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Research Article

# Bioequivalence Study of Molnupiravir 200 mg Capsules in Healthy Thai Volunteers

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## ABSTRACT

The antivirals inhibiting RNA-Dependent RNA Polymerase (RdRp) enzyme seem to be the most effective therapeutic solution for a Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Molnupiravir is a prodrug of N-Hydroxycytidine (NHC) which is subsequently phosphorylated to NHC triphosphate, thereby targeting RdRp and inducing error catastrophe. In response to the treatment demands within the national public health system during the COVID-19 pandemic, the Government Pharmaceutical Organization (GPO), Thailand had developed a generic product of molnupiravir 200 mg capsules. An open label, randomized, two-treatment, two-period, two-sequence, single oral dose, crossover study was designed to determine the bioequivalence of two molnupiravir 200 mg capsule formulations, MONOVIR® and LAGEVRIO® under fasting conditions. The plasma-concentration time profiles of NHC were used to characterize the rate and extent of absorption of molnupiravir as the parent compound was rapidly converted in plasma. The pharmacokinetics parameters were calculated using non-compartmental model. The 90% confidence intervals of geometric least squares mean ratio (test/reference) for ln-transformed parameters were within 80.00%-125.00% of bioequivalence criteria: 103.74%-111.46% for $AUC_{0\text{-tlast}}$, 103.73%-111.4% for $AUC_{0\text{-}\infty}$ and 101.98%-110.19% for $C_{max}$. Both products were well tolerated and no serious adverse events were reported. This study demonstrated bioequivalence between MONOVIR® and LAGEVRIO® supporting the interchangeability between these products.

**Keywords:** Molnupiravir; Bioequivalence; Pharmacokinetics; Antiviral; COVID-19

## INTRODUCTION

A coronavirus disease 2019 (COVID-19) is an infectious disease caused by a Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Since this disease has become a worldwide pandemic, several attempts have been made to find effective antiviral agents against SARS-CoV-2. The antivirals inhibiting RNA-Dependent RNA Polymerase (RdRp) enzyme seem to be the most effective therapeutic solution [1]. Molnupiravir is a prodrug of N-hydroxycytidine (NHC) (Figure 1), which has the activity against SARS-CoV-2 and other RNA viruses [2]. NHC is a ribonucleoside analogue which is subsequently phosphorylated to NHC triphosphate, thereby targeting RdRp and inducing error catastrophe [3]. The clinical studies demonstrated that molnupiravir was safe and tolerable for short-term use. At the dose of 800 mg twice daily, time to clearance of viral RNA and viral isolation were significantly reduced when compared to placebo [4]. Phase III clinical study also revealed that molnupiravir lowered the risk of hospitalization and death in mild to moderate COVID-19 patients [5]. Therefore, molnupiravir have been given an Emergency Use Authorization (EUA) for COVID-19 in Thailand, like in many other countries [6].


**Figure 1:** The structures of Molnupiravir and N-Hydroxycytidine (NHC).

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Following oral administration, molnupiravir was rapidly converted to NHC in plasma and the maximum concentration of NHC was achieved within an hour after dosing. There was also a trend of delayed $t_{max}$ with the increased dose. The extent of absorption between the solution and capsule dosage form was similar whereas the rate of absorption was slightly slower for the capsule formulation. There was no food effect on the extent of absorption of molnupiravir, thus it can be taken with or without food. The elimination half-life of NHC was between 0.91 and 1.29 hours for the dose range of 50-800 mg while the mean elimination half-life was increased with the higher dose. Following multiple dosing, no significant accumulation was observed [7].

In response to the treatment demands within the national public health system during the COVID-19 pandemic, the Government Pharmaceutical Organization (GPO), Thailand had developed a generic product of molnupiravir 200 mg capsules. The bioequivalence study was conducted to demonstrate the equivalence in biopharmaceutics quality between a generic product, MONOVIR® and a reference product, LAGEVRIO®.

## MATERIALS AND METHODS

### Study products

The test product used in this study was MONOVIR®, molnupiravir 200 mg capsules bearing lot no. S655045 manufactured by the Government Pharmaceutical Organization (GPO), Thailand. The reference product used in this study was LAGEVRIO® bearing lot no. 2568751 manufactured by MSD International GmbH (Peurto Rico Branch) LLC, United States of America.

