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Virtual Screening of M3 Protein Antagonists for Finding a Model to Study the Gammaherpesvirus Damaged Immune System and Chemokine Related Diseases

Ibrahim Torktaz[1], Mohaddeseh Behjati[2*], Sareh Arjmand[3,4]

[1]*Young Researchers and Elite Club, Central Tehran Branch, Islamic Azad University, Tehran, Iran*
[2]*Isfahan Cardiovascular Research Center, Isfahan cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran*
[3]*National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*
[4]*Department of Clinical Biochemistry, Tarbiat Modares University, Tehran, Iran*

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ABSTRACT

Introduction: M3 protein is a chemokine decoy receptor involved in pathogenesis of persistent infection with gammaherpesvirus and complications related to the latency of this pathogen. We proposed that antagonists of the M3 would provide a unique opportunity for studying new therapeutic strategies in disordered immune system, immune-deficient states and role of chemokines in pathogenesis development. ***Methods:*** Comparative modeling and fold recognition algorithms have been used for prediction of M3 protein 3-D model. Evaluation of the models using Q-mean and ProSA-web score, has led to choosing predicted model by fold recognition algorithm as the best model which was minimized regarding energy level using Molegro Virtual Docker 2011.4.3.0 (MVD) software. Pockets and active sites of model were recognized using MVD cavity detection, and MetaPocket algorithms. Ten thousand compounds accessible on KEGG database were screened; MVD was used for computer simulated docking study; MolDock SE was selected as docking scoring function and final results were evaluated based on MolDock and Re-rank score. ***Results:*** Docking data suggested that prilocaine, which is generally applied as a topical anesthetic, binds strongly to 3-D model of M3 protein. ***Conclusion:*** This study proposes that prilocaine is a potential inhibitor of M3 protein and possibly has immune enhancing properties.

Introduction

Chemokines are small molecular weight chemoattractant proteins which are main mediators for migration of inflammatory and non-inflammatory cells during immune surveillance, inflammation, and development. Chemokines perform their role through interaction with respective G-protein coupled receptors [GPCR].[1,2] Based on the number and arrangement of conserved cysteines, chemokines are divided into four structural groups named CC, CXC, C and CX3C.[3] Impaired functions of chemokines are associated with enhanced susceptibility to infections and autoimmune diseases.[4] Chemokines malfunctioning can be due to the chemokines' system subverting induced by large DNA viruses to escape detection and clearance by host immune responses.[5] *Murine gammaherpesvirus 68* (MHV68), closely related to *human herpesvirus 8* (HHV8) and *Epstein–Barr virus* (EBV), is an example of chemokine evading viruses usually served as a model for the study of gammaherpesvirus pathogenesis.[6] MHV-68 contains a gene encoding secreted 44-KD protein, named M3 which is considered as a powerful multi-chemokine blocker.[7] This protein binds to a broad range of chemokines, with a displayed selectivity for CXC class of chemokines. Dimerized M3 mimics elements of GPCR and generates a binding site for acidic N-terminal loop of chemokines.[8] This binding event blocks the interaction of chemokines with cellular receptors and disrupts chemokine signaling and subsequent antiviral inflammatory responses.[9,10] M3 drastically blocks lymphocyte recruitment induced by CCL2, CCL21 and CXCL13.[6] These properties made M3 an effective chemokine scavenger or decoy receptor with high affinity.[8] Scavenging characteristics of M3 are essential for diminished inflammation observed *in vitro*.[11] M3 displaces and removes chemokines from the site of inflammation, in a concentration dependent manner.[12] M3 protein is abundantly expressed during acute viral infection

***Corresponding author:** Mohaddeseh Behjati, Email: behjati{at}med.mui.ac.ir

as the product of an immediate-early transcript and seems to have inhibitory interference with virus proliferation, dissemination and pathogenesis.[3,7,12] Virus evasion from chemokine network, as an essential component of immune response, potentiated life-long viral latency and chronic persistence. The M3 gene is located in a region of MHV-68 genome that is transcribed during latency. The chemokine inhibitory action of M3 protein and its gene location in the virus genome, raises the possibility of its role in establishment of or reactivation from latency.[13] Moreover, chemokine scavenging ability of M3 works cooperatively with other viral proteins involved in the disease pathogenesis including lymphomas and arthritis of great vessels.[14]

