RESEARCH PAPER | Biotechnology | Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

Identification of Lead Molecule For 1TNF Receptor to Treat Tuberculosis and Type 2 Diabetes Mellitus

KEYWORDS: TNF-alpha, idock, TANAGRA tool, FlexX

Chaithra, D.R.	Tanusree Bhattacharya	Kusum Paul
Department of Biotechnology, The Oxford College of Engineering, Bangalore-560068, India	Department of Biotechnology, The Oxford College of Engineering, Bangalore-560068, India	Department of Biotechnology, The Oxford College of Engineering, Bangalore-560068, India

ABSTRACT *Tuberculosis (TB) is one of the life threatening diseases around the world infected by Mycobacterium tuberculosis. Mycobacteria are resistant to most commonly used antibiotics and drugs. The increasing emergence of drug resistant tuberculosis strains and is one of the common causes of poor response to anti-tubercular treatment these necessitates the development of novel drugs. Diabetes in tuberculosis increases drug resistance strains hence, lead to treatment failure and increases death rates. Previous studies explain that, TNF- alpha gene involved in both tuberculosis and diabetes mellitus type 2. The best ligand for the TNF-alpha was identified, that can act as a drug molecule to cure both the disease at a time.*

1. INTRODUCTION

Tuberculosis is one of the life threatening diseases about one third of the world population is infected with *Mycobacterium tuberculosis*. TB remains a major source of a diseased state and death rates throughout the world. This disease is responsible for about 1·5 million deaths annually [1]. India ranks second in top 10 countries account for 1\5th of tuberculosis cases. Type 2 diabetes mellitus is a chronic disorder associated with wide range of complication [2] and mainly characterized by hyperglycemia [3].

Several epidemiological studies describe that type 2 diabetes mellitus are 3 times more prone to getting the active tuberculosis compared with non diabetes patients [4]. Tuberculosis may lead to the development of mew diabetes cases due to impaired glucose tolerance, in patients with tuberculosis [5]. Previous studies reported that decreased pro-inflammatory cytokines in patients with after infection with *M.tb* [6, 7, 8]. In tuberculosis and type 2 diabetes mellitus IL10, IL12B, IL8, IL6, IL4, TNF, INF and TNFRSF1A genes are common in both disease. In one of the recent study reported that, the TNF-alpha play a vital role in both TB and type 2 diabetes mellitus. In one of the study reported the reduction of helper Th1 cytokines in tuberculosis infects the diabetes host [6, 9].

Patients with type 2 diabetes mellitus, in which many cytokines and chemokines are up-regulated [10] and inflammation caused by cytokines in response to tuberculosis infection, may cause an increase in insulin resistance thereby leading to hyperglycemia [11]. Several drugs available to treat the tuberculosis disease, diabetes in tuberculosis increases drug resistance hence, leads to treatment failure and increases rate of death [12]. In present study we identify the lead molecule, through combinatorial chemistry approach, that can be used as a drug to cure both of the diseases at the same time.

2. MATERIALS AND METHODs

2.1 Identification of genes

Genes was collected from NCBI, Databases for human genes and genetic variants associated with Tuberculosis http://genome.igib.res.in/hgvtb/index.html, and T2DM databases. The common genes obtained from the list were selected in both diabetes mellitus and tuberculosis.

2.2 Screening for ligand

The BINDING Database is a web accessible database, to find the protein and ligand, drug-like molecule interaction and binding affinities of the proteins. The binding affinities information of TNF-alpha gene collected from BINDING database. We selected the ligands and the receptor from the list obtained from the BINDING database. The different receptors obtained from the list were filtered by using the UNIPROT Database based on the reclusion factor and functions 1TNF selected as receptor and PDB file downloaded from the RCSB Database. The initial docking was done to screen the large number of ligands through the iDOCK server.

2.3 Screening ligand through TANAGRA tool

The TANAGRA software tool was used to screen the result obtained from the idock server. The Spearman's rho test was used to find the correlation between the variables. These tests transform the continuous variables into ranks, computes and test the correlation between these ranks.

2.4 Docking studies through FlexX

The FlexX docking tool was used to perform the docking for receptor 1TNF. The FlexX tool is best docking tool now available in BiosolveIT site. The FlexX performs the flexible docking of ligand and receptor.

3. RESULT AND DISCUSSION

3.1 Identification of genes

Genes was collected from NCBI, 268 genes obtained for tuberculosis and 502 genes obtained for type 2 diabetes mellitus from NCBI database. 438 genes obtained for tuberculosis from Databases for human genes and genetic variants associated with Tuberculosis (hgv&tb) database and 8 genes were collected for type 2 diabetes mellitus from T2DB database, 100 genes obtained from TBdatabase, and 57 genes from tb_human database. Among these genes 99 genes were present in both diseases. The TNF-alpha is the potential gene screened through the literatures.

3.2 Screening for ligand

The receptors were collected from the list obtained from the BINDING Database there were 7 receptors, we selected 1TNF as a receptor based on the function and the

Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

resolution using Uniprot database. The idock server was used to screen the ligand, among 11,630 total ligands best 1000 ligands obtained after docking with idock server based on the ADMET properties.

