

Human bioavailability studies demonstrates interchangeability between brands of pefloxacin tablets marketed in Nigeria having wide price margins

A Ogbonna, K Chukwu, C Esimone, B Ogbonna, C Ogbonna

Citation

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Abstract

Objective: Several brands of pefloxacin tablets with very wide price margins are prevalent in many developing countries. We set out here to evaluate whether three popular brands (peflotab, peflaxcin-400 and paflox-400) (average cost, USD 2.4 per sachet) are bioequivalent with the innovator brand, peflacin (average cost, USD 10 per sachet). **Methods:** Using the Latin Square cross-over design, twelve subjects were randomly assigned into four groups (three subjects per group). Subjects in each group received one tablet each of a particular brand of pefloxacin (each brand contains 400 mg pefloxacin) followed by a 10-day washout period before cross-over. Urine was collected at various intervals up to 42 hours postdosing and the pefloxacin content measured. The Fischers Least Significant Test was used to evaluate differences in urinary pharmacokinetic parameters between subjects receiving the different brands. **Result:** While the cumulative quantity of pefloxacin excreted within 42 hours and the maximum excretion rate of the less expensive brands (peflotab, peflaxcin-400 and paflox-400) were not significantly different from that of the expensive innovator brand, peflacin ($P>0.05$), only the Time for maximum urinary excretion rate for peflaxcin-400 was significantly different from that of the innovator brand (22 h for peflaxcin -400 and 6 h for peflacin). **Conclusion:** The three tested brands are bioequivalent and therefore interchangeable with the innovator brand, although they are more than 4 times cheaper. For developing countries, it is important to promote the use of cheaper alternative antibiotics that are bioequivalent with the often more expensive inovator brands to avoid non-compliance with prescribed medication as a result of non-affordability by the patients.

INTRODUCTION

It is often assumed that the more expensive brands of drugs are the most effective. This belief, coupled with the aggressive promotional strategies adopted by most multinational drug companies makes prescription of their often expensive branded drugs more or less the “only” choice for medical practitioners (1,2). This could result in patients not complying with the prescription because they cannot afford the drug (3,4). In most third world countries such as Nigeria, this has led to the misuse or abuse of antibiotics (5,6) because patients usually resort to alternative cheaper antibiotics (different from the class prescribed) or to reduced doses of the prescribed drug.

Overwhelming evidences relating to the influx of counterfeit and fake drugs, especially antibiotics from China and India into many developing and developed countries (7,8,9,10) has necessitated the generalisation that such cheap multisource antibiotics are substandard. However, recent empirical

invitro-based equivalence studies involving multisource ciprofloxacin (11,12), sulfadoxine-pyrimethamine combinations (13) and metformin (14) in Nigeria has revealed that some of the cheaper multisource drugs are indeed equivalent and hence interchangeable with the often expensive innovator drugs. Risha et al (15) have also shown that seven multisource ciprofloxacin tablets marketed in Tanzania were interchangeable with the inovator brand (Ciproxin, Bayer) based on in vitro studies. A selected brand was also shown to be bioequivalent with the innovator after an oral bioavailability study. The World Health Organisation (WHO) recognises that generics may be more adequate and accessible to developing nations because of their significantly lower costs, provided they are of good quality. Accordingly, the WHO has issued guidelines for global standards and requirements for the registration, assessment, marketing, authorization and quality control of generic pharmaceutical products (16).

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Pefloxacin is a synthetic bactericidal antibiotic, which belongs to the fluoroquinolones. It has a wide spectrum of activity, including *Escherichia coli* and various species of salmonella, shigella, enterobacter, *Neisseria* and camphylobacter (17). Clinically, it is employed in treating urinary tract infection, prostatitis, sexually transmitted disease, gastrointestinal and abdominal infection, respiratory tract infection, bone, joint and soft tissue infection, tuberculosis and AIDS-related opportunistic bacterial infections. Although several brands of pefloxacin are available in Nigeria, "Peflacin" (Rhone Poulenc) has almost become the traditional name for pefloxacin prescribed by medical practioners in Nigeria. While peflacin has demonstrated extensive therapeutic benefits in the treatment of severe bacterial infection, its high cost may deter patients from utilizing it. We therefore decided to carry out bioequivalence studies in order to sort out the cheaper brands of pefloxacin that are bioequivalent and hence interchangeable with the innovator brand, peflacin.

