Study on structural insight of the analysis of negative effects of opioids analgesics in naltrexone with TLR4 Mutations

Iqra Tariq[1], Ali Farhan[1], Usman Ali Ashfaq[1], Muhammad Qasim[1], Mahmood-ur-Rehman[1], Fahad Humayun[1], Masaoud Shah[2], Anum Munir[3] and Muhammad Shareef Masoud[1]*

[1]Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan
[2]Department of Molecular Science and Technology, Ajou University, Suwon, Korea
[3]Department of Bioinformatics and Biosciences Capital University of Science & Technology, Islamabad, Pakistan

Abstract: Chronic pain has been defined as the persistence that remained for more than three months. The extent of previous time duration with the normal time of natural healing phase becomes poor and results in reduced life quality and morbidity. Opioids are well recognized therapy for pain management and the clinical prescriptions based on opioids have been defined with increasing implicating behavior among patients suffering with chronic pain. The association between the pain and immunity has long been established since the involvement of interleukin-1ß (IL-1ß) in sickness that is considered with the induced hyperalgesia. In the context of pharmacodynamics Toll like receptors (TLRs) are involved in the negative effects of opioids as analgesics. The soluble factors released by immune cells as well as from the disruptive cells bind to TLRs. This binding leads the pre and post-synaptic ends on endothelial and microglial cells that exhibit the activation of complex inhibitory and excitatory process at the synapses site. In TLRs, TLR4 is mostly reported that is strongly associated in specifically in areas of T cells and macrophages. The current study is designed to investigate the structural insights of the opioids and TLR4 interactions by using computational approach in the aspect of recognizing the chemical combinatorial factors that are involved in the pain management. This study targets that how opioids interact with TLR4 and the process of chemical interaction that leads to negative effects of opioids at neuroimmune interface as well as to investigate the extent of particular naltrexone that mediates with the negative effects of opioids.

Keywords: Naltrexone, TLR4, opioids, analgesics.

INTRODUCTION

The relationship of pain with immunity has been recognized since the time of interleukin-1 involvement with sickness-induced hyperalgesia as well as hyperplasia-β (Roeckel & Glenn-Marie Le Coz, 2016) (Grace, *et al*., 2014). Pain that last for no less than a month, followed by usual healing time for any severe injury with non-curative wound. It is mainly defined as a pain that remains for minimum three months' time (Verhaak, *et al*., 1998). Most of the general published records have verified that the frequency of chronic and acute pain in local population is very high. Continuous pain is recognized to target the complexity of associated chemical bonding with TLR4.

Conventionally, the most common kinds of chronic pain are classified as "nociceptive", or pain due to ongoing motivation of pain receptors by the damage of tissues are called as "neuropathic". Neutropathic pain is related to harmness of peripheral or central nervous system. Such categorizes are really complicated where chronic and acute pain are connected to peripheral and central nervous system, which always engage with different and with severe pain modulating systems. The perturbations that in the end results in ache sensitivity are caused by neurons and physiological approaches and few other related mechanisms, as recent proof has started to focus on the function of nervous and immune stimulation on the tissue damage with vital mechanism in the improvement of continuous ache (DeLeo, 2006).

In the aspect of social means, the effect of naltrexone in pain management with alcohol and opioid abuse considered in humans in limiting way however it is aggressively influences in the factors of social norms (Havassy, *et al*., 1991). Pain-associated disorders and pain severances found to be related in social and mental factors, and patients with same sicknesses related with pain, that include in diseases have also been reported greatly in different articles with pain severance and pain associated disorders have been reflected (Rosenblum, *et al*., 2008).

The inhibition of mu receptors by naltrexone efficiently performed and more or less to delta opioid receptors (Smith, *et al*., 2011) it may lead to preventing the euphoric effect of alcohol and opioid. Naltrexone was synthesized as antagonist of orally competitive opioid receptor in 1963 (Younger, *et al*., 2013). The structural and functional behaviors of naltrexone are similar to the opioid antagonist naloxone (Resnick, *et al*., 1974). The commercial availability of Naltrexone for the treatment of

**Corresponding author:* e-mail: masoudshareef@gmail.com

opioid addiction was approved by FDA in 1984 (Verebey, 1975).

