The Off-target Effects of Oseltamivir on Influenza

Rishi Vijay

Abstract

Medicinal drugs and treatments are developed to cure patients of a specific virus or disease. Unfortunately, they can also have unintentional off-target effects on other biological processes, which can significantly disrupt the human body. I conducted an experiment analyzing the off-target effects of Oseltamivir, also known as Tamiflu, which is a drug meant to prevent and treat the influenza virus. At a molecular level, Tamiflu works by inhibiting the viral neuraminidase (NA) enzyme. The Tamiflu carboxylate stops the viral NA enzyme from cleaving sialic acid particles on infected cells. My experiment found that, in addition to the viral NA enzyme, human Sialidase-1 and Sialidase-2 may be unintentionally inhibited by Oseltamivir. This study evaluates similarities between the viral NA enzyme and humane sialidases and discusses the potential for side effects from Oseltamivir use due to inhibition of Sialidase 1 and 2.

Introduction

The human body contains millions of systems, many of which are composed of macromolecules called proteins. Each of these proteins plays a specific role in the human body, ranging from acting as enzymes, hormones, antibodies, or something entirely different (1). On the other hand, if everything in the process of constructing proteins isn't perfect, catastrophic consequences could happen. In the beginning, the fabrication of a protein always starts with a microscopic strand of deoxyribonucleic acid (DNA), which is a molecule with base pairs and a double helix structure. Through a process called transcription, the base-pair sequences in the genes of a DNA strand are encoded onto a strand of messenger ribonucleic acid (mRNA) (1). Afterward, each codon (a pair of three nitrogenous bases) in the mRNA, that was transcribed from the DNA, codes to a specific amino acid. In a process called translation, the codons in the mRNA connect with a ribosome and form a polypeptide chain made up of amino acids that correspond with the specific codons in the mRNA, and the complex structures of polypeptide chains eventually form proteins (1).

But within this process of transcription and translation, there is a lot of room for error. If each biological process within a polypeptide chain, or a chain of amino acids, does not correctly function, the entire protein could be faulty or completely broken. Similarly, drugs or outside substances inserted into the human body can affect how a protein functions by binding to the protein. When drugs are inserted into the human body, they are usually intended to bind to a particular protein. Off-target protein binding can lead to negative side effects in the protein.

Influenza and Oseltamivir

A drug that can have off-target effects is Oseltamivir's process in combating the Influenza Virus. The Influenza Virus is a viral infection that usually lasts for a week, targeting the nose,

bronchi, throat, and lungs (1). This infection is characterized by aching muscles, severe malaise, high fever, headache, dry cough, sore throat, and rhinitis. Influenza is transmitted via small particles or droplets of the coughs or sneezes of an infected person, and the virus is transferred easily. In most cases, people infected with influenza will recover without needing serious medical treatment. But there is a chance that the infection can lead to more severe complications such as pneumonia, or even death (2).

At a more molecular level, influenza has multiple steps in the process of infecting a cell. Firstly, the influenza virus binds to particular receptors of a respiratory epithelial cell (3). Afterward, through a process called endocytosis, the cell phagocytoses the virus in a vesicle called an endosome. Inside the endosome, the endosomal membrane and the viral membrane fuse together, which releases the viral genetic material of influenza into the cytoplasm of the host cell. The viral polymerase enzymes also transcribe and replicate the viral RNA; as a result, the new viral RNA segments are synthesized, and new viral proteins are produced (3). The synthesis of new viral proteins, such as hemagglutinin (HA) and neuraminidase (NA), happens using the host cell's machinery. The synthesized viral RNA segments and viral proteins assemble into new viral particles after moving to the cell's surface. Finally, the viruses are released from the infected cell through a process called budding, and with the help of the viral neuraminidase enzyme severing sialic acid residues on the cell surface (3).

Oseltamivir, also known as Tamiflu, works by inhibiting the viral NA enzyme in the influenza virus; as a result, the enzyme is not able to clear a path for influenza to leave the infected cell, so the virus does not spread (4). After being taken orally, Tamiflu competes with sialic acid (the natural substrate of NA) in binding to the viral NA enzyme. After binding to the active site of the viral NA enzyme, the Tamiflu carboxylate stops the viral NA enzyme from cleaving sialic acid particles on infected cells. By inhibiting this main function of the viral NA enzyme, influenza particles cannot be released, which contain influenza within just the infected cell (4).