Although immune enhancement through blocking chemokine blockers seems to be a potential remedy in deficient immune system, it is not yet clearly understood whether blockage of decoy receptors will result in immune enhancement. In the present study, we aim to investigate virtually novel chemicals that act as M3 antagonist, using bioinformatics tools and docking study. We propose that this antagonist could be considered as a valuable model for developing new therapeutic strategies against persistent infection with gammaherpesviruses and its related complications such as arthritis of great vessels, B-cell lymphoma and Kaposi's sarcoma. In addition, since M3 acts as a potent multi-chemokine blocker, its antagonism would help as an effective tool in studies for development of novel therapeutic strategies for the treatment of autoimmune diseases and damaged immune states. Indeed, using this model, more antagonist agents can be designed for immune enhancement purposes.

Materials and methods

M3 protein 3-D model prediction

Sequences of the M3 gene related to MHV-68 were retrieved from NCBI in FASTA format (accession number; 6625570). Comparative modeling based on one template and multi templates was used as the first approach to predict 3-D model of query. Automated mode of Swiss Model[15] and Geno3D[16] web servers were used, respectively, to meet this goal. The fold recognition algorithm which is used in Phyre2 web server was considered as the next approach[17] for theoretical prediction of M3 protein 3-D structure. Phyre2 generates query model based on similar folds from similar proteins. In other words, in this algorithm, alignment is performed between folds. Accuracy of predicted models was evaluated using Q-mean[18] and ProSA-web scoring.[19] Minimization of structural energy in the selected model was performed by Molegro Virtual Docker (MVD) software (version 2011.4.3.0). MVD cavity detection and MetaPocket algorithms[20] were applied to detect potential binding sites of the model for docking study as the next step.

Docking study

In order to find appropriate ligand structures, a library of KEGG compound containing 10000 ligands were derived. These ligands have been used for docking study against eight found cavities. Before docking operation, structure of protein and ligands were prepared using MVD software. For this purpose, calculated charge by MVD was added to ligands and protein structure; explicit hydrogen in models was created; flexible torsions in ligand was detected; and side chain flexibility for amino acids which were present in predicted binding sites was defined.

Derived ligands from KEGG library were docked for finding best compound with high affinity to the model cavities. MolDock score[21] was used as docking algorithm. Ten runs were performed for each ligand with permitted hydrogen bond between ligand and protein grid resolution of 0.3. Energy minimization procedure was performed after docking and hydrogen bonds were optimized. Energy threshold was defined 100 and similar poses were neglected.

Pharmacophore determination

Ligand Scout 3.02 was used for pharmacophore determination. Pharmacophore was designed for finding the best pose of ligand in cavity of M3 protein.

Results

Model prediction

In this study we tried to simulate *in vivo* condition. To this end, we used a flexible docking operation in the presence of solvent and neutralizing ions based on a comparative modeling retrieval structure using available M3 protein structures as templates (1ML0 and 1MKF). Automated mode of Swiss model server generated 3-D model of query based on the template d1mkfb, and modeling range was from amino acid 36 to 406. In Geno3D server, four templates (1mkfa, 1mkfb, 1m10a and 2nyza) were used for prediction of the final structure. Phyre2 server generates a 3-D structure of the query based on identical folds of similar structures. D1mkfb from family of viral chemokine binding protein was used as a basic model template for phyre2. This template had 100% identity with query in aligned regions. The accuracy of the predicted 3-D models was estimated using Q-mean and ProSA-web scoring. Q-mean has a parameter ranging from 0 to 1 and the best crystallographic structures reach approximately the score of 1.[18] ProSA-web uses a Z-score which indicates the overall model quality based on measuring the deviation of total energy of the structure as for an energy distribution derived from random conformations.[19] Table 1 describes Q-mean and Prosa score of models. Eight cavities were identified using MVD software as the first approach for finding pockets (Table 2). As an alternative approach, MetaPocket web server was used to identify potential binding sites of query.[20] Among output data, 20 amino acids were recognized as preset in catalytic sites (Table 3). Catalytic and ligand binding sites which were detected by MetaPocket had compliance with MVD results (Fig. 1).