It has been evident in number of studies that chemical association of naltrexone with Toll-like receptors 4 (TLR4) is well recognized for the complete action. Toll-like receptors critically involved in the central immune signaling process (Gold, *et al*., 1982). Multiple microbial ligands have been identified as activators of TLR4 (Austin & Moalem-Taylor, 2010).

Computational models for naltrexone and TLR4

There are *In silico* models that presented the structural insights of molecular docking responsible for naltrexone with TLR4 (Uematsu & Akira, 2008). In the light of emerging studies focused on the structural and functional realms of TLR4 action opioids are involved in the activation of non-neuronal cell and this type of activation significantly effects the behavior of opioids and may also some other drugs (Kawai & Akira, 2010) (Coller & Hutchinson, 2012). Activation of TLR4 is responsible in central nervous system (CNS) for the cause of releasing pro-inflammatory as well as neuroexcitatory cytokines, such as tumor necrosis factor- and interleukin-1 (20, 23 nih104).

The distribution of TLR4 and some other Toll-like receptors in the brain persists broadly and for necessary connection in it (Narita, *et al*., 2008). It has been reflected that all these innate immune receptors importantly expressed among the immunocompetent cells as microglia (Olson & Miller, 2004), astrocytes (Bowman, *et al*., 2003), with oligodendrocytes (Peterson & Lokensgard, 2007). Some experimental evidences show that TLR4 also exhibited in cortical CNS neurons (Tang, *et al*., 2007).

TLR4 effect in association with morphine

Morphine is well known and well triad opioid drug that metabolized in the liver sight and also in the CNS due to strong binding affinity traits in chemical significance (King & Tephly, 1999). Clinical studies reflect that in humans, about 44-55% of morphine is metabolized to morphine-3-glucuronide (M3G), and about to 9-15% morphine metabolized into morphine-6-glucuronide (M6G) (Kilpatrick & Smith, 2005).

Conversely, the clinical trials exhibited that M3G and M6G found in the CNS as lumbosacral cerebrospinal fluid (CSF) (Thunedborg *et al*., 1998; Dale, *et al*., 2007). Recent studies suggested that integration of μ-opioid receptor (MOR) and Morphine antagonists perform the inhibition of TLR4 activation (Hutchinson, *et al*., 2011).

The behavior of opioids in pain sensitivity of induced proinflammatory oppose the analgesic actions of morphine and it also enhance the neuronal excitation. Different *in vitro, in vivo, and in silico* studies have been performed to analyze the role of morphine, with its metabolite as M3G (Hutchinson, *et al*., 2009).

Structural insights of M3G and M6G are used to show the behavior of opioids in pain sensitivity through various experimental approaches. In this study the descriptive analysis of Naltrexone action sites with TLR4 have been focused with introducing the TLR4 mutations by using the computational approaches. However, previous *in silico* studies (Shah & Yesudhas Krishnan Choi, 2016) significantly targeted the structural dynamics of TLR4 with Naloxone and Morphine to analyze the behavior of opioids in pain sensitivity.

MATERIALS AND METHODS

Structures retrieval

Three Dimensional (3D) structures of TLR4 predicted by using Phyre2 the form of pdb files that is non-commercial protein structure prediction online server. The opioids (morphine, naltrexone) two dimensional (2D) structures were retrieved from PubChem https://pubchem.ncbi.nlm.nih.gov/compound/morphine#section=2D-Structure in SDF file format.