### Study subjects

The sample size was calculated by considering the maximum intra-subject variability for $C_{max}$ of molnupiravir about 29.8%, T/R ratio at 95%, significance level at 5%, power at ≥ 85% and bioequivalence limits of 80.00%-125.00% [7-9]. The calculation suggested that the sample size of 43 study subjects were sufficient to establish bioequivalence at the power greater than 85%. However, 58 study subjects were enrolled by estimating 25% dropouts.

The enrolled subjects were healthy Thai males and females at the age between 18 and 55 years, with a body mass index between 18.0 $kg/m^2$ and 30.0 $kg/m^2$. They were estimated to be healthy by assessment of medical history, physical and laboratory examinations. All of them were well informed and gave voluntary written informed consent before participation in the study at International Bio Service Co., Ltd., Golden Jubilee Medical Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. Female subjects were not pregnant or breastfeeding at all time of the study and were instructed to use acceptable birth control methods (non-hormonal method) throughout the study.

Study subjects were withdrawn if they met the study exclusion criteria including history of hypersensitivity to molnupiravir or any excipients of the study products, a recent history or presence of any disease, positive results for COVID-19 RT-PCR test, history of allergic reaction after taking medications, alcohol dependence, drug abuse, cigarette smoking, recent clinical trial participation or recent blood donation within 3 months prior to the start of the study. Consumption of any medication, vitamins, dietary supplement, xanthine containing products or grapefruit/pomelo/orange-based products was restricted prior to dosing and during the study.

### Study design

An open label, randomized, two-treatment, two-period, two-sequence, single oral dose, crossover study was designed to determine the bioequivalence of two molnupiravir 200 mg capsule formulations under fasting conditions. The study was conducted following the study protocol which was reviewed and approved by the Institute for the Development of Human Research Protections, Thailand. Fifty-eight study subjects were enrolled and randomly assigned to two groups, Test-Reference (TR) and Reference-Test (RT) according to the randomization schedule of receiving the product in each period of the study. After at least 10 hours overnight fasting, a capsule of the test or reference product was orally administered to each subject with 240 mL of water, followed by mouth and hand check to assess dosing compliance. The subjects were asked to remain in sitting or ambulatory posture for the first 2 hour after administration. After a washout period of 7 days, the procedure was repeated in the same manner to complete the crossover design. Adverse events were monitored throughout the study based on direct questioning, clinical and laboratory examinations.

### Blood sampling

A total of 21 blood samples were collected from each study subject in each period at pre-dose (0 hour), 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.5, 4, 4.5, 5, 6, 8, 10 and 12 hours post-dose [10]. Approximately 4 mL of blood at each time point was collected through an indwelling intravenous cannula in a forearm vein of the study subjects and transferred into vacutainers containing dipotassium ethylenediaminetetraacetate (K2EDTA) as an anticoagulant, and subsequently centrifuged at 3000 ± 100 relative centrifugal force (rcf) for 5 minutes at 4°C to separate plasma. Each plasma sample was transferred into tube containing buffer solution (formic acid: acetonitrile: water (20:40:40, v/v/v)) at 2% of total plasma volume. The plasma samples were stored upright at -55°C or colder until completion of analysis.

### Study sample analysis and incurred sample reanalysis

The study samples were assayed for N-Hydroxycytidine (NHC), active metabolite of molnupiravir using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) complying with the Principles of Good Laboratory Practice (GLP), in-house Standard Operating Procedures (SOPs), and EMA guideline on bioanalytical method validation [11]. The study samples of the same subject were determined for concentration of NHC in the same analytical run under the calibration range of 1.001-1506.557 ng/mL. NHC and the internal standard ([$^{13}C$, $^{15}N_2$]-NHC) were extracted from plasma using protein precipitation method. Briefly, working solution of 40 ng/mL of the internal standard was added into plasma samples, followed by cold acetonitrile. Thereafter, the samples were centrifuged at 4500 ± 100 rcf for 5 minutes at 10°C. The supernatant of each sample was transferred into pre-labeled tube and subsequently evaporated. The residuals were reconstituted with 0.05% acetic acid in acetonitrile: 0.05% acetic acid in water (1:99, v/v) and transferred into HPLC vials for subsequent analysis.