Connection to Broader Topic

While this example only focuses specifically on the unintended side-effects of Oseltamivir binding to the NA enzyme, the greater goal of the study is to generalize how drugs or medicine can have an unintentional effect on biological processes that weren't targeted. Furthermore, some drugs fixate on biological processes that already have an important functionality in the human body, so the loss of that system could have a negative impact.

Materials and Method

The experiment conducted consists of three parts: the identification of non-target proteins that Tamiflu might impact, the investigation of complicated protein interactions, and the

discovery of which parts of the body Tamiflu affects the most (5). Throughout the experiment, the only material I used was a computer.

Identifying Off-Target Proteins

Using the NCBI Gene Database website, I searched for “neuraminidase enzyme homo sapiens”, the enzyme that the drug “Oseltamivir” binds to (6). Next, I retrieved the amino acid sequence of the NA enzyme. I used NCBI Basic Local Alignment Search Tool (BLAST) to find other proteins with similar amino acid sequences (7). My entries were the NA enzyme protein sequence, “Neuraminidase Enzyme”, “Reference proteins (refseq_proteins)”, and “homo sapiens” (5). I then ran the BLAST program. Afterward, I went to the “Distance tree of results” page to see how similar the NA enzyme is to other proteins, and I took note of the two proteins closest to the NA enzyme. Finally, in the previous NCBI BLAST page, I went to the “Graphic Summary” tab. This displayed a diagram representing the degree of similarity for the sequences, with a color key at the top. The proteins with the highest degree of similarity to the NA enzyme were recorded.

Investigating Complicated Interactions

I went to the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database website and searched for “NA enzyme” (8). I then took note of the different processes the NA enzyme affects and how big or small of a role the NA enzyme has. Afterward, I repeated the same process but substituted “NA enzyme” with the other two proteins I found in the “Identifying Off-Target Proteins” part.

Possible Organs Targeted by Tamiflu

In the NCBI Gene Database, I searched for “neuraminidase enzyme homo sapiens” and clicked on the same “neuraminidase 1” result as before in Part 1 (6). I scrolled down to the section labeled “Expression” and wrote down the two organs or biological processes with the highest amounts of the NA enzyme as shown in the bar graph (5). Afterward, on the same website, I searched for the abbreviations of one of the two proteins I found in Part 1 using the Google website. I again went to the “Expression” section and noted down the two organs with the highest amount of that protein. I repeated this with the other protein I found as well.

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  1 mtgerpstal pdrrwgpril gfwggcrvwv faaiflllsl aaswskaend fglvqplvtm
 61 eqllwvsgrq igsvdtfrip litatpratl lafaearkms ssdegakfia lrrsmdqgst
121 wsptafivnd gdvpdglnlg avvsdvetgv vflfyslcah kagcqvastm lvwskddgvs
181 wstprnlsld igtevfapgp gsgiqkqrep rkgrlivcgh gtlerdgvfc llsddhgasw
241 rygsgvsgip ygqpkqendf npdecqpyel pdgsvvinar nqnnyhchcr ivlrsydacd
301 tlrprdvtfd pelvdpvvaa gavvtssgiv ffsnpahpef rvnltlrwsf sngtswrket
361 vqlwpgpsgy sslatlegsm dgeeqapqly vlyekgrnhy tesisvakis vygtl
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Figure 1: The protein sequence of Viral Neuraminidase obtained from NCBI Gene Database

Results

Identifying Off-Target Proteins

The NCBI Gene Database search for viral neuraminidase resulted in a genetic sequence that encodes a 415 amino acid protein sequence (Figure 1) (6). BLAST analysis of the amino acid sequence resulted in one primary and one secondary match for protein sequence similarity. The primary and secondary results encoded human Sialidase-1 and Sialidase-2 (7). They play a role in cleaving sialic acid residues from glycoproteins and glycolipids in lysosomes (3). Additionally, Sialidase-4 is another protein found in the human body similar to the viral NA enzyme, but Sialidase-1 and Sialidase-2 have more in common with the viral NA enzyme, which is why I didn't focus on Sialidase-4 in my later experiments.

