Biofarmasetics and Pharmacokinetics Study Analysis of Paracetamol Excretion Through Urine and Saliva

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Abstract

The purpose of this study was to determine that Paracetamol, also known as acetaminophen, is a commonly used analgesic and antipyretic drug to relieve pain and reduce fever. The process of metabolism and elimination of paracetamol from the body is important to understand, especially in the context of the safe use of this drug. This study aims to analyze the biopharmaceutics and pharmacokinetics studies of paracetamol excretion through urine and saliva as important indicators in understanding the metabolic and elimination processes of this drug. Paracetamol is rapidly and efficiently absorbed through the gastrointestinal tract, reaches the highest concentration in plasma in a short time, and is dispersed throughout the body fluids. The metabolic process of paracetamol mainly occurs in the liver, through two main phases: oxidation and conjugation with glucuronic and sulfuric acids. Excretion of drugs and their metabolites can occur through urine and saliva, with the kidney as the main organ in the excretion process. Qualitative analytical methods were used in this study to identify paracetamol metabolite compounds in urine and saliva samples and to evaluate the metabolic pathways involved. Glucoronide, sulfate, and phenol conjugate assays were used to detect the presence of paracetamol metabolites in the samples. The assay results showed that urine was more effective in excreting paracetamol compared to saliva. In conclusion, this study provides a better understanding of the metabolic process and elimination of paracetamol from the human body through the analysis of biopharmaceutics and pharmacokinetics studies. These results are important for understanding the therapeutic effects and toxicity of paracetamol as well as for developing more effective and safe drug use management strategies.

Keywords: Paracetamol, excretion, urine and saliva.

1. INTRODUCTION

Paracetamol, also known as acetaminophen, is often used as a painkiller or fever reducer. Paracetamol has analgesic and antipyretic activity, with little anti-inflammatory activity. Paracetamol has the same mechanism of action as aspirin, which inhibits prostaglandin synthesis. The analgesic effect of paracetamol is to eliminate or reduce mild to moderate pain. Paracetamol lowers body temperature by a mechanism thought to be based on central effects. Its anti-inflammatory effect is very weak, therefore paracetamol is not used as an antireumatic.

Paracetamol is absorbed quickly and completely through the gastrointestinal tract. The highest concentration in plasma is reached within half an hour and the plasma half-life is

between 1-3 hours. The drug is dispersed throughout the body fluids. Elimination of a drug can occur through biotransformation (metabolism) or excretion or excretion or a combination of both. Metabolism is the overall chemical reaction to transform both endogenous substances and exogenous substances that occur enzymatically. Drug metabolism has the basic purpose of changing the drug from active to ineffective from less polar to polar so that it can be easily excreted through urine.

Most metabolism occurs in the liver, although it can also occur in the skin, tissues, lungs, gastrointestinal tract and kidneys. Drug excretion is the final elimination of a drug or metabolite from systemic circulation through the kidneys with urine, through bile and saliva into the intestines with feces, through sweat, through the skin and breast milk. Drugs that are poorly soluble in water are difficult to be excreted through the above routes. They are first metabolized into polar form and then excreted.

Analysis of biopharmaceutics and pharmacokinetics studies on paracetamol excretion through urine and saliva is important in understanding the metabolism and elimination of this drug from the human body. Paracetamol, also known as acetaminophen, is one of the most commonly used analgesic (pain relieving) and antipyretic (fever-reducing) drugs worldwide. Although paracetamol is considered a relatively safe drug in recommended doses, overdose can cause serious liver damage and can even be fatal.

Biopharmaceutics and pharmacokinetics studies help in evaluating the effectiveness of treatment with paracetamol. By understanding how paracetamol is absorbed, distributed and excreted, researchers and medical practitioners can determine whether the administered dose has reached the desired therapeutic concentration. Each individual can have differences in response to paracetamol based on factors such as metabolism, body weight, or certain medical conditions. Through the study of biopharmaceutics and pharmacokinetics, it may be possible to develop individualized pharmacokinetic profiles that allow for more precise treatment tailored to each patient's needs. Physiological variations such as age, gender or certain health conditions may affect the way the body absorbs, distributes and excretes paracetamol. Biopharmaceutics and pharmacokinetics analysis helps in understanding how these factors affect the use and effects of the drug, and helps in determining the appropriate dosage. Pharmacokinetic studies also make it possible to evaluate drug interactions, especially if the patient is taking more than one drug simultaneously. This study can reveal whether there is a potential increase or decrease in the effects of paracetamol when combined with other

medications. Analysis of paracetamol in urine and saliva requires sensitive and specific analytical methods. This study may assist in the development of more sophisticated and efficient analytical methods to detect and quantify paracetamol concentrations with high accuracy, which is important for overdose diagnosis or therapeutic monitoring. Biopharmaceutics and pharmacokinetics studies should also pay attention to ethical and privacy aspects. Collection of urine and saliva samples from research subjects requires appropriate consent and adequate privacy protection to ensure compliance with research ethics and individual rights.

