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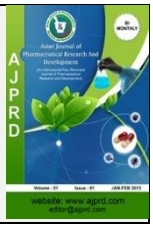

Open Access Research Article

# Randomized, open-label, two-way crossover bioequivalence study of Novirax (Drug International Ltd, Bangladesh) compared with Zovirax (Glaxo Wellcome, UK) – two brands of Acyclovir – in healthy male volunteers

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## ABSTRACT

**Objectives:** A crossover-randomized bioequivalence study of two oral formulations of acyclovir 200 mg tablet was accomplished with 16 adults and physically fit male volunteers from Bangladesh. **NOVIRAX**™ (Drug International Ltd, Bangladesh) was the test variant whereas **ZOVIRAX**™ (GlaxoWellcome) was the reference one.

**Methods:** After a night fasting each volunteer was subjected to take a single dosage of tablet with 150 mL of water. After a week for complete washing out, a second dosage was applied. After that, blood samples were collected at a serial interval of a 24 hours period to observe the plasma concentration by HPLC technique. Several pharmacokinetic parameters like $C_{max}$, $T_{max}$, $AUC_{0\rightarrow 24h}$, $t_{1/2}$, and $K_{el}$ were estimated.

**Results:** The mean (± SD) $AUC_{0\rightarrow 24h}$ for acyclovir of test variant **NOVIRAX**™ for 16 participants was 1057.5 ± 358.9 ng/hr/mL whereas it was 1134.9 ± 467.2 ng/hr/mL for acyclovir of **ZOVIRAX**™. The relative bioavailability (**NOVIRAX**™/**ZOVIRAX**™ ratio) was 93.2%. The $C_{max}$, $t_{max}$, half-life of elimination ($t_{1/2}$) and the rate of elimination ($K_{el}$) of acyclovir of test drug were 207.0 ± 86.9 ng/mL, 2.4 ± 0.4 hours, 3.2 ± 1.8 hour and 0.0898 respectively. The $C_{max}$, $t_{max}$, half-life of elimination ($t_{1/2}$) and the rate of elimination ($K_{el}$) of acyclovir of reference drug were 230.7 ± 107.2 ng/mL, 2.0 ± 0.9 hours, 2.8 ± 1.2 hour and 0.0921 respectively.

**Conclusion:** Depending on these statistical speculations; it was culminated that a **NOVIRAX** tablet is bioequivalent to a **ZOVIRAX** tablet.

**Keywords:** Bioequivalence, Acyclovir, HPLC, Pharmacokinetics, Drug International Ltd.

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## INTRODUCTION

The term bioequivalence (BE) is quite often used in studying the pharmacokinetics of a newly launched drug (generic, test T) with reference to practicing or commercially established one (reference R). If the new drug coincides with the reference pharmacokinetic properties such as $AUC_{0-t}$, $C_{max}$, $t_{1/2}$, $K_{el}$ etc. lie in the range between 80-125%, then the T drug will be said bioequivalent with the R drug. BE also requires similar bioavailabilities, efficacy and safety of the two preparation in the above-mentioned range (80-125%). Typically healthy and physically fit volunteers are chosen for BE experiment in a specially designed trial unit. Mainly in BE trial, the

comparison of $C_{max}$ and $AUC_{0-t}$ are the key features. Those values give an idea of concentration-time profile in the serum for a single dose of drug administration, either the test or reference one. The relative study was attained by performing/knowing the ratio

$$\theta = M_T/M_R \quad \ldots\ldots(1)$$

where, M denotes various distinguished metrics e.g., $AUC_{0-t}$, $C_{max}$, $t_{1/2}$, $K_{el}$ etc.

The statistical analysis of the observed data generates an estimated value, called confidence interval (CI) and it propounds a range of likely values of unacquainted metrics. During this statistical survey clinical trial protocol was followed properly, which is usually formed and regulated by a group of experts of different disciplines.