An ExionLC™ UPLC system coupled with a Triple Quad® 4500 mass spectrometer (AB Sciex, Singapore) was used for study sample analysis. The sample analysis was performed on AQUITY UPLC®

BEH C18 1.7 µm (2.1 mm × 50 mm) analytical column maintained at 30°C. A gradient elution by changing the composition of 0.05% acetic acid in acetonitrile and 0.05% acetic acid in water at a flow rate of 0.35 mL/minute. The transition of precursor to product ion was monitored in positive mode at m/z 260.20 to 128.20 for NHC and m/z 263.20 to 131.10 for the internal standard. Data acquisition and evaluation of chromatographic data was performed using Analyst® version 1.7.0 (AB Sciex, Singapore).

According to EMA guideline on bioanalytical method validation, study samples at the concentrations close to maximum concentration and in the elimination phase of each subject in each period were chosen for incurred sample reanalysis in separate analytical runs. The concentration from incurred samples was not used for pharmacokinetic calculation.

### Pharmacokinetic and statistical analysis

Non-compartmental model of Phoenix WinNonlin software version 6.4 (Pharsight Corporation, USA) was used to characterize the pharmacokinetics of NHC. The maximum plasma concentration ($C_{max}$), area under the concentration-time curve from time zero to the last sampling time point ($AUC_{0\text{-tlast}}$) and area under the concentration-time curve from time zero to infinity ($AUC_{0\text{-}\infty}$) were considered as the primary pharmacokinetic parameters, whereas the time to reach $C_{max}$ ($t_{max}$), elimination rate constant ($\lambda_z$) and half-life ($t_{1/2}$) were considered as the secondary parameters.

The statistical analysis was carried out using PROC GLM of SAS® version 9.4 (SAS Institute Inc., USA). The analysis of variance (ANOVA) was used to determine the effects of the formulation, period, and sequence on ln-transformed primary parameters ($AUC_{0\text{-tlast}}$, $AUC_{0\text{-}\infty}$ and $C_{max}$). The ANOVA model included sequence, formulation and period as fixed effects and subject nested within sequence as a random effect. Sequence effect was tested using subject nested within sequence as an error term. The 90% Confidence Intervals (CIs) for the ratio of the geometric least squares mean (test/reference) of ln-transformed primary parameters were computed. Wilcoxon signed rank test was performed to compare the median $t_{max}$ of the test and reference products. All statistical calculations were executed at a significance level of 5% ($\alpha$=0.05).

## RESULTS

### Demographic characteristics of study subjects

In period I, 58 subjects were enrolled and randomly divided into TR and RT group equally. The mean ± SD of age, height, weight and BMI of enrolled subjects were 36.02 ± 8.87 years, 1.64 ± 0.07 m , 62.37 ± 9.57 kg and 23.07 ± 2.68 kg/m$^2$, respectively. Four subjects were withdrawn by the principal investigator before check-in of period II due to the conditions as per the exclusion criteria. There was one drop-out subject due to personal reason. Therefore, 53 subjects completed the study and their plasma concentration data were used for statistical analysis.

### Study sample analysis and incurred sample reanalysis

All study samples from 58 study subjects were completely analyzed. The correlation coefficient of each analytical run constructed from 8 calibration standards was more than 0.99. Four levels of quality control samples were used to demonstrate the precision and accuracy in each analytical run. The inter-day Coefficient of Variation (CV) and accuracy of quality control samples ranged from 2.0%-2.9% and 98.7%-101.3%, respectively. A total of 190 samples were chosen to establish the reproducibility of the analytical data *via* incurred sample reanalysis, and 96.8% of them had percent difference between the original and reanalyzed concentrations less than ± 20%.

