AT-121 as a Potential Opioid Replacement

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Abstract

The mu opioid Receptor (μ-Receptor) is the neural structure involved in interpreting pain signals. An opioid acts as an agonist that provides pain relief by binding to a large number of these receptors and preventing pain signals from being processed by the brain. Over prescription of addictive opioids in America has led to a rise in addiction in recent decades. To reduce addiction rates, we sought to research a new drug that has the potential to block pain signals without causing dependence and see what sets it apart from common opioids. A ligand supposedly matching this description has been identified in AT-121. We used computational docking methods and structural analysis to determine if AT-121 poses a legitimate solution to opioid addiction. To determine if docking was successful, we relied on a complementary study to identify key ligands, and their residues involved with neurochemical opioid interactions. Our results indicate that AT-121 interacted with the residue that is essential for a conformational change to the binding cavity. Given this, human testing should be carried out to further assess the agonist's effectiveness at reducing addiction to opioids. If testing results show positive results, AT-121 could pose as a beneficial drug for helping to cease the US opioid epidemic.

Introduction

United States Specific Crisis

Humans have cultivated opium for centuries, its use dating back as far as 3400 B.C. in Mesopotamia. In those times, opium was cultivated for its euphoric effects that are still linked to opioid abuse today. Later, it led to conflict between Britain and China during the opium wars, after Britain sold many opioids in China. The increase in Chinese opioid use led to a surge of addiction and death in the 19th century (Pletcher 2020). Opium-related deaths have skyrocketed in recent years and has led to heavy regulation of the substance in the United States of America. On average, 128 American deaths are caused by opioid use each day, and those numbers are continuing to rise. All of this is due to three main waves of opioid use that dramatically changed America forever (CDC 2020). In 1996, Purdue Pharma patented OxyContin, a powerful opioid-based pain pill. Through aggressive marketing and promotions, Purdue was able to grow sales from $48 million to around $1.1 billion in 2000. This feat unleashed the first wave epidemic in the US (Van Zee 2009). The second wave is attributed to the introduction of heroin to the American streets in the early 2000's. Heroin proved to be a cheap alternative to its prescribed counterpart, but the various additives in it make overdoses much more likely. Finally, fentanyl is responsible for the third and most recent wave of the epidemic (CDC 2020). Fentanyl is a compound that is 50 to 100 times more potent than morphine. It has led to a 10% increase in synthetic related opioid deaths from 2017 to 2018 (CDC 2020). With the pharmaceutical and illegal production of addictive opioids growing rapidly, this issue will only worsen in the United States.

Benefits of finding a treatment

Finding an alternative to opioids for pain management that is not associated with physical dependence, would be extremely beneficial for the United States. Introducing a drug into the market of that description would cause a surge of economic growth due to the manufacturing process creating new jobs and sales going globally (Su 2018). It would also likely lead to a more effective allocation of resources from mainly producing addictive opioids and drugs like Narcan that revive overdosed patients, to this drug that positively benefits society by lowering overdose related deaths (NIDA 2019).

Opioid addiction in the brain

After opiates enter the body, they dissolve into the bloodstream where the chemicals they contain are dispersed. The bloodstream transports the pain-relieving endorphins to most organs throughout the body, including the brain. The endorphins attach to mu, delta, and kappa opioid receptors. This has many effects on a large number of brain structures, one of them being the mesolimbic reward system (Kosten 2002). The mesolimbic reward system activates signals that release dopamine in the brain causing the user to experience feelings of pleasure. The brain records the circumstances that lead to the feelings of happiness as a memory (Kosten 2002). With repeated and consistent opioid use the association between pills and pleasure increases in strength and a dependence is developed. The brain adapts to the influx of endorphins and the opioids' effect on the body lessen creating the need for an increased dosage to attain the same levels of pleasure. If the user continues to increase their dosage, his or her body will begin to function abnormally without the opioids. When the body can no longer function normally

without opioids the dependence evolves into an addiction (Kosten 2002). Long-term opioid addictions lead to changes in bodily function and brain structure. As these changes take place the withdrawal symptoms become more severe (Kosten 2002). This is important to understand when discussing opiate addiction.

