HIGH SCHOOL EDITION
Journal of Student Research
Volume 13 Issue 3 (2024)

The Effect of Non-Steroidal Anti-Inflammatory Drugs on Protein Digestion

Shriya Deshpande

Mills E. Godwin High School, USA

ABSTRACT

The purpose of this experiment was to identify and investigate the potential harm caused by the intake of orally administered drugs on the digestion of protein. All around the world, pain relieving drugs such as Advil and Aleve are taken for common infections or mild pain. Continually consuming medication such as non-steroidal anti-inflammatory drugs (NSAIDs) can result in interference with the gastrointestinal (GI) tract, causing dangerous side effects. A total of 400 mg of Ibuprofen or Naproxen were used for each test tube in twenty-five trials. Albumin, a protein made by the liver to contain fluid, was used as a sample of other proteins in the stomach. The recommended initial dose for adults was used to calculate the percentage of protein digested. A positive and negative control were both used for comparison of the results. It was hypothesized that when Naproxen was administered it would exert a higher inhibitory effect on protein digestion. The results showed that Ibuprofen did not interfere with the digestion process compared to Naproxen. According to multiple t-tests, the only trial that resulted in significant data was the positive control versus negative control. In conclusion, the data for the experiment was not statistically significant because only 1 out of 6 t-tests resulted in significant data. It is believed that this was due to the data points maintaining a substantial difference as well as being due to chance. This research could further apply to investigating effects of antibiotics such as penicillin on the liver.

Introduction

Throughout the world, pain relieving drugs such as Advil and Aleve are frequently used without prescriptions for maladies or common infections. However, regularly consuming medication such as non-steroidal anti-inflammatory drugs (NSAIDs) can interfere with the gastrointestinal (GI) tract, causing dangerous side effects. For adults, an initial dose of 400 mg is permitted. In addition, follow-up doses range from 200 – 400 mg every four hours (Blahd Jr., 2021). Contact with NSAIDs or other orally administered medicine, produces a risk for severe bleeding in the GI tract, as well as a higher risk of stroke or heart-related death (Marks., 2015). The significance of this experiment is to identify the potential harm produced by the intake of orally administered drugs on essential body processes.

A substantial amount of sickness and infections existing in today's world have driven the study of non-prescription drugs, otherwise known as NSAIDs. Furthermore, due to the outbreak and growth of Covid-19, and its rapid spread, it has infected around 636 million people worldwide (World Health Organization., 2022). In addition, the numerous strains of Covid-19 such as Omicron and Delta, as well as viruses such as Influenza, continue to decrease the world population. As a result, the usage of non-prescription medicine has increased. Solely in the United States, NSAID usage grown 23% overall between 2017 to 2021 (Da Costa., 2021). The abundant use of orally administered drugs such as NSAIDs could have a detrimental effect on the human body.

Non-steroidal anti-inflammatory drugs are a group of painkillers used to ease pain from mild to moderate ranges. These medications are most commonly used in the world today and do not require a prescription. The variety of NSAIDs include aspirin, ibuprofen, and naproxen. Although acetaminophen is not classified as

an NSAID, its property to reduce pain and fever mirrors an NSAID but excludes its anti-inflammatory properties. Unlike amoxicillin, which is used for bacterial infections, NSAIDs are consumed for immediate relief such as body aches, fever, or swelling (Cleveland Clinic., 2020). The performance of NSAIDs is reliant on the main mechanism of action—the inhibition of the enzyme cyclooxygenase. The enzyme converts arachidonic acid to various types of eicosanoids, such as thromboxane and prostaglandin (Ghlichoo, 2022). Eicosanoids are signaling molecules that contribute to neural facilities comprised of long-term potentiation, synaptic plasticity, accretion of inflammation, and neuroprotective bioactivity (Tassoni., 2008). Due to the lack of eicosanoids, the performance of NSAIDs is enhanced. The independent variables applied to this experiment were Ibuprofen and Naproxen. According to National Health Service (NHS), Ibuprofen and Naproxen were the two most widely used NSAIDs (National Health Service, 2022).

