In vitro – In vivo Correlation Study of Colon-targeted Metronidazole Microparticle in Corncob Hemicellulose Capsule

Gabena Indrayani Dalimunthe[1] and Ida Fauziah[2]
[1]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Universitas Muslim Nusantara Al Washliyah, Medan, Indonesia*
[2]*Department of Biology, Faculty of Science and Technology, Universitas Medan Area, Medan, Indonesia*

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Abstract: Colon-targeted therapy requires a strategy to keep the medicine pass the stomach and release in the colon, which is hard to reach by conventional dosage form of metronidazole in gelatin capsule. In this study, microparticles dosage form of metronidazole which was placed inside corncob-hemicellulose capsule shell need to be compared with conventionally gelatin capsule of metronidazole preparation to examine whether it meets medications standards. The aim of this study was to find out the in vitro and in vivo assay correlation of metronidazole microparticle which covered by corncob hemicellulose capsules. In vitro test was carried out to observe the profile of differences in the percent release of metronidazole from various formulations with various media and times. It was performed using a dissolution tester, in an artificial stomach medium of pH 1.2 for 6 hours in artificial intestinal medium of pH 7.4 for 10 hours, and in artificial colonic medium of pH 8 for 10 hours. The in vivo test design was conducted using six rabbits. The drug released in plasma was measured by HPLC using 1% glacial acetic acid solvent in aqua bidest and methanol-water with a ratio of 80: 20. The test was performed using the cross over design method. Metronidazole microparticle capsules were administered orally according to the test design (metronidazole in microparticles and metronidazole in conventional forms). Based on the plotted graph (data retrieval was started from the drug released in the colon because the drug began to be absorbed at that time), a correlation value was obtained ($R^2 = 0.8785$). It can be stated that there is a correlation between the formulations tested in vitro and in vivo because the correlation value was greater than 0.8, it is assumed to have a correlation.

1 INTRODUCTION

Gelatin capsule has been used for decades in various therapy as well as for gastrointestinal diseases. The emerging problems are including the enzymatic reactions in the upper gastrointestinal tract which induce premature release of medicine before it reaches the colon (Nicholas et al. 2011, Lee et al. 2020). Colon Drug Delivery Systems (CDDS) has become a focus in in drug research and development resulting in modifications of capsules by using animal and plant origins material in order to improve drug bioavailability in the colon (Amidon et al. 2015, Oladzadabbasabadi et al. 2017, Yang et al. 2020). Plant products has become more preferable in recent years in contrast with products that are made from animal origin material which could be due to numerous reasons including consumers views and beliefs (Cliceri et al. 2018).

The phenomenon has no exception for medications. Therefore, rapid drug development is required to enhance efficacy and fit consumers and patient's preference. In vitro and in vivo investigations are paramount in drug development to confirm the effectiveness which were carried out by examining the relationship between dissolution and bioavailability, resulting in the concept of in vitro in vivo correlation. In the last few years, the concept and application of in-vitro-in vivo correlation for pharmaceutical dosage forms has become a major focus of attention of the pharmaceutical industry, academics and the regulatory sector (Maity et al. 2016).

From a biopharmaceutical perspective, in vitro-in vivo (IV-IVC) correlation is a mathematical prediction that describes the relationship between the in vitro nature of the dosage form (drug release rate) and the relevant in vivo response (plasma drug

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concentration, urine, amount of drug absorbed). IVIVC is a tool for the development of drug dosage forms, since IVIVC can assist in the selection of drug formulations with suitable and acceptable dissolution criteria, these predictions can be used as estimates or substitutes for further bioequivalence studies (Emami, 2006; Qiu and Duan, 2017)

The in vitro test was carried out to find out the profile of differences in the percent release of metronidazole from various formulas with various mediums and times with a stirring speed of 100 rpm, medium volume of 900 ml at 370.5°. Meanwhile, in vivo testing is a test that is carried out using experimental animals to determine the metabolism of a compound in the body. Animals used in in vivo experiments must be from mammals, because the results can be applied to humans (Chow et al. 2003).