### Pharmacokinetic and statistical analysis

After oral administration, molnupiravir was rapidly converted to NHC in plasma with maximum plasma concentration ($C_{max}$) of NHC achieved within 2.5 hours. The mean $C_{max}$ of NHC was 1277.337 ng/mL and 1194.589 ng/mL for the test and reference products, respectively. The mean $AUC_{0\text{-}\infty}$ of the test product was slightly higher than the reference product. However, in general, the pharmacokinetic parameters of NHC were comparable between the test and reference products (Table 1). The mean plasma concentration-time profiles of NHC for the test and reference products are illustrated in Figure 2.

**Table 1:** Pharmacokinetic parameters of NHC following oral administration of test and reference products.

| Parameters | Mean ± SD, N=53 | |
|---|---|---|
| | Test product | Reference product |
| $AUC_{0\text{-tlast}}$ (ng.hr/mL) | 2951.260 ± 778.195 | 2726.456 ± 655.477 |
| $AUC_{0\text{-}\infty}$ (ng.hr/mL) | 2957.147 ± 780.687 | 2732.124 ± 657.252 |
| $C_{max}$ (ng/mL) | 1277.337 ± 341.214 | 1194.586 ± 287.987 |
| $t_{max}$ (hr, in median (min,max)) | 1.5 (0.75 , 2.5) | 1.25 (0.75 , 2.25) |
| $\lambda_z$ (1/hr) | 0.584 ± 0.100 | 0.559 ± 0.097 |
| $t_{1/2}$ (hr) | 1.237 ± 0.304 | 1.311 ± 0.359 |
| Extrapolated AUC (%) | 0.192 ± 0.086 | 0.205 ± 0.097 |

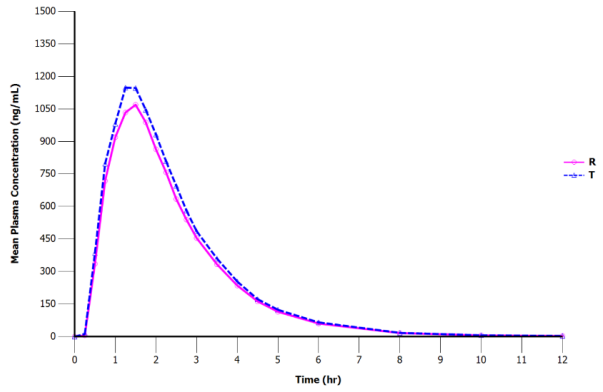


**Figure 2:** The mean NHC plasma concentration-time profiles after administration of the test and reference products (N=53). **Note:** (—) R, (- - -) T.

The data obtained from 53 subjects who completed the entire study were used for statistical analysis and the results are represented in Table 2. The ANOVA showed no significant effects of period and sequence on ln-transformed primary pharmacokinetic parameters, but significant formulation effect was observed on all tested parameters. The 90% CIs for the ratio of the geometric least squares mean (test/reference) of ln-transformed $AUC_{0\text{-tlast}}$, $AUC_{0\text{-}\infty}$ and $C_{max}$ were within the bioequivalence criteria of 80.00%-125.00%. Wilcoxon signed rank test showed insignificant difference in

median $t_{max}$ between the two products (p-value=0.6894).

**Table 2:** Results of statistical comparison of primary parameters of NHC between test and reference products.

| Parameters | Ratio (90% confidence interval) | Power (%) | Intra-subject CV (%) | ANOVA (p-value) | | |
|---|---|---|---|---|---|---|
| | | | | Formulation | sequence | period |
| ln $AUC_{0\text{-tlast}}$ | 107.5 (103.74 -111.46) | 100 | 11.0 | 0.0014 | 0.1798 | 0.6120 |
| ln $AUC_{0\text{-}\infty}$ | 107.5 (103.73 -111.45) | 100 | 11.1 | 0.0014 | 0.1797 | 0.6138 |
| ln $C_{max}$ | 106.0 (101.98 -110.19) | 100 | 11.9 | 0.0148 | 0.2078 | 0.4906 |

## Tolerability

Total of 38 adverse events reported in 30 subjects after oral administration of Molnupiravir. There were 16 adverse events and 22 adverse events reported in the test group and the reference group, respectively as listed in Table 3. All adverse events were mild in intensity but were reported to the Institute for the Development of Human Research Protections, Thailand in a timely manner. The mainly observed adverse events for both products were related to hematologic changes such as an increase or decrease in lymphocyte, neutrophil and white blood cell count. There was no serious adverse event reported in this study.