MATERIALS AND METHODS

CULTURE MEDIA

The following media were prepared as specified by the manufacturers:

Muller- Hinton Agar, Nutrient agar and Nutrient broth (oxoid).

DRUGS

Four brands of film coated pefloxacin tablets (400mg) marketed in Nigeria were purchased from Pharmacy shops in Ebonyi State, Nigeria. Details of each brand are presented below:

Figure 1

Table 1: Country of origin, manufacture and batch number of pefloxacin brands.

Brand Name	Manufacturer	Batch Number
Peflacin	Rhone Poulenc, France	105
Peflotab	Vs International, Mumbai, India	VPF-106
Peflaxcin-400	Vardhman Export, Mumbai, India	KD-271
Paflox-400	Milan Labs, Panvel, India	ME11-289

SELECTION OF SUBJECTS

Twelve healthy, adult male volunteers between the ages of 20-35years who were within 10% of their ideal body weight were selected for the study. Informed signed consent was

obtained from each subject. Prior to initiation of the study, the subjects were given thorough physical examination and their medical history taken. Subjects were not permitted to take any drug two weeks before trials and during the trials.

STUDY DESIGN

The experimental design employed to determine relative bioequivalence of the four brands of pefloxacin tablet was the Latin square crossover design. Twelve subjects were randomly assigned to four different group (three per group). Each group received a particular treatment (brand). The treatments were separated by a 10-day washout period and the design was balanced over weeks.

The Volunteers were fasted overnight prior to and for 3h immediately after administration of a 400mg pefloxacin tablet. No beverage such as coffee, milk or diet drink was permitted during the fasting periods. The tablet was administered with 300mls of water. An additional 100ml of water was given each hour for the first three hours after dosing. The subjects were ambulatory for the first twenty-four hours treatment, and for the remaining 18hours were permitted to proceed with their normal daily routine in so far as possible considering their availability for urine collection. The subjects were not permitted to engage in any strainous or athletic activities on the period of this study.

URINE SAMPLING AND ASSAY OF DRUGS

Urine samples were collected (total void) at 0-4, 4-8, 8-12, 12-16, 16-20, 20-24, 24-36 and 36-42 hours following drug administration. The total volume of each interval was recorded and a 10ml sample was frozen until assay.

The microbiological assay was used for the analysis of the urine samples. Molten Mueller-Hinton Agar (MHA) seeded with a standardized inoculum (0.5 MacFarland Standard) of *E. coli* ATCC 11775 was allowed to solidify. Thereafter, 5 mm holes were bored on each MHA using a sterile cork borer. Various concentrations of a standard solution of pefloxacin (31.25-1000µg/ml) and the various urine samples obtained from each subject at different intervals were randomly introduced into different holes (40 µl per hole). After allowing for 30 minutes pre-diffusion at room temperature, the plates were then incubated at 37°C for 24 hours. The inhibition zone diameters (IZDs) were measured. The IZDs of the standard was used to construct a dose-response plot from which the concentration of pefloxacin in each urine sample was extrapolated by fitting their respective IZDs into a regression equation derived from the

standard dose-response plot.