Acyclovir is a drug of antiviral class [1], usually a synthetic nucleoside analog. It is used to treat cold sores (in mouth), herpes simplex virus [2], shingles and chicken pox, Epstein-Barr virus (EBV). This drug is available in various forms e.g., oral dose, cream and injection and noted efficacious in infectious mononucleosis treatment for which EBV is responsible [3,4]. It does not cure such types of viral infections because these infections keep continuing to alive in the host body. Rather it reduces the symptom by healing the sores faster, keeping new sores from forming and lessens the pain or itching. Actually, it helps by decreasing the infection severity and out breaking time period. It works faster if used just after symptoms arise, delayed treatment may not work well. Generally, doctors recommend using it 2-5 times a day with or without food. Acyclovir was first confirmed in 1981 for medical use [5]. In case of eye herpes, acyclovir is assumed to be less harmful than idoxuridine [6]. It is also reported as safe for breast feeding mothers [7, 8], but advised to avoid breastfeeding if herpes lesions appear near or on the breast [9,10]. In pregnancy, it has no adverse effect [11].

Nausea, vomiting, headache, diarrhea are commonly reported side effects in taking this drug. Besides having an allergy to it, kidney/liver problem or weakened immune system, patients must tell the doctor because rarely it causes life-threatening disorder [12]. Hallucination might take place due to over-dosing. It is advised to complete the prescribed time length even though symptoms may get decreased after taking a few doses. Synergic effect has been observed with ketoconazole, though not clinically proved [13]. Increased half-life and decreased urinary excretion of acyclovir have been reported when administered along with probenecid [14]. Patients need to be cautious about taking acyclovir while receiving IV because of the synergistic effect. Zovirax is the brand name and prototype of acyclovir and available in three different forms [15].

***Objective of the investigation***

To estimate the bioequivalence of a generic product NOVIRAX™ (acyclovir 200 mg per tablet, produced by Drug International Ltd, Bangladesh) with the innovator product ZOVIRAX ™ (acyclovir 200 mg per tablet; GlaxoWellcome, UK) by measuring the plasma concentrations using HPLC, after that to calculate the bioequivalence parameters.

***Protocol***

Randomized, open-label, two-way crossover bioequivalence studies were continued with a washout period of more than 7 days. Sufficient precautions were adopted to avoid any major aberration from the approved protocol.

## MATERIALS AND METHODS

***Study subjects***

We obtained participants with the help of volunteer bank and finally, it was executed through individual counseling. A total of sixteen participants between the age limit of 27.0 ± 5.5 years; height was in the range of 165.2 ± 6.6 cm and the weight was between 54.7 ± 4.1 kg during the screening period. The collection of blood samples from the participants was done in two phases after drug administration. In Phase I**:** 9/10/2012 to 10/10/2012 and 17/10/2012 to 18/10/2012; in Phase II**:** 11/10/2012 to 12/10/2012 and 19/10/2012 to 20/10/2012. Blood specimens were assessed from 6/11/2012 to 14/1/2013.

***Study medication***

Treatment A (generic formulation): NOVIRAX™ Batch No 02, Manufacturing date: February 2012, Expiry date: February 2014; Manufacturer: Drug International Ltd, Bangladesh. Treatment B (reference formulation): ZOVIRAX™ Batch No 5770, Manufacturing date: April 2011; Expiry date: April 2016; Manufacturer: GlaxoWellcome, UK.

***Dosage regimen***

Each of the participants inherited a single dose of both types of treatments following a randomized scheme as per the protocol keeping a washout span of more than 7 days.

***Institutional review board***

The protocol and the ethical aspect of this study were approved by the Institutional review board of Khwaja Yunus Ali Medical College Hospital, Enayetpur, Sirajgonj, Bangladesh, which comprises of 7- members including a lawyer, local religious leader (Imam) and a woman representative. Some minor adjustments were made to finalize the protocol. This trial was carried out heeding the International Conference of Harmonization (ICH). Good Clinical Practice (CGP) regulation was endorsed by the

European Agency for the evaluation of Medicinal Products (EMEA).