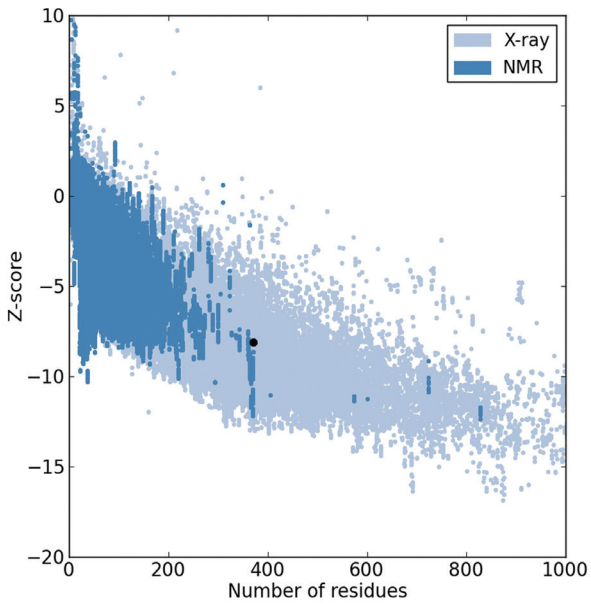

Fig. 1. ProSA score of phyre2 model

Table 1. ProSA and Q-mean scores for each model

Model name	Q-mean score	ProSA score
Swissmodel	0.713	-8.39
Phyre2	0.779	-8.1
Geno3D	0.494	-5.45
1mkf	0.733	-8.2

Table 2. Characteristics of identified cavities using MVD

Cavity number	Volume	Surface
1	23.040	88.32
2	21.504	92.16
3	20.480	89.60
4	16.896	70.40
5	16.896	76.80
6	16.384	57.60
7	14.336	69.12
8	10.230	51.20

Finding cavities and docking study

Catalytic and ligand binding sites which were predicted by MetaPocket had compliance with MVD results. For this reason, identified cavities by MVD software were used for further molecular docking analyses (Fig. 2). Based on virtual screening data, docking energy level for three top poses of each cavity is described in table 4. The best binding affinity belonged to cavity 8 with a MolDock score of -365.540. Interestingly, all of the most negative scores in all cavities were related to one chemical which was identified as prilocaine in PubChem database. The overall pharmacophore properties of prilocaine are described in Fig. 3 and Table 5.

Discussion

Selecting the most representative 3-D model for M3 protein

Swiss model output reached the best ProSA-web score, but because of low coverage (amino acids 36-406), its Q-mean score was less than that of Phyre2 model (Incomplete models penalized). Since this model had 34 missing residues, it was not used for further studies. Geno3D model had weak scores in both ProSA-web and Q-mean. Phyre2 output gained the best scores in both Q-mean and ProSA-web, indicating that the quality of this predicted model is in the zone of X-Ray crystallography. Also, we found that our modeled structure by fold recognition algorithm, after optimization has more precious structure than 1mkf (Table 2). Phyre2 server extracted model for M3 was used as a basic one for next studies.