PubChem that is recognized as the database of chemical molecules and their activities to use for the analysis of biological assays and can be visualized from Discovery studio visualizer. These structures exhibited the clue for finding the representative active sites for the interaction of associated drugs with TLR4 protein molecule. Visualization of each structure was performed by Discovery studio version 2016. The binding sites for each structure were analyzed on the standard orientation of structural parameters established in *in silico* techniques. Molecular structural dynamics methodologies for TLR4 active sites were also considered in this study.

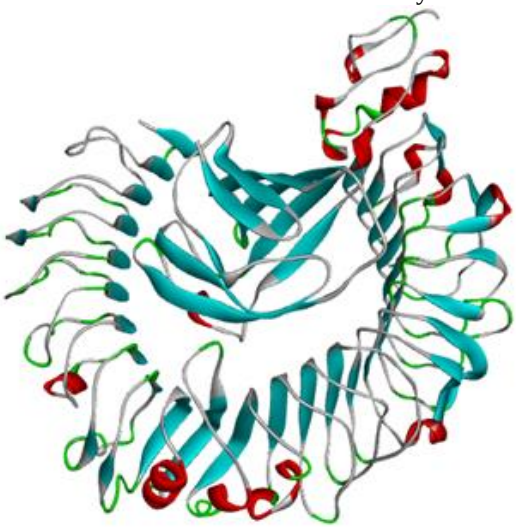

Fig. 1: The 3D structure of TLR4 Protein, the blue color represents beta sheets, red color represents alpha helices, and coils are represented by grey color.