***Informed consent***

The motive of the trial was demonstrated to each volunteer in the local language (Bengali) by the medical officer before getting started. Only when they conceded to take part in the trial, each participant signed a consent form after perusing it as a written document. A doctor was deployed to clarify the queries and doubts that arose among the volunteers. A Xerox copy of the signed consent paper was affixed in the protocol.

***Hospital admission***

After performing the screening process, volunteers were taken at the hospital ward (12 bedded and well maintained for such study) 24 hours prior to the study started. The compartment was good enough and facilitated with a color TV for watching various recreational programs and participants also had the option of playing carom and cards. During this trial the total number was divided into two groups, 8 in each were taken into the compartment at a time.

***Drug administration and sample collection***

In this study, each subject was given a single dose of reference or test formulation of acyclovir with 250 mL water following a full night starving (9 h), after 2 h of the dosing they were allowed to drink water, after 4h breakfast was provided and then lunch and dinner keeping a time scheme. Volunteers were under direct medical observation at the study place. The blood samples were taken immediately before and at 0.3, 0.6, 1, 1.5, 2, 3, 4, 8, 12 and 24 hours after administering acyclovir (2 mL in each time). EDTA tube was used to collect sample (blood) and plasma was separated by centrifuging at 4000 rpm about 10 min then the plasma was preserved at –80°C in Eppendorf tube. After seven days, the experiment was performed once again in a similar way to finish the crossover design.

***Chromatographic condition for drug analysis***

We used HPLC equipped with a UV-Visible detector to analyze the plasma concentration of acyclovir. The HPLC was calibrated and prepared prior we started the study accompanying international guidelines [16]. The solvents chosen in the study were HPLC grade; while other chemicals and reagents were of analytical grade.

In this study the HPLC system was from Agilent 1200 series, Germany and it consisted of a degasser, solvent delivery binary pump, autosampler, a column and a diode array detector; ChemStation software was utilized to perform integration. Chromatographic separation was done using C18 column (4.6 mm×150 mm) having particle size 5 µm (Sigma Aldrich). The mobile phase consisted of 98% of 50 mmol sodium dihydrogen phosphate having pH 4.50 and 2% methanol. The flow rate of mobile phase was 0.6 ml/min. The effluent was monitored using a wavelength of 254 ±8 nm. The retention time of acyclovir was 19 min. The limit of detection (LOD) was 3 ng (3SD) whereas limit of quantification (LOQ) was 9 ng (10SD). The $AUC_{0\rightarrow 24h}$ value constructed the extent of absorption of acyclovir, the $C_{max}$ and $t_{max}$ value simultaneously generated the rate of absorption. Half-life of elimination ($t_{1/2}$) and the rate of elimination ($K_{el}$) values of acyclovir also helped in complete mapping of the pharmacokinetic outcome.

***Sample preparation for HPLC injection***

A volume of 100 µL Sodium dihydrogen phosphate buffer (pH 6.5) added to 100 µL plasma and was vortexed for 1 min. After that 1 mL methanol was added and was again vortexed for 3 min and centrifuged for 5 min at 13500 rpm. The supernatant was evaporated by nitrogen gas and water bath at 45°C and the residue was reconstituted with 400 µL mobile phase and were vortexed for 1 min and was injected 20 µLin the HPLC system.

***Statistical analysis***

Pharmacokinetic parameters under the curve ($AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \alpha}$,), peak exposure ($C_{max}$), time-to-peak exposure ($T_{max}$), half-life ($t_{½}$) and elimination rate constant ($K_{el}$) for the two formulations (test and reference products) were calculated by two-way analysis of variance (ANOVA) procedure using Thermo Kinetica 2000 software[17]. The 90% confidence interval was calculated using online software.