Investigating Complicated Interactions

The KEGG Pathway Database search for viral neuraminidase resulted in a picture of the viral NA enzyme found in the influenza virus, while Sialidase-1 and Sialidase-2 are found in all human cells. When I searched for Sialidase-1 in the KEGG Pathway Database, I found it was specifically located in the lysosomes of cells; Sialidase-2 is found in the cytosol and plasma membrane of cells (8).

Possible Organs Targeted by Tamiflu

After searching for the NA enzyme in the NCBI Gene Database and scrolling down to the "Expression" section, I discovered that the NA enzyme was found most prevalent in the thyroid and placenta (6). The abbreviation I found for Sialidase-1 after using Google was NEU1, and the abbreviation for Sialidase-2 is NEU2. When I searched for NEU1, I discovered it was also found most common in the thyroid and placenta, while NEU2 was usually found in the testis and prostate gland.

Discussion

Sialidase-1 and Sialidase-2 could be two proteins that are possible unintended targets of Oseltamivir, because of their resemblance to the viral NA enzyme as revealed by the BLAST results. The crucial role of Sialidase-1 in the human body is mainly to remove sialic acid residues from glycoproteins and glycolipids (9). Specifically, sialidase-1 hydrolyzes the glycosidic bond between sialic acid and the carbohydrate chain of glycoproteins and glycolipids. This process is necessary to regulate the levels of sialic acid on the cell surface and ensure the appropriate regulation for cell adhesion and signal transduction. Similar to Sialidase-1, Sialidase-2 also plays an essential role in the human body. But unlike Sialidase 1, Sialidase-2 acts specifically on glycolipids, particularly gangliosides, by catalyzing the hydrolysis of gangliosides (9).

The processes that depend on Sialidase-1, or the organs Sialidase-1 is found most common in, are the thyroid and placenta. Furthermore, Sialidase-2 is found to be most common

in the testis and prostate glands. When the influenza virus infects a cell, Oseltamivir tries to inhibit the specific viral NA enzyme of influenza. But, if Sialidase-1 and Sialidase-2 are also in a cell, they could pose as potential targets of Oseltamivir. If the roles of Sialidase-1 and Sialidase-2 are inhibited, the following effects on the human body could be catastrophic (9). For example, there is an uncommon metabolic disorder called sialidosis, which is characterized by a neuraminidase deficiency (10). Although they are found in different parts of the human body, both Sialidase-1 and Sialidase-2 play a role in cleaving sialic acids on cells, so if the role of a sialidase enzyme is removed, sialic acids could accumulate, which can lead to cellular function impairment and overall serious damage depending on the area targeted (9).

In 2011, a study was conducted regarding the effectiveness of Oseltamivir on the NA enzyme, and how scientists could minimize the chance of possible off-target effects Oseltamivir might have (11). The study revealed two key factors about Oseltamivir. Firstly, the recognition of substrates by human NA relies heavily on the importance of the C7-C9 pocket, which is a specific domain in the human NA that plays an important role in substrate binding. Human NA inhibitory activity was found to be limited when compounds containing non-polar groups, such as 3-pentyl, were situated in this area. Secondly, designing drugs that target the recently identified 150-pocket within viral NA enzymes, which is situated near the substrate binding site, presents a significant chance to enhance inhibitor selectivity (11). Certain compounds were effective at inhibiting viral NA, but had no impact on human NA. This observation supports the notion that inhibitors designed for viral NA with non-polar side-chains in the C7-C9 pocket are less likely to affect human NA. In the end, the scientists found that targeting these specific structural parts of the viral NA and human NA significantly reduced the possibility of off-target effects (11).

Conclusion

Oseltamivir is a solid example of a drug with possible unintentional side effects because there is more than just one protein in the human body with a similar structure to the viral NA enzyme. Oseltamivir focuses on inhibiting the viral NA enzyme in the influenza virus, but two sialidase enzymes with a similar structure to viral NA are expressed in all human cells. Because of their similarity to the viral NA enzyme, it is very possible that Oseltamivir can bind to human sialidases and cause unintentional, damaging side effects to the human body. Studies have already shown that exploiting certain parts of the viral NA can lead to a more selective drug that minimizes the chance of off-target effects. Further work should be done to develop drugs selective for viral NA based on the findings described here.

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