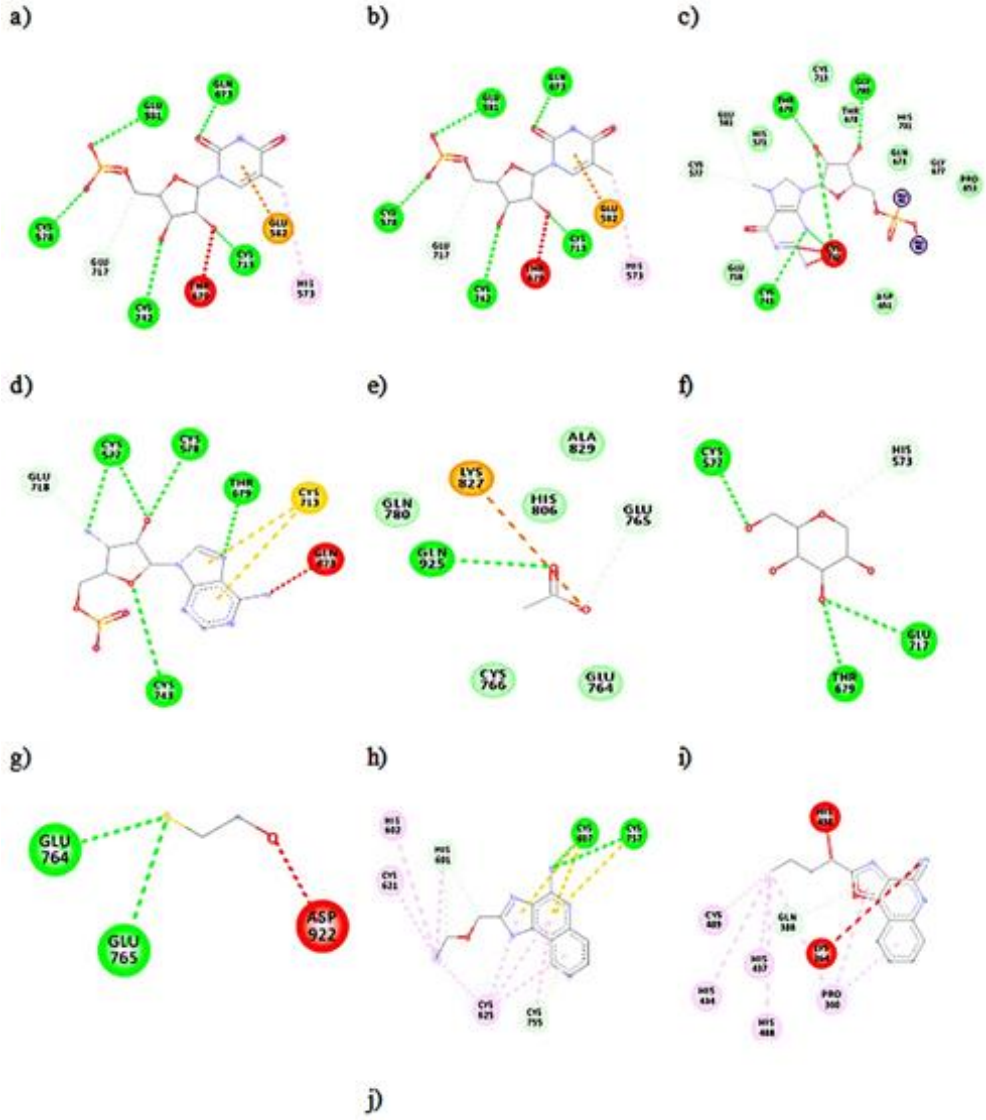

Fig. 2: The docking results of 10 ligand molecules with the TLR4 protein, a – j represents the individual non-therapeutic frames with TLR4 protein.

Comparative modeling

The comparative approach was used to predict the models based on genetic mutation analysis for TLR4 and each mutated structure was further incorporated for the molecular docking with most chemically associated drug ligands. Statistically each three-dimensional structure was used to determine the high accuracy values for conventional molecular dynamics involved in TLR4 with naltrexone.

Analysis of physiochemical properties

In order to determine the chemical excitation of TLR4 the physiochemical properties were retrieved from ProtParam tool that is recognized for the computation of various chemical and physical properties of protein parameters. For physiochemical properties of naltrexone and Morphine Protparam that is freely available to analyze the particular instant of physical and chemical relationship of targeted compounds.

Central interacting molecules that were chemically enhanced through protein ligand docking approach also showed the flexibility in structural dynamics in molecular visualization of naltrexone with TLR4 binding affinity sites. All conventional statistical means covered in this descriptive study.

RESULTS

The 3D structure of mutated TLR4 was retrieved from the PDB database. The TLR4 protein is encoded by the TLR4 gene and involves in intracellular signaling pathways, in

its activated state it recognizes the foreign pathogens and activates the responses of innate immune system against them (Stefano & Kream, 2012). The molecular properties of TLR4 are shown in table 1, while the physical and chemical properties of TLR4 are shown in table 2.

Table 1: Molecular data of TLR4 based on 3D Structure

Molecular weight	95680.13
Formula	$C_{4334}H_{6734}N_{1122}O_{1246}S_{37}$
Total number of atoms	13473
Number of amino acids	839
Theoretical pI	5.88
Ext. coefficient	90520
Estimated half-life	30 hours
Instability index	43.05
Aliphatic index	101.86
Average of hydropathicity	0.033

Table 2: TLR4 physical and chemical properties

Molecular weight	18545.54
Formula	$C_{845}H_{1300}N_{210}O_{237}S_{11}$
Total number of atoms	2603
Number of amino acids	160
Theoretical pI	8.80
Ext. coefficient	19285
Estimated half-life	30 hours
Instability index	37.81
Aliphatic index	82.81
Average of hydropathicity	-0.084

Table 3: Morphine physical and chemical properties

Molecular weight	341.407 g/mol
Chemical formula	$C_{20}H_{23}NO_4$
H-bond acceptors	5
H-bond donors	3
Log P	2.07
Molar refractivity	91.5 $m^3 \cdot mol^{-1}$
Rotatable bonds	2
Polar surface area	71
Physiological Charge	1
Polarizability	35.97 $Å^3$
melting point	168-170 °C

DISCUSSIONS

In comparative approach to investigate neuropathic oriented pain models are subjected in lasting the tetrasynapse projections (Grace & Maier, 2015). The computational modeling aspects discussed in this study that have been associated with the structural insights of negative opioids are more conducive for microglia and spinal cord demonstrations in clinical perspective. The analgesic effects as addressed by (Williams, *et al*., 2013) the most significant feature in this concentration is the exposure of opiates. This study has remarkable key factors with the *in silico* techniques to identify the most significant structural sites that paly roles in molecular functional investigations to manage the chronic pain incidences.