***Pharmacokinetic analysis***

The pharmacokinetic parameters $C_{max}$, $T_{max}$, $AUC_{0\rightarrow 24h}$, $t_{1/2}$, and $K_{el}$ were attained. The mean (± SD) $AUC_{0\rightarrow 24h}$ for acyclovir of test drug **NOVIRAX**™ for 16 volunteers was 1057.5 ± 358.9 ng/hr/mL whereas it was 1134.9 ± 467.2 ng/hr/mL for acyclovir of **ZOVIRAX**™. The relative bioavailability (**NOVIRAX**™/**ZOVIRAX**™ ratio) was 93.2%. The $C_{max}$, $t_{max}$, half-life of elimination ($t_{1/2}$) and the rate of elimination ($K_{el}$) of acyclovir test drug were 207.0 ± 86.9 ng/mL, 2.4 ± 0.4 hours, 3.2 ± 1.8 hour and 0.0898 respectively and those of the acyclovir reference drug were 230.7 ± 107.2 ng/mL, 2.0 ± 0.9 hours**,** 2.8 ± 1.2 hour and 0.0921 respectively.

**Table I:** Pharmacokinetic parameters following oral administration of NOVIRAX™ (test) and ZOVIRAX ™ (reference)

| Parameters | NOVIRAX™ | | | | ZOVIRAX ™ | | | |
|---|---|---|---|---|---|---|---|---|
| | mean | SD | 90%CI | | Mean | SD | 90%CI | |
| AUC (ng/hr/mL) | 1057.5 | 358.9 | 96.5 | 103.5 | 1134.9 | 467.2 | 95.7 | 104.2 |
| $C_{max}$ (ng/mL) | 207.0 | 86.9 | 95.7 | 104.3 | 230.7 | 107.2 | 95.2 | 104.8 |
| $T_{max}$ (hour) | 2.4 | 0.4 | 95.7 | 104.3 | 2.0 | 0.9 | 95.0 | 104.9 |
| half-life (hour) | 3.2 | 1.8 | 86.4 | 123.6 | 2.8 | 1.2 | 82.4 | 117.6 |
| $K_{el}$ | 0.0898 | | 86.9 | 113.0 | 0.0921 | | 94.0 | 106.0 |

$AUC_{0\rightarrow 24h}$ = Area under the plasma concentration–time curve from zero hours to 24 hours; $C_{max}$ = maximal plasma concentration; $t_{max}$ = time for the maximal plasma concentration; $t_{1/2}$ = half-life; $K_{el}$ = elimination rate constant.

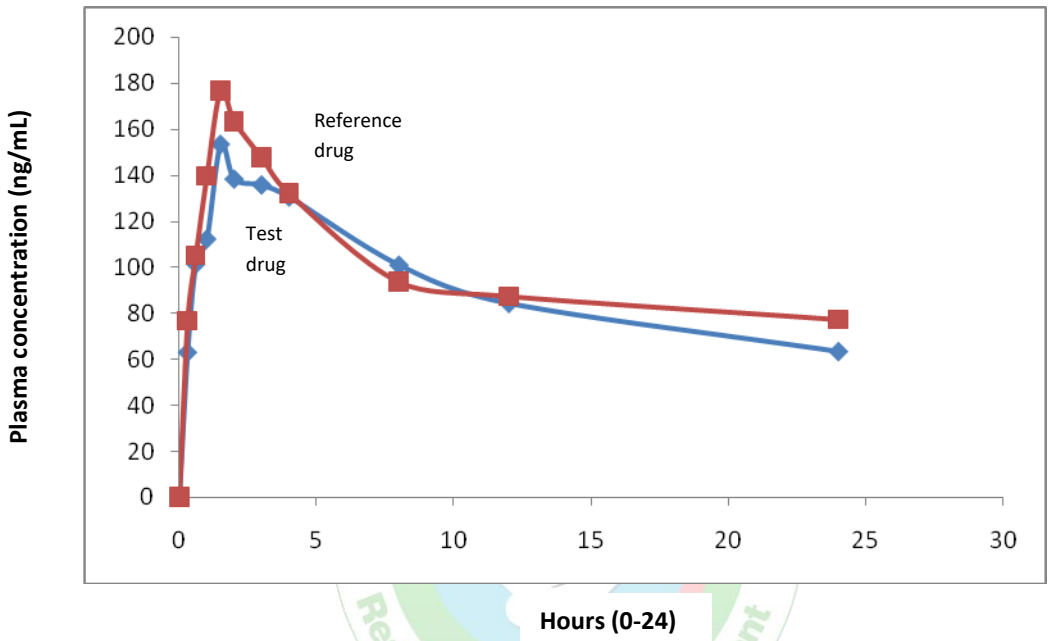


**Figure 1:** Mean plasma concentrations of acyclovir in 16 human volunteers

**Tolerance**

The unit dose of acyclovir (200 mg) of both variants was satisfactorily admissible among the participants.