Allowing the experimental group to only cater to a majority of the population provided increasingly accurate results. To compare results, two controls were used: positive and negative. In addition, baking soda was used to illustrate the negative trials. Biuret reagent, an aqueous solution used to test the presence of peptide bonds in a given analyte, provides a negative reaction when it lacks the presence of a protein. When baking soda was added, no protein was existent. Therefore, no reaction occurred. These controls were necessary for the authentication of the independent variable trials.

The dependent variable for this experiment was protein digestion. Digestion of food is the breakdown process for large food particles into smaller, absorbable units required for nutrition. Energy production, body growth, and cellular repair allow the body to function. The digestion of food molecules takes place in numerous parts of the body such as the GI tract, the stomach and the intestines. In the tract, there are two principal forms of digestion, namely mechanical, and chemical. Mechanical digestion is the physical process of breaking down the large particles of food. This degradation allows the digestive enzymes to be accessed for chemical digestion, a process which entails the splitting of proteins, carbohydrates, and lipids, into miniature amino acids, sugars, and fatty acids to enable the process of mastication (Heda., 2022). When a piece of food is consumed and reaches the stomach, a type of enzyme called proteases and hydrochloric acid break down the food into small chains of amino acids (Dix., 2021). Pepsin, another enzyme in the stomach, aids in the disintegration of the protein. In this experiment, pepsin was used as the main enzyme due to its frequency of usage in the body. Protein digestion was measured by a colorimetric assessment. In colorimetric tests, a substance is mediated based on a color, subsequently, a corresponding percentage is calculated or statistical results. Protein digestion allows the body to maintain cell functions, muscle repair, and immune function.

The purpose of this experiment was to identify and investigate the potential harm caused by the intake of orally administered drugs on the digestion of protein. It was hypothesized that when Naproxen was administered it would have a higher inhibitory effect on the protein digestion process. Naproxen was chosen due to its reoccurring gastric irritation and interactions with food. Compared to Ibuprofen, Naproxen is more unreliable from the health perspective. Non-steroidal anti-inflammatory drugs are used to provide mild pain relief. Due to its accessibility, NSAIDs are taken unnecessarily and/or frequently, which can result in the interference of natural body processes. As seen with antibiotic usage, in the GI tract, toxin levels can start to rise. Toxic environments in the GI tract induce enzyme insufficiency, excess mucus production, and structural damage (Gelberg., 2017). The main function of the GI tract is to digest and break down nutrient macromolecules, such as protein, to be absorbed into the systemic circulation of the intestine wall. However, when excessive amounts of medicine are consumed, the myriad of enzymes strengthening the GI tract are disturbed (Murea., 2017).

Procedure

Throughout this experiment, all regular lab safety rules were followed along with precautions. Paper towels were kept nearby and gloves as well as goggles were worn. Two different non-steroidal anti-inflammatory drugs

(NSAIDs) were used, ibuprofen and naproxen. The digestion of an essential nutrient, protein, was being evaluated. By measuring 2.5 mL of pepsin, 2.5 mL of a 0.1 M hydrochloric acid solution, 0.2 mL of biuret reagent, and 0.1 g of albumin powder the positive control was created. This was to prove that the solution was capable of displaying the correct results when the drugs were added. The negative control experiment included 0.1 g of baking soda as well as the previous materials. The negative control demonstrated a condition that would happen if the experiment did not occur correctly. It also produced a situation that should not occur during the experiment or be hypothesized.

To create the solutions containing the drugs, 2.5 mL of pepsin, 2.5 mL of a 0.1 M hydrochloric acid solution, 0.2 mL of biuret reagent, 0.1 g of albumin powder, and 0.4 g of the crushed tablet of the specific drug was processed. Once the albumin power dissolved, the presence of peptide bonds was visible by the color reaction. The reactant and the biuret reagent combined to create a solution which could be assessed by the colorimetric assessment. The colorimetric assessment was used to compare the experimental test tubes with the color which represented the percent of protein digested. This is decided based off the experimenter's gauge of the color in the test tube which could be identified as a source of error. Once the experiment was completed, the test tubes were diluted with water and rinsed out in the sink for a safe disposal method.