Analysis of the μ-Receptor (mu-Receptor)

The μ-Receptor is the only opioid receptor that is linked to addictive tendencies out of the three: mu, delta, and kappa (Zaki 1996). In another study regarding rats who were given morphine, those who were genetically modified to not have the μ-Receptor did not show the reinforcing properties that morphine possessed in terms of additivity (ECNP 2007). The reason opioids are so effective is due to the μ-Receptor's large binding cavity. The largeness of the active sight allows binding of a wide range of molecules. The receptor works by having an initial molecule, like morphine, activate the receptor via binding and leads to the blocking of potassium channels which in return stop action potentials from occurring. Those action potentials are what cause the signal of pain to be sent to the brain (Jamil 2017). This reaction takes place all over the body, connecting with the central nervous system and peripheral nervous system, which makes it effective.

How AT-121 compares to opioids

Opioids provide pain relief by binding to the μ-Receptors throughout the body and brain. In order to develop a non-addictive treatment for pain, we must use a substance that binds to these same receptors without activating the brain's reward system. The small molecule that fits this description is AT-121. This ligand is an agonist of the μ-Receptor and another subtype of opioid receptor known as the nociceptin receptor (Ding 2018).

However, the more important prospect of the solution is that it has shown to not display the same addictive properties of opioids. In primate experiments, AT-121 was tested against oxycodone and has been shown to have a much lower reinforcing effect (Ding 2018). Additionally, AT-121 was administered to monkeys in relatively large doses to assess if it could potentially compromise biological functions. These tests indicate that AT-121 did not cause substantial depression of the respiratory and heart rate of the primate subjects (Ding 2018). If the effects of the substance translate to humans, AT-121 could be the ligand that helps reduce the rates of opioid addiction.

Methods

Selection of receptor and ligand structure models

We selected the PDB ID 5C1M (Huang 2015) from the RCSB Protein Data Bank (RCSB). The protein structure model represents the active form of μ-OR with the morphine agonist (ligand ID 4VO). The prior study indicated that the receptor specific residues are: Glu229, Lys303, and Trp318 (Parras et al. 2019). We used UCSF Chimera (Pettersen et al. 2004) to remove duplicate residues. Next, Chimera was used to add hydrogen atoms to the protein and ligand structures, and they were saved as two different PDB files. Having isolated the μ-OR structure model, we identified and downloaded the 3-D structure of the experimental agonist, 3-Oxo-1'-(4-propan-2-ylcyclohexyl)-2-[2-

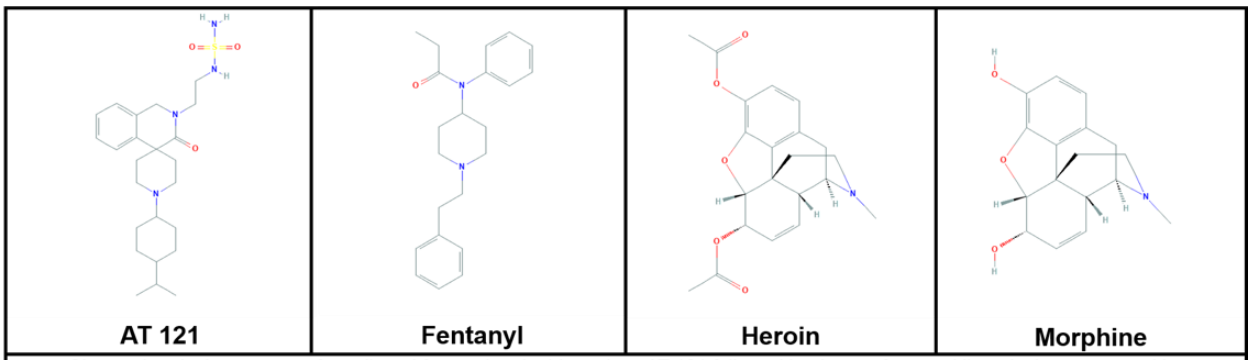

Fig 1. Shows chemical structures of one ligand focused on in this project: AT 121 followed by three referenced ligands that were used in a similar research study: Fentanyl, Heroin, and Morphine. All ligands chosen are known to interact with the key residues of the μ-opioid receptor. Representations were created using PubChem and Microsoft Office PowerPoint.