## RESULTS AND DISCUSSION

To be bioequivalent of any newly launched variant with a reference or practicing product is necessary to eliminate any clinically significant difference in the rate and extent at which the active entity of the drug becomes available at the site of action. The study was confined by the involvement of healthy and fit male volunteers and each volunteer was administered a single dose in the fasted state. The study contained some shortcomings that should be taken into consideration. There was no unpredicted occurrence that could have altered the study result. No discontinuation was allowed and all the participants who took part in the experiment continued till the end and were released from the study center in sound health. The reported analytical method was manifested sensitive and accurate for the determination of acyclovir in plasma. The retention time of acyclovir was 19 min. The plasma assessment methodology was endorsed. The limit of detection (LOD) was 3 ng (3SD), whereas limit of quantification (LOQ) was 9 ng (10SD). Examining the pharmacokinetic properties and bioequivalence of two variants of acyclovir in well and fited Bangladeshi male participants was our main purpose. Comparable pharmacokinetic properties between the two forms were mirrored from the obtained data and calculation [18]. Acyclovir was readily absorbed for both formulations from the gastrointestinal tract and acyclovir was quantifiable at the first sampling time (0.33 h) for most of the volunteers. The mean concentration-time profiles of the study is shown in Figure 1 and indicating that the mean plasma drug concentration profiles of the two formulations were nearly similar. Peak concentrations maximum were achieved at 2.0-3.0 h after drug administration and then diminished reasonably but the acyclovir was noticeable until the last blood sample. All calculated pharmacokinetic parameters were in good concurrence with reported values. Table 1 shows the pharmacokinetic parameters for two studies. The extent of absorption is a key characteristic of a drug formulation, and therefore AUC, Cmax and Tmax are important parameters for bioequivalence study and could affect the therapeutic use of a drug [19]. The relative

bioavailability (**NOVIRAX**$^{TM}$/**ZOVIRAX**$^{TM}$ ratio) was 93.2%. The $C_{max}$, $t_{max}$, half-life of elimination ($t_{1/2}$) and the rate of elimination ($K_{el}$) of acyclovir of test drug were 207.0 ± 86.9 ng/mL, 2.4 ± 0.4 hours, 3.2 ± 1.8 hours and 0.0898 respectively. The $C_{max}$, $t_{max}$, half-life of elimination ($t_{1/2}$) and the rate of elimination ($K_{el}$) of acyclovir of reference drug were 230.7 ± 107.2 ng/mL, 2.0 ± 0.9 hours**,** 2.8 ± 1.2 hours and 0.0921 respectively. The mean and standard deviation of $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \alpha}$, Cmax, Tmax, $t_{1/2}$ and Kel of the two products did not differ remarkably. They recommend that the blood profiles produced by Novirax are comparable to those produced by Zovirax. Analysis of variance (ANOVA) for these parameters manifested no statistically significant variance between the two sorts. 90% confidence intervals also demonstrated that the ratios of $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \alpha}$, Cmax, Tmax, $t_{1/2}$ and Kel of the two formulations lie within the FDA acceptable range of 80%–125%.

## CONCLUSION

NOVIRAX$^{TM}$ (test) is bioequivalent to ZOVIRAX$^{TM}$ (reference) supported by the rate and extent of absorption and can be deployed alternatively in a clinical approach. The 90% confidence interval for the NOVIRAX$^{TM}$ (test) and ZOVIRAX$^{TM}$ (reference) satisfied the acceptance range of 80–125%.

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