Molecular docking simulation

MVD performs flexible ligand docking so that the optimal geometry of the ligand is determined during the docking. MVD includes MolDock score for evaluating docking solutions. In this study, docking results were evaluated on the basis of the MolDock score. MolDock algorithm combines differential evolution with a cavity prediction algorithm. The MolDock scoring function is based on a piecewise linear potential (PLP) and takes the directionality and charges of hydrogen bonding into consideration. Docking scoring function used in this study is defined as:

$E_{score} = E_{inter} + E_{intra}$

E_{inter} is ligand-protein interaction energy and calculated as follows;

$$E_{\text{inter}} = \sum_{i \in ligand} \sum_{j \in protein} \left[E_{PLP}(r_i) + 332.0 \frac{q_i q_j}{4 r_j^2} \right]$$

Where E_{PLP} is a piecewise linear potential. The summation encompasses all heavy atoms in the protein and the ligand as well as any cofactor atoms and water molecule atoms. The second term points up the electrostatic interactions between charged atoms.

E_{intra} is the internal energy of the ligand that describes the electrostatic interactions between charged atoms and is calculated as follows:

$$E_{intra} = \sum_{i \in ligand} \sum_{j \in ligand} E_{PLP}(r_i) + \sum_{\substack{flexible \\ bonds}} A[1 - \cos(m.\theta - \theta_0)] + E_{clash}$$

Where θ is the torsional angle of the bonds, and E_{clash} is punishment for infeasible ligand conformations by assigning a penalty of 1000 for distances less than 2.0 A between two heavy atoms. Among 10000 chemicals which were used for virtual screening purpose, prilocaine could provide the strongest avidity to m3 protein. Interestingly, it could bind to several positions of the protein surface

Table 3. Predicted binding site in MetaPocket server

Binding site 1:					**Binding site 3:**				
ATOM	1382	OG	SER	213	ATOM	836	CA	GLU	147
ATOM	1385	C	SER	214	ATOM	837	C	GLU	147
ATOM	1389	N	THR	215	ATOM	838	O	GLU	147
ATOM	1390	CA	THR	215	ATOM	839	CB	GLU	147
ATOM	1391	C	THR	215	ATOM	840	CG	GLU	147
ATOM	1392	O	THR	215	ATOM	841	CD	GLU	147
ATOM	1393	CB	THR	215	ATOM	842	OE1	GLU	147
ATOM	1394	OG1	THR	215	ATOM	844	N	PHE	148
ATOM	1395	CG2	THR	215	ATOM	845	CA	PHE	148
ATOM	1396	N	PHE	216	ATOM	846	C	PHE	148
ATOM	1397	CA	PHE	216	ATOM	847	O	PHE	148
ATOM	1400	CB	PHE	216	ATOM	848	CB	PHE	148
ATOM	2359	O	ALA	343	ATOM	849	CG	PHE	148
Binding site 2:					ATOM	851	CD2	PHE	148
ATOM	388	CG	GLN	87	ATOM	855	N	TYR	149
ATOM	389	CG	GLN	87	ATOM	1048	O	SER	172
ATOM	390	OE1	GLN	87	ATOM	1052	CA	ASP	173
ATOM	391	NE2	GLN	87	ATOM	1053	C	ASP	173
ATOM	432	CE	LYS	93	ATOM	1054	O	ASP	173
ATOM	433	NZ	LYS	93	Binding site 4:				
ATOM	754	CD	GLU	136	ATOM	190	O	LEU	61
ATOM	756	OE2	GLU	136	ATOM	1153	CE	LYS	184
ATOM	1317	CG	LYS	205	ATOM	1154	NZ	LYS	184
ATOM	1318	CD	LYS	205	ATOM	1360	C	PRO	211
ATOM	1319	CE	LYS	205	ATOM	1361	O	PRO	211
ATOM	1320	NZ	LYS	205	ATOM	1362	CB	PRO	211
ATOM	1333	OG1	THR	207	ATOM	1377	N	SER	213
ATOM	1444	CD	GLU	221	ATOM	1378	CA	SER	213
ATOM	1445	OE1	GLU	221	ATOM	1379	C	SER	213
ATOM	1446	OE2	GLU	221	ATOM	1380	O	SER	213