3.3 Screening of result through TANAGRA tool

The TANAGRA software tool was used to filter out the ligands and to select the best 10 ligands for the final docking based on the statistical values obtained. 10 ligands were selected based on the values obtained from the spearman's rho test. The statistical values for the different attributes are shown in table 1.

Figure 1 Indicates the statistical values obtained from the TANAGRA software. Y and X represents attributes provided in the input and output parameters respectively. Pr (>|t|) =0.0000 indicates the highly confident value.

Table 1: The statistical values obtained from the spearman's rho test.

Y	X	r	r^2	t	Pr(>|t|)
Molecular weight(g/mol)	Free energy(Kcal/mol)	0.0939	0.00088	8.8482	0.0000
Molecular weight(g/mol)	Ligand efficiency (kcal/mol)	0.0847	0.0072	7.9699	0.0000
Molecular weight(g/mol)	RF-score(pK)	0.1040	0.0108	8.8070	0.0000
Molecular weight(g/mol)	Consensus score(pK)	-0.0928	0.0036	-8.7384	0.0000
Partition coefficient xlogP	Free energy(Kcal/mol)	0.0091	0.0001	0.8531	0.3936
Partition coefficient xlogP	Ligand efficiency (kcal/mol)	0.0115	0.0001	1.0808	0.2798
Partition coefficient xlogP	RF-score(pK)	0.0513	0.0026	4.8212	0.0000
Partition coefficient xlogP	Consensus score(pK)	-0.0077	0.0001	-0.7202	0.4714
Apolar desolvation(kcal/mol)	Free energy(Kcal/mol)	0.3656	0.1337	36.8356	0.0000
Apolar desolvation(kcal/mol)	Ligand efficiency (kcal/mol)	0.3409	0.1162	34.0085	0.0000
Apolar desolvation(kcal/mol)	RF-score(pK)	0.5736	0.3291	65.6733	0.0000
Apolar desolvation(kcal/mol)	Consensus score(pK)	-0.3526	0.1244	-35.8356	0.0000
Polar surface area tPSA(A^2)	Free energy(Kcal/mol)	0.1336	0.0719	12.6435	0.0000
Polar surface area tPSA(A^2)	Ligand efficiency(kcal/mol)	0.1380	0.0190	13.0668	0.0000
Polar surface area tPSA(A^2)	RF-score(pK)	0.1378	0.0190	13.0436	0.0000
Polar surface area tPSA(A^2)	Consensus score(pK)	0.0174	0.0174	-12.4680	0.0000

3.4 Docking studies through FlexX

The final docking was done to select the best ligand among the 10 ligand list. The FlexX docking tool provides the best ligands for the receptor 1TNF. The SDF files for the ligands were downloaded from the ZINC Database. After collecting the ligands SDF files we prepared the ligands library and then we prepared the receptor by adding the hydrogen, water molecule and interacting molecules. The chain was selected and the active site residues were selected for docking. The ligand were loaded and run the docking to get best 20 ligands based on the number of pose sites. Hyde assessment was done after docking for all the 20 ligands. The ligand which gives the less ΔG values and high ligand efficiency value were selected as a best ligand. The docking result for the ligand ZINC56103771 and ZINC16085141 were shown in the figure 1 and 2 respectively. We docked the ligand ZINC56103771 and ZINC16085141 with receptor 1TNF for top 20 pose, from the docked list ZINC56103771_019 and ZINC16085141_001 was the best ligand which was having the best ligand efficiency and these ligands can be used as a best ligand to cure both tuberculosis and type 2 diabetes mellitus at a same time.

Figure 1 displays the docking result for receptor 1TNF and ligand ZINC56103771 through FlexX docking tool, the ligand in white colour in the center figure obtained after docking. The residues Ser99, Ile118, Lys98 and Ile97 which were the interacting residues with the ligand molecule and the dotted lines indicate the interacting molecules. The

Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

Figure 1: Docking result for receptor 1TNF and the ligand ZINC56103771.

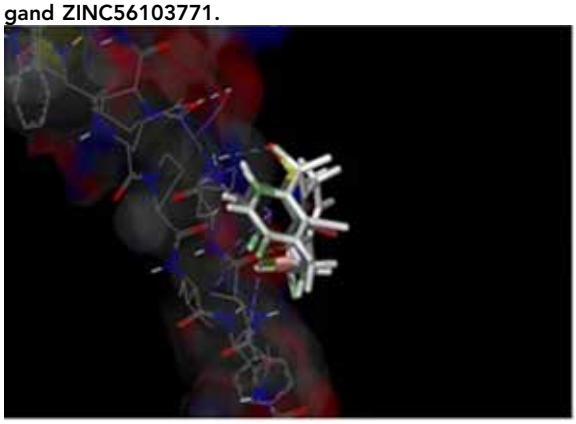
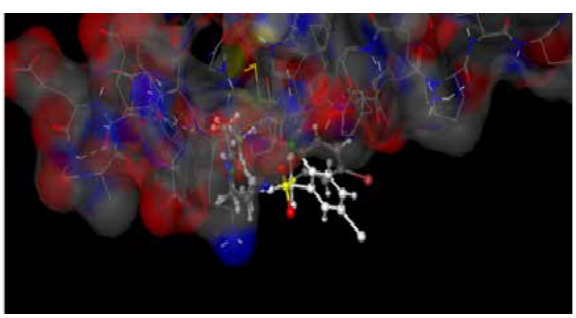