Results

The effects of non-steroidal anti-inflammatory drugs (NSAIDs) on protein digestion was studied. The results of the statistical analysis are displayed in table 2 and graph 1. A research hypothesis was created that when Naproxen would be administered, it would have a higher inhibitory effect on protein digestion. The mean for independent variable levels was determined. The average percent of protein digested for Ibuprofen (72%), in comparison with the average of Naproxen (33%), showed that Naproxen had the highest inhibitory effect. Upon further analysis, it was shown that the hypothesis was supported by the data. The percentages of Naproxen being lower than Ibuprofen indicated that they did not interfere with the digestion process. The variance and standard deviation were calculated for the independent variable levels. The standard deviation for Ibuprofen was higher than the other independent variable levels. Although this would indicate outliers, given the high original data points this suggests that the data was in a close and tight group. There were no outliers for the 4 independent variable levels due to all the data points fitting between the SD 2 range.

A t-test was performed on the data at a level of significance of 0.05 and the degrees of freedom at 48. A null hypothesis was formulated that there would be no significant difference between the percent digested for Ibuprofen and Naproxen. The calculated t value for Naproxen versus Ibuprofen (0.716), Ibuprofen versus positive control (0.549), and the negative control test (1.415) were all lower than the critical t value (2.021). For the remaining trials, Naproxen versus the positive control (0.488) and Naproxen versus negative control (0.205) were both lower than the critical t value as well. This implies that the null hypothesis should not be rejected and there is no significant difference between the given comparisons. The probability of the results being due to chance is greater than 0.05 and implies that the results of the experiment are not due to the independent variable. Given the fact that the lower the numerical value of the t-test the more protein digested, these results show that the drugs did not have a significant effect on protein digestion.

Table 1. Statistical Analysis on the Effect of Non-Steroidal Anti-Inflammatory Drugs on Protein Digestion

Descriptive Information	**Estimated percent (%) of protein digestion**			
	Ibuprofen	**Naproxen**	**Positive Control**	**Negative Control**

Mean	72%	33%	97%	6%
Range	72%	60%	30%	25%
Maximum	100%	70%	100%	25%
Minimum	28%	10%	70%	0%
Variance	4.767	2.648	0.411	0.673
Standard Deviation	21.833	16.274	6.411	8.202
SD 1	0 - 22.553	0 – 16.604	0 – 7.381	0 – 8.262
SD 2	0 - 44.386	0 – 32.878	0 – 13.792	0 – 16.464
SD 3	0 - 66.219	0 – 49.152	0 – 20.203	0 – 24.666
Number	25	25	25	25

