ABSTRACT

The bioequivalence of metronidazole tablet 200mg reference (Flagyl®) and another brand of metronidazole 200mg (Brand AB) were compared in 6 healthy male volunteers. With mean aged of 23.5±2.0 years and mean body weight of 65.5± 4.5kg. A randomized cross-over design with two weeks wash-out period between each dose of 400mg oral dose was applied. Salivary sample before dosing and at various appropriate time interval of 15 mins, 30 mins, 1, 2, 3, 4, 5, 6 hours were analyzed by validated double beam U.V. spectrophotometer method, with 96.1% extraction recovery. Pharmacokinetic parameters for bioequivalence evaluation $C_{\text{max}}$, $T_{\text{max}}$ and AUC were determined, the values of reference tablet. Flagyl® was $C_{\text{max}}$ 7.20 ±0.58 μg/ml, $T_{\text{max}}$ 65.00 ±0.49 mins and AUC 69.50± 1.15μg/ml/hr. Brand AB (test) was $C_{\text{max}}$ 7.00 ± 0.43 μg/ml, $T_{\text{max}}$ 70.00±0.41 mins and AUC 65.50±0.89 μg/ml/hr. The test Brand AB over reference ratios of the pharmacokinetic parameters obtained was found to be within bioequivalence acceptable range of 0.8 – 1.25, indicating (Brand AB) was bioequivalent to Flagyl® (Innovator brand).

Key words: Bioequivalence, pharmacokinetics, metronidazole

INTRODUCTION

The Bioequivalence of two formulations of the same drug denotes equivalence with respect to their rate and extend of absorption, the Area Under Concentration time curve (AUC) generally serves as the indicator for extent of absorption while peak concentration ($C_{\text{max}}$) and the time of its occurrence ($T_{\text{max}}$) reflects the rate of absorption.

With the phenomenal increase in the availability of generic drug e.g. of metronidazole in recent years the issues of bioavailability and bioequivalence have received a greater attention, in order for a drug product to be interchangeable with the innovator brand it must be bioequivalent to the brand name product.

Bioequivalence is usually assessed by a single in-vitro study in healthy volunteers (Donald et al 2003). Thus, the two predominant issues involved in the assessment of bioequivalence are the pharmacokinetic parameters that best characterize the rate and extent of absorption and the most appropriate method of statistical analysis of the data. Since the AUC is directly proportional to the amount of drug absorbed, this pharmacokinetic parameter is most commonly used to characterized the extent of absorption, the choice of an appropriate pharmacokinetic characteristic for the rate of Absorption is still being discussed however, the most commonly used parameters are peak concentration ($C_{\text{max}}$) and time to peak concentration ($T_{\text{max}}$) (Donald et al 2003).

Metronidazole (2-(2-methyl-5-nitro-1 – imidazole 1-yl) ethanol) is a nitromidazole antimicrobial drug. Chemically it is $C_6H_9N_3O_3$ with a molecular weight of 171.2. Metronidazole is completely and promptly
absorbed after oral intake, reaching concentrations in plasma to about 6 to 12 μg/ml after a single oral dose of 250 and 500mg (James et al, 2001) respectively. It is widely distributed, appearing in most of the body tissues and fluids and less than 20% of the drug is bound to plasma proteins. The half-life of metronidazole in plasma is about 8 hours, and the volume of distribution is approximately that of total body water. The liver is the main site of metabolism by side chain oxidation and glucuronide conjugation. Majority of the dose of the drug is excreted in the urine, largely as metabolites. (James et al 2001).

The purpose of this study was to evaluate bioequivalence of generic brand of metronidazole (Brand AB) with innovator brand Flagyl®.

MATERIALS AND METHOD

Study Products
The test formulation was Brand AB (200mg metronidazole tablet) Batch No. SGE- 008, expiry date February, 2008, and the reference product Flagyl® tablet Batch No. IV 1014, expiry date August, 2010 manufactured by Aventis Pharma.

Study Subject
Six healthy male adult volunteers (Age 23.5±2.0 years and weight 65.5±4.5kg) participated in this study at Faculty of Pharmaceutical science, ABU, Zaria, Nigeria. The volunteers were non-smokers and non-drinkers and were free from liver disease. They were instructed to abstain from taking any drugs, for 2 weeks prior to and during the study.

Drug Administration and Saliva Collection
After an overnight fast, the volunteers were made to rinse their mouth, and (chew gums) to stimulate saliva secretion. Volunteers were then administered a single oral dose (400mg) of reference tablet is about 100m of water (phase 1) no food was allowed until three (3) hours after dose administration. About 5m of saliva sample was collected at time 00hrs 15 min, 30 min 1, 2, 3, 4, 5, and 6 hours respectively. Samples was then stored in refrigerator at -4°C before analysis. After a wash out period of 2 weeks (phase 2) the (test) tablets was administered to same volunteers and sample was collected at same time intervals and then store in refrigerator at -4°C before analysis.