PHARMACOKINETIC CALCULATIONS

To analyse the urine concentration data, we assume that pefloxacin kinetics after oral administration could be described by a one compartment open model with linear kinetics. The concentration time data for the study period for 42 hrs after completion of the pefloxacin administration can be expressed in equation one.

$$C = C_0 \cdot e^{-kt} \dots\dots\dots 1$$

Where C is the concentration at time t, C₀ is concentration when t=0

For each subject, a plot of the cumulative amount of pefloxacin excreted in urine, against sampling time was constructed. The amount of pefloxacin recovered in each of the tests was calculated from the cumulative urinary excretion and % recovery determined. The cumulative amount of pefloxacin excreted was obtained by adding the amount of drug excreted up to that sampling time. The excretion rate for each subject was calculated using equation 2.

$$\text{Excretion rate} = dA_e / dt \dots\dots\dots 2$$

Where A_e is the cumulative amount of pefloxacin excreted at each sampling time.

T = sampling time.

Other pharmacokinetic parameters calculated include time for maximum excretion rate and % drug recovery.

STATISTICAL ANALYSIS

The results are expressed as means +/- standard deviation. The Urinary pharmacokinetic variables after the various treatments (administration of the different brands of pefloxacin) were compared by the Fischers Least Significant Test. Differences were regarded as significant if p< 0.05.

RESULTS AND DISCUSSION

When drugs are administered orally and assessed by urinary collection method, pharmacokinetics features of interest include cumulative quantity excreted in urine over time, maximum excretion rate and time for maximum excretion rate (18, 19). Accordingly, graphs of the cumulative amount of drug excreted in urine versus time and the urinary excretion rate versus time for peflacin, peflotab, peflaxcin-400 and paflox-400 are presented in Figures 1 and 2 respectively.

Figure 2

Figure 1: Cummulative Quantity Excreted Versus Midpoint Time of Pefloxacin

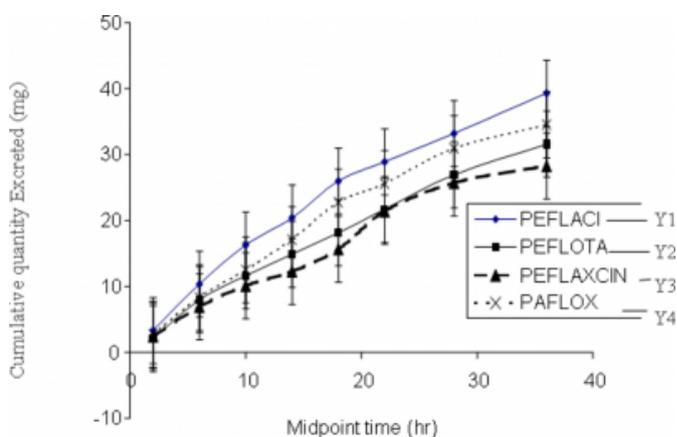
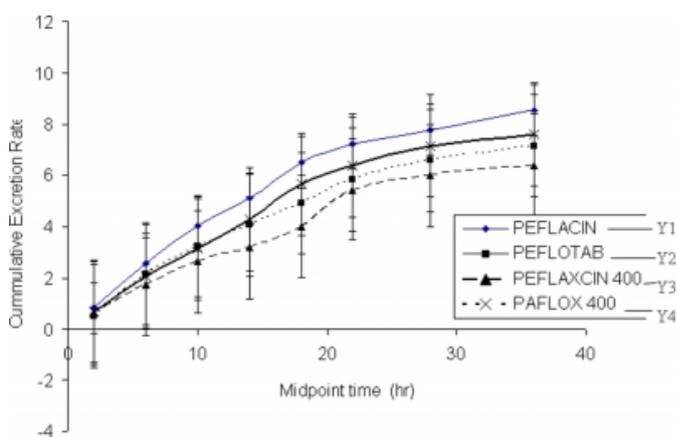


Figure 3

Figure 2: Graph of cummulative excretion rate versus Midpoint time for Pefloxacin



A summary of these relevant urinary pharmacokinetic parameters derived from these graphs for peflacin, peflotab, peflaxcin-400 and paflox-400 is presented in Table 2.