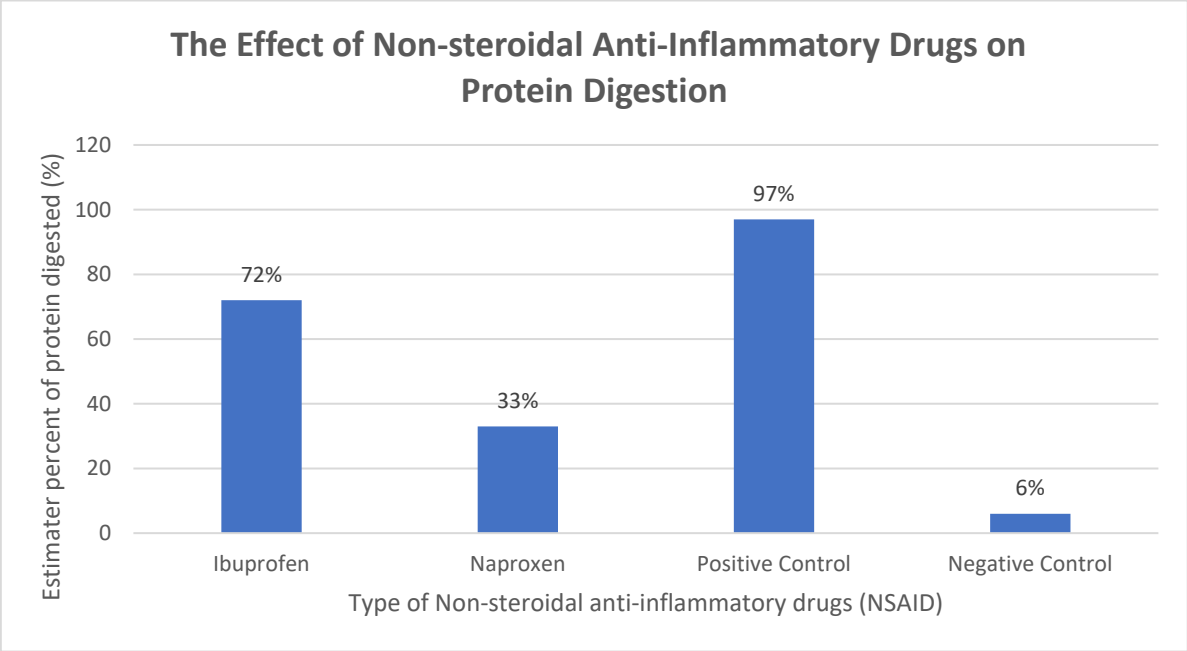
Results of t-test	Ibuprofen vs Naproxen	t= 0.716	p>0.05
	Ibuprofen vs Positive Control	t= 0.549	p>0.05
	Ibuprofen vs Negative Control	t= 1.415	p>0.05
	Naproxen vs Positive Control	t= 0.488	p>0.05
	Naproxen vs Negative Control	t= 0.205	p>0.05
	Positive Control vs Negative Control	t= 4.370	p<0.05
	At df= 48 ; a= 0.05 ; t=2.021 for significance		

The value of the calculated t-test for the positive control versus negative control (4.370) was the only statistical test which proceeded a significant value. This value was greater than the critical t of 2.021 at the degrees of freedom of 48. This means that the null hypothesis should be rejected and there was a significant difference between the trials. The probability of the results being due to chance was less than 0.05 and they are due to solely the independent variable. Overall, the data for non-steroidal anti-inflammatory drugs having an effect on protein digestion is not statistically significant with the exception of the positive control versus negative control trial. This depicts that the data was most likely due to chance revealing that a typical dose of medication does not harm the natural processes of the human body.

Conclusion

The purpose of this experiment was to identify and investigate the potential harm caused by the intake of orally administered drugs on the digestion of protein. In total, 400 mg of Ibuprofen or Naproxen were used for each test tube in twenty-five trials. A biuret test was conducted to identify the estimated percent of protein digestion. Once the albumin was disintegrated, the color of the test tube was noted and compared to a colorimetric assessment chart for a semi-quantitative method of measurement. A research hypothesis was formulated that when Naproxen was administered it would exert a higher inhibitory effect on protein digestion. Furthermore, this hypothesis was proven significant due to the percentages of Naproxen being lower than Ibuprofen.

To find the statistical significance of the results, multiple t-tests were performed. In the experiment, the only trial which was significant was the positive control versus negative control. The reason for the significant result was that neither of the trials were given the drug. This allowed for no outside force impacting the results. Given the substantial gap between the numbers, the product of the t-test was bound to be statistically significant. The remaining trials were all not statistically significant, signifying that the digestion of the protein was not affected by the doses of NSAIDs used.



Graph 1. The Effect of Non-Steroidal Anti-Inflammatory Drugs on Protein Digestion

While used frequently for its anti-inflammatory and analgesic effects, non-steroidal anti-inflammatory drugs (NSAIDs) are responsible for many upper gastrointestinal (GI) peptic ulcer diseases. In an experiment performed in 2017, the effects of oral antibiotics were observed in relation to gastrointestinal (GI) side effects. The antibiotic was delivered using disks placed in the test tubes. The data was evaluated in a semi-quantitative manner, similar to this experiment (Murea., 2017). In another study, protection of the small bowel against NSAID-injury in rats was explored. While the use of NSAIDs is associated with adverse reactions involving the kidneys, liver, and cardiovascular system, the gastrointestinal (GI) tract becomes affected as well. Life-threatening complications such as peptic ulcers and epigastric discomfort can occur when NSAID usage is not controlled (Fornai., 2016).