2 MATERIALS AND METHOD

2.1 Materials

Metronidazol (E Merck), NaOH 0.1 N, (E Merck), $CaCl_2$ (E Merk), KH_2PO_4 (E Merk), HCl (E Merck), NaOCl 5% (E Merk), talcum (Yuanfen), asam asetate glasial (E Merck), trichoroasetat acid (TCA) 20% , Alkohol 96%, heparin, Metanol for HPLC (E.Merck), Aquabidestilata (PT. Ikapharmando Putramas), Metronidazol BPFI (Badan POM RI) metronidazole tablet (indofarma), all of materials used in the study are in pro analysis standard (Ahuja, et al, 2005).

Spectrophotometer (Shimadzu UV-1800), disintegration tester (Erweka), disolution tester, HPLC (Agilent 1120 Compact LC), Colom ODS C-18, solvent container (oberol), vial (agilent), animal box, vakum pump (Gast DO), sonicator (branson), paper membrane filter *cellulosa nitrate* 0,45 µm (whatman), paper membrane filter nylon 0.45 µm (whatman), PTFE 02 µm (whatman) (Muchlisyam, 2014).

2.2 Dissolution Test

Dissolution test was performed using a dissolution apparatus type 2 (paddle), with 900 mL medium pH 1.2, pH 7.4and pH 8 and temperature of 37 ± 0.5 ° C with a rotation speed of 100 rpm. At certain time intervals of 5, 15, and 30 minutes until 600 minuts, the sample solution was taken 5 mL and measured at a wavelength of 320 nm (United State Pharmacopeial convention. 2008; USP, 2009).

2.3 Animal Experiment

Animal test used in this study were male rabbits weighing 1.5-2 kg, which has been conditioned to the environment and feeded for 1 week with kale and carrots during the study. Blood sampling time is 10 minutes after drug administration.

2.4 Plasma Preparation

Rabbits were fasted at least 8 hours prior to the experiment. Weighed and cleaned fur ears clean. The blood was taken from 2 male rabbits approximately 5 ml each, divided into 4 tubes which had contained 2 drops of heparin, added 2 ml TCA 20%, then centrifuged at 3000 rpm for 10 minutes. Each supernatant was taken and used as a blanko and a calibration curve (Kemenkes RI, 2014).

2.5 *In vivo* Test

The test was conducted using six rabbits. The administration of metronidazol rabbits with this method can be seen in Table 1.

Table 1: Microparticle Metronidazole and conventional metronidazole capsules were administered to rabbits using the cross-over design method (Chilukuri, et al. 2007).

Treatment I		2 Weeks break	**Treatment II**	
Rabbit	**Dosage Form**		**Rabbit**	**Dosage Form**
1	A		1	B
2	A		2	B
3	A		3	B
4	B		4	A
5	B		5	A
6	B		6	A

A= Metronidazole microcapsule capsules, B = Metronidazole capsules (conventional)

At first, the rabbits were fasted for about 12 hours, then the metronidazole microparticles and the conventional metronidazole capsules were administered orally, the dosage forms were administered based on the procedure according to Table 3.3. Furthermore, rabbit blood was taken through the marginal vein at certain time intervals, namely: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15, hours using a 1.0 mL syringe. The syringe was initially rinsed with heparin. The blood was put into a centrifuge tube containing 2 drops of heparin. Then 1.0 mL of 20% TCA was added to the tube and vortexed until homogeneous. The tube was put into a centrifuge and centrifuge at 3000 rpm for 10 minutes and the supernatant was taken. Each supernatant was filtered using a 0.2 µm PTFE filter membrane and the levels were measured using a HPLC device by injecting 10 µL of supernatant (Ahuja et al, 2005 and Kemenkes RI. 2014).

Rabbits were fasted for 12 hours before orally administered with FCL-6, the design could be in the Table 1. After shaving the hair around rabbits ears, the blood was taken through the marginal vein at specified time intervals are: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 dan 15, hours using 1.0 mL syringe. Syringes rinsed beforehand with heparin. Blood inserted into the centrifuge tube which already contains 2 drops of heparin. Then TCA 20% as much as 1.0 mL tubes were added and shaked using vortex apparatus until homogeneous. The tube was centrifuged at 3000 rpm for 10 minutes and the supernatants were collected. Each supernatant was filtered using a 0.2 µm PTFE membrane filter and the metochlopramide concentrations measured using HPLC instrument by injecting as much as 10 uL supernatant (Kemenkes RI, 2014).