**Table 3:** List of adverse events.

| Adverse events | Reported incidence (N) | |
|---|---|---|
| | Test product | Reference product |
| Neusea | 1 | 1 |
| Decreased/increased lymphocyte | 3 | 2 |
| Decreased/increased neutrophil | 4 | 1 |
| Decreased/increased white blood cell count | 1 | 4 |
| Increased hemoglobin and hematocrit | 0 | 1 |
| Decreased hemoglobin and hematocrit | 0 | 3 |
| Increased platelet count | 3 | 1 |
| Increased total protein | 2 | 2 |
| Increased albumin | 0 | 3 |
| Increased alkaline phosphatase | 0 | 1 |
| Increased ALT | 0 | 2 |
| Others | 2 | 1 |
| Total | 16 | 22 |

# DISCUSSION

Since molnupiravir is rapidly converted to NHC in plasma thereby making molnupiravir undetectable in plasma sample, the bioequivalence was assessed based on the pharmacokinetics of NHC. The bioequivalence study was carried out under fasting conditions although food effect on $C_{max}$ was reported. However, the extent of absorption was similar, thus molnupiravir can be taken with or without food [10]. It is justifiable to conduct the study under fasting conditions considering that this is the most sensitive condition to detect formulation differences [8]. Pharmacokinetic parameters and profiles were comparable between the test and reference products as demonstrated in the concentration-time profiles of both products. While comparing with the reported data, the pharmacokinetics of NHC in this study was slightly different [7]. The extent of exposure assessed by the $AUC_{0\text{-}\infty}$ and the $C_{max}$ were higher in this study. At the study dose, half-life was approximately 1.2-1.3 hours. As evidenced by undetected concentration in any pre-dose samples of period II, seven days of washout period was sufficient for complete drug elimination.

NHC was not considered as a highly variable compound since the intra-subject variability of primary pharmacokinetic parameters were less than 30% of the CV. With 53 subjects who completed the study, the bioequivalence was established with the power of 100% for all primary pharmacokinetic parameters. Due to overestimating the dropout rate, it might lead to statistical overpower of the study. From ANOVA results, there were no significant effects of period and sequence on the ln-transformed primary pharmacokinetic parameters. However, the ANOVA model showed a significant formulation effect on all primary pharmacokinetic parameters ($p<0.05$). These might be due to low intra-subject variability on each parameter and high number of subjects providing data for statistical comparison. This formulation effect did not interfere in the results of this study since the bioequivalence was concluded by the 90% CIs for the ratio of the geometric least squares mean of ln-transformed $AUC_{0\text{-tlast}}$, $AUC_{0\text{-}\infty}$ and $C_{max}$ which were within the acceptance criteria of 80.00%-125.00% [12]. Moreover, Wilcoxon signed rank test showed no significant difference between two formulations in median of $t_{max}$. The results in this study indicated bioequivalence between two molnupiravir capsule formulations in the terms of rate and extent of absorption.

Based on the prescribing information of the reference product, LAGEVRIO®, the most commonly reported adverse event is diarrhea which was not observed in this study [13]. The hematologic changes observed in this study was not clinically relevant with no further follow-up required. However, it is important to note that the study dose was much lower than the recommended dose at which the adverse events should be monitored carefully. In addition, the tolerability should be further investigated.

# CONCLUSION

The test product, MONOVIR® and reference product, LAGEVRIO® were bioequivalent as evident from the statistical comparison of $AUC_{0\text{-tlast}}$, $AUC_{0\text{-}\infty}$ and $C_{max}$ between these products. The 90% CIs of the geometric least squares mean ratio between the formulations for ln-transformed primary pharmacokinetic parameters were within the acceptance range of 80.00%-125.00%. The test and reference formulations were well tolerated by the study subjects and there were no serious adverse events reported in this study. The bioequivalence study in healthy adult Thai volunteers under fasting conditions supported the use of MONOVIR® as a generic substitute of LAGEVRIO®.

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