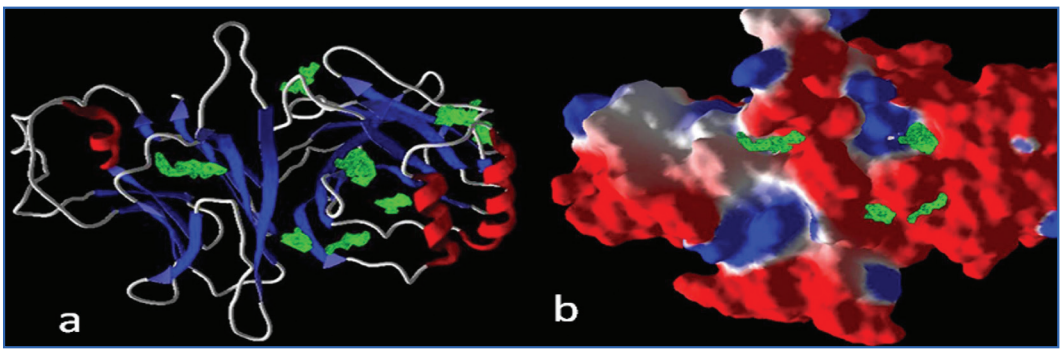

Fig. 2. a) Position of cavities in secondary structure view, b) Position of cavities in surface view

Table 4. Energy level of top three poses in each cavity

Ligand	MolDock Score	Re-rank Score	HBond	Cavity number
Prilocaine	-356.980 -349.104 -345.582	43.0553 -124.228 -115.803	-7.7415 -7.8688 -13.036	1
Prilocaine	-361.229 -357.244 -327.136	-47.611 -66.265 -29.045	-7.9991 -7.9952 -5.5254	2
Prilocaine	-344.705 -343.468 -336.690	12.033 -64.867 -36.121	-11.6066 -10.7700 -9.8470	3
Prilocaine	-304.612 -290.074 -287.586	-12.074 202.315 101.489	-2.5582 -6.4359 -11.0539	4
Prilocaine	-303.233 -290.259 -285.108	114.053 99.8484 113.27	-12.394 -8.2107 -2.1942	5
Prilocaine	-350.304 -347.1 -344.108	-70.3644 1.14145 -21.6572	-9.0216 -7.0731 -12.723	6
Prilocaine	-347.3 -342.062 -332.5	-54.5662 -56.6849 -6.79349	-3.7293 -5.8988 0	7
Prilocaine	-365.540 -351.460 -345.444	-15.7661 -110.394 -112.001	-14.0746 -17.4707 -15.0899	8

with strong binding avidity (Table 4). The structure of M3 in complex with prilocaine was determined after docking simulation and was used for pharmacophore designing purpose and determination of contact bonds.

Designing pharmacophore

Results of pharmacophore designing indicated two important hydrogen bonds between NH of prilocaine and threonin 215 of the protein and another hydrogen bond between C=O of prilocaine and valin 45 of the protein. Moreover, there are important hydrophobic interactions between aromatic ring of prilocaine and its CH3 moiety with a hydrophobic pocket formed by threonin 319, threonin 356 and alanin 343 of M3 protein. Other important hydrophobic interactions are formed between a propyl side chain of prilocaine and leucin 49, leucin 89 and threonin 217 of M3 protein. Overall and summarized pharmacophore model of the best compound with high affinity to the best cavity of protein was shown in Fig. 3. Also, properties of different pharmacophore classes of prilocaine are described in Table 5.