Table 4: Naltrexone physical and chemical properties

Molecular weight	315.369 g/mol
Chemical formula	$C_{18}H_{21}NO_4$
H-bond acceptors	5
H-bond donors	1
Log P	1.04
Molar refractivity	84.04 $m^3 \cdot mol^{-1}$
Rotatable bonds	1
Polar surface area	59 $Å^2$
Physiological Charge	1
Polarizability	32.8 $Å^3$
melting point	219 °C

The structure of TLR4 consists of two protein chains A and C having 599 amino acid residues in chain A and 135 residues in chain C, it consists of 90% of the beta sheets and only 2 alpha helices. The 3D structure of TLR4 is shown in fig. 1.

Table 3 exhibits the physical and chemical properties of Morphine that enables the structural analysis to compare the significant sites in conventional and therapeutic dynamics in the context of opioids activities. As the current study has prior descriptive analysis with targeting the naltrexone other than naloxone thus table 4 shows the physical and chemical properties of naltrexone that are addressed in the differences of more compact forms of structural dimensions that are not modeled with the template of naloxone chemical structure with the *in silico* subject as described in the studies (Anwar, *et al*., 2016).

TLR4 and MD-2 form a heterodimer that recognizes LPS (lipopolysaccharide) from Gram-negative bacteria. In this study MD2 complex targeted with the mutations of TLR4 that may express the core binding sites for the inhibition of drug effects. In this work, the molecular docking studies are performed for both the MD2 complex and the retrieved ligands. Three-dimensional structure predictions of the genes/proteins endorse that it might be employed more to understand the potential component of disease growth and part of these proteins in bringing up abnormalities (Vaure & Liu, 2014) (Bronte, *et al*., 2010). The docking results of ten ligand compounds with TLR4 are shown in fig. 2.

From fig. it is observed that in all the docking results the amino acids HIS, GLU, GLN and CYS are common interacting amino acids of the docked pockets. Molecular docking is the process of fitting a ligand inside the active site of a receptor and involves perusing for the low-

energy binding modes. The docking can help to productively investigate the binding space of a ligand.