(sulfamoylamino)ethyl] spiro[1H-isoquinoline-4,4'-piperidine] (PubChem CID 129188444) (AT-121), from PubChem (Kim et al. 2019). AT-121 served as the test ligand for this study.

Docking Procedure

Using the AutoDock Tools (ADT) software suite (Morris et al. 2009), we prepared the protein and ligand structure files for docking. The PDB files saved in Chimera were converted to PDBQT files by adding charges for each atom type in the structure file. The next step was to determine the search area for docking, which is done using the grid box tool. The center for our grid box was (2.000, 15.612, - 58.700) with dimensions of 20 Å by 22 Å by 18 Å. The charged PDBQT files and grid box parameters were used with AutoDock Vina (Trott and Olson 2010) to re-dock 4VO and dock AT-121 into the 5C1M structure model. In our docking results, we found nine potential poses with the first one being the best due to its free energy of binding being the most negative value.

Analysis of Docking Results

Methods explained extensively in previous docking studies were used as a guide for analysis in this study (Lewis 2011). Analysis included an assessment of potential

interactions between the protein and ligand, which were identified using distance measurements between heavy atoms. Docking results were assessed to identify hydrophobic and hydrogen bond interactions. A hydrophobic interaction is categorized as a configuration of carbon atoms on a ligand and a non-polar amino acid side chain within a distance of 3.9 Å (Lewis 2011). A distance less than or equal to 3.3 Å between two charged or polar atoms, such as an interaction between an amine group on a ligand and a carboxyl group on a residue, is classified as a hydrogen bond (Lewis 2011). Calculation of distance measurements and visual inspection of the docking results were carried out using Chimera.

Results

Redocking

After successfully finding an appropriate grid box, we were able to redock the 4VO ligand known as morphine back into 5C1M protein using Autodock Vina (Trott and Olson 2010). We were only able to generate one pose for the redocked morphine agonist. The redocking was successful with the original ligand overlapping the redocked pose almost symmetrical as seen in figure 2.

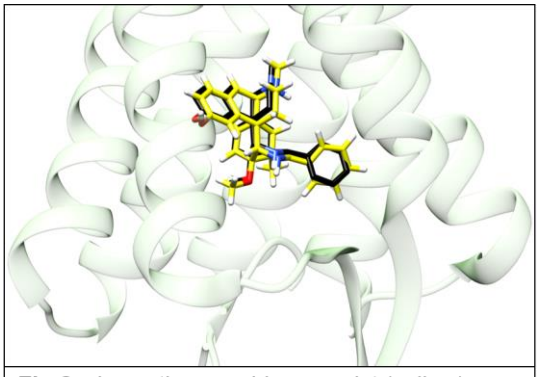

Fig 2. shows the morphine agonist (yellow) native to the 5C1M protein (mint green) superimposed by the lowest free energy of binding pose of morphine (black).