Importance of Concentration Measurement: The concentration of paracetamol in urine and saliva is a direct reflection of the amount of drug excreted from the body. Therefore, analyzing the concentration of paracetamol in these two fluids is important to understand how efficient the body is in excreting the drug. This information is important for evaluating the metabolism and elimination of drugs from the body.

Measurement of paracetamol concentration in urine and saliva is also used as a tool to detect overconsumption or overdose of the drug. Paracetamol overdose can cause serious or even fatal liver damage, therefore, it is important to be able to detect overdose as quickly as possible to provide timely medical intervention. Measurement of paracetamol excretion through urine and saliva also helps in understanding individual variability in drug elimination. Individuals can vary in their ability to excrete paracetamol from the body, which can be influenced by factors such as metabolism, kidney function, or genetic factors. Understanding this variability is important for determining the correct dosage and for accounting for individualized factors in medication management. Certain physiological and pathological conditions may affect paracetamol excretion through urine and saliva. For example, impaired renal function may slow down the elimination of the drug, while conditions such as asamuria (a condition where urine has a low pH) may affect the formation and excretion of paracetamol metabolites. Therefore, analysis of biopharmaceutics and pharmacokinetics helps in understanding how a person's health condition may affect drug processing and elimination. Paracetamol concentrations in urine and saliva can also serve as biomarkers to evaluate drug exposure and monitor response to therapy. In the context of long-term use of paracetamol, monitoring concentrations in urine and saliva may provide insights into patient adherence to treatment and effectiveness of therapy. The characteristics of paracetamol excretion in urine and saliva, such as excretion rate and time-dependent profile, also provide additional

information on the drug elimination mechanism. For example, slow or unstable excretion patterns may indicate the presence of metabolic problems or disturbances in the excretory system.

2. METHODS

This study is a qualitative study. Research analysis of biopharmaceutics and pharmacokinetics studies on paracetamol excretion through urine and saliva, qualitative methods can be used to provide a deeper understanding of the metabolic process and elimination of this drug from the body. Qualitative methods can be used to identify paracetamol metabolite compounds present in urine and saliva samples. Techniques such as liquid chromatography-gas chromatography (KCKG) with a mass detector or tandem mass spectrometry (MS/MS) can be used to separate and identify metabolite compounds resulting from the metabolism of paracetamol. researchers can identify the metabolic pathways involved in the breakdown of paracetamol into its metabolites. For example, the use of mass spectrometry techniques can help in identifying key metabolic pathways, such as conjugation with glucuronic or sulfuric acids, as well as the formation of toxic metabolites such as N-acetyl-p-benzoquinone imine (NAPQI). Qualitative methods can also be used to evaluate possible drug interactions that may affect paracetamol metabolism. By comparing metabolite profiles in urine and saliva samples from individuals taking paracetamol alone with those taking paracetamol together with other drugs, researchers can identify differences in metabolic patterns suggestive of drug interactions. Qualitative analysis can help in evaluating potential biomarkers associated with paracetamol use. This research can find out if there are certain compounds in urine and saliva samples that can be used as indicators of exposure or therapeutic effects of paracetamol. Qualitative methods are also important in the validation of analytical methods used in research. This involves testing the methods to ensure the accuracy, repeatability and reliability of the results obtained. Validation of qualitative analytical methods ensures that the data generated is reliable and relevant for interpretation. In addition to compound identification and metabolic pathways, qualitative methods can also be used to qualitatively analyze data. This involves a deeper understanding of the patterns and trends observed in the metabolite profiles of paracetamol in urine and saliva samples, as well as interpretation of the clinical significance of the findings.

Sample

a. Each group had 2 volunteers who were assigned the day before the experiment.
b. On the day of the experiment, the volunteers drank 2 glasses of water 2 hours before the experiment.
c. Before the drug is taken the bladder is emptied and urine is collected for qualitative analysis.
d. Each volunteer only takes 1 type of drug with the help of 250 cc of water. Urine samples are taken every 30 minutes for 3 hours and saliva samples are taken every 15 minutes for 90 minutes. Perform qualitative tests on each sample in the same way as in point.
e. Qualitative test results are expressed with - (negative) and + (positive) signs.
f. Based on the above results, make a table of sampling time and qualitative test results.