Figure 4

Table 2: The summary of the bioavailability data of the four brands of pefloxacin derived from invivo study

PARAMETER	Peflacin	Peflotab	Peflaxcin-400	Paflox-400
Cumulative Qty. excreted in urine for 42 hrs. (mg)	39.30	31.56	28.86	34.56
Maximum urinary excretion rate (mg/hr)	1.75	1.65	1.43	1.39
Time for maximum urinary excretion rate (hr)	6	6	22	6

The average cumulative quantity of pefloxacin excreted in the urine was 39.3, 31.56, 28.8 and 34.56 mg for subjects receiving peflacin, peflotab, peflaxcin-400 and paflox-400 respectively. This translates to about 9.83, 7.9, 7.2 and 8.6 %

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recovery respectively. These values, although lower than those reported by Naber et al (20), could be said to be consistent with our experimental protocol. Naber et al reported 34 % recovery when 800 mg of pefloxacin was administered over a 6 day urinary sampling period. On the other hand, we used half of this dose (400 mg) with a 42 hour (about 4 half lives) urine sampling period. This was because subject compliance to the study after 48 hours was generally very difficult; this is a well known limitation in bioavailability studies. For each brand, the urinary excretion data obtained from the various subjects (Table 3) was subjected to statistical analysis using the Fischers Least Significant Test, to assess whether there were significant differences in the percentage recoveries of the drug within and across subjects.

Figure 5

Table 3: Cumulative quantity excreted (mg) and their corresponding % recovery per subject

SUBJECT	Peflacin	Peflotab	Peflaxcin-400	Paflox-400
A1	9.12 (2.295%)	22.93 (5.73%)	47.87 (11.98%)	24.45 (6.11%)
A2	22.86 (5.71%)	22.88 (5.71%)	73.15 (18.29)	18.76 (2.19%)
A3	12.81 (3.20%)	34.78 (8.70%)	45.64 (11.41%)	15.25 (3.81%)
A4	23.59 (5.79%)	16.09 (4.02)	16.88 (4.22%)	39.80 (9.95%)
A5	25.62 (6.41%)	22.16 (5.54%)	25.56 (6.44%)	38.63 (9.66%)
A6	18.58 (4.65%)	19.47 (2.37%)	38.88 (10.26%)	93.71 (23.43%)
A7	47.12 (11.78%)	20.10 (5.02%)	15.65 (3.91%)	45.62 (11.41%)
A8	75.14 (18.79%)	11.81 (2.95%)	11.69 (2.92%)	77.05 (19.26%)
A9	66.50 (16.63%)	20.77 (5.19%)	6.04 (1.51%)	42.95 (10.74%)
A10	48.92 (12.23%)	68.19 (17.05%)	28.71 (7.18%)	11.11 (2.78%)
A11	70.96 (17.74)	85.15 (21.29)	19.35 (4.34%)	16.50 (4.13%)
A12	50.28 (12.57%)	48.12 (12.03%)	13.93 (3.48%)	6.56 (1.64%)

Result shows that there were statistically significant inter-subject differences ($P < 0.05$) in urinary recoveries. Even though Latin square crossover design minimizes the subject/subject variability in bioavailability studies, variability cannot be said to be completely eliminated. This variability can be said to be inherent in human subjects and results from inter-subject differences in human GIT physiology, rate of distribution, excretion and metabolism of drug. Further statistical comparison of the brands using Fishers least significance test ($P < 0.025$) shows that there was no statistically significant difference among the brands. Therefore, the studied brands could be said to be bioequivalent to the innovator brand (peflacin) and therefore interchangeable. The urinary excretion rate profile is presented in Figure 2. The maximum urinary excretion rate generated from this plot was shown to be 1.75, 1.65, 1.43 and 1.39 mg/hr for peflacin, peflotab, peflaxcin-400 and paflox-400 respectively (Table 2). Again, subjecting the data from the various subjects to statistical analysis revealed considerable inter-subject variation. However, no

statistically significant difference among the brands was observed ($P < 0.025$). This again confirms the studied brands are bioequivalent and hence interchangeable.