Based on the pharmacophore model, which is presented in this study it is suggested that the most reactive groups of prilocaine are NH and O, which can establish hydrogen bonds, and the aromatic ring and its CH3 moiety, which can interact with hydrophobic pockets of target protein. Prilocaine is a local anesthetic of the amino amide type that because of its low cardiac toxicity is commonly used for intravenous regional anesthesia (IVRA). As prilocaine

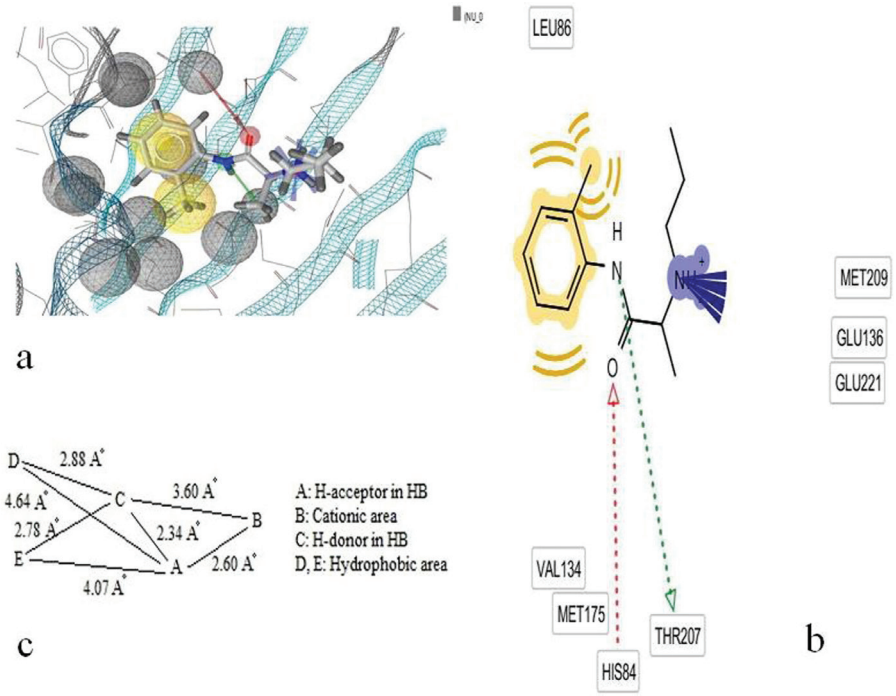

Fig. 3. Pharmacophore model for prilocaine in best inhibitory position. a) prilocaine in cavity of M3, b) interactions between aromatic ring of prilocaine and M3, c) interaction model of prilocaine with M3.

Table 5. Pharmacophore properties of prilocaine

Pharmacophore Class	x	y	z	Radius
Aromatic	65.40	-16.59	57.60	1.10
HydrogenDonor	65.49	-19.36	57.56	0.50
HydrogenDonor	64.02	-21.89	55.90	0.50
HydrogenAcceptor	64.02	-21.89	55.90	0.50
HydrogenAcceptor	63.29	-20.01	58.03	0.50
Hydrophobic	65.40	-16.59	57.60	1.00
Hydrophobic	67.07	-17.96	59.52	1.00
Hydrophobic	66.28	-21.52	56.59	1.00
Hydrophobic	63.59	-24.33	54.11	1.00

is a common drug, it is probable that its engineered metabolites would have less toxicity in human body. Beside the data, we have obtained by in silico analysis in this study that additional data based on experimental studies are required to reveal the inhibitory effects of the prilocain in M3 protein and to elucidate its mechanism of action. Furthermore, it is probable that any similar chemicals with described specifications can interact with M3 protein with efficient binding affinity. This in silico study may shed lights on designing new and potent inhibitors to be used as next generation of anti-viral drugs.

Conclusion

Prilocaine is a local anesthetic agent usually used as a mixture with lidocaine.[22,23] Based on MolDock score unit, this chemical can bind to M3 protein with noticeable efficiency of -365.54. Binding of prilocaine to the M3 structure leads to 3D occupation of binding sites in M3 protein; and therefore M3 cannot perform its role as a chemokine decoy receptor, anymore. This phenomenon is expected to inhibit chemokine malfunctioning following viral infection. Since M3 protein binds to both mouse and human chemokines,[3] herein we introduced prilocaine, for the first time, as a candidate chemical for experimental studies on a wide spectrum of viral and non-viral complications in which hemokines and chemokine receptors act as mediator.

Ethical issues

Non to be declared.

Competing interests

The authors declare no conflict of interests.

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