Test Docking

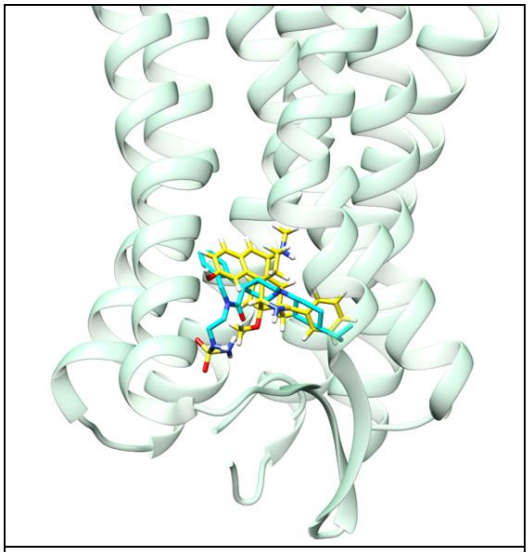

Fig 3. shows the original agonist, Morphine (yellow) superimposed by AT-121 (cyan blue) after a successful test docking of Morphine.

Figure 3 shows the test-docking results of our agonist and our experimental ligand. The similarity in orientation indicated that AT-121 would likely be successful in binding to the 5C1M structure. The slight differences in position likely stem from the increased number of atoms in the molecular formula of AT-121.

Comparison of AT-121

Docking of the AT-121 ligand into the 5C1M structure model provided docking results that were distinctly different to what was observed in the previous study. We previously believed that AT-121 had potential for binding with Tyr326, Asp147, and Tyr14,8 which are the key residues identified in the previous study (Parras et al. 2019). However, when AT-121 was docked, it showed no signs of any form of interaction with Tyr326, Asp147, and Tyr148. The lack of hydrogen bonds with the ligand's amine groups are shown by the distances greater than 3.3 Å as shown in figure 4.

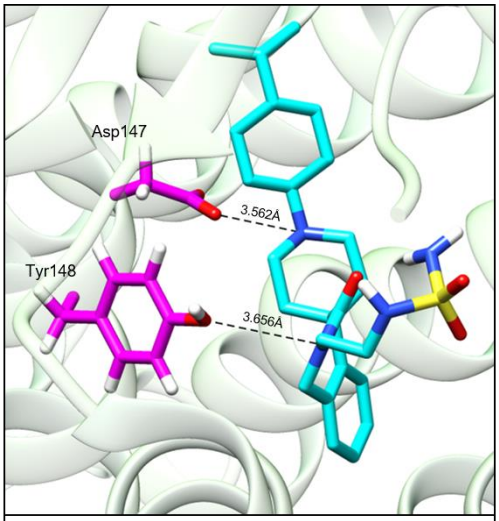

Fig 4. shows key residues: Asp147 and Tyr148 (magenta) not forming a hydrogen bond with AT-121 (cyan blue) due to the distances of 3.562Å and 3.656Å being greater than 3.3Å.

The residues that the complimentary study found that are specific to the μ-OR are Glu229, Lys303, and Trp318. While many common opioids did not show any interactions with these residues, it is important to note that AT-121 did (Figure 5). The docked pose for AT-121 has two oxygen atoms, which are attached to the sulfur atom, positioned near the opening of the cavity. The proximity of these oxygen atoms to Lys303 (2.862 Å) suggests the formation of a hydrogen bond might occur between the protein and ligand at this position. The location of the ligand at the opening of the binding cavity could mean that the ligand serves as a gatekeeper for the binding site.

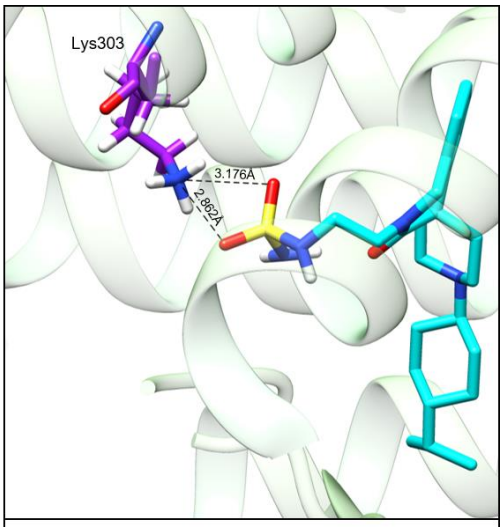

Fig 5. Shows AT-121 (cyan blue) forming hydrogen bonds with unique μ-Receptor residue Lys303 (purple). Distances show as 3.176Å on the top and 2.862Å below