Figure 2 displays the docking result for receptor 1TNF and ligand ZINC16085141 through FlexX docking tool, the ligand in white colour in the figure obtained after docking.

Figure 2: Docking result for receptor 1TNF and the ligand ZINC16085141.



Acknowledgement
We would like to thank the management of The Oxford College of Engineering for their support. Extends our thanks to Principal Dr. Rajendra Prasad for providing necessary resources and we also tank all the faculty members of Biotechnology department for their support.

REFERENCE

1. Meghan, A. B., Anthony, D. H., Christie, Y. J., Jessica, E. H., Anil, K., Knut, L., Salah-Eddine, O., Sunali, D.G., and Megan, B. M.(2011).Impact of Diabetes on Tuberculosis Treatment Outcomes: A Systematic Review. BMC Medicine,81(9). Doi: 10.1186/1741-7015-9-81. | 2. Mallikarjun, V. J., Vinay, K. M., and Murigendra, B. H.(2013).Bidirectional Screening of Tuberculosis Patients for Diabetes Mellitus and Diabetes Patients for Tuberculosis. Diabetes Metab J, 37(4), 291–295. Doi: 10.4093/dmj.2013.37.4.291. | 3. Lin, Y., Sun, Z. (2010).Current views on Type 2 Diabetes. J Endocrinol, 204(1), 1-11. Doi: 10.1677/JOE-09-0260. | 4. Jeon, C. Y., Murray, M. B. (2008).Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. PLoS Med, 5(7), 1091-1101. Doi: 10.1371/journal.pmed.0050152. | 5. Lonnroth, K., Raviglione, M. (2008).Global epidemiology of tuberculosis: prospects for control. Semin Respir Crit Care Med, 29(5), 481-491. Doi: 10.1055/s-0028-1085700 | 6. Nathella, P. K., Rathinam, S., Vaithilingam, V. B., Mohideen, S. J., Michael, P. F., Thomas, B. N., and Subash, B. (2013).Type 2 Diabetes Mellitus Coincident with Pulmonary Tuberculosis Is Associated with Heightened Systemic Type 1, Type 17, and Other Proinflammatory Cytokines. Ann Am Thorac Soc, 10(5), 441–449. Doi: 10.1513/AnnalsATS.201305-112OC. | 7. Al-Attiyah, R. J., Mustafa, A. S. (2009). Mycobacterial antigen-induced T helper type 1 (Th1) and Th2 reactivity of peripheral blood mononuclear cells from diabetic and non-diabetic tuberculosis patients and Mycobacterium bovis bacilli Calmette-Guérin (BCG)–vaccinated healthy subjects. Clin Exp Immunol. 158(1), 64–73. Doi: 10.1111/j.1365-2249.2009.04000.x | 8. Stalenhoef, J. E., Alisjahbana, B., Nelwan, E. J., Van der Ven-Jongekrijg, J., Ottenhoff, T. H., van der Meer JW, Nelwan RH, Netea, M. G., and Van, C. R.(2008).The role of interferon-γ in the increased tuberculosis risk in type 2 diabetes mellitus. Eur J Clin Microbiol Infect Dis. 27(2), 97–103. | 9. Vallerskog, T., Martens, G. W., Kornfeld H. (2010).Diabetic mice display a delayed adaptive immune response to Mycobacterium tuberculosis. J Immunol, 184(11), 6275–6282. Doi: 10.4049/jimmunol.1000304 | 10. Rashmi, S., Chaman, L. S. (2007).TNF-alpha inhibitors: current indications. Indian J Crit Care Med, 11(3), 139-148. Doi: 10.4103/0972-5229.35087. | 11. Susan, P. Fisher-Hoch. (2014).Worldwide increase in diabetes: implications for tuberculosis control. Research and Reports in Tropical Medicine, 2014(5), 35-44. Doi: http://dx.doi.org/10.2147/RRTM.S45082. | 12. Vadim, M., Benoit, L., Ming, Z., Joao, N., Astrid, M. S., Susanne, A. R., Ruben, C. H., Olga, B. R., Anthony, V., Laurent, A. D., Nicolas, W., Thierry, B., Wilbert, B., Koen, A., Florence, P., Paul, J. D., and Stewart, T. C. (2014).Towards a new combination therapy for tuberculosis with next generation benzothiazinones. EMBO Mol Med, 6(3), 372–383. Doi: 10.1002/emmm.201303575. |