This experiment has many explanations for the results. The majority of the t-test results were not significant. However, one trial, the positive control versus negative control had statistically significant results. Although the numerical values of the other t-tests were not significant, the results proved a positive outcome. Due to the low values, it was proved that the drugs did not have a standing effect on protein digestion and the recommended doses did not interfere with natural body processes. Nevertheless, during the preliminary testing, when doses too high were used, the percentage decreased. This revealed that the digestion process was being interfered with. As stated in PubMed, “up to 70% of patients with long-term NSAID ingestion have endoscopic abnormalities such as ulceration and subepithelial hemorrhage (Tai., 2021).” This shows that continuous usage of NSAIDs can disturb normal processes in the body which can lead to severe complications.

In this experiment, there were numerous sources of error as well as limitations that could have been improved for a more successful experiment. The semi-quantitative manner of measurement restricted the numerical values as the results were estimated based on the colorimetric assessment chart. This could have been improved by measuring the concentration of amino acids or peptide bonds in the solutions. Another source of error would have been that the solution after albumin was added was kept too long before collecting results. With this error the results would have been delayed, causing the previous data to be overlooked. For future study, the investigation of the way antibiotics such as penicillin interact with different parts of the body could be studied. Furthermore, this would allow insight into prescription medication impacting digestion processes.

Acknowledgments

I would like to thank my advisor for the valuable insight provided to me on this topic.

References

Da Costa, B. (2021). Effectiveness and safety of non-steroidal anti-inflammatory drugs and opioid treatment for knee and hip osteoarthritis: network meta-analysis. TheBmj, 375: 2321. Retrieved November 26, 2022, from doi: 10.1136/bmj.n2321

Fornai, M. (2016). Small bowel protection against NSAID-injury in rats: Effect of 6ifaximin, a poorly absorbed, GI targeted, antibiotic. Science Direct, 104: 186-196. Retrieved January 3, 2023, from doi: 10.1016/j.phrs.2015.12.031

Gelberg, H. (2017). Pathophysiological Mechanisms of Gastrointestinal Toxicity. National Library of Medicine, 139-178. Retrieved November 26, 2022, from doi: 10.1016/B978-0-12-801238-3.10923-7

Ghlichloo, I. (2022). Nonsteroidal Anti-inflammatory Drugs (NSAIDs). National Library of Medicine. Retrieved November 26, 2022, from https://www.ncbi.nlm.nih.gov/books/NBK547742/

Heda, E. (2022). Physiology, Pepsin. National Library of Medicine. Retrieved November 26, 2022, from https://www.ncbi.nlm.nih.gov/books/NBK537005/

Maseda, D. (2020). NSAID – Gut Microbiota Interactions. National Library of Medicine, 11: 1153. Retrieved November 26, 2022, from doi: 10.3389/fphar.2020.01153

Murea, E. (2017). The Effect of Antibiotics on Nutrient Digestion. Journal of Emerging Investigators. Retrieved July 29, 2022, from https://emerginginvestigators.org/articles/the-effects-of-antibiotics-on-nutrient-digestion/pdf

Tai, F. (2021). Non-steroidal anti-inflammatory drugs and the gastrointestinal tract. National Library of Medicine. 21 (2): 131-134. Retrieved January 4, 2023, from doi: 10.7861/clinmed.2021-0039

Tassoni, D. (2008). The role of eicosanoids in the brain. Asia Pac J Clin Nutr. 17 Suppl 1:220-8. National Library of Medicine. Retrieved November 26, 2022, from https://pubmed.ncbi.nlm.nih.gov/18296342/