A slightly varied pattern was observed when the time for maximum excretion rate for the tested brands was evaluated. While peflacin, peflotab and paflox-400 peaked at 6h, peflaxcin-400 peaked at a significantly higher time of 22h. It shows that with peflaxcin-400, a significantly longer time was required for pefloxacin to accumulate in the body. This could possibly be due to the type of film coating materials employed in the manufacture of the tablets. Some film coating materials take longer time to dissolve in the GIT, with the result that the release of drug from the dosage form and absorption may be delayed. We also observed that although the maximum excretion rate of peflaxcin-400 (1.43mg/hr) is higher than that of paflox-400 (1.39mg/hr), the cumulative quantity excreted for paflox-400 (34.56mg) is higher than that for peflaxcin-400 (28.86mg). This could be due to the longer time it took brand peflaxcin-400 to reach maximum excretion rate (22 h). However, it appears this long time does not negatively affect its bioavailability since the other pharmacokinetic parameters were not statistically different from that of the other brands.

While the average retail price of the innovator brand (peflacin) is approximately \$10 per sachet, the average retail price of the tested brands is about \$2.4 per sachet, reflecting a more than 4-fold difference in price. The price of medicaments is a principal factor that affects patient compliance and hence encourages drug abuse and /or misuse in many developing countries (4,5,6). Although cheaper drugs, especially from Asia have consistently been shown to be substandard (8,9,10), there is still need to characterise multisource drugs prevalent in any local market to ascertain whether cheaper and bioequivalent brands exist. Risha et al (15) demonstrated that a cheaper generic brand of Ciprofloxacin (U.S.\$ 0.09 per tablet) from India was bioequivalent with the innovator brand (Ciproxin, Bayer) which costs U.S.\$ 2 per tablet. They concluded that many times, it is wrongly assumed that the cheaper generic products have been produced without a careful preformulation study or using inferior additives. Therefore, our observation here that the cheaper brands (also from Asia) are bioequivalent with the innovator brand further highlights the need to really characterise multisource tablets and make the findings available to the National Health Authorities for onward dissemination to all health

institutions nation-wide. This will greatly harmonize the prescription pattern of such brands and thus enhance patient compliance in cases where high prices hinder compliance. The need to regularly test multisource drugs is further necessitated by the fact that biopharmaceutical properties of most drugs may be adversely affected by the tropical climatic conditions of developing countries (21, 22). In fact, the influence of climatic conditions in tropical countries on the quality of essential drugs has been of concern to the WHO, which has accordingly recommended that such drugs be tested for stability under class IV conditions (40°C, 75% relative humidity) (15, 22). Earlier studies on the interchangeability of multisource fluoroquinolones (11, 12) or other tablets (13, 14) in Nigeria have mainly been based on in vitro dissolution testing. Although in vitro dissolution testing has been shown to be a valuable predictor of the in vivo bioavailability and bioequivalence of oral solid dosage forms (17, 23), the need for an in vivo confirmatory bioequivalent /bioavailability study can never be over-emphasized.

CONCLUSION

The present study has utilised a simple in vivo design, based on urinary pharmacokinetics to demonstrate interchangeability between peflotab, peflaxcin-400, paflox-400 and peflacin. These multisource pefloxacin film coated tablets are more than 4 times cheaper than the innovator brand, peflacin. For a developing country like Nigeria, it is important to promote the use of cheaper alternative antibiotics that are bioequivalent with the often more expensive innovator brands to avoid non-compliance with prescribed medication as a result of non-affordability by the patients.

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Author Information

A. Ogbonna, M. Pharm.

Department of Pharmaceutical Services, Federal Medical Centre

K.I. Chukwu, Ph.D.

Dept. of Pharmaceutical Technology and Industrial Pharmacy, University of Nigeria

C.O. Esimone, Ph.D.

Dept. of Pharmaceutics, University of Nigeria

B.O. Ogbonna, M.Sc.

Dept. of Clinical Pharmacy, College of Medicine, University of Lagos

C.I.O. Ogbonna, B.Pharm.

Dept. of Pharmacology and Toxicology, University of Nigeria