METHOD

In-vitro studies identification test, Assay, disintegration, and dissolution test were performed according to (Bp 2002) required. Analytical method by Double beam U.V spectrophotometer was developed and validated for both within-day and between-day precision, using a workable λmax of 277nm with acetonitrile as solvent.

The Calibration curve was linear between 10 μg/ml-50 μg/ml prepared by serial dilution from stock lmg/ml of metronidazole standard powder and correlation – coefficient was (r=0.978). The method of extraction with 96.1 percent extraction recovery indicated reproducibility for intended application.

Extraction Procedure
Sampled collected was extracted and analysed by Double beam UV spectrophotometer pharmacokinetic parameters for bioequivalence studies Cmax and T_max was generated by residual method using mean concentration values while the AUC, was determined by Tripezoidal rule. The bioequivalence ratio of test/reference was determined and compared with the acceptable range of 0.8 - 1.25 levels of bioequivalence.

RESULT

From the results of in-vitro identification test, weight variation, Assay, disintegration rate test and dissolution rate tests in O.IN HcL medium at temperature of 37°C± 0.5°C (as specified by B.P 2002) indicated that both the (Brand AB) and the reference drug passed the tests. The mean saliva concentration time of Flagly® and the Brand AB are shown in figure IThe mean salivary concentration – Time profile of Flagyl® and Brand AB (Fig.1).
Table 1: Mean Pharmacokinetic parameters and SEM (n = 6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Flagyl®</th>
<th>Brand AB</th>
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</thead>
<tbody>
<tr>
<td>C_max (ug/ml)</td>
<td>7.20±0.58</td>
<td>7.00±0.43</td>
</tr>
<tr>
<td>T_max (Mins)</td>
<td>65.00±0.49</td>
<td>70.00±0.41</td>
</tr>
<tr>
<td>AuC_{0→6} (ug/ml/hr)</td>
<td>69.93±1.15</td>
<td>65.50±0.89</td>
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The evaluation of the pharmacokinetic parameters with respective point estimate of the ratio of test and reference are given in table 2 with the acceptable range of 0.8 – 1.25.

Table 2: Bioequivalence Ratio of Test and Reference

<table>
<thead>
<tr>
<th></th>
<th>C_max</th>
<th>AUC</th>
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<tbody>
<tr>
<td>Brand AB (Test)</td>
<td>7.00±0.43</td>
<td>65.50±0.89</td>
</tr>
<tr>
<td>Flagyl® (Reference)</td>
<td>7.20±0.58</td>
<td>69.93±1.15</td>
</tr>
<tr>
<td>point estimate of ratio</td>
<td>0.972</td>
<td>0.937</td>
</tr>
<tr>
<td>Conclusion</td>
<td>Bioequivalent</td>
<td>Bioequivalent</td>
</tr>
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</table>
DISCUSSION

From the in-vitro studies result of identification, assay, weight variation, disintegration; and dissolution tests, as specified by BP 2002. The two brands had passed the test indicating pharmaceutical equivalence giving the opportunity of initiating bioequivalence trial.

The in-vivo study conducted showed that the values of (test brand) C_{max} 7.00±0.43 and (reference) C_{max} 7.20±0.58 with point estimate ratio of 0.972 was within the bioequivalence acceptable range of 0.8 – 1.25 and the values of (test brand) AUC 65.50±0.89 and reference AUC 69.93±1.15 with a point estimate ratio of 0.937 was equally within the bioequivalence acceptable range of 0.8 – 1.25 which indicates Brand AB was bioequivalent to reference Flagyl®.

In a bioequivalent evaluation comparing two brands of metronidazole 250mg tablets, the ratio of the test over the reference (Flagyl®) was calculated to be within the acceptable limit of 0.80 – 1.25. It was therefore concluded that the generic metronidazole tablet was bioequivalent to flagyl (Jaber et al., 2006). In another bioequivalence evaluation of two brands of Aceclofenac tablets (100mg) (Aceclofar and Bristaflam) a non – steroidal anti inflammatory drug, the pharmacokinetic parameters comparing the two brands indicated insignificant difference i.e. Bristaflam is bioequivalent to Aceclofar (Najib et al., 2004). Also in a bioequivalence study comparing a new paracetamol solution for injection and proparacetamol after single intravenous infusion in healthy subjects the pharmacokinetic parameters were found to fall within the range of 0.8 - 1.25 which showed that proparacetamol is bioequivalent to paracetamol (Luthy et al., 2004).

CONCLUSION

Based on the pharmacokinetic parameters and statistical analysis, in the bioequivalence studies, it was found that Brand AB showed no significance difference with the reference drug (Flagyl®). Both the generic and reference products may be considered interchangeable in medical practice.

REFERENCES