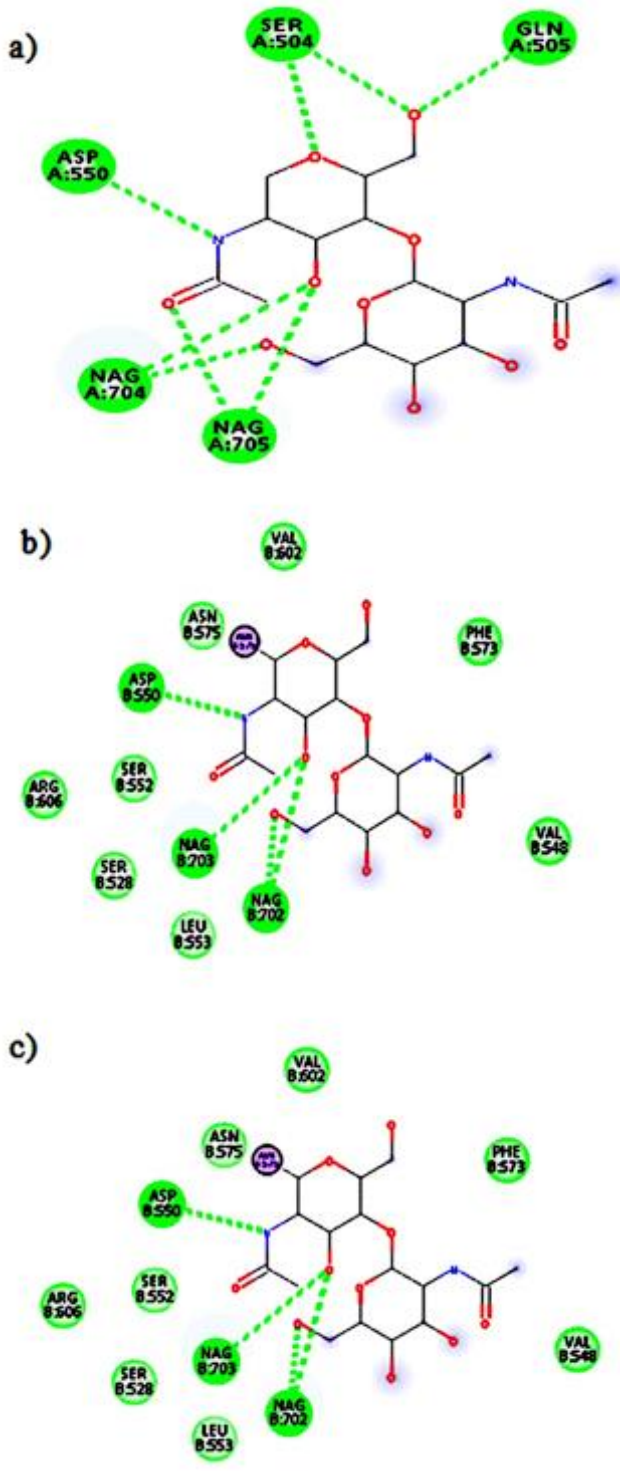

Fig. 3: The docking results of the commercial drugs with TLR4 protein, a) the docked complex of TLR4 and Pethidine, b) the docked complex of Oxycodone with TLR4 and c) the docking results of TLR4 and Naltrexone.

Thus, it is in charge of assessing the binding affinity once the right binding pose is identified (Vaure & Liu, 2014). Different types of bonding were observed in the docked complexes shown in fig. 2, such as conventional carbon-hydrogen bonds represented by green dotted lines, the orange dotted lines show Pi alkyl bonding, purple lines represent pi- sulfur bonding and red dotted lines represent bump.

Three different commercial drugs; pethidine, Oxycodone, and naltrexone, already used to cure the mutations of TLR4 were downloaded from the PubChem database and docked with TLR4 to compare the docking results of 10 ligands and commercially available drugs. The docked results of the commercial drugs are shown in fig. 3.

From fig. 3 it is observed that all three drugs interact with ASP and NAG residues. The 10 ligands interacted with larger number of pocket residues as compared to the commercial drugs. Among all the compounds docked with TLR4, the three drugs only made conventional carbon-hydrogen bonding with the amino acid residues of TLR4 protein. The 6 of the 10 ligands represented bumps. Bumps refer to the collision of molecules to each other; the minimum acceptable number of bumps is five. The bumps are denoted by red dotted line. The chemical features of compounds discovered by the interaction are taken into interpretation into the best docked models, as well as the interactions between the target receptor and the ligand molecule.

CONCLUSION

The current study is designed to investigate the structural insights of the opioids and TLR4 interactions using computational approach in the perspective of pain management. The study focused on the interaction of opioids with TLR4 and the interaction that leads to negative effects of opioids at neuroimmune interface and how specifically naltrexone mediates the opioids associated negative effects. The previous studies have been critically used the Naloxone as targeted compound. The opioids and naltrexone are docked with TLr4 protein.

All three drugs interact with ASP and NAG residues. The 10 ligands interacted with larger number of pocket residues as compared to the commercial drugs, but also represented bumps during docking. In future this research work can be further utilized as a part of clinical trials to test its adequacy and social advantages.

The Morphine is a pain reliever and found naturally in many plants and animals it basically targets the central nervous system to relieve pain (Stefano *et al.*, 2012). The molecular data of morphine is shown in table 3 while physical and chemical properties are shown in table 4.

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