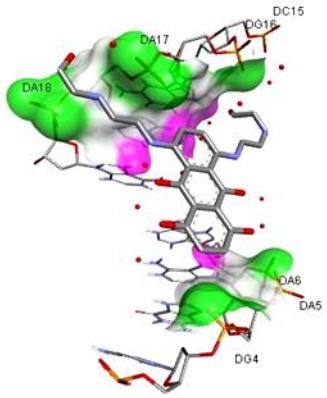
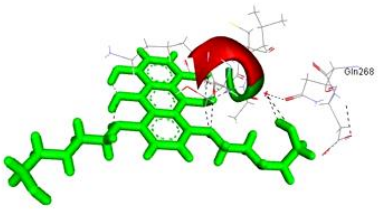
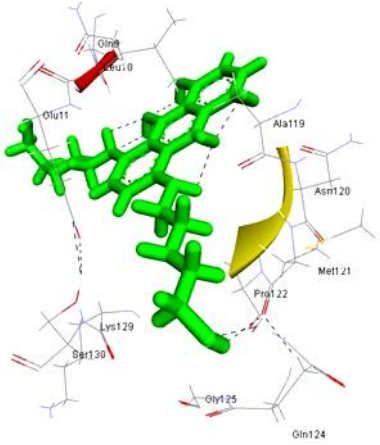
MTX binds stat4 with a binding energy of -3.38. Ligand-receptor interactions were stabilized by the presence of amino acid residues – MET (121), GLN (124), GLU (11), PRO 9122), LEU (10), ALA (119), LEU (116), ASN (120), GLN (6), GLN (9), and SER (130). A pi- sigma bond is formed between LEU (10) and the central ring of the chromophore. Side chain of the ligand is bound to MET (121), GLN (124), SER (130), GLU (11), ALA (119) and PRO (122). (Table 1)

Using Schrodinger:

The binding takes place with a docking score of -5.375. The amino acid residues involved in the interaction are MET (121), GLN (124), SER (130), GLU (11), ALA (119), PRO (122), LEU (10), LEU (116), ASN (120). Some amino acid residues are present in the vicinity of the interaction, GLN (9), GLU (6), PHE (14) and GLU (128).

The functional groups NH and OH present in the side chain of Mitoxantrone form hydrogen bond with GLU (11) and ALA (119) AND pro (122) respectively (Table 2).

Table 1: Docking results of Mitoxantrone with DNA, HSA and STAT4 using Autodock 4.2

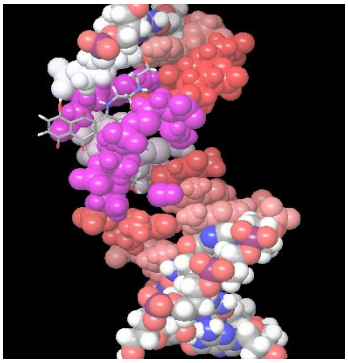
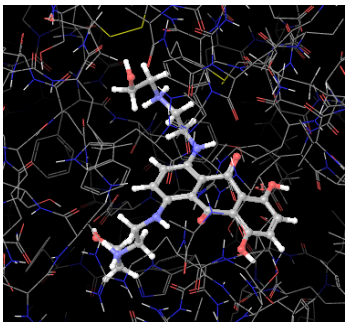
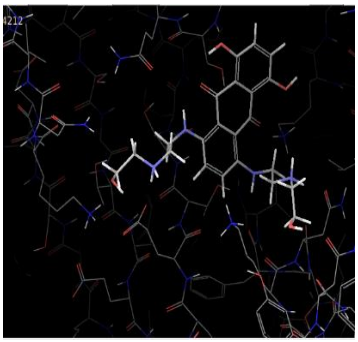
Receptors	Binding Energy	Interaction
DNA	-2.19	
HSA	-1.84	
STAT4	-3.38	

DISCUSSION

The docking studies provide an insight into the interactions taking place between the ligand and receptors. The mode of interaction cannot be deduced

by molecular modeling but it gives an idea of the site to which the ligand binds the receptor.

Table 2: Docking results of MTX with DNA, HSA and STAT4 using Schrodinger

Receptors	Docking score	Interaction
DNA	-9.773	
HSA	-7.179	
STAT4	-5.373	

Mitoxantrone is an effective anti-cancer Anthracycline with the advantage of causing less cardiotoxicity compared to other Anthracyclines drugs such as Daunomycin and Adriamycin. Its interaction with the DNA, HSA and STAT4 could be studied further and exploited for therapeutic purposes. The dockings of Mitoxantrone with DNA, HSA and STAT4 using Autodock 4.2 show negative binding energies indicating that the interaction is thermodynamically favorable and spontaneous. The same is validated by the negative docking score obtained in the docking results of Schrodinger.

Docking result of MTX with DNA shows two different binding patterns. When the docking was done using Autodock 4.2, MTX was seen to bind DNA in the major groove with a good affinity (binding energy = -2.19). This result is in consent with the majority of previous works which state that Mitoxantrone shows preference for the major groove of the DNA. However, contrasting results were obtained in Schrodinger, where DNA- MTX adduct was formed at the minor groove (docking score =-9.733). The Autodock 4.2 results also reveal that there is partial intercalation of the chromophore ring in the DNA. Based on these results, it seems that MTX can bind DNA both in the major as well as minor groove though binding preference may be more for the former. And there could be partial intercalation mode of binding as proposed earlier. Moreover, the findings of Hajihassan et al reveal that histone proteins could be selected as candidates for chemotherapy drug development. The results of docking of human serum albumin and Mitoxantrone reveal that MTX binds human serum albumin at site III in domain 1. This is a non-classical and high affinity binding as MTX binds HSA at only one site. The residue numbers of the interacting amino acids indicate that the binding is different from the typical site III binding in which the ligand fits entirely in the hydrophobic cavity. The result reinforce that the interaction is not only stabilized by hydrophobic interactions but ionic and hydrogen binds are also formed between the ligand and receptor. The docking studies of STAT4 reveal the interaction is thermodynamically favored indicating that Anthracycline drugs can bind STAT4 to give stable adduct. The value of binding energy and docking score indicate the high affinity displayed by MTX in binding STAT4. The results show the involvement of side chains in stabilizing this interaction. This furthers the prospect of selecting STAT4 as target for drug development in anti-cancer therapy. Although the mode of interaction and binding characteristics are not clear from the docking, further intensive study of the interaction like CS spectroscopy or fluorescence studies could form a clear picture.

CONCLUSION

Docking studies of Mitoxantrone with DNA, HSA and STAT4 established the fact that these can be very potential drug targets for anticancer activity. The study

revealed the binding affinity of the Mitoxantrone towards DNA, HSA and STAT4 for future studies.

CONFLICT OF INTEREST: None

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