Test for Glucoronide conjugates

0.5 mL urine/saliva 2 mg solid naphthoresinol and 1 mL concentrated HCL. Heat for 3 min and wait to cool. Add 3 mL of ethyl acetate and then shake homogeneously until a purple color forms on the organic layer. Positive if a purple color forms on the organic layer.

Barium Chloride assay for sulfate conjugate

Set the ph / urine 0.5 mL until it is in the range of 4-6. Add 2% BaCl2 as many drops into a test tube containing 0.5 mL of concentrated HCl and then boil in a fume hood for 3 minutes. Positive if a yellowish precipitate forms.

Iron (III) Chloride test for phenols

Set the ph of urine / saliva to 7. add 3 drops of fecl3 2%. positive if the color changes brownish yellow.

3. RESULTS AND DISCUSSION

Results

Urine data (minutes)	Conjugate test glucoronide	Sulfate conjugate assay	Phenol test
Urine before	Yellow turbidity precipitate (-)	No sediment (-)	Brownish yellow (+)
PCT 30 menit	Yellow turbidity	No sediment (-)	Clear (-)

	precipitate (-)		
PCT 60 menit	Yellow turbidity precipitate (-)	No sediment (-)	Clear (-)
PCT 90 menit	Yellow turbidity precipitate (-)	There is sediment turbidity	Brownish yellow (+)
PCT 120 menit	Yellow turbidity precipitate (-)	There is sediment turbidity	Brownish yellow (+)

Discussion

Drugs are generally in the form of a xenobiotic (foreign substance) for the body that is unwanted because it can damage cells and interfere with body functions. Therefore, the body will make changes to the chemical structure of the drug (biotransformation or metabolism) into metabolites that are hydrophilic. This will facilitate the excretion process by the kidneys that excrete drug metabolites through urine.

Paracetamol as a p-aminophenol derivative drug that has properties as an analgesic and antipyretic. Paracetamol has the main function as a medicine to reduce body heat caused by infection or other causes. Paracetamol can also be used to relieve pain symptoms with mild to moderate intensity. Paracetamol works by affecting the pain threshold by inhibiting the cyclooxygenase (COX) enzyme involved in the formation of pain mediators (prostaglandins). Paracetamol is safe to consume in standard doses but because it is easily available there are often cases of intentional or accidental paracetamol overdose. Paracetamol is absorbed quickly and completely through the gastrointestinal tract. The highest concentration in plasma is reached within half an hour and the plasma half-life is between 1-3 hours. The drug is dispersed throughout the body fluids. Binding of this drug to plasma proteins is variable, only 20%-50% may be bound at concentrations found during acute intoxication. After a therapeutic dose, 90%-100% of the drug is found in the urine during the first day, mainly after hepatic conjugation with glucoronic acid (about 60%), sulfuric acid (about 35%) or cysteine (about 3%), small amounts of hydroxylated and deacetylated metabolites have also been detected.

Paracetamol is rapidly absorbed through the small intestine, therefore anything that affects the speed of gastric emptying can also affect the absorption effectiveness of paracetamol. The presence of food in the stomach will slightly slow down the absorption of paracetamol preparations. This also greatly affects the results of the experiment because slow

absorption makes the presence of paracetamol longer in the body. This also has an impact on the metabolic process which becomes a little slow and not as it should be (in the absence of food).

Elimination of a drug can occur through biotransformation (metabolism) or excretion or a combination of both. Metabolism is a whole host of chemical reactions transforming both endogenous substances and exogenous substances that occur enzymatically. Drug metabolism has the basic goal of changing the drug from active to ineffective from less polar to polar so that it can be easily excreted through urine. Most metabolic processes occur in the liver, although they can also occur in the skin, tissues, lungs, gastrointestinal tract and kidneys.

Drug excretion is the final elimination of the drug or metabolites from the systemic circulation through the kidneys with urine, through bile and saliva into the intestines with feces, through sweat, through the skin and breast milk. Drugs that are less soluble in water, difficult to be excreted through the above pathways, they are first metabolized so that they turn into polar forms and then excreted. The kidney is the most important organ for excretion of drugs and their metabolites. There are three renal excretion mechanisms, namely glomerular filtration, tubular active secretion, and tubular reabsorption.