Discussion

Analysis of Common Opioids

The results of this study were compared to those of a previous study in which morphine, heroine, and fentanyl were docked into the 5C1M structural model (Parras et al. 2019). The previous group identified Asp147, His297, and Tyr326 as important residues for ligand binding based on assessment of their docking results (Parras et al. 2019). This study found that morphine possessed an amine group that was positioned within hydrogen binding distance to Asp147 and Tyr326. The oxygen atom that connects the two carbon rings also formed a hydrogen bond with Tyr148. Similarly, heroin showed

hydrogen bonding between Asp147 and Tyr326 and its amine group, as well as between the center oxygen atom and Tyr148 (Parras et al. 2019). We noted, as did the previous group, that heroin and morphine share a core chemical structure, which explains the similarities in docking for the two compounds. Fentanyl, comparatively, has a different chemical structure that is planar and has a hydrophobic phenyl tail. The docked pose for fentanyl was shown to form a hydrogen bond with Asp147 and the oxygen on the ligand (Parras et al. 2019). Furthermore, fentanyl had hydrophobic interactions with residues His297 and Val143 (Parras et al. 2019). It was also observed that in all of the ligands tested in the complementary study, oxygen atoms pointed downwards towards the opening of the binding site (Parras et al. 2019). This was also true when AT-121 was docked.

All of the ligands tested in the prior study exhibited hydrogen bonding with the μ-opioid receptor. This can also be said about the AT-121 ligand. Fentanyl showed a potentially hydrophobic interaction when docked into 5C1M (Parras et al 2019). AT-121 has the smallest bonding distances between the two ligand oxygen atoms, attached to the sulfur and residue Lys303. These distance values were 2.862 Å and 3.176 Å (figure 5). Morphine showed the second smallest bond distances at 3 Å, 3.1 Å, and 3.3Å as found in the complementary study. The bond distances between the fentanyl and the residues were 3 Å, 3.4 Å, and 3.6Å. The heroin molecule had distances of 3 Å, 3.1 Å, 3.3 Å, 3.4 Å and 3.6 Å and 3.8. Å between itself and residues of the 5C1M structure (Parras et al 2019) The distances between AT-121 and the residues have the lowest values. To us, this explains why the binding is still successful despite bonds only forming with one residue from the μ-opioid receptor.

Mutagenesis of Lys303

A mutagenesis study performed by Bonner, Meng, and Akil genetically altered the μ-opioid receptor structure and changed Lys303 into three different amino acids. After a mutagenesis was performed, ligand-binding affinities were not significantly reduced. The researchers who performed this study describe lys303 as a ligand with a large binding cavity. Meaning, it will bind with non-selective opioid ligands as well as opioid ligands that are specific to the mu opioid receptor (Bonner et al. 2000). This aspect of the residue is what we believe is the underlying reason AT-121 was able form a hydrogen bond with the residue.

Conclusion

The complementary study that docked commonly abused opioids with the μ-opioid receptor indicated the significant residues for binding are Tyr326, Asp147, and Tyr148 (Parras et al. 2019). It is our contention that these residues are also linked to the addictiveness of these opioids (Noori 2014). Also identified were residues specific to the μ-opioid receptor : Glu229, Lys303, and Trp318 (Parras et al. 2019). Our docking results indicate AT-121 formed Hydrogen bonds with Ly303. The results from the mutagenesis study (Bonner et al. 2000) indicate AT-121 would maximize pain relief by occupying the large binding cavity of lys303 and forming a hydrogen bond with the residue (Bonner et al. 2009). This hydrogen bond allows for the ligand to block pain signals. However, since the ligand does not interact with the addiction-associated residue, it does not possess the same addictive properties most opioids do. These factors make AT-121 an ideal

substance for reducing the effects of opioid addiction. AT-121's unique binding properties show potential for future human studies to combat opioid addiction.

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