2.6 Correlation of *In vitro* and *In vivo*

Correlation of in vitro and in vivo was determined by using a level A correlation that explains the relationship between the rate of drug release (% cumulative drug apart) in vitro and speed of drug release in vivo (plasma drug concentration).

2.7 Analysis of Blood Plasma Level

Rabbits that have been granted in accordance with the oral drug bioequivalence trial design that can be seen in Table 1. At intervals; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 dan 15, hours, rabbit blood drawn with the help of 1.0 mL syringe that has been rinsed with heparin, was transferred to a centrifuge tube which already contains heparin, and add 2 drops of 20% TCA 1 mL, centrifuged at 3000 rpm for 10 min, the supernatant was taken, filtered with a 0.2 µm PTFE membrane filter and assayed using HPLC.

3 RESULTS AND DISCUSSION

3.1 Correlation Test

Chilukuri, et al. (2007) stated the correlation between *in vitro* assay with *in vivo* assay can be explained by using the correlation IVIVC level A which is a relation between the cumulative percent of the drug released of in vitro assay and the percent amount of absorbed drug in blood plasma of in vivo assay. The release of metronidazol mikroparticle and konventional metronidazol for the *in vitro test.*

In vitro release of metronidazole and the average level of metronidazole absorbed in plasma (in vivo) can be seen in Figure 1. The presence of metronidazole measured in artificial colon and in the plasma showed a sustain release pattern. The optimal in vivo measurement was depicted at 7 hours after administration.

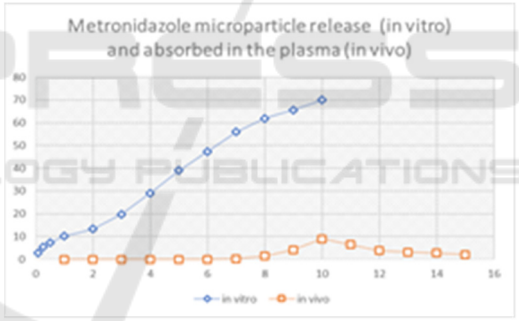

Figure 1: Cumulative percent of metronidazole microparticles (in vitro) and average percent of metronidazole microparticles in plasma (in vivo).

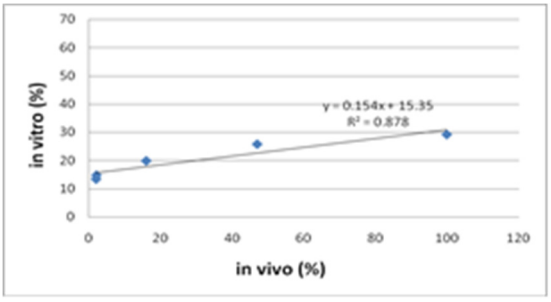

Figure 2: In vitro and in vivo correlation of metronidazole microparticles.

Based on the plotted graph (data retrieval was started from the drug released in the colon because the drug began to be absorbed at that time), a correlation value was obtained (R^2 = 0.8785). It can be stated that there is a correlation between the formulations tested in vitro and in vivo because the correlation value was greater than 0.8, it is assumed to have a correlation (Dalimunthe et al. 2019, Shargel 1988).

The in vivo test was carried out for 15 hours, but the new drug was released at 7 hours, which was about 0.1888 µg / ml, this means that it can be proven that the capsule is not destroyed in the in vivo test either in the stomach or in the intestine, but the intestines have begun to expand, and you can see that the drug begins to release and there is an increase in the percentage of drug release at 8 hours.

Based on the reaction kinetics, the in vitro test (dissolution) shows that the drug follows order 1, order zero, and higuchi but it tended to be order zero because the release is relatively constant.

4 CONCLUSION

Corncob hemicellulose capsule considered as altenate carrier for colon-targeted drug, it was recorded that the drug release was occurred at pH 8 and 7 hours post oral administration. Therefore, it has distinctive properties with conventional gelatin capsule which is vulnerable to stomach acidic conditions. In vitro in vitro correlation (IVIVC) study also exhibites a strong relationship which was indicated by the value of R^2 = 0.8785. It demonstrated that in vitro dissolution test of metronidazole microparticle in corncob hemicellulose capsule is of high relevance for in vivo assay.

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