Urine is a residual fluid excreted by the kidneys which will then be removed from the body through the process of urination. Urine excretion is basically necessary to remove residual molecules in the blood filtered by the kidneys and to maintain body fluid homeostasis. Saliva is an exocrine secretory fluid in the mouth that comes into contact with the mucosa and teeth, originating mainly from three pairs of major salivary glands and minor salivary glands on the oral mucosa. Drug levels in saliva are similar to free drug levels in plasma, so saliva can be used to measure drug levels. urine pH affects drug excretion. urine pH varies from 4.5 to 8. Acidic urine increases the elimination of drugs that are weakly alkaline.

Urine tests have long been done and are often performed because samples are easy to obtain and the test technique is not so difficult. Urine tests (urinalysis) aim to show the presence of substances that are not normally present in the urine or show changes in the levels of substances that are normally present in the urine. Saliva can provide signs of disease and metabolic status. Saliva reflects the host's response to various disease developments.

The naphthoresinol assay is performed to test for the presence of acetaminophen glucoronide conjugates in urine samples. Naphthoresinol will react with acetaminophen glucoronide conjugate to produce a complex compound characterized by a purple coating. The test results obtained in the urine sample from the initial minute to minute 120 did not contain acetaminophen glucoronide conjugate which was marked by a yellow color change and no purple layer was formed.

The naphthoresinol test was conducted to test for the presence of acetaminophen glucuronide conjugates in urine and saliva samples. Naphthoresinol will react with acetaminophen glucuronide conjugate to produce a complex compound characterized by a purple layer. The test results obtained in saliva samples from the 15th minute to the 90th minute did not contain acetaminophen glucuronide conjugate which was indicated by the absence of a purple layer. This color comes from the reaction of complex compound formation between naphthoresinol and glucoronide from the sample. Conjugation with glucoronic acid or glucoronidation is a common way of conjugation in metabolic processes. The drug undergoes this process with the help of the enzyme UDP-glycoronitranferase. Sulfate conjugation to increase the solubility of the compound in water and make the compound non-toxic. Conjugation using glutatin compounds from glutathione conjugate plays a crucial role in the detoxification process of reactive electrophile compounds. Kidneys are used to excrete metabolic waste in the form of urine. The process of urine formation goes through three stages including filtration, reabsorption and secretion.

The barium chloride test aims to determine the presence of acetaminophen sulfate conjugate in the sample. Positive results are characterized by the formation of a precipitate or cloudy solution resulting from the reaction between the sulfate group in the sample with barium ions from the reagent to produce insoluble compounds. This is because when urine and saliva samples in which there are sulfate groups (SO_4^{2-}) will bind to $BaCl_2$ to form $BaSO_4$ (barium sulfate).

The iron (III) chloride test was conducted to identify the presence of unmetabolized paracetamol (intact paracetamol) in the sample. Positive results are indicated by the presence of a brownish yellow color in the tested sample which comes from the complexation reaction between ferric ions (Fe^{3+}) from the reagent with phenol groups on intact paracetamol. The test results obtained from urine samples at the 90th minute to the 180th minute contained phenol

groups from paracetamol or in other words, paracetamol was only slightly metabolized into its conjugates.

Positive results in the phenol test indicate metabolic disorders in the body. Phenolic compounds are dangerous pollutants with a high level of toxicity. This phenol compound is toxic, this toxic compound should conjugate with glutaion or GSH in the body which can turn the compound into a non-toxic one. GSH is an antioxidant compound found in the body. Failure to synthesize with GSH can be caused by the influence of free radicals that interfere with the body's metabolism.

The normal metabolism that should occur when someone takes paracetamol is that paracetamol undergoes biotransfomation (metabolism), most of which about 90% is conjugated with glucuronic acid to form acetaminophen glucuronide and a small portion with sulfuric acid to form acetaminophen sulfate. The remaining 10%, and 5% of them through the oxidation pathway by cytochrome P-450 form toxic or toxic metabolites in the form of NAPQI (N-acetyl-p-benzoquinone imine) and (3-5%) will be excreted from the body in the form of intact paracetamol.

4. CONCLUSION

The metabolic process of paracetamol occurs through two phases, namely phase by oxidation and phase II by glucuronidation and sulfation pathways. The process of excretion of paracetamol based on the test results in urine samples is more effective than excretion in saliva samples. The content of paracetamol in urine and saliva can be done by glucoronide conjugate test, barium sulfate test for sulfate conjugate, and iron (III) chloride test for phenol. The glucoronide test shows positive results with the formation of purple clouds, the barium chloride test with the formation of a precipitate, while the phenol test with the formation of brownish yellow warrants. The metabolic products of paracetamol are acetaminophen glucuronide, acetaminophen sulfate and NAPQI (N-acetyl-p-benzoquinone imine).

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